The effect of age on post-activation depression of the upper limb H-reflex

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Abstract

Purpose

Postactivation depression (PaD) refers to the inhibition of the H-reflex induced by a preceding conditioning stimulus able to activate the afferents mediating the H-reflex itself. PaD can be investigated assessing the frequency-related depression of the H-reflex. This parameter, which is highly correlated to the severity of spasticity, has been used in the longitudinal assessment of spastic patients, in particular to assess the effect of drugs and rehabilitation over the years. However, in such longitudinal assessment, changes observed might be age- and not only disease-related. The aim of this study was to investigate the possible age effects on PaD.

Methods

The frequency-related depression of the *flexor carpi radialis* (FCR) H-reflex was examined in two groups of young (20 subjects; 28±3 years) and aged (20 subjects; 70±7 years) healthy subjects. PaD was evaluated by comparing the H-reflex amplitudes obtained with a stimulation frequency of 0.1Hz with those obtained using higher frequencies (0.33-0.5-1-2Hz).

Results

The results showed that frequency-related depression of the FCR H-reflex reflecting PaD is similar in young and elderly subjects at all frequencies, with the exception of 2 Hz.

Conclusion

Our study shows that ageing does not affect the frequency-related depression of the FCR H-reflex at the frequencies of 1Hz or lower, supporting the reliability of this method to assess PaD in the clinical practice, particularly for the longitudinal assessment of spasticity. A decrease of GABA-ergic presynaptic inhibition seems to be the more likely explanation for the age-related changes that we observed at the frequency of 2Hz.

Introduction

Postactivation depression (PaD) refers to the inhibition of the test response (either H-reflex or stretch reflex), elicited in a given muscle (test muscle) at rest, induced by a preceding conditioning stimulus able to activate the afferents mediating the test response (e.g. a tap or a vibration applied to the tendon of the test muscle, a passive lengthening or a voluntary contraction of the test muscle, an electrical stimulation of the mixed nerve supplying the test muscle) (Crone and Nielsen 1989; Hultborn et al. 1996). In practice, two methods have been used to assess PaD in humans: the frequency-related depression of the *soleus* and the *flexor carpi radialis* (FCR) H-reflex (Aymard et al. 2000; Lamy et al. 2009; Rossi-Durand et al. 1999), often reported as low-frequency depression (Ishikawa et al. 1966), or the *soleus* H-reflex depression following passive stretch of the same muscle (Hultborn et al. 1996; Schieppati and Crenna 1984).

As far as the mechanisms underlying PaD are concerned, the following two points are largely accepted. First, PaD is a presynaptic phenomenon acting on the Ia terminals. In fact, during PaD of the *soleus* H-reflex, both voluntary contraction (Schieppati and Crenna 1984) and motor potentials evoked by transcranial magnetic stimulation (Hultborn et al. 1996) are unchanged, thereby arguing against motoneuron excitability decrease as the cause of H-reflex inhibition. Second, PaD differs from the "classical" presynaptic inhibition, which does not depend on the previous activation of the tested muscle and consists of a GABA-ergic depolarization of the Ia terminals (Eccles et al. 1963). This presynaptic inhibition lasts only 500ms and is widely distributed among the afferent fibres of the stimulated limb (Eccles 1964; Hultborn et al. 1996). On the contrary, PaD lasts several seconds (usually up to 10s) and it is confined to the afferents stimulated by the conditioning stimulus (Hultborn et al. 1996; Kohn et al. 1997).

On the basis of these characteristics, PaD is thought to correspond to the homosynaptic depression described in animals (Hultborn et al. 1996; Lundbye-Jensen and Nielsen 2008; Nielsen et al. 1993),

considered an intrinsic neuronal property associated with a decreased probability of transmitter release from the repetitively activated afferents (Capek and Esplin 1977; Curtis and Eccles 1960). However, the molecular mechanisms responsible for homosynaptic depression are still an open issue (Kohn et al. 1997).

The efficacy of GABA-ergic presynaptic inhibition decreases with increasing age (Butchart et al. 1993). When tested as *soleus* H-reflex depression from passive stretch, also PaD decreases in elderly people (Robertson and Koceja 2003), fitting well with animal studies showing that ageing affects synaptic efficacy (Kanda et al. 1989). However, this method studies not only PaD but also spindles excitability, which is known to decrease with ageing in animal models (Miwa et al. 1995). Therefore, we believe that the H-reflex frequency-related depression is the more appropriate parameter to assess the effect of ageing on PaD.

Key words
H-reflex
ageing
postactivation depression
frequency-related depression
homosynaptic depression
presynaptic inhibition

Methods

Subjects

The subjects were 38 right-handed healthy volunteers, divided into two groups according to age. In the young group, the 20 subjects (10 females) were aged between 24 and 33 years (28±3 years). In the aged group, the 18 subjects (9 females) were aged between 60 and 82 years (69±6 years).

To assess their usual level of physical activity, subjects filled out a questionnaire. They had to report their job and they were asked what kind of training they practised (jogging, cycling, training with treadmill...), how many times a week they trained and how long a single training session lasted.

None of the subjects practised competitive sport activities. The young group comprised six medical doctors, six office workers, five students and three nurses. In the aged group were instead recruited seven medical doctors, eight office workers and three nurses. As all these jobs did not require relevant physical activity, subjects were grouped as “sedentary” or “active” only according to the frequency of exercising (less or more than two training sessions a week with a minimum duration of 30 minutes for each single session). In the young group, eight subjects were active (six practised jogging and two aerobics) and twelve sedentary. In the aged group, eight subjects were active (three practised jogging, three cycling and two dancing) and ten subjects were sedentary.

All subjects gave informed consent according to the Declaration of Helsinki. The study was approved by the local ethical committee.

H-reflex recruitment curve and postactivation depression

The subjects were seated in an armchair. The shoulder was in slight abduction (60°), the elbow semi-flexed (110°) and the forearm pronated and supported by the arm of the chair. Special care was taken to assure that the subjects were at complete rest. All subjects were tested on both sides in two

separate sessions, with the exception of one patient in the aged group (male, 82 years), who was tested only on the right side.

EMG was recorded through bipolar surface preamplified electrodes (TSD150B, Biopac Systems Inc, USA) positioned over the FCR muscle, 3cm below the elbow. EMG signals were amplified (×3000), band-pass filtered (10Hz–1kHz, -6dB/octave), analog-to-digital converted at a 2kHz frequency.

To elicit H-reflexes in FCR, the median nerve was stimulated at the cubital fossa with bipolar surface electrodes (1cm half-balls 2cm apart). Rectangular pulses of 1ms duration were administered by means of a constant-current stimulator (model DS7A, Digitimer, UK).

M-wave and H-reflex peak-to-peak amplitudes were evaluated by means of the Acqknowledge software (Biopac Systems Inc, USA).

At the beginning of the experimental session, a soleus H–M recruitment curve was built up using a stimulation frequency of 0.1Hz. At this stimulation frequency, H-reflex depression is thought to be absent or minimal (Hultborn et al. 1996). The electrical stimulation intensities producing the H-reflex with the maximal amplitude (H-max) and the M-wave elicited by a supramaximal stimulus (M-max) were found and the H-max/M-max ratio was calculated. Afterwards, frequency-related depression was evaluated by comparing the H-reflex amplitude obtained with a stimulation frequency of 0.1Hz (H-test) with those obtained using higher frequencies (from 0.33Hz to 2Hz), at whom the depression is known to be present (Hultborn et al. 1996). The method can be explained as follows. First of all, using a stimulation frequency of 0.1Hz, the intensity of stimulation was carefully adjusted to produce H-reflexes on the ascending limb of the input-output curve with an amplitude ranging from 40% to 60% of H-max. After recording a train of 25 H-reflexes at the frequency of 0.1Hz, five trains of 20 reflexes each were evoked in a fixed order at the following stimulation frequencies: 0.33Hz, 0.5Hz, 1Hz and 2Hz. Then, a second train of 25 reflexes at 0.1Hz

was obtained. The minimum time between two subsequent trains was 1 minute. If the amplitudes of the 25 H-reflexes of the last train were different compared to those of the 25 reflexes of the first train (2 tailed t-test for unpaired data), the stimulation conditions were considered unstable and the experiment not accepted.

Concerning the trains obtained at the frequencies higher than 0.1Hz, we excluded the first H-reflex of each train, since it was not influenced by a previous stimulation. The amplitudes of the remaining 19 H-reflexes were measured and their mean value was calculated. Frequency-related depression was quantified as the ratio between the mean H-reflex amplitude obtained at the tested frequency (from 0.33Hz to 2Hz) and the mean amplitude of the 50 H-reflexes obtained at 0.1Hz (H-test). This is referred to as the H_{ratio} at different stimulation frequencies: the greater the ratio, the smaller the depression. Trials showing EMG activity in the pre-stimulus period were rejected.

Statistical analysis

H-max, M-max, H-max/M-max ratio, H-test/H-max ratio, H-test/M-max ratio and H_{ratio} at each stimulation frequency have been analysed using a factorial ANOVA with GROUP (aged/young) and SIDE (right/left) as main factors. Furthermore, H_{ratio} values have been analysed using a repeated measures ANOVA with H_{ratio} as within-subjects factor and GROUP (aged/young) and SIDE (right/left) as main factors. Analyses were considered significant for $p<0.05$. All the measures of variability are expressed as standard deviation (SD).

Results

The young subjects showed a significantly higher H-max ($F[1,71]=13.5$, $p=0.0005$; young 3.3±2.1mV, aged 1.9±1.1mV), M-max ($F[1,71]=8.5$, $p=0.005$; young 8.4±3.5mV, aged 6.3±2.7mV) and H-max/M-max ratio ($F[1,71]=5.6$, $p=0.02$; young 0.40±0.17, aged 0.31±0.13) compared to the aged group, without differences between the right and left side and without interaction SIDE X GROUP.

Conversely, the H-test/H-max ratio was similar between the young group (0.50±0.06) and the aged group (0.51±0.09) ($F[1,71]=1.5$, $p=0.2$), without differences between the right and left side and without interaction SIDE X GROUP. The H-test/M-max ratio was not significantly different between age-groups ($F[1,71]=3.4$, $p=0.07$; young 0.19±0.09, aged 0.15±0.07).

Repeated measures ANOVA showed that H_{ratio} values decreased as the stimulus frequency was increased ($F[3,213]=117.7$, $p<0.0001$), without difference between the two groups of subjects ($F[1,71]=3.6$, $p=0.06$), without difference between the two sides ($F[1,71]=0.2$, $p=0.6$) and without interaction SIDE X GROUP ($F[1,71]=0.7$, $p=0.4$). This increase of H-reflex depression with increasing frequencies was similar in all conditions as shown by the lack of interaction H_{ratio} X SIDE ($F[3,213]=1.2$, $p=0.3$), H_{ratio} X GROUP ($F[3,213]=2.3$, $p=0.8$) and H_{ratio} X SIDE X GROUP ($F[3,213]=0.6$, $p=0.6$). The post-hoc test showed that H_{ratio} values obtained at each stimulation frequency were different among them ($p<0.0001$) (Figure 1).

No difference of H_{ratio} values was found between the two groups of subjects at 0.33Hz ($F[1,71]=0.003$, $p=1.0$), 0.5Hz ($F[1,71]=1.3$, $p=0.3$) and 1Hz ($F[1,71]=2.7$, $p=0.1$). On the contrary, at 2Hz H_{ratio} values were higher in the aged group of subjects ($F[1,71]=9.7$, $p=0.003$) (Figure 2).

Discussion

Our results show that frequency-related depression of the FCR H-reflex does not change between young and aged subjects, with the exception of the 2Hz frequency. At this frequency of stimulation, H_{ratio} values are higher in the aged group, indicating lower H-reflex depression.

PaD is thought to be the sole mechanism inducing frequency-related depression of the H-reflex at stimulation frequency of 1Hz or lower (Clair et al. 2011; Meunier et al. 2007). Studies in animals showed that the "classical" GABA-ergic presynaptic inhibition can last up to 500ms (Hultborn et al. 1996). Therefore, GABA-ergic presynaptic inhibition may have a role in the frequency-related depression tested at 2Hz or higher frequencies (Clair et al. 2011; Meunier et al. 2007). Indeed, the recent works showing the pivotal role of PaD in the pathophysiology of spasticity have been performed assessing the frequency-related depression of the H-reflex at the frequency of 1 Hz (Achache et al. 2010) or at lower frequencies (Aymard et al. 2000; Lamy et al. 2009).

The present results, showing that frequency-related depression tested at 1Hz and at lower frequencies is similar in young and aged subjects, strongly suggest that PaD is not influenced by age. Theoretically, the reduced depression in aged subjects found at 2Hz could reflect a decrease of both GABA-ergic presynaptic inhibition and PaD. However, considering the present results obtained at lower frequencies and previous findings showing a decrease in GABA-ergic presynaptic inhibition with increasing age (Butchart et al. 1993), it seems that a decrease of GABA-ergic presynaptic inhibition is the more likely explanation for the age-related changes that we observed at the frequency of 2Hz.

The lack of an age effect on frequency-related depression tested at the frequencies of 1Hz, 0.5Hz and 0.33Hz, which are known to be specific for PaD assessment, could appear in contrast with previous results. In 2003, Robertson and Koceja investigated PaD in the soleus muscle in two groups of healthy subjects with different age. They found that PaD was decreased in the aged

subjects, who had on average 16.1% less H-reflex depression in comparison with the young subjects (Robertson and Koceja 2003). This discrepancy may be due to the different investigated muscles. It has been shown that the dymamics of transmitter release in Ia terminals targeting the fast FCR motoneurons are dissimilar from those targeting the slow soleus motoneurons (Rossi-Durant et al. 1999). Therefore, the present findings showing that ageing does not change postactivation depression could not be reproduced when postactivation depression is tested in the soleus muscle. A further study is necessary to examine this issue. Furthermore, this discrepancy may be due to the different method used to assess PaD. We used the frequency-related depression of the FCR H-reflex, whereas Robertson and Koceja (Robertson and Koceja 2003) used the *soleus* H-reflex depression following passive stretch of the *soleus* muscle (Schieppati and Crenna 1984). In this method, the reflex depression depends on the Ia discharge induced by muscle stretching, which could be itself an age-dependent phenomenon. Indeed, a histological study in humans has shown that ageing increases the thickness of the muscle spindles' capsule (Swash and Fox 1972). This morphological modification could lead to an increased stiffness of the capsule, thus reducing the extension of Ia spirals and their discharge during muscle stretching. In line with these results in humans, Miwa et al. were able to demonstrate a decreased response of Ia fibers to a muscle stretch in aged rats (Miwa et al. 1995). Considering these previous observations, it seems likely that the difference in PaD between young and elderly reported by Roberston and Koceja was due, at least in part, to a decreased Ia discharge after muscle stretching in the group of aged subjects, as correctly discussed by the same Authors (Robertson and Koceja 2003).

In our study, on the contrary, the conditioning Ia discharge causing the reflex depression is probably similar in the young and aged subjects, as we matched the size of the test H-reflex (i.e. H-reflex obtained at 0.1Hz) in the two groups. It must be said, however, that the number of motor units as well as their synaptic characteristics can differ between the two age groups, owing to the difference

in the size of the H reflex expressed relative to M-max. In this context, our findings suggest that the intrinsic properties of the Ia fibers underlying PaD are not affected by ageing.

In patients, it has been demonstrated that diminished PaD is highly correlated to the severity of spasticity, stating the role of PaD in the pathophysiology of increased muscle tone (Achache et al. 2010; Aymard et al. 2000; Calancie et al. 1993; Grey et al. 2008; Lamy et al. 2009; Nielsen et al. 1993). Furthermore, PaD has been used in the longitudinal assessment of spasticity (Schindler-Ivens and Shields 2000; Trimble et al. 1998), in particular to assess the efficacy of drugs and rehabilitation therapy over years (Shields et al. 2011). It is clear that this type of longitudinal assessment could be impaired by discrepancies attributable to age-related changes. Although caution is needed when extrapolating ageing effects from a cross-sectional study, the present finding, showing that ageing does not affect the frequency-related depression of the FCR H-reflex at the frequencies of 1Hz or lower, supports the reliability of this method to assess PaD in the clinical practice.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Achache V, Roche N, Lamy J, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R (2010) Transmission within several spinal pathways in adults with cerebral palsy. Brain 133:1470-1483.

Aymard C, Katz R, Lafitte C, Lo E, Pénicaud A, Pradat-Diehl P, Raoul S (2000) Presynaptic inhibition and homosynaptic depression: a comparison between lower and upper limbs in normal human subjects and patients with hemiplegia. Brain 123:1688-1702.

Butchart P, Farquhar R, Part NJ, Roberts RC (1993) The effect of age and voluntary contraction on presynaptic inhibition of soleus muscle Ia afferent terminals in man. Exp Physiol 78:235-242.

Calancie B, Broton JG, Klose KJ, Traad M, Difini J, Ayyar DR (1993) Evidence that alterations in presynaptic inhibition contribute to segmental hypo- and hyperexcitability after spinal cord injury in man. Electroencephalogr Clin Neurophysiol 89:177-186.

Capek R, Esplin B (1977) Homosynaptic depression and transmitter turnover in spinal monosynaptic pathway. J Neurophysiol 40:95-105.

Clair JM, Anderson-Reid JM, Graham CM, Collins DF (2011) Postactivation depression and recovery of reflex transmission during repetitive electrical stimulation of the human tibial nerve. J Neurophysiol 106:184-192.

Crone C, Nielsen J (1989) Methodological implications of the post activation depression of the soleus H-reflex in man. Exp Brain Res 78:28-32.

Curtis DR, Eccles JC (1960) Synaptic action during and after repetitive stimulation. J Physiol 150:374-398.

Eccles JC (1964) The physiology of synapses. Springer, Berlin.

Eccles JC, Schmidt R, Willis WD (1963) Pharmacological studies on presynaptic inhibition. J Physiol 168:500-530.

Grey MJ, Klinge K, Crone C, Lorentzen J, Biering-Sørensen F, Ravnborg M, Nielsen JB (2008) Post-activation depression of soleus stretch reflexes in healthy and spastic humans. Exp Brain Res 185:189-197.

Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H (1996) On the mechanism of the post-activation depression of the H-reflex in human subjects. Exp Brain Res 108:450-462.

Ishikawa K, Ott K, Porter RW, Stuart D (1966) Low frequency depression of the H wave in normal and spinal man. Exp Neurol 15:140-156.

Kanda K, Hashizume K (1989) Changes in properties of the medial gastrocnemius motor units in

aging rats. J Neurophysiol 61:737-746.

Kohn AF, Floeter MK, Hallett M (1997) Presynaptic inhibition compared with homosynaptic depression as an explanation for soleus H-reflex depression in humans. Exp Brain Res 116:375-380.

Lamy J, Wargon I, Mazevet D, Ghanim Z, Pradat-Diehl P, Katz R (2009) Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients. Brain 132:734-748.

Lundbye-Jensen J, Nielsen JB (2008) Immobilization induces changes in presynaptic control of group Ia afferents in healthy humans. J Physiol 586:4121-4135.

Meunier S, Kwon J, Russmann H, Ravindran S, Mazzocchio R, Cohen L (2007) Spinal use-dependent plasticity of synaptic transmission in humans after a single cycling session. J Physiol 579:375-388.

Miwa T, Miwa Y, Kanda K (1995) Dynamic and static sensitivities of muscle spindle primary endings in aged rats to ramp stretch. Neurosci Lett 201:179-182.

Nielsen J, Petersen N, Ballegaard M, Biering-Sørensen F, Kiehn O (1993) H-reflexes are less depressed following muscle stretch in spastic spinal cord injured patients than in healthy subjects. Exp Brain Res 97:173-176.

Robertson CT, Koceja DM (2003) Post-activation depression of the soleus H-reflex in the elderly. Electromyogr Clin Neurophysiol 43:103-111.

Rossi-Durand C, Jones KE, Adams S, Bawa P (1999) Comparison of the depression of H-reflexes following previous activation in upper and lower limb muscles in human subjects. Exp Brain Res 126:117-127.

Schieppati M, Crenna P (1984) From activity to rest: gating of excitatory autogenetic afferences from the relaxing muscle in man. Exp Brain Res 56:448-457.

Schindler-Ivens S, Shields RK (2000) Low frequency depression of H-reflexes in humans with acute and chronic spinal-cord injury. Exp Brain Res 133:233-241.

Shields RK, Dudley-Javoroski S, Oza PD (2011) Low-frequency H-reflex depression in trained human soleus after spinal cord injury. Neurosci Lett 499:88-92.

Swash M, Fox KP (1972) Techniques for the demonstration of human muscle spindle innervation in neuromuscular disease. J Neurol Sci 15:291-302.

Trimble MH, Kukulka CG, Behrman AL (1998) The effect of treadmill gait training on low-frequency depression of the soleus H-reflex: comparison of a spinal cord injured man to normal subjects. Neurosci Lett 246:186-188.

Abbreviations

PaD – Postactivation depression

FCR – Flexor carpi radialis muscle

Figure legends

Figure 1

Effects of the rate of stimulation on H-reflex amplitude obtained in all the 40 subjects in the left (A) and in the right (B) *flexor carpi radialis* (FCR) muscle. Diamonds show the rate effect on aged subjects and squares show the rate effect on young subjects. At each stimulation frequency reported in the abscissa, the amplitude of the H-reflexes is expressed as a fraction of the amplitude of the H-reflexes obtained at 0.1Hz (H_{ratio}). Each square (in A e B) and each diamond in B represents the average of 20 H_{ratio} values. Each diamond in A represents the average of 19 H_{ratio} values (one subject in the aged group was not tested in the left side). The error bars represent magnitude of standard deviation (SD).

Figure 2

Effects of the rate of stimulation on H-reflex amplitude obtained in all the 40 subjects at 0.33Hz (A), 0.5Hz (B), 1Hz (C) and 2 Hz (D). Data from the right and left *flexor carpi radialis* (FCR) muscles were pooled together. The bars represent the amplitude of the H-reflexes expressed as a fraction of the amplitude of the H-reflexes obtained at 0.1Hz (H_{ratio}). The bars for the young subjects represent the average of 40 H_{ratio} values (20 from the right side and 20 from the left side). The bars for the aged subjects represent the average of 39 H_{ratio} values (20 from the right side and 19 from the left side; one subject in the aged group was not tested in the left side). The error bars represent magnitude of standard deviation (SD). H_{ratio} values were different between young and aged subjects only at the stimulation frequency of 2Hz (asterisk means $p<0.05$).

Figure 1

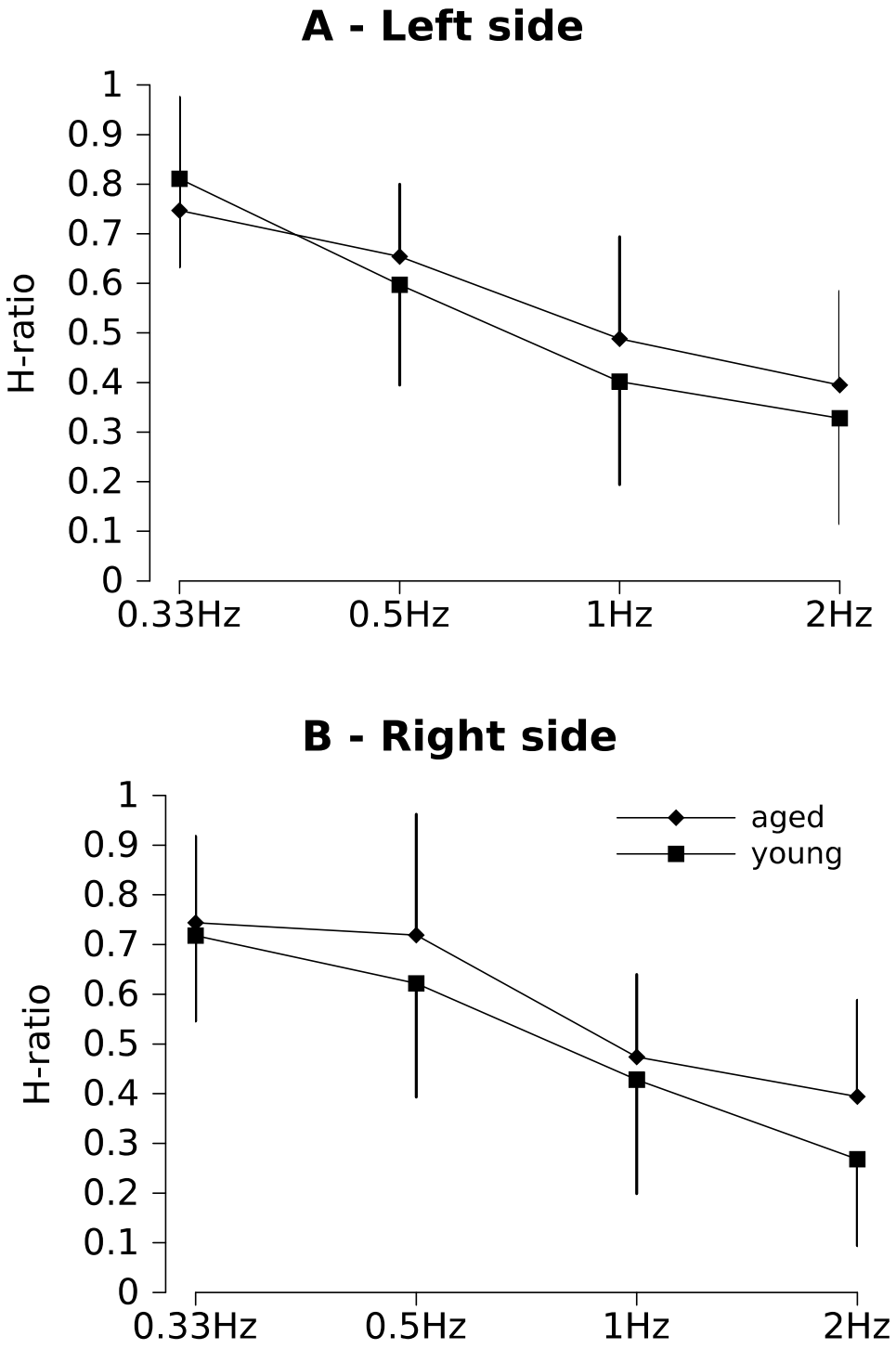
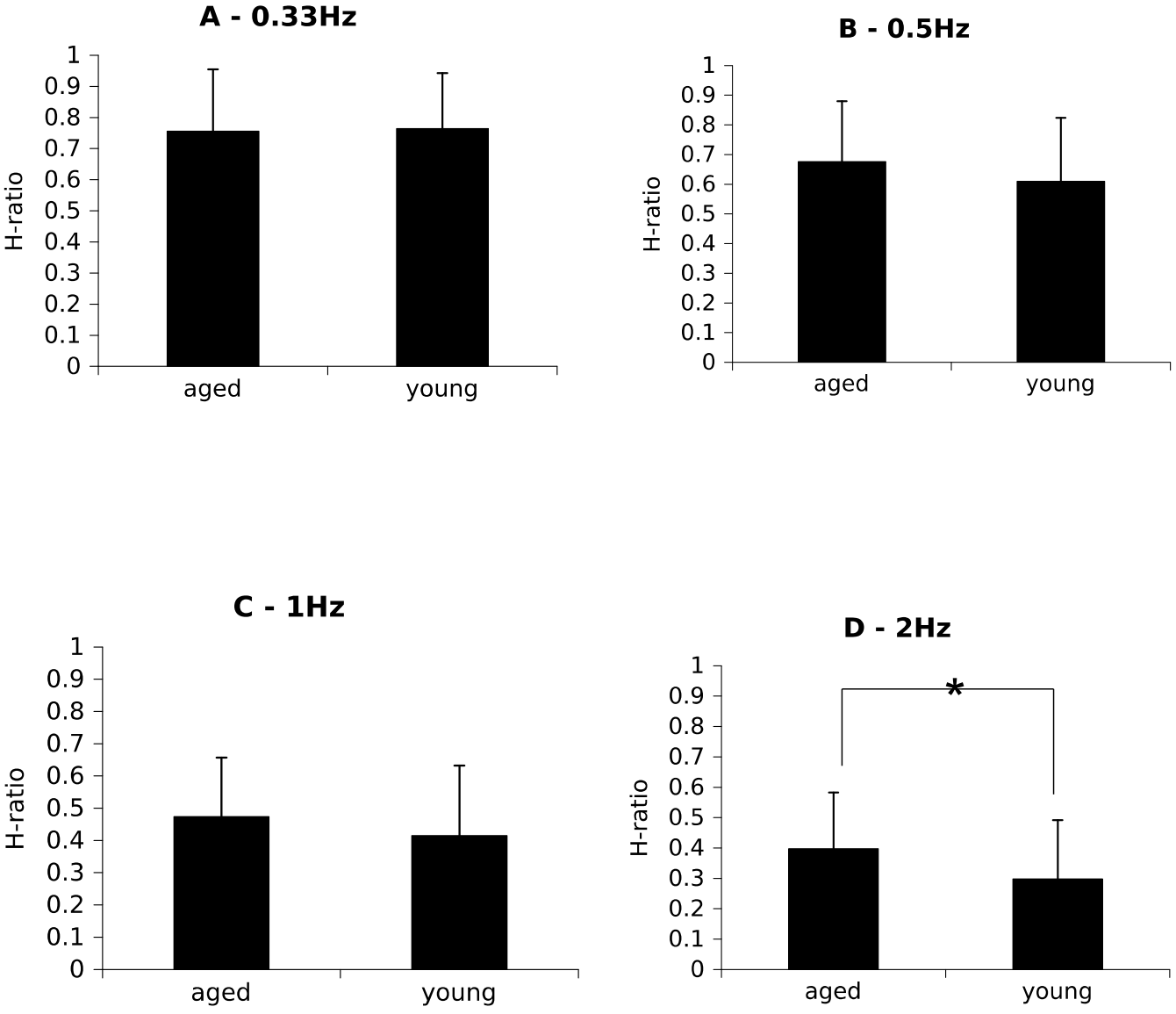

A - Left side
H-ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.33Hz
0.5Hz
1Hz
2Hz
B - Right side
H-ratio
aged
young
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.33Hz
0.5Hz
1Hz
2Hz

Figure 2


A - 0.33Hz
B - 0.5Hz
C - 1Hz
D - 2Hz
H-ratio
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
aged
young
*