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A startling acoustic stimulation (SAS)-TMS approach to assess the reticulospinal system in healthy and stroke subjects

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ABSTRACT

Reticulospinal (RS) hyperexcitability is observed in stroke survivors with spastic hemiparesis. Habituated startle acoustic stimuli (SAS) can be used to stimulate the RS pathways non-reflexively. However, the role of RS pathways in motor function and its interactions with the corticospinal system after stroke still remain unclear. Therefore, the purpose of this study was to investigate the effects of conditioning SAS on the corticospinal system in healthy subjects and in stroke subjects with spastic hemiparesis. An established conditioning SAS- transcranial magnetic stimulation (TMS) paradigm was used to test the interactions between the RS pathways and the corticospinal system. TMS was delivered to the right hemisphere of eleven healthy subjects and the contralesional hemisphere of eleven stroke subjects during isometric elbow flexor contraction on the non-impaired (or left) side. Conditioning SAS had similar effects on the corticospinal motor system in both healthy and stroke subjects, including similar SAS-induced motor evoked potential (MEP) reduction at rest, but not during voluntary contraction tasks; similar magnitudes of TMS-induced MEP and force increment and shortening of the silent period during voluntary elbow flexor contraction. This study provides evidence that RS excitability on the contralesional side in stroke subjects with spastic hemiparesis is not abnormal, and suggests that RS projections are likely to be primarily unilateral in humans.

1. Introduction

The brainstem motor system, in particular, the reticular system and its reticulospinal (RS) pathways, can influence the motor output from cortical motor areas. It can facilitate motor initiation in healthy subjects [1–3] and after stroke [4], and increase the magnitude of voluntary muscle contraction in healthy subjects and Parkinson's patients [1,5]. The RS pathways have been demonstrated to compensate for damage of corticospinal pathways for motor recovery after stroke in animal models [6–11]. Direct assessment of brainstem activities in humans is not available even with most advanced brain imaging technologies [12–16]. A combined startling acoustic stimulation (SAS) and transcranial magnetic stimulation (TMS) represents the best available non-invasive approach to examine the contribution of the brainstem motor system and its interaction with the corticospinal system [17–20]. In this paradigm, a TMS stimulus is delivered at different times (30–120 ms) to the motor cortex to examine the SAS-induced effect on the corticospinal motor system.

An unexpected loud sound, i.e., SAS, usually causes startle reflex responses. This acoustic startle reflex is mediated by a relatively simple reflex circuit. The reflex circuit in humans includes the cochlear nucleus, the caudal pontine reticular nuclei, the motoneurons of the brainstem and the spinal cord activated via the medial RS pathway [21–23]. Acoustic startle reflex is commonly habituated after a few SAS trials [19,20]. Ensuing SAS continues to stimulate the brainstem reticular system and RS pathways, but not cause reflex responses. However, SAS imposes different effects on the motor cortex and the spinal motor neurons. In healthy subjects at rest or during minimal voluntary activation, it is known that SAS causes transient effects: an early cortical inhibition at 30-60 ms [17] and a later general cortical excitation for another 50 ms after the stimulation [24]. This early inhibition of the motor cortex is thought to be mediated by reticulo-thalamo-cortical polysynaptic inhibition [19]. This early cortical inhibition effect is not observed when the motor cortex is already activated during 10% of

Abbreviations: RS, reticulospinal; SAS, startling acoustic stimuli; TMS, transcranial magnetic stimulus; MEP, motor evoked potential; ASR, Acoustic startle reflex; SES, subcortical electrical stimulation; $REST_{TMS}$, TMS at rest without SAS; $REST_{SAS\text{-}TMS}$, TMS at rest with a conditioning SAS; $VOLT_{TMS}$, TMS during voluntary muscle contractions without SAS; $VOLT_{SAS\text{-}TMS}$, TMS during voluntary muscle contractions with a conditioning SAS

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Table 1
Stroke patient characteristics. (Ting, please make sure MAS of 0 for elbow flexors is accurate for these patients).

Subject	Age	Gender	History of Stroke (Months)	Paretic Side	Dominant Side	Elbow Flexor MAS	Wrist Flexor MAS	Finger Flexor MAS	Impaired biceps MVC (N-m)	Non-impaired biceps MVC (N-m)	Lesion type and site
Stroke_1	59	F	100	L	R	1	0	1	16.4	26.9	Hemorrhagic, right MCA
Stroke_2	44	F	63	L	R	1	1	2	34.4	37	Ischemic, Right MCA
Stroke_3	70	M	66	R	R	1+	2	2	16.6	48.5	Ischemic, left frontotemporal
Stroke_4	72	M	64	L	R	1+	2	3	6.2	23.6	Ischemic, right caudate
Stroke_5	70	M	84	R	R	1	2	0	9.3	37.4	Ischemic, left caudate
Stroke_6	63	F	171	L	R	1	1	2	7.4	30.1	Ischemic, right MCA
Stroke_7	62	M	7	L	R	2	1	1	16	44.6	Ischemic, right MCA
Stroke_8	72	M	82	R	R	1	0	1	26	39	Hemorrhagic
Stroke_9	68	F	112	R	L	1	0	1	9.5	30	Ischemic, left basal ganglia and thalamic
Stroke_10	56	M	27	L	R	1	0	1	10	29	Ischemic, right MCA
Stroke_11	85	M	26	L	R	1	1+	1+	5	20	Hemorrhagic

MAS: Modified Ashworth Scale. MVC: Maximum Voluntary Contraction. MCA: Middle Cerebral artery; Nm: newton meter; F: female; M: male; L: left; R: right.

maximum voluntary contraction (MVC) tasks [20]. In other words, the effect of SAS on the motor cortex depends on its pre-existing excitability. In contrast, SAS imposes excitatory effects on spinal motor neurons both at rest and during 10%MVC tasks as reflected by shortening of the silent period in TMS studies [20]. This excitatory effect is mediated by stimulation of RS pathways by SAS.

Neural plasticity plays an important role in motor recovery as well as development of motor complications, such as spasticity after stroke [25]. Post-stroke plastic changes occur in ipsilesional (iM1), contralesional (cM1) motor cortices, and subcortical areas, primarily pontomedullary reticular formation (PMRF) [9,19,26–30]. iM1 plasticity primarily contributes to recovery of motor function [31,32]. There is increased cM1 activation during movement of the paretic side in patients with motor impairment [33,34]. As a result, the heightened cM1 activation leads to an abnormally high inhibitory drive from the cM1 to iM1 areas [35]. The cM1 activation decreases if good motor recovery occurs [36]. Therefore, the increased cM1 activation in patients with motor impairment is considered as maladaptive plasticity [37]. As a result of damage to iM1 and its descending pathways and subsequent unmasking of inhibition, there is increased excitability in the brainstem reticular system and descending RS pathways. This RS hyperexcitability is a possible mechanism mediating post-stroke spasticity [25,30,38,39]. Although RS hyperexcitability is generally considered to be maladaptive, for some severely impaired patients the activation of cM1 and cortico-reticulo-spinal pathways could potentially improve motor function [29].

The RS system has been shown to compensate for the loss of, or damage to corticospinal system after stroke in animal models [6,9,11,40]. However, the role of RS system in post-stroke motor recovery remains controversial. Motor rehabilitation programs designed to improve initiation and pacing of voluntary movements have been reported, where acoustic stimuli in the forms of rhythmic cueing or music therapy have been integrated [41–44]. The RS system is likely activated through acoustic stimuli. However, Aluru et al. [45] reported that auditory rhythmic cueing improved motor performance in stroke patients with severe spastic paresis of wrist flexors, but not in those patients with minimal impairment or spastic co-contractions. It is known from animal studies that the brainstem reticular motor system receives bilateral corticoreticular projections and sends bilateral reticulospinal projections [9]. However, weakness and spasticity occur primarily only on the paretic side clinically. The role of RS pathways in spasticity and motor function and its interaction with the corticospinal system needs further investigation.

Accordingly, we use an established combined SAS-TMS paradigm to examine the relations between reticular system and corticospinal pathways after stroke in this study. The details of the paradigm were described in our previous work [20]. The primary aim was to compare the effects of conditioning SAS in healthy subjects and in stroke subjects with spastic hemiplegia. It was hypothesized to observe greater SAS-induced inhibition during TMS stimulation to the contralesional M1 in stroke subjects as compared to healthy subjects.

2. Methods

2.1. Participants

Eleven healthy adults (30.5 yrs. ± 5.3 yrs.; 2 women) and eleven stroke subjects (65 yrs. ± 10.9 yrs.; 4 women) participated in this study. All healthy subjects were right-handed with no known neuromusculoskeletal impairments. Inclusion criteria for the stroke subjects were: 1) hemiplegia secondary to an ischemic or hemorrhagic stroke; 2) at least 6 months post-stroke; 3) residual voluntary elbow flexion force, at least be able to antigravity; 4) spastic hypertonia of the upper limb of the impaired side was rated as < 3 on the Modified Ashworth Scale (MAS); and 5) able to understand and follow instructions related to the experiment. Exclusion criteria for the stroke subjects included: 1) a

history of multiple strokes or bilateral involvement; 2) presence of contracture that would limit full elbow range of motion on the impaired side; 3) visuospatial neglect; and 4) taking any medication which could alter the severity of muscle spasticity. The detailed information of the stroke subjects is listed in Table 1. The Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston approved the procedures of this study. All participants provided written informed consent before participating in the study.

2.2. Experimental tasks

The purpose of this study was to examine the differences of SAS-induced effects in left biceps of healthy subjects and non-impaired biceps of stroke subjects using our recent experimental protocol [20]. There were four tasks: 1) TMS at rest ($REST_{TMS}$); 2) a conditioning SAS followed by a single pulse of TMS at rest ($REST_{SAS\text{-}TMS}$); 3) TMS during voluntary muscle contractions ($VOLT_{TMS}$); 4) a conditioning SAS followed by single pulse of TMS during voluntary muscle contractions ($VOLT_{SAS\text{-}TMS}$). Prior to the four tasks, the TMS hotspot was carefully localized, and maximum voluntary contraction (MVC) force of targeted biceps muscle was estimated.

The hotspot for the target biceps muscle was first detected and localized. A single-pulse TMS stimulus (BiStim2, MagStim Corp., UK) was set at an intensity of 75% of the maximum stimulator capacity (equal to 84.75% maximum stimulator output on MagStim 200^2, MagStim Corp., UK) while subjects held their forearm in 90° of elbow flexion. TMS was delivered over the targeted primary motor cortex using a figure-of-8 shaped stimulation coil (a 70-mm mean diameter of each wing, Model: BiStim^2, MagStim Corp., UK). The hotspot was determined when the largest increment in elbow flexor contraction was produced in 3 consecutive trials. We used a gel ink pen to mark the spot on the scalp.

MVC force was estimated from 3 elbow flexion MVC attempts. The subjects were asked to produce a maximum elbow flexion force and to maintain the force for 3–5 s. The highest force among 3 attempts was considered the MVC force to predefine the target force in the main experiment. At least one-minute of rest was provided between consecutive MVC attempts. In order to ensure habituation of the startle reflex, 6–8 startling acoustic stimuli were delivered at 100 dB with at least a 20-s interval between two consecutive stimuli after MVC tasks [19,46].

All subjects were asked to perform the following four main tasks in a randomized order after the MVC attempts and habituation of acoustic startle reflex.

1) $REST_{TMS}$: A single pulse of TMS was delivered to the hot spot of the target biceps muscle. TMS was randomly delivered between 7 and 11 s during a 12-s trial.
2) $REST_{SAS\text{-}TMS}$: Similar to the $REST_{TMS}$ task, but a 100 dB SAS was delivered 50 ms prior to the delivery of TMS.
3) $VOLT_{TMS}$: Healthy subjects were asked to perform left elbow isometric flexion, while stroke subjects were asked to perform the task with their non-impaired elbow. Before a trial began, a horizontal red line on the monitor was provided as the target (10% of MVC). The real-time force signal was provided as a white line running from left to right on the monitor during each 12-s trial. Subjects were asked to match the white line with the red line as precisely as possible by increasing and then maintaining elbow flexion force. Subjects were asked to generate force one second after a trial started to show the baseline force. At least one practice trial was given to the subjects for the purpose of familiarization. TMS was delivered randomly between 7 and 11 s during a 12-s trial.
4) $VOLT_{SAS\text{-}TMS}$: Similar to the $VOLT_{TMS}$ task, but a 100 dB SAS was delivered 50 ms prior to the TMS delivery.

The hotspot was verified intermittently throughout the experiment to ensure the correct spot was stimulated. Five trials were collected in $REST_{TMS}$ task, and six trials were collected in other three tasks. To minimize possible fatigue effects during the experiment, subjects were allowed to have adequate rest breaks.

2.3. Experimental set-up

Subjects were seated comfortably on a height adjustable chair in an upright position. Subjects were asked to put the test arm in a customized device. The arm was secured against two adjustable metal plates with a padded strap approximately 2–4 in. proximal from the wrist. Subjects were asked to keep their two arms at symmetrical positions with the following arrangement: the shoulder joint was placed approximately in 30° of abduction and 45° of flexion, while the elbow was flexed to 90°. The wrist and fingers were kept naturally relaxed. An adjustable height table was provided for the resting arm in order to keep symmetrical position.

Subjects faced a 20-inch computer monitor (Model: 2001FP, Dell Computer Corp., Texas, USA), which was located approximately 1 m in front at each subject's eye level. The monitor was used for visual display using a custom-written computer program (LabView®, National Instrument™ Inc., Austin, Texas, USA). A horizontal red line indicated the target force, while a white trace ran from left to right to reflect the real-time force produced by isometric elbow flexor contraction. All subjects confirmed that they could see the display clearly. A loud sound (Microsoft system warning sound, 1 kHz tone of 50 ms) was generated by the computer through a sound card (Model: Sound Blaster Extreme, Creative Technology Ltd.) and a speaker (Model: HS50M, YAHAMA Corp., Hamamatsu, Japan) at 100 dB. The speaker was placed 30 cm behind the subject at the ear level in the subject's midline.

2.3.1. Force measurement

The elbow flexion force exerted during MVC, $VOLT_{TMS}$, $VOLT_{SAS\text{-}TMS}$ tasks was measured with a torque sensor (Model: TRS-500, Transducer Techniques, Temecula, CA, USA). The sensor was located in line with the center of the rotation of the targeted elbow joint. The elbow flexion torque signal was sampled at 1000 Hz with a NI-DAQ card (Model: PCI-6229, National Instruments, Austin, TX, USA).

2.3.2. EMG measurement

Muscle activity was recorded with a Bagnoli EMG system (Delsys Inc., Boston, MA, USA) from the target biceps muscle according to the European Recommendations for Surface Electromyography [47]. The EMG signals were band-pass filtered from 20 to 450 Hz, amplified 1000 times, and then sampled at 1000 Hz using the same NI-DAQ card. All collected data were stored on a personal computer.

2.4. Data analysis

Data were analyzed off-line with custom-written Matlab® programs (Math Works™ Inc., Natick, Massachusetts, USA). The raw force signal was low-pass filtered at 10 Hz with a fourth-order, zero-lag Butterworth digital filter before further analysis. For each trial, we extracted the force and EMG signals 100 ms before and 400 ms after the TMS onset from the raw data. The following parameters were calculated in the same way as in our recent study [20].

- Background EMG. Background EMG was quantified as the root mean square (RMS) of the EMG calculated over the 100-ms window prior to the TMS delivery.
- Background force. Background force was calculated as the mean force over a 100-ms window prior to the TMS delivery.
- MEP latency. MEP latency was quantified as the interval between the TMS delivery to the time point when the EMG signal exceeded 2 standard deviations of the EMG during the 100-ms window before the TMS delivery [48].
- MEP amplitude. To calculate the MEP amplitude, we quantified the

peak-to-peak EMG amplitude within the time window from the MEP onset to 50 ms after the TMS delivery.

- Silent period. Before calculating the silent period, the EMG signals were further processed as follows: 1) high-pass filtered at 65 Hz with a fourth-order, zero-lag Butterworth digital filter in order to remove slow oscillations [49]; 2) rectified; 3) low-pass filtered at 30 Hz with a fourth-order, zero-lag Butterworth digital filter to smooth the EMG signals; 4) averaged all the processed EMG signals for each task and each subject. The silent period was quantified as the interval between the TMS delivery to the time when the EMG signal reached the background EMG level again [50].
- TMS-induced force increment. During the $VOLT_{SAS\text{-}TMS}$ and $VOLT_{TMS}$ tasks, TMS-induced force increment was quantified as the difference between the background force and the peak force after the TMS delivery.

2.5. Statistical analysis

In this study, we calculated the following dependent variables: 1) background EMG; 2) background force; 3) MEP latency; 4) MEP amplitude; 5) silent period; 6) TMS-induced force increment. Paired t-tests were used to compare the background EMG and background force for healthy subjects and stroke subjects separately. Two-way mixed ANOVAs were used to compare the effect of SAS on TMS-induced MEP responses, with a between-group factor of GROUP (Healthy or Stroke) and a within-group factor of SAS (with and without) at rest and during 10% voluntary contraction, respectively. Two-way mixed ANOVAs were used to test the effect of SAS on the silent period and TMS-induced force with factors of GROUP and SAS. The alpha level for all statistical tests was set at 0.05. Data are reported as mean ± SD within the text and as mean ± SEM in the figures. Only the significant main effects are presented, unless otherwise noted.

3. Results

3.1. MVC and background EMG and force

For healthy subjects, the average MVC force was 37.2 ± 8.17 Nm. The background force was not significantly different ($p > .05$) between the $VOLT_{SAS\text{-}TMS}$ task (3.66 ± 0.87 Nm) and the $VOLT_{TMS}$ task (3.67 ± 0.85 Nm). The background EMG was not significantly different ($p > .05$) between the $REST_{SAS\text{-}TMS}$ task (0.0033 mV ± 0.001 mV) and the $REST_{TMS}$ task (0.0033 mV ± 0.003 mV). The background EMG was also not significantly different ($p > .05$) between the $VOLT_{SAS\text{-}TMS}$ task with (0.01 mV ± 0.006 mV) and the $VOLT_{TMS}$ task (0.01 mV ± 0.005 mV).

For stroke subjects, the average MVC force was 33.3 ± 8.80 Nm. The background force was not significantly different ($p > .05$) between the $VOLT_{SAS\text{-}TMS}$ task (3.46 ± 0.88 Nm) and the $VOLT_{TMS}$ task (3.41 ± 0.89 Nm). The background EMG was not significantly different ($p > .05$) between the $REST_{SAS\text{-}TMS}$ task (0.0073 mV ± 0.008 mV) and the $REST_{TMS}$ task (0.0066 mV ± 0.008 mV). The background EMG was also not significantly different ($p > .05$) during voluntary contraction tasks with and without SAS conditioning ($VOLT_{SAS\text{-}TMS}$ task: 0.024 mV ± 0.021 mV vs. $VOLT_{TMS}$ task: 0.023 mV ± 0.02 mV).

3.2. The effects of SAS at rest

The effects of conditioning SAS were similar between healthy subjects and stroke subjects at rest. There was no change in the MEP latency with conditioning SAS (all $p > .05$). Representative trials from one healthy subject and one stroke subject were depicted in Fig. 1 B and C, respectively. For healthy subjects, the MEP latency was similar between the $REST_{TMS}$ task (15.1 ms ± 1.21 ms) and the $REST_{TMS}$ task (15.2 ms ± 0.92 ms) task. For stroke subjects, the MEP latency was

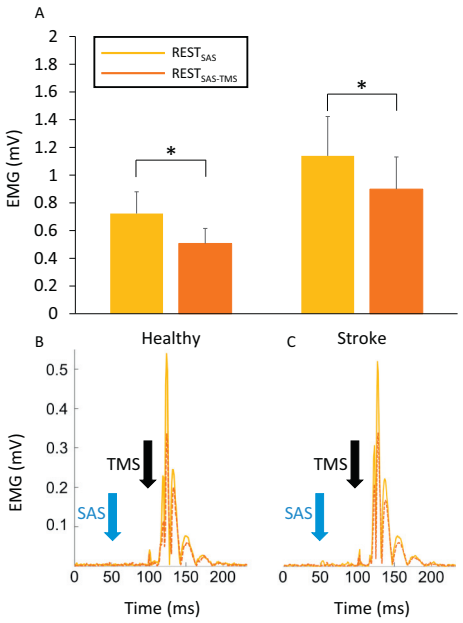

Fig. 1. (A)The average MEP amplitude was smaller during the $REST_{SAS\text{-}TMS}$ task compared with the $REST_{TMS}$ task for both healthy subjects and stroke subjects. Representative trials of MEP responses from (B) a healthy subject and (C) a stroke subject. *Indicates statistical significant difference ($p < .05$).

15.1 ms ± 1.36 ms for the $REST_{TMS}$ task and 15.0 ms ± 1.42 ms for the $REST_{SAS\text{-}TMS}$ task. In contrast, SAS conditioning led to a significant reduction in the MEP amplitude. Two-way ANOVA tests revealed a main effect of SAS ($F_{(1,20)} = 22.22$, $p < .001$; Fig. 1A).

There were no significant main effects of GROUP nor GROUP × SAS interactions for the MEP amplitude (both $p > .05$). For healthy subjects, the MEP amplitude was smaller during the $REST_{SAS\text{-}TMS}$ task (0.51 mV ± 0.36 mV) than during the $REST_{TMS}$ task (0.72 mV ± 0.52 mV; $p = .008$). For stroke patients, the MEP amplitude was also significantly reduced during the $REST_{SAS\text{-}TMS}$ task (0.89 mV ± 0.78 mV) compared with the $REST_{TMS}$ task (1.14 mV ± 0.94 mV; $p = .03$).

In order to further compare the SAS-induced reduction of MEP amplitude between healthy subjects and stroke subjects, the reduction of MEP amplitude was normalized using the following equation:

$$\text{Normalzied reduction of MEP amplitude}(\%) = 100 \times \frac{REST_{SAS-TMS}\ \text{MEP amplitude} - REST_{TMS}\ \text{MEP amplitude}}{REST_{TMS}\ \text{MEP amplitude}}$$

The MEP reduction was 25.5% ± 17.7% for healthy subjects and 27.2% ± 11.0% for stroke subjects. They were not significantly different ($p > .05$).

3.3. The effect of SAS during voluntary contractions

The effects of conditioning SAS were similar between healthy subjects and stroke subjects during voluntary contractions. For MEP latency, there were no significant effects of SAS or GROUP nor their interactions during voluntary contractions ($p > .05$). For healthy subjects, the MEP latency was similar between the $VOLT_{SAS\text{-}TMS}$ task (14.2 ms ± 1.64 ms) and the $VOLT_{TMS}$ task (14.2 ms ± 1.05 ms). For stroke subjects, the MEP latency was also similar between the $VOLT_{SAS\text{-}TMS}$ task (15.2 ms ± 1.42 ms) and the $VOLT_{TMS}$ task (14.6 ms ± 1.78 ms).

There was also no significant change in the MEP amplitude with conditioning SAS in both healthy and stroke subjects ($p > .05$). For healthy subjects, the MEP amplitude was similar during the $VOLT_{SAS\text{-}}$

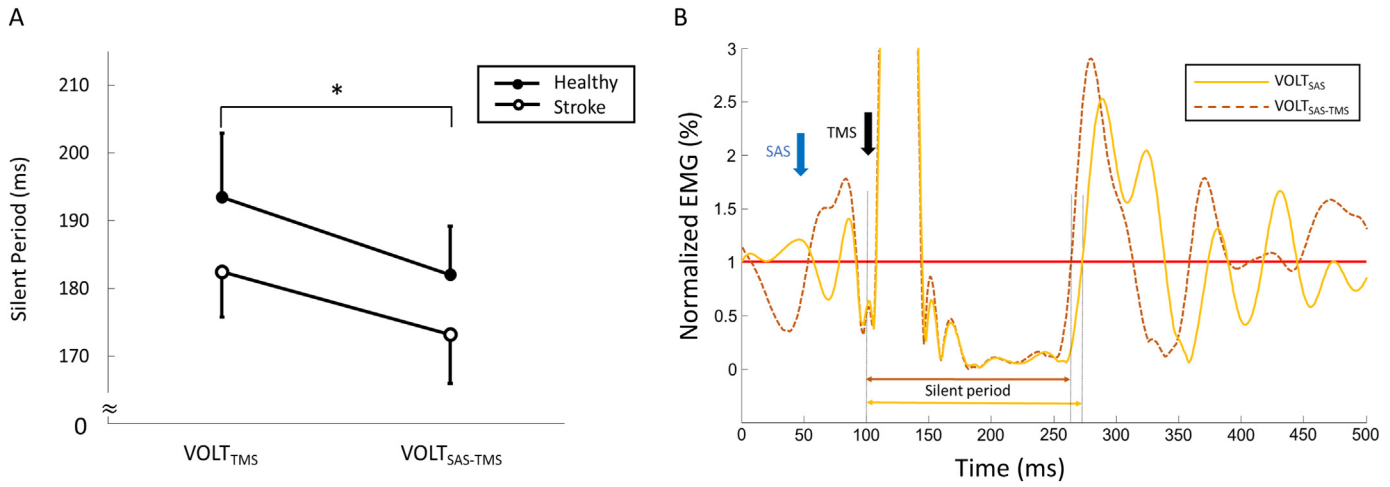

Fig. 2. (A) The silent period was shorter during the VOLT$_{SAS-TMS}$ task compared with the VOLT$_{TMS}$ task for both healthy subjects and stroke patients. (B) Silent period demonstration from a representative stroke subject during VOLT tasks. *Indicates statistical significant difference ($p < .05$).

$_{TMS}$ task (1.24 mV ± 0.86 mV) compared with the VOLT$_{TMS}$ task (1.18 mV ± 0.88 mV). For stroke subjects, the MEP amplitude was also similar between the VOLT$_{SAS-TMS}$ task (2.18 mV ± 1.77 mV) and the VOLT$_{TMS}$ task (2.24 mV ± 2.01 mV).

There was a significant effect of SAS conditioning on the silent period ($F_{(1,20)} = 5.71$, $p = .03$) (Fig. 2A). Fig. 2B demonstrated comparisons of silent periods between VOLT$_{TMS}$ and VOLT$_{SAS-TMS}$ tasks in a representative stroke subject. On average, the silent period was significantly shortened during the VOLT$_{SAS-TMS}$ task than during the VOLT$_{TMS}$ task for both healthy (VOLT$_{SAS-TMS}$: 182.0 ms ± 23.8 ms; VOLT$_{TMS}$: 193.5 ms ± 31.3 ms) and stroke subjects (VOLT$_{SAS-TMS}$: 171.0 ms ± 21.6 ms; VOLT$_{TMS}$: 182.8 ms ± 22.3 ms). There were no main effects of GROUP nor significant GROUP × SAS interactions for silent period (both $p > .05$).

Similar to the lack of change in the MEP amplitudes, there were no significant main effects or interactions for TMS-induced force. Specifically, the TMS-induced force was similar between the VOLT$_{SAS-TMS}$ task and the VOLT$_{TMS}$ task for both healthy (VOLT$_{SAS-TMS}$: 2.95 Nm ± 1.86 Nm; VOLT$_{TMS}$: 3.48 Nm ± 1.31 Nm) and stroke (VOLT$_{SAS-TMS}$: 3.66 Nm ± 1.48 Nm; VOLT$_{TMS}$: 3.97 Nm ± 1.9 Nm) subjects.

4. Discussion

The purpose of this study was to determine whether conditioning SAS imposed different effects in healthy and stroke subjects. TMS was delivered 50 ms after conditioning SAS to the right motor cortex of healthy subjects and the contralesional primary motor cortex (cM1) of stroke subjects at rest and during 10% MVC elbow flexor contraction tasks on the left side or the non-impaired side, respectively. Conditioning SAS significantly decreased MEP values at rest and shortened the silent period during voluntary contraction. However, there was no significant difference in these changes between healthy and stroke subjects.

In this study, conditioning SAS was delivered binaurally. The RS systems were stimulated in both ipsilesional and contralesional sides simultaneously. TMS was only applied to one hemisphere (cM1in stroke subjects). Since SAS imposes inhibition to ascending reticulo-cortical projections via polysynaptic connection and facilitation to descending reticulospinal projections concomitantly, results of similar conditioning SAS effects suggest that the RS excitability and its interaction with cM1 and its descending corticospinal system in stroke subjects are similar to those in healthy subjects, i.e., the RS excitability on the contralesional side is likely within the normal limits. A greater SAS-induced MEP reduction via reticulo-cortical inhibition would have been observed at rest in stroke subjects with spastic hemiparesis, if there existed a heightened RS excitability on the contralesional side. Therefore, the result of similar MEP reduction between healthy and stroke subjects suggests that reticulocortical inhibition is mainly mediated by contralateral polysynaptic projections from the contralesional side, given known RS hyperexcitability on the lesional side in spastic stroke patients [51,52].

The finding of normal reticulospinal excitability on the contralesional side in stroke subjects with chronic spastic hemiparesis is not trivial. Burford et al. (Fig. 1 in [9]) summarized previous findings from animal studies that the brainstem reticular system receives bilateral corticoreticular projections and sends bilateral RS projections. However, recent animal studies showed that cortico-reticulo-spinal projections also have laterality dominance [53–55], similar to corticospinal projections [7]. Our study represents a first step in understanding the interactions between the RS and corticospinal system on the contralesional side in chronic stroke survivors. As schematically illustrated in Fig. 3, the reticular system is hyperexcitable on the lesional (paretic) side, and has normal excitability on the contralesional (non-paretic)

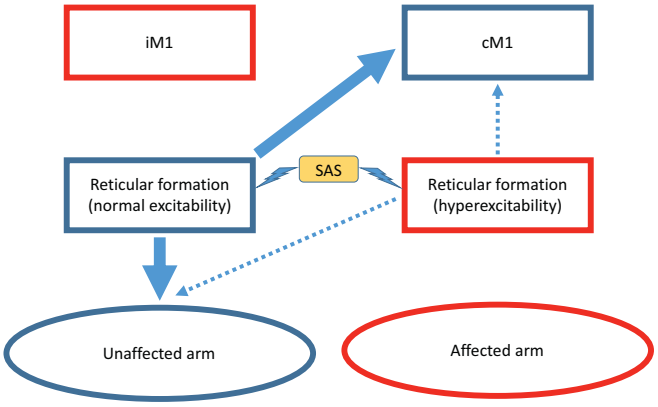

Fig. 3. Schematic representation of the proposed interactions between corticospinal and cortico-reticulo-spinal systems in stroke subjects. Red shapes indicate the affected side, and blue shapes indicate the unaffected side after stroke. Based on our results, reticulo-cortical projections are primarily contralateral, while reticulospinal projections are primarily ipsilateral. Solid arrows indicate primary connections. Dashed arrows indicate possible connections. iM1: ipsilesional primary motor cortex. cM1: contralesional primary motor cortex. SAS: startling acoustic stimuli. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

side. The reticular system has predominant ipsilateral reticulospinal projections (bold solid arrow), and contralateral reticulocortical projections (bold solid arrow). As such, when the reticular system on the contralesional side with normal excitability is stimulated by SAS non-reflexively, similar results between healthy subjects and stroke subjects are observed. As observed in this study, these results include facilitation of silent period via reticulospinal projections and MEP reduction via reticulocortical projections. This pattern of results does exclude projections from hyperexcitable reticular system on the lesioned side (dashed arrows).

The proposed relations can explain findings of normal acoustic startle reflex (ASR) responses in the non-paretic side and exaggerated ASR responses on the spastic side of stroke survivors in a previous study [52]. However, the results of similar SAS conditioning effects are not able to provide evidence whether the cM1 excitability is increased or within normal limits. When the primary motor cortex excitability is slightly elevated during very light activation in healthy subjects [17–19,56], the SAS-induced MEP reduction is similar to those when healthy subjects are at rest [20]. Similar MEP reduction could occur at normal or increased cM1 excitability. In a recent meta-analysis study on iM1, cM1 and interhemispheric inhibition, McDonnel and Stinear [57] concluded that there is no clear evidence for cM1 hyperexcitability or imbalanced interhemispheric inhibition. This report supports our interpretation that the excitability of the reticular system is likely within the normal limits.

There are some limitations. Subjects with different locations of stroke and different levels of impairment were enrolled. A recent animal study [11] reported that changes in the brainstem reticular system and RS pathways and their contributions to motor recovery differed depending on severity of cortical ischemic injury. Another limitation is that only the contralesional M1 was stimulated in this study. Application of TMS to ipsilesional M1 could advance our understanding on how the RS system compensates for the corticospinal damage and contributes to motor recovery after stroke. Finally, ages of healthy subjects and stroke subjects were not matched, though commonly recommended. Given significant differences in ages between groups in this study, lack of significant differences in the conditioning SAS effects between two groups suggests that age did not play an important role. To our knowledge, this is the first study using the combined SAS-TMS approach to investigate the role of RS system and its interactions with the corticospinal system in stroke subjects. The findings help advance our understanding of the interactions between the corticospinal and cortico-reticulo-spinal systems.

5. Conclusion

In summary, a combined SAS-TMS approach was used to examine the role of RS system and its interactions with the corticospinal system in stroke subjects with chronic spastic hemiparesis as compared to healthy subjects. TMS was applied to the contralesional M1 in stroke subjects. Conditioning SAS at 50 ms prior to TMS had similar ascending cortical inhibition at rest, but not during voluntary contraction in both healthy and stroke subjects. Furthermore, conditioning SAS led to similar descending RS facilitation of spinal motor neurons during voluntary activation in both healthy and stroke subjects. These results suggest that RS projections are likely to be primarily unilateral in humans. Following stroke, further studies are needed to investigate the RS system as it responds to activation of either the non-lesioned or the lesioned motor cortex.

Data availability statement

The datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest and any disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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