Title: Local muscle pressure stimulates the principal receptors for proprioception

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ABSTRACT

Muscle spindles are considered the principal receptors for proprioception, sensing stretch and signaling limb position and velocity. Here, we show that local muscle pressure is an adequate stimulus for human spindles in isometric conditions, and that pressure enhances spindle responses during stretch, as hypothesized due to compression of the spindle capsule. Our findings redefine basic muscle feedback and urge reassessment of proprioception's role in sensorimotor function and various neuromuscular conditions.

MAIN TEXT

It is well established that intramuscular fluid pressure (IMP) increases with contractile force and passive muscle elongation[1-3]. IMP has proven a better index of muscle force than muscle electrical activity (Electromyography: EMG), especially so in the case of eccentric contractions, muscle fatigue and recovery[4,5]. It is also known that single motor unit activity involves sharp increases in pressure within the motor unit territory, with no effect at more distant sites in the muscle, and that IMP influences the degree of actively produced muscle force[6]. It follows that access to sensory feedback concerning muscle pressure gradients would empower more robust sensorimotor function, such as by directly incorporating the muscle's fluidic behavior in segmental reflex arcs and more global muscle activation strategies[7]. However, with regard to skeletal muscle proprioception, it is generally believed that the nervous system receives major sensory feedback only about stretch and global tension via muscle spindles and Golgi tendon organs, respectively (e.g., [8,9]).

In the early 1960's, Bridgman and Eldred put forward a hypothesis that the secondary ending of the muscle spindle organ may be able to function as a pressure-sensing mechanism[10]. Specifically, they argued that pressure acting on the expanded equatorial area of the spindle organ will compress and elongate the fluid-filled capsule, stretching the intrafusal fibers terminating inside the capsule (the more numerous "nuclear chain" fibers[11,12]), and stimulating the spindle receptor ending. However, there has been no systematic investigation of the ability of spindles to encode locally applied pressure *in vivo*. Here, we assess an updated version of the original hypothesis, namely that both primary and secondary spindle receptors of awake humans are affected by local muscle pressure (i.e., both endings engage nuclear chain fibers[13]). We show that local pressure is an adequate stimulus for spindles in isometric conditions, and that pressure enhances spindle responses during stretch.

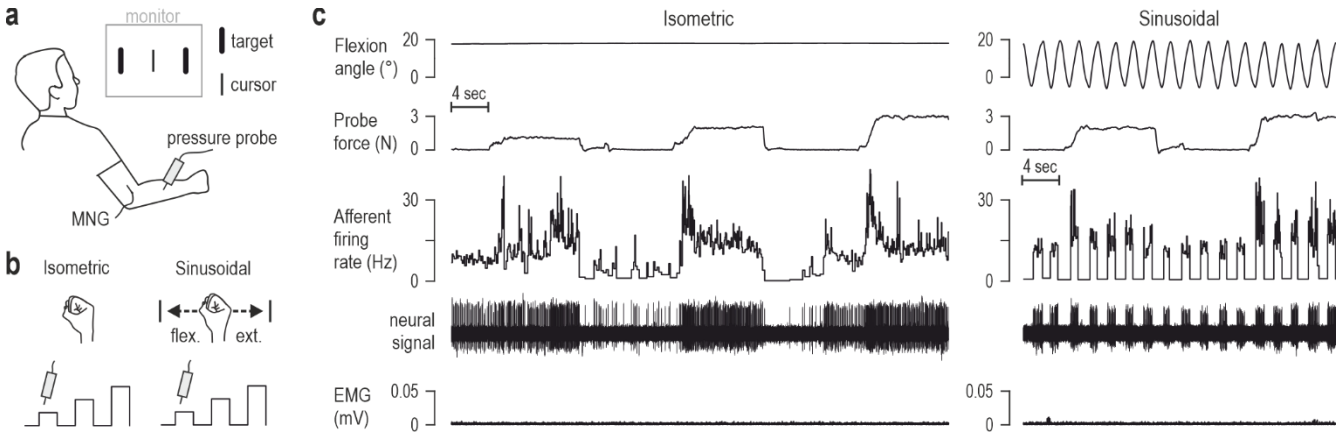

Fig 1. Experimental set-up and exemplary spindle afferent responses.

a) Microneurography (MNG) was used for recording from muscle spindle afferents (n=14) in the right radial nerve of 8 awake individuals, while a probe applied pressure on a small part of the forearm (circular tip, 5mm diameter; see Methods section for more details). A kinematic sensor attached to the dorsum of the participant's hand tracked movement about the wrist in the flexion-extension dimension. Surface EMG electrodes recorded muscle activity of wrist and finger extensor muscles from which the recorded spindle afferents originated. A monitor displayed two immobile visual targets and a cursor that tracked hand movement. **b)** The experimental task involved two conditions. In the first condition ('isometric'), the semi-pronated hand was immobile whereas in the 'sinusoidal' condition the experimenter moved the participant's hand continuously in the flexion-extension dimension so that the cursor reached the visual targets at a 0.5 Hz pace. In both conditions, three target levels of press force (1 - 3N) were sequentially applied at 10 sec intervals, at the location on the forearm where the spindle afferent was most responsive to brief taps. Three of the participants also performed sinusoidal movements actively, for comparison of spindle behavior. **c)** Left: raw data of a spindle afferent from the extensor carpi radialis muscle during the isometric condition. Right: data from the same afferent/participant during the latter parts of the sinusoidal condition.

Specifically, we applied pressure percutaneously on one of three major extensor muscles of the forearm using a hand-held probe (5mm tip diameter) while recoding spindle afferent activity from this muscle, as well as monitoring skeletal muscle activity (EMG) and hand kinematics (Fig. 1a). Three target levels of press force were sequentially applied when the hand was immobile ('isometric' condition) and when the participant's hand was moved rhythmically about the wrist by the experimenter in the flexion-extension dimension at a 0.5 Hz pace ('sinusoidal' condition; Fig. 1b). We also present three spindle afferent recordings where the participants performed sinusoidal movements with and without the muscle press, for comparison.

The left panel of Figure 1c displays the responses of an exemplary spindle afferent recorded during the 'isometric' condition. There is vigorous afferent firing in response to locally applied pressure on the spindle-bearing muscle. The right panel of Figure 1c shows that the same afferent responds with higher firing rates during wrist flexion (i.e., muscle stretch) when pressure is applied. Figure 2a-c displays the responses of three additional muscle spindle afferents during the isometric condition, with each afferent originating from a different muscle. All three respond to the locally applied pressure stimulus, with a dynamic component in their responses, which could possibly reflect the stimulation of not just nuclear chain fibers i.e., some nuclear bag fibers also terminate near the polar ends of the capsule[14]. Nevertheless, the afferent response profiles look very similar to those previously demonstrated during isometric contraction[15], with spindle responses in those cases believed due to co-activation of alpha and gamma motor neurons[16]. As muscle contraction is accompanied by increases in IMP[1-3], it is quite possible that spindle responses during isometric contraction result largely from increased levels of IMP (i.e., compression of the spindle capsule) rather than arise due to neural control of spindles.

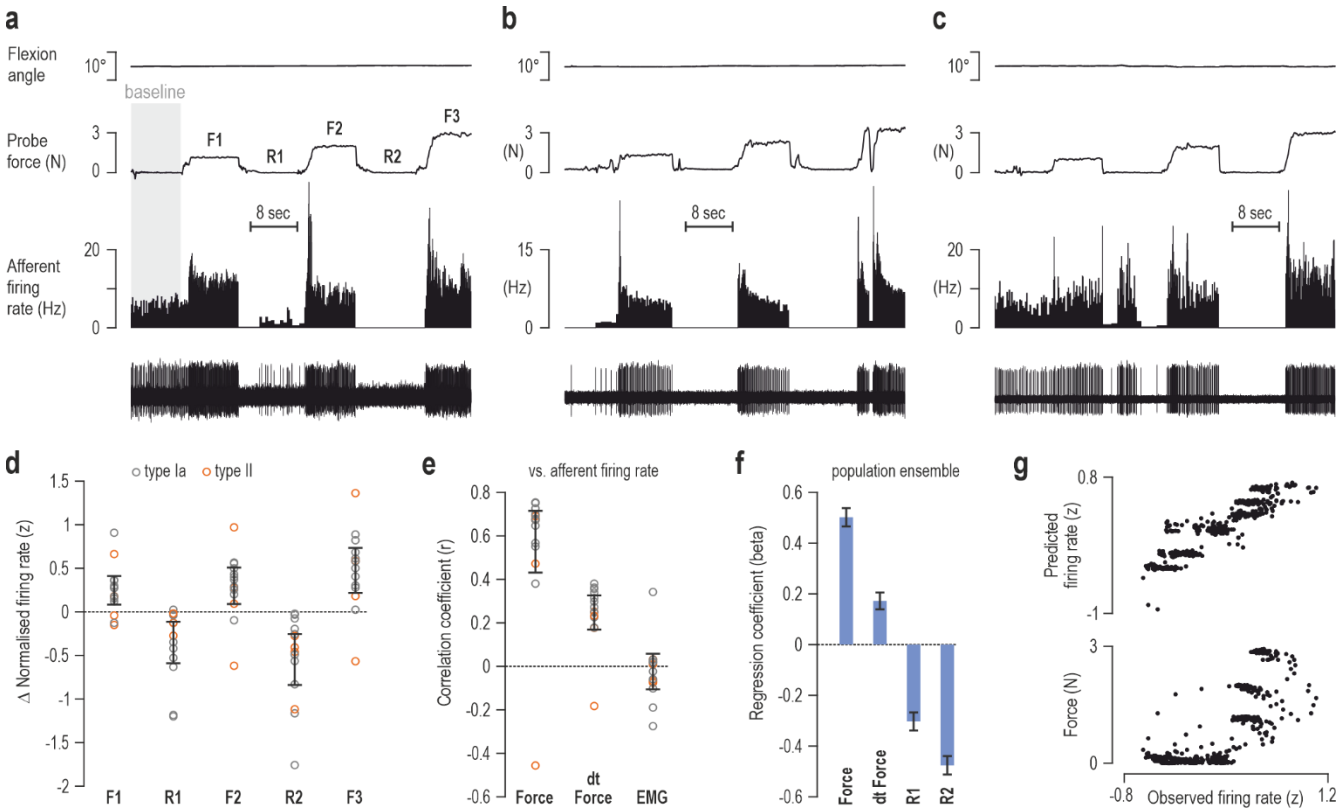

Fig 2. Local muscle pressure is an adequate stimulus for spindle receptors in the isometric condition.

a) A spindle afferent from the common digit extensor muscle displaying vigorous responses to locally applied muscle pressure. For analysis purposes, the latter 8 seconds before application of the first press stimulus was designated as the baseline epoch, and the latter 8 seconds of the subsequent press and release epochs were coded as illustrated (i.e., F1-F3). **b)** As 'a' but representing a spindle afferent from the extensor carpi radialis muscle. **c)** As 'a' but representing a spindle afferent from the extensor carpi ulnaris muscle. **d)** Mean change in afferent firing rate (from baseline) in the five epochs of the isometric condition, as designated in 'a'. Each circle outline corresponds to a single afferent (N=14). Error bars represent 95% confidence intervals. **e)** Full data sets during the isometric condition (e.g., see 'a'-'c') were down-sampled with a moving average window of 100 ms and correlations performed at the level of individual afferents. Error bars represent 95% confidence intervals, that were Fisher z transformed and then reversed to r. Only one afferent displayed a negative relationship between its firing rate and press force or the first derivative of force ('dt force'; see also Suppl. Fig. 1). **e)** Statistically significant beta (scaleless) regression coefficients following a forward stepwise regression to predict afferent population ensemble responses. Error bars represent 95% confidence intervals. **g)** Above: the regression coefficients in 'f' produced a very good reconstruction of the observed ensemble firing rate, with adjusted r^2=0.85, $p<10^{-5}$. Below: a significant relationship between ensemble firing rate and the ensemble press force signal, with r=0.75 and $p<10^{-5}$.

Figure 2d shows the mean change in firing rate across all recorded afferents for the different epochs of the 'isometric' condition (see also Fig. 2a). Data for this comparison were generated by first normalizing spindle firing rate (z-transform), and then subtracting the mean firing rate observed during the epoch before any pressure was applied i.e., 'baseline' epoch, at the level of single afferents. The recorded afferents were classified as primary ('type Ia') or secondary ('type II'; see Methods for more details), although no systematic differences were subsequently observed as a function of afferent type. Single-sample t-tests indicated that spindle firing rate was significantly different from baseline during all subsequent epochs, with local press inducing stronger responses (F1: t_{13}=3.24, p=0.0064; F2: t_{13}=3.08, p=0.0086; F3: t_{13}=3.97, p=0.0016) and 'release' epochs associated with lower firing rates than baseline (R1: t_{13}=-3.19, p=0.007; R2: t_{13}=-4.03, p=0.0014). A repeated-measures ANOVA test of the three press epochs revealed a significant impact of pressure level ($F_{2,26}$=7.4, p=0.003), with Tukey's HSD test indicating significantly higher firing rates during the F3 epoch (vs. F1: p=0.003; vs. F2: p=0.022).

Moreover, a dependent-samples t-test indicated significantly lower firing rates in the R2 epoch vs. R1 (t_{13}=2.27, p=0.04). All relevant tests were two-tailed. These results suggest that local pressure is an 'adequate' stimulus for muscle spindles in the isometric condition, with significantly higher firing rates for higher press forces (~2N resolution in our study; e.g., Fig. 2d), and removal of pressure is associated with lower firing rates than baseline.

To complement these findings, we also analyzed continuous raw data profiles of single afferents (see e.g. Fig. 2a-c), down-sampled with a moving average window of 100 ms. As illustrated in Figure 2e, the firing rate of all but one afferent displayed a positive relationship with press force (95% CI in r [0.71, 0.43]) and with its first derivative (95% CI in r [0.33, 0.17]). Although participants were instructed to remain relaxed during the isometric task, some minor EMG activity was occasionally evident in the spindle-bearing muscle of some participants. The relationship with EMG activity was therefore also examined as a proxy for alpha-gamma co-activation[16], as done previously (see Methods). However, as expected, there was no systematic relationship between EMG and spindle firing across the recorded afferents (95% CI in r [0.06, -0.11]).

The down-sampled profiles were also collapsed across afferents (averaged) to create population ensemble signals for a forward-stepwise regression. Specifically, press force and its first derivative, as well as dummy variables representing the release epochs ('R1' and 'R2') and spindle-bearing muscle EMG were entered as predictors to reconstruct the afferent ensemble responses. A very good reconstruction of ensemble firing rate was achieved with all predictors except EMG exerting a significant impact (force β=0.5, $p<10^{-5}$; force dt β=0.18, $p<10^{-5}$; R1 β=-0.3, $p<10^{-5}$; R2 β=-0.47, $p<10^{-5}$; EMG β=0.02, p=0.19; see Fig. 2f-g). A strong positive relationship is also evident between the population firing rate and press force (Fig. 2g, bottom). Performing the above analyses using data down-sampled with a larger moving average window (500 ms) produced virtually identical results (Suppl. Fig. 2). Taken together, our results demonstrate that in isometric conditions spindle neurons are strongly affected by and can encode locally applied muscle pressure.

Figure 3a displays the responses of a typical spindle afferent recorded during the 'sinusoidal' condition (see also Fig. 1c, right). In this condition, there is no increase in background firing throughout periods of pressure; rather, the afferent responses to muscle stretch (wrist flexion) are stronger during periods of pressure. Figure 3b shows the mean change in afferent firing rates across all recorded afferents for the different epochs of the 'sinusoidal' condition. Data for this comparison was generated as per Figure 2d. Single-sample t-tests confirmed that spindle firing rates were significantly higher than baseline during all epochs where pressure was applied (F1: t_{13}=2.9, p=0.012; F2: t_{13}=2.97, p=0.011; F3: t_{13}=3.83, p=0.002). Moreover, a repeated-measures ANOVA of the three press epochs again produced a significant impact of pressure level ($F_{2,26}$=6.3, p=0.006), with Tukey's HSD test indicating significantly higher firing rates in the F3 epoch vs. F1, with p=0.004. In contrast to the isometric task, however, single-sample t-tests indicated no systematic difference in firing rate during the 'release' epochs (R1: t_{13}=-0.45, p=0.66; R2: t_{13}=-0.32, p=0.75). As expected, performing single-sample t-tests as above for wrist angle, angular velocity and muscle EMG indicated no significant difference from baseline values in any epoch (all p>0.05).

We also analyzed continuous data profiles of single afferents (see e.g., Fig. 3a), down-sampled with a moving average window of 100 ms. However, since it was clear from the raw data profiles that vigorous afferent responses were present only during muscle stretch, these specific analyses were confined to periods of wrist flexion. As indicated in Figure 3c, the firing of the majority of afferents displayed a positive relationship with flexion angle (95% CI in r [0.63, 0.26]), angular velocity (95% CI in r [0.52, 0.34]), press force (95% CI in r [0.37, 0.14]) and its first derivative (95% CI in r [0.22, 0.14]), but there was no systematic relationship between afferent firing and EMG (95% CI in r [0.1, -0.03]). Comparing the r^2 values obtained for the relationship between spindle firing and press force in the isometric condition (Fig. 2e) with those obtained for angle (i.e., hand position) and

velocity above (Fig. 3c) indicated a significantly stronger relationship for the former (Suppl. Fig. 3). These results further reinforce that local pressure should be considered an 'adequate' stimulus for spindles in isometric conditions. Population ensemble signals pertaining to the variables named in Figure 2c were used to reconstruct the afferent population firing rate (see Methods for more details). Following a forward stepwise regression, a very good reconstruction of ensemble firing rates was achieved (adjusted r^2=0.95, $p<10^{-5}$) with joint angle, angular velocity and press force emerging as significant predictors (angle β=0.77, $p<10^{-5}$; velocity β=0.7, $p<10^{-5}$; force β=0.31, $p<10^{-5}$). As shown in Figure 3d, the impact of pressure manifested as a positive offset in afferent responses during wrist flexion; there were otherwise no significant differences in r values between spindle firing rate and angle or velocity as a function of press i.e., angle red r (0.61) vs. angle blue r (0.7), p=0.52; velocity red r (0.63) vs. velocity blue r (0.55), p=0.65. Last, we recorded from three spindle afferents during active sinusoidal movement and pressure again resulted in enhanced spindle responses during stretch (Suppl. Fig. 4).

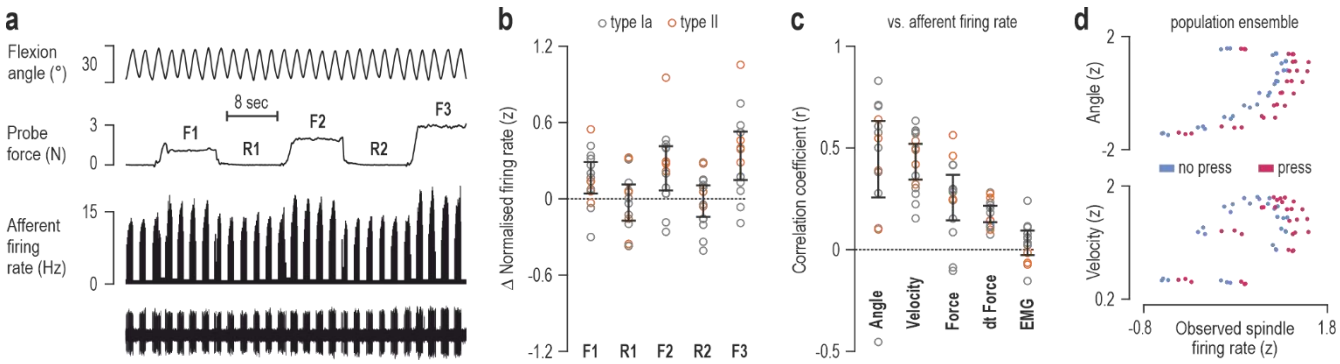

Fig 3. Local muscle pressure enhances spindle responses during stretch.

a) Exemplary responses from a spindle afferent during the sinusoidal condition. Despite the same sinusoidal movement of the hand, higher firing rates are evident in response to muscle stretch (wrist flexion) when local press was applied on the muscle. As per the isometric condition, the latter 8 seconds before application of the first press stimulus was designated as the baseline epoch, and the latter 8 seconds of the subsequent press and release epochs were coded as illustrated (i.e., F1-F3). **b)** Mean change in afferent firing rate (from baseline) in the five epochs of the sinusoidal condition, as designated in 'a'. Each circle outline corresponds to a single afferent (N=14). Error bars represent 95% confidence intervals. **c)** Full data sets in the sinusoidal condition were down-sampled with a moving average window of 100 ms and correlations were performed at the level of individual afferents. Error bars represent 95% confidence intervals that were Fisher z transformed and then reversed to r. **d)** Normalized ensemble population signals during muscle stretch in the sinusoidal condition. Red dots correspond to datapoints during the three press epochs (F1-3) and the blue dots represent the equivalent for the three epochs with no press (i.e., baseline and R1-2). At the population ensemble level (and single afferent level as well; see e.g., 'a'), the impact of muscle pressure on spindles manifested primarily as a positive offset in afferent responses.

In summary, we demonstrate that local muscle pressure is an adequate stimulus for muscle spindles in isometric conditions, and that pressure enhances spindle responses during muscle stretch. While we used percutaneous press to stimulate spindle receptors in our study with fully-alert humans, evidence in support of the hypothesis that compression of the spindle capsule does occur during muscle contraction have been outlined long ago[10]. More invasive animal studies can elucidate further the impact of IMP on spindle organ compression in healthy and diseased muscle. Nevertheless, IMP associates naturally with both muscle contraction and percutaneous compression, such as when sitting or leaning on our muscles in everyday life. Our findings therefore serve to redefine naturalistic proprioceptive feedback. The ability of spindles to encode local muscle pressure urges a reassessment of the role of muscle feedback in sensorimotor function[9,17,18] (especially in the context of postural maintenance) and in various neuromuscular conditions[19] including muscle cramps[20].

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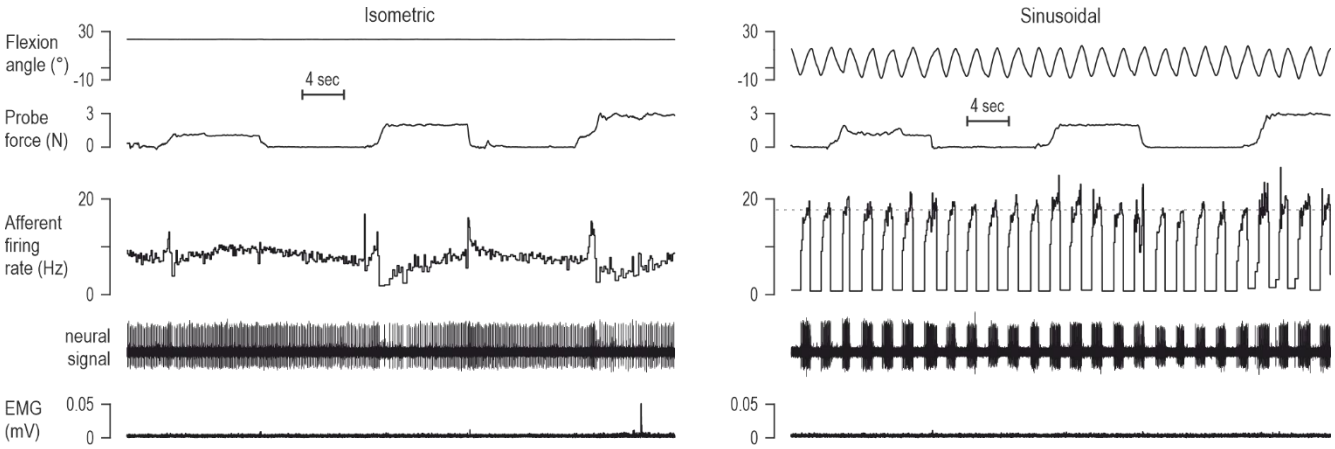
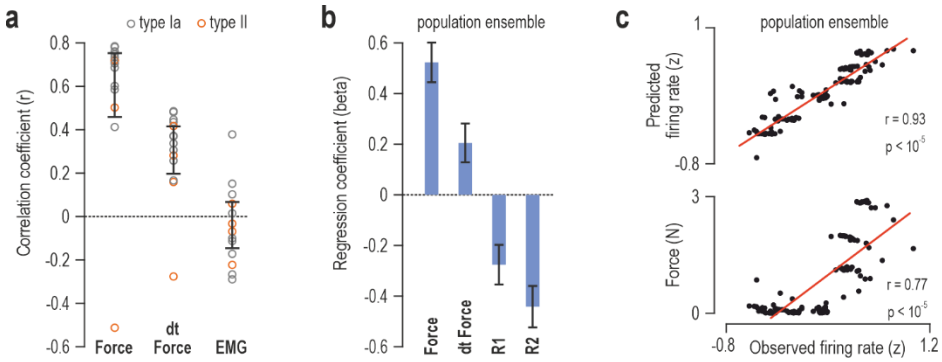
SUPPLEMENTAL FIGURES

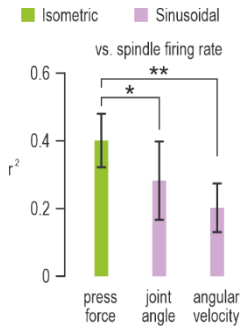


Supplemental Fig 1. The only recorded spindle afferent with a net negative relationship to muscle pressure in the isometric condition. Left: responses of the only recorded spindle afferent that displayed a negative overall relationship with press force in the isometric condition. Note, however, the brief increase in firing rate at the onset of each of the three press stimuli (i.e., the afferent did respond to brief taps at the location of the muscle where the probe subsequently pressed). Right: during the sinusoidal condition, the same afferent responded consistently to wrist flexion (i.e., muscle stretch) with some minor increase in firing rate during the early movement cycles when the 2N and 3N press force was applied.



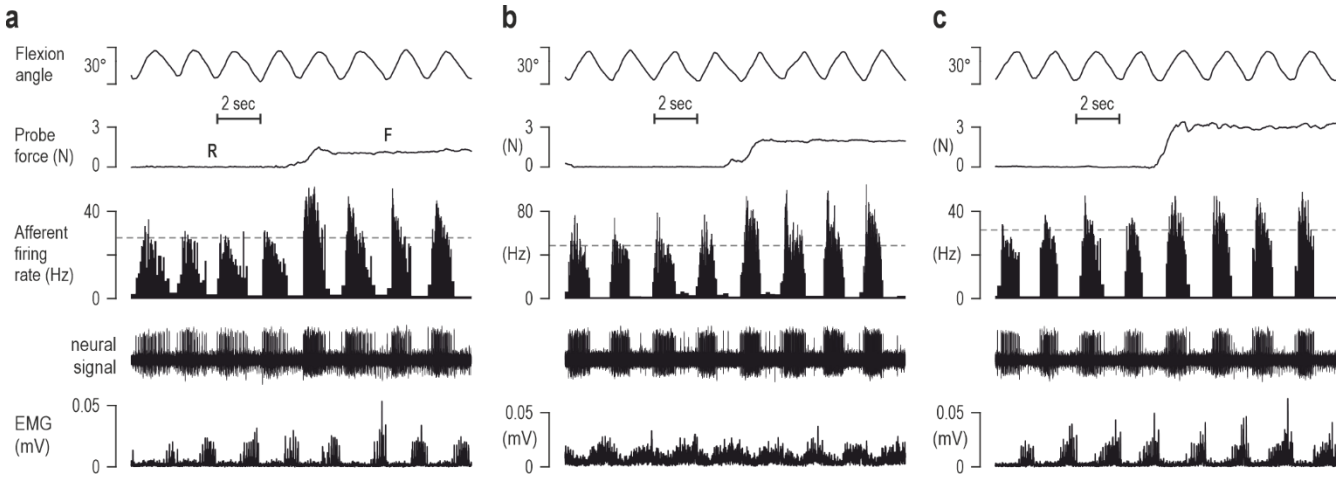
Supplemental Fig 2. A larger down-sampling window produces qualitatively the same results.

a-c) As Figure 2e-f but using a larger moving average time window (500 ms) to down-sample the continuous data. Qualitatively the same results were obtained.



Supplemental Fig 3. The closest observed relationship is between spindle firing and press force in the isometric condition.

Mean r^2 values across afferents (generated from the r values shown in Fig.2e and Fig. 3c) describing the relationship between spindle afferent firing and press force in the isometric condition (green) and the relationship between spindle firing and joint angle/velocity in the sinusoidal condition (pink). Error bars represent 95% confidence intervals. Dependent-samples t-tests indicated that press force in the isometric condition was more strongly related to spindle firing than joint angle (t_{13}=2.38, p=0.033) or angular velocity (t_{13}=3.64, p=0.003) during sinusoidal movement. Shapiro-Wilk tests indicated that the distribution of r^2 values relating to press force did not depart from normality (W=0.91, p=0.16), nor did the r^2 values relating to angle (W=0.96, p=0.81) and angular velocity (W=0.93, p=0.35). Single star signifies statistical significance at alpha=0.05, and double star signifies statistical significance at alpha=0.01.



Supplemental Fig 4. Local muscle pressure enhances spindle responses to stretch in active movement.

a-c) Responses from three separate spindle afferents during active movement of the hand, as per the sinusoidal condition. Despite very similar movement of the hand in each case, higher firing rates are evident in response to muscle stretch (wrist flexion) when local press was applied on the muscle ('F').

METHODS

Participants

Eight neurologically healthy adult human volunteers participated in the current study (mean age 24.5 years ± 4.7 standard deviations; half self-identified as female and half as male). The participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki, and were financially compensated. No statistical method was used to determine sample size, but the number of participants (and single afferents) in the current study is similar or exceeds the number used in equivalent studies (see e.g.,[21,22]). Our study was conducted as part of a research programme approved by the Umeå Regional Ethics Committee.

Muscle afferent recordings

Microneurography (MNG) was used to record single spindle afferent activity in the radial nerve of the right arm[23,24,], as previously done in our lab[21,25,26]. Afferent recordings were obtained from either the radial wrist extensor (extensor carpi radialis), the ulna wrist extensor (extensor carpi ulnaris) or the common digit extensor (extensor digitorum communis). The recorded action potentials were identified as originating from primary or secondary muscle spindles (vs. e.g., Golgi tendon organ or skin receptors) according to standard procedures described in detail elsewhere[27-29]. In short, these procedures include examining the afferent responses to ramp and hold stretches of the relaxed spindle-bearing muscle, the response to taps on the muscle (vs. skin of the hand) and assessing the variability in background firing rate[30]. In total, 14 muscle spindle afferents were recorded (10 'type Ia' and 4 'type II'). Each participant contributed with at least one afferent recording. The number of afferents recorded here is similar or exceeds the number of afferents recorded in previous MNG studies with human participants[21,26,27,31].

Electromyography (EMG)

Custom-made surface EMG electrodes, with a diameter of 2 mm and an internal distance of 12 mm were attached to three forearm muscles. Specifically, recordings were made from the extensor carpi ulnaris, extensor carpi radialis and extensor digitorum communis. Electrode placement was selected using a hand-held stimulator probe and isometric contraction/relaxation maneuvers. Prior to placement the EMG electrodes were coated with conductive gel, and then attached with double-sided tape. A ground electrode was placed on the lateral epicondyle of the humerus bone.

Experimental set-up and design

Participants sat on an adjustable dental chair with their right arm resting on a padded cushion. The chair was tilted backward and adjusted to fit the participant's stature and shape. The forearm was secured in place using a padded wooden vise proximal to the wrist, and this allowed the hand to move freely while the arm was stabilized to prevent displacement of the MNG electrode. Visual feedback was provided by a monitor placed opposite the participant at approximately eye-level (see Fig. 1a). Two vertical targets (26.5 cm long and 0.8 cm wide, located 8.5 cm from the origin) were continuously displayed on the monitor, and hand position was represented by a vertical cursor/line (25.5 cm long and 0.3 cm wide). Hand kinematics were recorded using a FASTRAK® sensor attached to the dorsal surface of the hand with double-sided tape and fixated with surgical tape. Movement of the semi-pronated hand about the wrist in the flexion-extension dimension controlled the 1D cursor position along the horizontal axis (i.e., between the visual targets).

The current study involved two experimental conditions (Fig. 1b). In the first condition ('isometric'), the participant's hand was relaxed and immobile, resting on a cushion in a lightly flexed position at the wrist. Note

that the label 'isometric' refers here to the lack of any externally perceptible movement of the participant's hand. Before the onset of the second 'sinusoidal' condition, the experimenter first grasped the lightly fisted hand of the participant and then moved the participant's hand in the initial position. In the initial position, the participant's hand (i.e., third metacarpal joint) was aligned with the longitudinal axis of the forearm (i.e., zero flexion/extension and ulna/radial deviation), and this corresponded to the cursor being in the center of the screen, between the two visual targets. The sinusoidal task then followed, which involved the experimenter moving the participant's lightly fisted hand rhythmically about the wrist in the flexion-extension dimension at a 0.5 Hz pace. The speed of hand movements was guided by a metronome and movement amplitude was guided by the visual targets i.e., the experimenter moved the cursor so that it alternated in reaching each of the two targets. One three occasions, single afferent recordings were made while the participants performed the sinusoidal movements actively, also using the metronome and visual targets to control the pace and amplitude of their movements (see Suppl. Fig. 4). Throughout, a rotation of one degree at the wrist corresponded to a 0.7 cm movement of the cursor along the horizontal axis of the monitor. Because the distance between the center of the screen and the center of a target was 8.5 cm, a wrist rotation of ~12 degrees was required for reaching the middle of each target from the initial position.

During each experimental condition, we applied pressure percutaneously on one of three major extensor muscles of the forearm using a hand-held probe. Specifically, the press force on the muscle was applied using a probe with an embedded six-axis force/torque transducer (Nano F/T transducer; ATI Industrial Automation), and a flat circular contact area of 5mm diameter. As indicated by the schematic at the bottom of Fig. 1b, after an idle period of 10 seconds (corresponding to the 'baseline' epoch; see e.g., Fig. 2a) three sequential target levels of press force (1, 2, and 3N) were applied in each experimental condition. The onset and offset time for applying a pressure stimulus, as well as the magnitude of the stimulus for any press, was indicated to a second experimenter (other than the one moving the hand in the sinusoidal condition) by a message shown on a second monitor. The second monitor was placed across the second experimenter, and outside the visual field of the participant. Each of the 'press' message on the second monitor lasted for 10 seconds, as did the 'release' messages. Some minor discrepancy was to be expected between the duration of the visual messages and that of the actual press epochs, due to the reaction time of the second experimenter. However, in addition to performing complementary analyses with complete continuous data profiles (e.g., Fig. 2e-g), analyses were confined to the latter 8 seconds of each epoch where necessary (e.g., Fig. 2d), as the experimenter's reaction time never exceeded 2 seconds. Concerning the magnitude of the applied press force, the experimenter received continuous real-time feedback in the form of a horizontal flat bar moving vertically along a background scale shown on the second monitor, with increases in force corresponding to movement of the bar towards the top of the scale.

Before inserting the MNG electrode, participants were familiarized with the two experimental conditions, including experiencing a dry run of the sinusoidal condition and performing the sinusoidal movement themselves. The participants were asked to remain relaxed and passive during the isometric and sinusoidal conditions. On three occasions where the afferent recording seemed particularly stable as the end of the passive sinusoidal movement (hence likely not to be lost during active movement), the participants performed the sinusoidal movement actively under pressure and release for comparison (Suppl. Fig. 4).

Data sampling and pre-processing

Data sampling was performed using SC/ZOOM™. With SC/ZOOM™ single action potentials were identified semi-automatically under visual control. The simultaneously recorded EMG channels were high-pass filtered with a 30 Hz cutoff and then root-mean-square processed with a symmetrical +-3.0 ms window. They were digitally

sampled at 4800 Hz. All data used for analyses, including EMG, were extracted from SC/ZOOM™ at a 1000 Hz rate. To facilitate comparison of EMG and afferent recordings across muscles and participants, the raw data were normalized by z-transformation using a procedure that has been described elsewhere[26,27,32]. In brief, for each individual afferent/participant, and each data variable, a grand mean and grand standard deviation was generated across all continuous datapoints. The z-transformed data was obtained by subtracting the grand mean from the measured value and dividing the difference by the grand standard deviation. To generate the down-sampled data that were entered into correlations and regression analyses, a moving average window of 100 ms was used on the recorded raw data profile of each afferent (e.g., Fig 2a-c & 3a). To generate the population ensemble signals of the isometric condition, the down-sampled profiles were collapsed (averaged) across afferents (N=14). All afferents were used in this procedure, including the one with a net negative relationship to muscle pressure (Suppl. Fig. 1). Using a larger down-sampling window produced equivalent correlation and regression results (Suppl. Fig. 2). To generate the population ensemble signals pertaining to the sinusoidal condition (i.e., Fig. 3d), a different approach was taken. For each single afferent separately, the raw data for each movement cycle was identified and re-sampled to 2 sec (corresponding to the target pace of movement: 0.5 Hz), and then collapsed (averaged) across the number of repetitions for each epoch (i.e., 6 epochs: baseline and F1-F3 as designated in Fig. 3a). The data for each of the 6 averaged cycles per afferent where then down-sampled with a moving average window of 100ms (resulting in 20 data points per cycle), and finally averaged data cycles were collapsed across afferents to create the population ensemble signals, totaling 120 data points for each variable (6 epochs x 20 points each cycle; Fig. 3d). Throughout, data pre-processing was performed using Matlab® (R2019a, MathWorks, Natick, MA, USA).

Statistical analyses

To assess the impact of press force on spindle afferent firing, all statistical analyses were two-tailed, where applicable. These involved single-sample t-tests to assess significant deviation in firing rate from baseline levels, as well as repeated-measures ANOVA to assess the effect of pressure level, along with Tukey's posthoc analyses and dependent-samples t-tests to compare firing rates across epochs (Fig. 2d & 3d). Single-sample t-tests were also employed to confirm the lack of significant deviation in wrist angle, velocity and EMG from baseline as a function of task epoch. Pearson's correlation coefficients ('r') were calculated to assess the relationship between firing rate and various independent variables, both at the single afferent level and at the population level (Fig. 2e,g & 3c). Dependent sample t-tests were used to contrast the strength of relationship between firing rate and press force in the isometric condition and that between firing rate and kinematic variables in the sinusoidal condition (Suppl. Fig. 3). Forward-stepwise regressions were used for identifying the significant predictors of afferent ensemble firing rate, using z-transformed data (Fig. 2g & 3d). Beta coefficients are reported which represent the standard deviation change in the dependent variable (here afferent firing rate) for 1 standard deviation change in the predictor variable. To assess data normality, Shapiro-Wilks test was used for sample sizes <50 data points, otherwise the Lilliefors test was used. Throughout, the level of statistical significance was 0.05. Correlation analyses at the level of single afferents were performed using Matlab® (R2019a, MathWorks, Natick, MA, USA), whereas all other statistical analyses were performed using STATISTICA® (StatSoft Inc, USA).

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Author contributions

M.D. conceived the study; M.D. and F.T. collected the data; M.D. and F.T. analyzed the data; M.D. and F.T. interpreted the data; M.D. and F.T. wrote the paper; M.D. supervised the study.

Competing interests

The authors declare no competing interests.

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