AGE-RELATED CHANGES IN THE HOFFMANN-REFLEX PATHWAY OF THE FLEXOR CARPI RADIALIS

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Abstract

Ageing is accompanied by numerous changes within the sensory and motor component of the muscle spindle pathway. To further document these age-related changes, this study compared the characteristics of the Hoffmann (H) reflex and M wave, evoked with several pulse durations, between young and old adults. The H-reflex and M-wave recruitment curves were analysed from recordings performed at rest in the flexor carpi radialis of 12 young (21-36 yrs) and 12 old adults (62-80 yrs). For each pulse duration (0.05-ms, 0.2-ms, and 1-ms), the maximal H-reflex amplitude (H\text{MAX}), the associated M wave (M\text{Hmax}), and the H-reflex amplitude for a stimulus intensity evoking an M-wave of 5% M\text{MAX} (H\text{M5%}) were measured. The strength-duration time constant and response threshold were estimated from the charge/stimulus-duration relation for H reflex and M wave. The main results indicate that varying pulse duration mainly induces similar effect on H-reflex and M-wave recruitment curves between young and old adults. However, regardless of pulse duration, old adults had lesser H\text{MAX} (p = 0.029) but greater H\text{M5%} (p<0.001) and M\text{Hmax} (p<0.001). The strength-duration time constant was lesser in old than young adults for the H reflex (p=0.048) but not the M wave (p=0.21). The H-reflex and M-wave response thresholds were greater in old than young adults (p=0.003). These results suggest greater age-related changes in the sensory than motor component of the H-reflex pathway, which may be indicative of a greater loss of sensory than motor axons or alterations of synapses between Ia afferents and motor neurones.

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ABSTRACT
Ageing is accompanied by numerous changes within the sensory and motor component of the muscle spindle pathway. To further document these age-related changes, this study compared the characteristics of the Hoffmann (H) reflex and M wave, evoked with several pulse durations, between young and old adults. The H-reflex and M-wave recruitment curves were analysed from recordings performed at rest in the flexor carpi radialis of 12 young (21-36 yrs) and 12 old adults (62-80 yrs). For each pulse duration (0.05-ms, 0.2-ms, and 1-ms), the maximal H-reflex amplitude (H_{MAX}), the associated M wave (M_{Hmax}), and the H-reflex amplitude for a stimulus intensity evoking an M-wave of 5% M_{MAX}(H_{M5%}) were measured. The strength-duration time constant and response threshold were estimated from the charge/stimulus-duration relation for H reflex and M wave. The main results indicate that varying pulse duration mainly induces similar effect on H-reflex and M-wave recruitment curves between young and old adults. However, regardless of pulse duration, old adults had lesser H_{MAX} (p = 0.029) but greater H_{M5%}(p<0.001) and M_{Hmax} (p<0.001). The strength-duration time constant was lesser in old than young adults for the H reflex (p=0.048) but not the M wave (p=0.21). The H-reflex and M-wave response thresholds were greater in old than young adults (p=0.003). These results suggest greater age-related changes in the sensory than motor component of the H-reflex pathway, which may be indicative of a greater loss of sensory than motor axons or alterations of synapses between Ia afferents and motor neurones.

Keywords: upper limb, H reflex, peripheral nerve stimulation, electromyography, electrophysiology, ageing

Introduction
The Hoffmann (H) reflex is an electromyographic response triggered by the electrical stimulation of muscle spindle afferents, mainly Ia afferents, which recruit spinal motor neurones (Burke, 2016). The H reflex is assumed to assess the effectiveness of Ia afferents to discharge motor neurones. A classical approach in motor control studies consists of recording the recruitment curves (RC) of the H-reflex and compound muscle action potential (M-wave) by progressively increasing the current intensity from an intensity that does not induce an H reflex to an intensity that evokes the maximum amplitude of the M wave (M_{max}). Paillard already reported in the soleus that a 1-ms pulse duration favoured the H-reflex amplitude relative to M-wave amplitude compared with a briefer (0.1 ms) pulse duration (Paillard, 1955). This was later confirmed for the same muscle (Lagerquist & Collins, 2008; Lin et al., 2002) and the flexor carpi radialis (FCR) in young adults (Lin et al., 2002; Panizza et al., 1989). Furthermore, increasing pulse duration from 0.05 to 1 ms induces a leftward shift of the H-reflex RC relative to the M-wave RC (Lagerquist & Collins, 2008; Panizza et al., 1989).

Lin and colleagues investigated the biophysical properties of afferent and motor axons involved in the H reflex and M wave, as well as the properties of cutaneous afferents, by stimulating the median nerve at the wrist with different pulse durations (Lin et al., 2002). Their results indicated a longer strength-duration time constant (SDTC) and a lower rheobase for the H reflex and cutaneous afferents compared with the M wave, with no difference between the H reflex and cutaneous afferents. The SDTC measures the rate of decline of the threshold current as the stimulus duration increases, whereas the rheobase is the threshold current for a stimulus of infinite duration (Bostock et al., 1983; Mogyoros et al., 1998). A lower rheobase implies that less current is required to generate action potentials, while a longer SDTC indicates that a long pulse duration...
(1 ms) is more effective in triggering action potentials. Such differences result in a greater excitability of Ia axons compared with motor axons for long pulse duration (Lagerquist & Collins, 2008; Panizza et al., 1989).

Mogyoros and colleagues showed a briefer SDTC for cutaneous afferents in the median nerve in old compared with young adults, without age-related difference for motor axons (Mogyoros et al., 1998). Considering that cutaneous and Ia afferents share biophysical properties (Lin et al., 2002), a similar decrease in SDTC of Ia axons with ageing should reduce their excitability to long pulse duration and minimise the difference between the excitability of sensory and motor axons for briefer pulse duration. Accordingly, a lesser leftward shift of the H-reflex RC relative to the M-wave RC should be observed in old adults when increasing the pulse duration. Indices which document H-reflex amplitude relative to M-wave response – such as the H-reflex maximal amplitude (H_max) relative to M_max (H_MAX/M_MAX ratio), the amplitude of the H reflex for a given amplitude (5% of M_max) of the M wave (H_M5%) and the amplitude of the M wave associated with H_max (M_Hmax) (Lagerquist & Collins, 2008; Scaglioni et al., 2003) – should be particularly suitable to document age-related changes of sensory relative to motor components of the spindle-reflex pathway. Indeed, a lesser change in these parameters from 0.05- to 1-ms pulse duration should indicate a lesser leftward shift of the H-reflex RC with increasing pulse duration, suggesting thereby more changes in the sensory relative to the motor component of the pathway. This approach is partly supported by the greater amplitude of the M wave evoked at the intensity eliciting the maximal H-reflex amplitude (M_Hmax) in old compared with young adults when using a 1-ms pulse duration (Scaglioni et al., 2003).

Therefore, this study compared the effect of pulse duration on the H reflex and M wave between young and old adults. To this end, the H-reflex and M-wave RC were recorded in the FCR by stimulating the median nerve at rest with pulse durations of 0.05, 0.2 and 1 ms. We hypothesised a lesser leftward shift of the H-reflex RC relative to the M-wave RC in old adults when increasing the pulse duration.

**Methods**

Twenty young [mean (SD); 27 (5) yrs] and 22 old adults [72 (7) yrs] participated in this study. They did not report Parkinson’s disease, multiple sclerosis, diabetes, stroke, cardiac history or neuropathology. In addition, participants with orthopaedic problems in their dominant upper limb within 12 months prior to the study were not included. Approval for the study was obtained from the local Ethics Committee, and all procedures used in this project conformed to the Declaration of Helsinki.

*2.1 Experimental setup*

Participants were comfortably seated in a chair with the forearm of the dominant arm (assessed using the Edinburgh Handedness Inventory; Veale, 2013) placed in an adjustable custom-made ergometer (Henry et al., 2022). The position of the participant was standardised so that the wrist was in the neutral position (0° of flexion), the forearm positioned midway between pronation and supination, and the shoulder and elbow flexed at about 25° and 40-50° (full elbow extension: 0°), respectively.

*2.2 Electromyography*

The surface electromyography was recorded from the FCR of the dominant arm with adhesive electrodes (3M Red Dot TM). Before attaching the electrodes, the skin was shaved when necessary and cleaned with a solution of alcohol, ether, and acetone to reduce the impedance at the skin-electrode interface. The electrodes were placed over the muscle belly in a bipolar configuration at the proximal third of the forearm, with an inter-electrode (centre to centre) distance of 2 cm. The location of the electrodes placed on the muscle belly was determined by palpation and ultrasound imaging (DP-6900Vet; Shenzhen Mindray Bio-Medical Electronics), using a 6-cm width linear-array probe (7.5-MHz; 75L60EA, Shenzhen Mindray Bio-Medical Electronics), during wrist flexion, extension and abduction. The reference electrode was placed over the olecranon. The EMG signal was amplified (200×) and band-pass filtered (10–1000 Hz) prior to A/D sampling at 4 kHz (Power 1401, 16-bit resolution, Cambridge Electronic Design, UK).

*2.3 Median nerve stimulation*
Electrical stimuli were applied to the median nerve via a constant current stimulator (DS8R, Digitimer, Hertfordshire, UK) connected to two surface electrodes (silver-silver chloride electrodes of 8 mm diameter) attached to the skin on the dominant arm with adhesive tape. The anode was placed a few centimetres above the epicondyle on the lateral side of the arm. The position of the cathode was determined by moving a pen electrode (cathode) along the nerve path until the site eliciting an H reflex with the largest amplitude at a given intensity was identified.

2.4 Protocol

Participants were asked to refrain from consuming caffeine for 12h before testing. They were also asked not to perform intensive physical activity for 72h before testing. The H-reflex and M-wave RC were recorded with three pulse durations: 0.05 ms (RC\(_{0.05}\)), 0.2 ms (RC\(_{0.2}\)) and 1 ms (RC\(_{1}\)). The RC started with a current intensity set below the H-reflex threshold (intensity evoking 3 responses out of 5 stimulations) and was gradually increased until the M\(_{\text{max}}\) was reached. Three stimuli were delivered at each intensity with an interstimulus of 10 s (Stein et al., 2007). Participants were asked to keep the same position and relax while recording the RC. As the decrease in H-reflex amplitude in the FCR observed for pulse duration greater than 1 ms should not be related to the biophysical properties of axons (Panizza et al., 1989), the longer pulse duration used in the present study was set at 1 ms.

2.5 Data reduction

The peak-to-peak amplitude of the H reflex and M wave was computed from the raw EMG signal. The amplitude of the three responses recorded for each current intensity was averaged before being normalised to the M\(_{\text{MAX}}\). The H-reflex RC [up to H\(_{\text{MAX}}\)(ascending part of the RC)] and M-wave RC were fitted with a Boltzmann sigmoid function (Klimstra & Zehr, 2008; Penzer et al., 2015) to determine relevant model-based variables (Figure 1): 1/ H\(_{\text{MAX}}\) and M\(_{\text{MAX}}\); 2/ the intensities evoking a response of half H\(_{\text{MAX}}\) (I\(_{H50}\)) and M\(_{\text{MAX}}\) (I\(_{M50}\)); 3/ H\(_{\text{MAX}}\); and 4/ M\(_{\text{MAX}}\) (Lagerquist & Collins, 2008; Scaglioni et al., 2003).

Due to difficulties in detecting the onset of the H-reflex and M-wave responses, the latency was measured from the moment of the stimulation to the first peak of the respective response. The latency was then expressed relative to the length of the arm (ms.cm\(^{-1}\)) as the latency of the H reflex in the FCR has been shown to be a function of the length of the arm of the participants (Burke, 2016; Khosrawi et al., 2015; Schimsheimer et al., 1985). Even though this variable may present some limitations (exact location of the recording and stimulating electrodes), such a normalisation can be used to compare the response latency between young and old adults (Scaglioni et al., 2003). The arm length was measured as the distance between the acromion and the styloid process of the radius.

We also estimated the SDTC and response threshold (reflecting the rheobase) for the H reflex and M wave using Weiss’ law (Kiernan & Kaji, 2013; Lin et al., 2002; Mogyoros et al., 1996). The I\(_{H50}\) and I\(_{M50}\) were converted into threshold charges (current intensity × duration, expressed in mA.ms) and plotted against the corresponding duration (0.05 ms, 0.2 ms, and 1 ms) (Lin et al., 2002). The SDTC was then determined by the negative intercept on the duration axis, whereas the response threshold was given by the slope of the regression line (Figure 1E). Although this method is commonly performed with more than three pulse durations, using three durations should not have influenced our results as no difference in the SDTC calculated from two, five, 12 or 50 data points was observed (Mogyoros et al., 1996).

An example of the fitted RC for one young and one old adult is illustrated in Figure 2. The ability to fit the data with a Boltzmann function for each pulse duration significantly differed between age groups [chi\(^2\) (3, 40) = 8.4, p = 0.037]. We could elicit H reflexes with a pulse duration of 1 ms in 37 participants (19 young and 18 old adults). Among these 37 participants, data from 1 young and 6 old adults had to be removed due to H-reflex amplitude being too small (<3% M\(_{\text{max}}\)) or inconsistent to be fitted by the Boltzmann function for briefer pulse duration. Therefore, the RC of only 18 young and 12 old adults has been retained for analysis. To have similar sample sizes for statistical analysis, we matched the 12 old participants [height:
1.67 (0.08) m; weight: 65.6 (8.8) kg, BMI: 23.7 (2.6)] with 12 young adults [height: 1.72 (0.10) m; weight: 66.6 (7.3) kg; BMI: 22.5 (2.1)] based on gender (8 women and 4 men), BMI and the kind of physical activity regularly practiced at the time of their participation (endurance, strength or mobility activities). Matching participants based on BMI contributed to controlling for large differences in body composition that could influence EMG recordings (Petrofsky, 2008) or induce long-term neurophysiological adaptations to excessive body weight (Maffiuletti et al., 2021). As a sedentary lifestyle can speed up the effects of ageing (Shadyab et al., 2017), physical activity was also considered as a matching factor. In addition, the kind of physical activity practiced by participants was considered to limit possible activity-dependent influences on the H reflex and M wave (Toïen et al., 2023).

[INSERT FIGURE 2 HERE]

2.6 Statistics

The statistical analysis was performed using JASP open-source software (version 0.13.1, Amsterdam, Netherlands). Before comparing each dependent variable, the normality of the data distribution was confirmed with the Shapiro-Wilk test. The effect of age (young vs old) and pulse duration (RC\textsubscript{0.05}, RC\textsubscript{0.2} and RC\textsubscript{1}) on the H-reflex and M-wave variables were investigated using two-way ANOVAs with repeated measures for pulse duration (age × pulse duration). The effect of age and pathway (H reflex vs M wave) was assessed on the SDTC and the response threshold using two-way ANOVAs with repeated measures for the pathway (age × pathway). When sphericity assumptions were violated (Mauchly’s test of sphericity), the Greenhouse-Geisser correction was applied. When appropriate, post-hoc tests with the Bonferroni correction were used to identify differences between means. The effect of age on the H-reflex and M-wave latency was investigated using Student t-tests. The level of statistical significance was set at p ≤ 0.05 for all comparisons. Values are expressed as mean (SD) in the text and tables and mean (SEM) in the figures.

Results

3.1 M wave

\( M_{\text{max}} \) amplitude was lesser in old than in young adults (age effect; p = 0.002; Figure 3A) for all pulse durations (pooled data; young: 5.0 mV; old: 2.8 mV) and did not change with pulse duration (p = 0.23; Table 1). A significant age x pulse duration interaction was observed for \( I_{M50} \) (p = 0.027). The associated post-hoc analysis indicated a greater \( I_{M50} \) for RC\textsubscript{0.05} compared with RC\textsubscript{0.2} and RC\textsubscript{1} in both age groups (p < 0.001), and a greater \( I_{M50} \) in old compared with young adults for RC\textsubscript{0.05} (p < 0.001; Figure 3C). \( M_{\text{max}} \) latency was similar in young and old adults [young: 0.08 (0.02) ms.cm\textsuperscript{-1}; old: 0.09 (0.02) ms.cm\textsuperscript{-1}; p = 0.55].

3.2 H reflex

\( H_{\text{max}} \) amplitude was lesser in old than young adults (age; p = 0.029; pooled data: young: 30.9% \( M_{\text{max}} \); old: 16.6% \( M_{\text{max}} \)) and changed with pulse duration in both groups (p = 0.045; Figure 3B). \( H_{\text{max}} \) amplitude was lesser for RC\textsubscript{0.05} than RC\textsubscript{1} (p = 0.032). A significant age x pulse duration interaction for \( I_{H50} \) (p = 0.004) and post-hoc analysis indicated a greater \( I_{H50} \) for RC\textsubscript{0.05} compared with RC\textsubscript{0.2} and RC\textsubscript{1} in both age groups (p < 0.001), and a greater \( I_{H50} \) for old than young adults for RC\textsubscript{0.05} (p < 0.001) (Table 1). The \( I_{H50}/I_{M50} \) ratio was greater for old than young adults (p = 0.025) and decreased with the increase in pulse duration (pulse duration: p < 0.001). The \( H_{\text{max}} \) latency was greater for old [0.34 (0.03) ms.cm\textsuperscript{-1}] than young adults [0.32 (0.02) ms.cm\textsuperscript{-1}; p = 0.008].

The \( H_{M5\%} \) was lesser for RC\textsubscript{0.05} than RC\textsubscript{1} in both age groups (p values [? ] 0.028), while RC\textsubscript{0.05} differed from RC\textsubscript{0.2} (p = 0.006) and RC\textsubscript{0.2} (p = 0.016) in young adults only (Table 1; Figure 3D). Moreover, \( H_{M5\%} \) was lesser in old compared with young adults for RC\textsubscript{0.2} (p < 0.001) and RC\textsubscript{1} (p < 0.001) but not for RC\textsubscript{0.05} (p = 0.36). \( M_{H\text{max}} \) was greater in old than young adults and varied with pulse duration (p < 0.001), with a decrease in \( M_{H\text{max}} \) from RC\textsubscript{0.05} (p < 0.001) and RC\textsubscript{0.2} (p = 0.021) to RC\textsubscript{1} in both groups (Figure 3C; Table 1).

[INSERT FIGURE 3 HERE]
3.3 Response threshold and SDTC

The response threshold was greater in old than young adults for the H reflex and M wave (p = 0.003). It was also greater for the M wave than the H reflex (p < 0.001; Figure 4A) in young [M wave: 6.0 (1.4); H reflex: 3.1 (0.5) mA] and old adults [M wave: 12.3 (1.8); H reflex: 7.5 (1.1) mA]. SDTC was briefer in old than young adults for the H reflex [young: 860.5 (71.7); old: 617.9 (49.2) μs; p = 0.009] but not the M wave [young: 555.4 (48.9); old: 436.1 (33.4) μs; p = 0.24]. The SDTC was shorter for the M wave than the H reflex in both groups (p < 0.001; Figure 4B).

Discussion

This study aimed to compare the effect of pulse duration on the H reflex and M wave between young and old adults with the rationale that it may contribute to further understanding the effect of ageing on the H-reflex pathway. The results indicate that varying pulse duration has mainly a similar effect on H-reflex and M-wave RC in young and old adults. However, if these results do not support our hypothesis (lesser effect of pulse duration on H-reflex recruitment curve in old adults), the analysis of specific parameters of the H-reflex RC (H_max, H_M5%, M_Hmax and I_H50) for the three pulse durations allows to step further in the effect of ageing on the neural part of the muscle spindle pathway. Indeed, the decrease in H_max and H_M5% and the increase in M_Hmax regardless of pulse duration suggest greater age-related changes in the sensory than the motor component of the H-reflex pathway that may reflect a greater loss of sensory than motor axons or alterations of synapses between Ia afferents and motor neurones.

4.1 Effect of pulse duration on recruitment curves in young and old adults

Varying pulse duration from 0.05 to 1 ms induces an increase in the H_max and H_M5% amplitude, and a decrease in M_Hmax amplitude (Figure 3). This agrees with previous work reporting a leftward shift of the H-reflex RC relative to M-wave RC (Panizza et al. 1989; Lagerquist and Collins, 2008) and supports differences in the biophysical properties of axons involved in the H reflex and M wave (Lin et al., 2002). In contrast with the absence of a pulse-duration effect on H_MAX in the soleus (Lagerquist & Collins, 2008), we observed an increase in H_MAX amplitude with the increase in pulse duration, as also reported in the FCR (Panizza et al., 1989). This can reflect the decrease in M_Hmax, which indirectly reflects the magnitude of the antidromic volley, as its decreased amplitude with the increase in pulse duration may reduce collisions between orthodromic and antidromic volleys (Schieppati, 1987). However, only a very weak negative association between the H_max amplitude and the M_Hmax was observed when data from both groups and the three pulse durations were pooled (r² = 0.11, p = 0.005), indicating that H_max may not primarily depend on the M_Hmax. Although the group I volley (Ia and Ib) induced by the stimulus is dominated by the monosynaptic Ia volley, the disynaptic Ib pathway from the Golgi tendon organ likely limits the amplitude of the H reflex (Marchand-Pauvert et al., 2002). Expecting similar biophysical properties between Ia and Ib afferents, the magnitude of the inhibition induced by Ib afferent stimulation should follow the recruitment of Ia afferents, therefore being greater for long pulse duration. This could counteract the lesser antidromic volley for long pulse duration, thereby contributing to the only weak relation between H_max and M_Hmax. Yet, this remains speculative and further studies are required to unravel the mechanisms of action that limit H_max amplitude and the influence of pulse duration on H_max.

4.2 Age-related changes highlighted by the effect of pulse duration on RC

The I_M50 decreased with the increase in pulse duration in both groups, indicating the increase in electrical charge with longer pulse durations. However, I_M50 was significantly greater for old compared with young adults for the 0.05-ms pulse duration. Furthermore, the response threshold for the M wave was greater in old adults while STDC did not change, as already observed in the median nerve when stimulated at the wrist (Jankelowitz et al., 2007). These age-related differences may be indicative of thicker subcutaneous tissue in old adults, thereby reducing the current flow from the electrode to the nerve, more so for brief pulse duration (0.05 ms) (Petrofsky, 2008). Changes in axonal excitability can also result from the preferential loss
of large-diameter motor axons (more excitable), which belong to high-threshold motor units characterised by a greater innervation ratio, with ageing (Hepple & Rice, 2016; Larsson et al., 2019). Basser and Roth (Basser & Roth, 1991) predicted that the response threshold is inversely proportional to the square of the fibre diameter by using a simulated model. Accordingly, the increase in the response threshold for the M wave in old adults may support a loss of large-diameter motor axons in the aged group. This is further supported by the consistent observation (Baudry et al., 2010; Kido et al., 2004; Scaglioni et al., 2003), of a lesser $M_{\text{max}}$ amplitude in older adults, even though a decline in the integrity of the neuromuscular junction (Hepple & Rice, 2016) can also contribute to decreasing the $M_{\text{max}}$ amplitude.

A common observation reported in the present study and previous work (Baudry et al., 2010; Kido et al., 2004; Scaglioni et al., 2003) is the lesser $H_{\text{max}}$ amplitude in old compared with young adults. Increased presynaptic inhibition of Ia afferents could reduce $H_{\text{MAX}}$ amplitude, although the lack of consensus on an increased presynaptic inhibition with ageing questions this possibility (Butchart et al., 1993; Morita et al., 1995; Baudry et al., 2010; Baudry & Duchateau, 2012). The age-related decrease in $H_{\text{MAX}}$ amplitude can also result from alterations in the synaptic transmission between Ia afferents and motor neurones. In rhesus monkeys and mice, ageing is accompanied by a loss of synaptic input onto motor neurones (Maxwell et al., 2018), while a decrease in the rate of rise of the Ia-induced excitatory postsynaptic potentials was observed in cats (Chase et al., 1985; Boxer et al., 1988). Together, these changes should reduce the effectiveness of Ia afferents in causing action potential in motor neurones (Fetz & Gustafsson, 1983). Finally, an age-related loss of Iaafferents has been observed, more so for the large-diameter afferents (Kim et al., 2007; Vaughan et al., 2016). In addition to decreasing the $H_{\text{max}}$, this may explain the increase in the H-reflex response threshold in old compared with young adults (figure 4). This is further suggested by the greater H-reflex latency in old adults, which may account for a slower conduction velocity. Even though nerve demyelination decreases conduction velocity (Ian McDonald, 1962), it is unlikely to be a major alteration of afferent fibres in this study, as it should increase rather than decrease the SDTC (Bostock, 1983). Furthermore, if the greater $I_{\text{M50}}$ in older adults could partly be due to thicker subcutaneous layers, a similar effect on $I_{\text{H50}}$ is unlikely as the difference remains even after being normalised to $I_{\text{M50}}$, supporting age-related changes involving the sensory components of the H-reflex pathway.

Interestingly, the change in H-reflex SDTC is not accompanied by a lesser leftward shift of the H-reflex RC relative to the M-wave RC. A decrease in SDTC suggests that sensory axons are less excitable for a 1-ms duration but relatively more excitable for a briefer pulse duration. Accordingly, the leftward shift induced by longer pulse durations should have been less pronounced in old adults as biophysical properties between sensory and motor axons were expected to be closer (no change in SDTC for motor axons). However, the almost similar changes in $H_{\text{M50}}$ and $M_{\text{max}}$ from $R_{0.05}$ to $R_{1}$ between young and old adults do not support a lesser leftward shift of the H-reflex RC relative to M-wave RC. Nonetheless, the lesser $H_{\text{M50}}$, greater $M_{\text{Hmax}}$ and $I_{\text{H50}}/I_{\text{M50}}$, and briefer SDTC in old adults regardless of pulse duration highlight a rightward shift of the H-reflex RC relative to the M-wave RC with ageing. Although changes in the M-wave pathway were also observed (decreased $M_{\text{max}}$, increased $I_{\text{M50}}$), this rightward shift suggests a greater age effect on the sensory than motor components of the H-reflex pathway (Bouche et al., 1993; Scaglioni et al., 2003). A loss of large-diameter sensory axons and synaptic alterations can support the absence of age-related changes in the effect of pulse duration on $H_{\text{M50}}$ and $M_{\text{Hmax}}$. In contrast with changes in biophysical properties of Ia afferent axons, a decreased number of Ia afferents and a decrease in synaptinc efficacy would have a constant effect on the H-reflex amplitude (decrease in $H_{\text{max}}$ and $H_{\text{M50}}$) across pulse durations.

4.3 Methodological considerations.

Our estimates of SDTC and response threshold do not strictly reflect the biophysical properties of afferent and motor axons. The M wave results from the recruitment of motor axons, the activation of the muscle fibres at the neuromuscular junction, and the propagation of action potentials along the sarcolemma. However, in the steepest portion of the RC (around $I_{\text{M50}}$), the M-wave amplitude can be assumed to be indicative of the recruitment properties of motor axons (Funase et al., 1994). Furthermore, the neuromuscular junction should not be a limitation as the safety margin permits an action potential conveyed by the axon to trigger an action.
potential on the sarcolemma (Paton & Waud, 1967). Other factors than the number of active motor units influence the M-wave amplitude (dispersion of their innervation zones, the thickness of the subcutaneous tissue layers, the orientation of the detection system with respect to the muscle fibres, and the intracellular action potential shape). However, these factors should only marginally influence the effect of pulse duration on the M wave as the EMG recording conditions were similar for the three pulse durations. Therefore, we expect that the response threshold and SDTC for the M wave provide an indirect estimate of the biophysical properties of motor axons.

The H-reflex amplitude is a more complex response, which depends on the recruitment of afferent axons, as well as presynaptic and postsynaptic mechanisms. As the SDTC measured from the H reflex in the median nerve did not differ when recorded at rest and during contraction (Lin et al., 2002), it is unlikely that mechanisms influencing the excitability of the motor neurone pool (recurrent inhibition and non-reciprocal group I inhibition) affect the measured parameter as these mechanisms change during voluntary contraction. Furthermore, Ia presynaptic inhibition should not influence our measures because it lasts less than 500 ms (Mizuno et al., 1971), whereas the inter-stimulus interval used in the present study was 10 s. Accordingly, the SDTC and response threshold for the H reflex likely represents Ia afferents’ biophysical properties, as suggested by Lin and colleagues (Lin et al., 2002).

4.4 Clinical implications

The H reflex is a valuable tool for assessing acute or long-term changes in the neural component of the excitatory pathway originating from muscle spindles with ageing (Burke, 2016). Although the current results should be interpreted cautiously, they highlight several interesting features that can be useful in clinical practice. First, the results confirm that a long pulse duration (1 ms) is recommended to evoke the largest H-reflex amplitude while minimising the associated M wave, regardless of age. Second, this study indicates that variables other than the \( H_{\text{MAX}} \) and \( M_{\text{MAX}} \) can provide relevant information about age-related neural changes. In particular, the \( I_{\text{H50}}, H_{\text{M5}}% \) and the \( M_{\text{Hmax}} \) can indirectly inform on the neural sensory components of the H-reflex pathway. In old adults, greater \( I_{\text{H50}} \) and \( M_{\text{Hmax}} \) but lesser \( H_{\text{M5}}% \) likely reflect greater changes in sensory than motor components of the reflex. Third, this study suggests that using different stimulus durations may provide indirect cues on structural (loss of large diameter Ia afferents) and functional (decrease in synaptic efficacy) alterations within the H-reflex pathway. This approach could then be relevant to document the effects of chronic neuromuscular conditions (spinal cord injury, stroke, etc.).

Conclusion

This study investigated age-related changes in the H-reflex pathway by using different pulse durations in young and old adults. The results indicate greater age effects on the sensory than the motor component of the H-reflex pathway and suggest that part of these effects relies on the loss of afferent axons and alteration in synaptic efficacy.

Conflict of Interest Statement

None of the authors have potential conflicts of interest to be disclosed.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References


Table 1. Mean (SD) of the main RC variables and results of the ANOVA

Results of the main variables of interest: the maximal M wave (M_{MAX}) and H-reflex amplitude (H_{MAX}), the M-wave amplitude associated with H_{MAX}(M_{Hmax}), the H-reflex amplitude associated with an M wave of 5% M_{MAX}(H_{M55}) and current intensity associated with a M-wave and H-reflex amplitude of 50% M_{MAX}(I_{M50}) and H_{MAX}(I_{H50})

Figure legends

Figure 1. Schematic illustration of the main variables of interest obtained from the recruitment curves of one old adult. A. Maximal amplitude of the M wave (M_{MAX}) and H reflex (H_{MAX}), M-wave amplitude associated with H_{MAX}(M_{Hmax}), H-reflex amplitude associated with an M wave of 5% M_{MAX}(H_{M55}) and current intensity associated with a M-wave and H-reflex amplitude of 50% M_{MAX}(I_{M50}) and H_{MAX}(I_{H50}). These data were extracted from the H-reflex and M-wave recruitment curves fitted by a Boltzmann sigmoid function. B, C and D. Illustration of the M_{MAX}, H_{MAX}, M_{Hmax}) and H_{M55}. E. Response threshold and strength-duration time constant (SDTC), extracted from the threshold-charge vs stimulus duration relation.

Figure 2. H-reflex and M-wave recruitment curves (RC) in one young (23 yrs; left panels) and one old adult (67 yrs; right panels) recorded with pulse duration of 0.05 ms (top panels), 0.2 ms (middle panels) and 1 ms (bottom panels). The H-reflex and M-wave amplitude are expressed as percentage of the maximal amplitude of the M wave (M_{MAX}), while the current intensity is expressed relative to the intensity evoking an M wave of 50% of M_{MAX}(I_{M50}). Open and filled circles represent H-reflex and M-wave data, respectively. Continuous lines represent the fitting of the experimental data (ascending part only for the H reflex) by a Boltzmann sigmoid function.

Figure 3. Effect of pulse duration on M_{MAX} (A), H_{MAX} (B), M_{Hmax} (C) and H_{M55} (D) in young and old adults. # indicates a difference between young and old adults (p < 0.05). *, ** and *** indicates significant differences between pulse durations at p < 0.05, p < 0.01 and p < 0.001, respectively. Bars and error bars represent means and SEM, respectively.

Figure 4. Effect of age on the response threshold and SDTC of the H reflex and M wave of young and old adults. # indicates a difference between young and old adults (p < 0.05). *** indicates significant differences between the M-wave and H-reflex pathways (p < 0.001). Bars and error bars represent means and SEM, respectively.

Graphical abstract
This study compared the characteristics of the H-reflex and M-wave recruitment curves (RC) evoked with 0.05, 0.2 and 1-ms pulse durations in young and old adults. Increasing pulse duration from brief (0.05 ms) to longer (1 ms) duration mostly induced a similar leftward shift of the H-reflex and M-wave RC in both groups. Regardless of pulse duration, the results indicate that H-reflex outcomes were impacted by ageing, suggesting a greater loss of sensory than motor axons or alterations of synapses between Ia afferents and motor neurones.

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