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学　位　論　文

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（運動皮質への 4 連発磁気刺激に対する反応の多様性）

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Summary

【導入】

非侵襲的脳刺激法(non-invasive brain stimulation: NIBS)は様々な脳科学分野のみならず臨床分野での応用が期待されている．NIBS は，反復刺激の後にも持続する効果を誘導する事ができ，その生理学的特徴や効果が NMDA 受容体拮抗薬により阻害されることから，NIBS はヒト脳にシナプス可塑性を誘導していると考えられている．しかし多くの NIBS プロトコールにおいて，正常被験者間でもその効果が一定しない，多様性（被験者により違った効果がでる）という問題がある．PAS(paired associative stimulation)，TBS(theta burst stimulation)，TDCS(transcranial direct current stimulation)のようなプロトコールは，被験者間の個人差のため，30-50%で想定される効果が得られないとされている．

我々が発明した単相性 4 連発磁気刺激法(quadripulse stimulation: QPS)は 4 つの単相性経頭蓋磁気刺激パルスからなり，それぞれのパルス間隔（inter-stimulus interval: ISI）を 1.5-100ms とし，バースト間の間隔（inter-burst interval: IBI）を 5 秒として 30 分間刺激する刺激法である．ISI を変化させることによって，QPS は大脳皮質の興奮性を双方向性に変化させることが可能である．短い ISI(1.5ms-10ms)により運動誘発電位(motor evoked potential: MEP)を増大させ，長い ISI(30ms-100ms)では MEP を低下させる．過去の検討では QPS の刺激強度は運動収縮時の閾値(active motor threshold: AMT)の 90%と 130%で違いがなく，刺激時間は 15 分間では効果が得られず，30 分間の刺激が必要であった．

しかし，IBI と総刺激パルス数については最適なパラメータがまだわかっていない．また，単相性反復磁気刺激は二相性刺激に比して効果が高いことが知られているが，二相性刺激の QPS についても検討されていない．そこで本研究では QPS の最適な刺激パラメータを設定し、被験者による効果のばらつきの有無について検討した．

【方法】

35 人の文書による同意を得た右利きの被験者（女性 8 名，男性 27 名，20-53 歳)で実験が行われた．全ての実験で，MEP は磁気刺激装置（Magstim 200^2：Magstim 社製）に 8 の字型刺激コイルを接続し，左運動野内右第一背側骨間筋(FDI)の motor hot spot を刺激し，QPS 前後で右 FDI の MEP を記録した．ベースラインの MEP に対する記録 MEP の振幅比を効果の指標とした．QPS は 4 台の磁気刺激装置を連結し左運動野の FDI motor hot spot を刺激部位として行った．これまでの報告で最も強い増強効果が得られた ISI 5ms の QPS(QPS5)および抑圧効果が得られた ISI 50ms の QPS(QPS50)を用いた．実験 1 では 2 つの異なる IBI（2.5 秒，5 秒）を比較した．パルス数を 1440 に固定し，刺激時間をそれぞれ 15 分および 30 分とした．実験 2 は 3 つの異なる IBI（5 秒，7.5 秒，10 秒）を比較した．刺激時間を 30 分に固定し，総刺激パルス数をそれぞれ 1440，960，720 とした．実験 3 では単相性 QPS と二相性 QPS の効果を比較した．実験 4 では実験 1-3 で得られた最適な刺激条件下に QPS の効果の個人によるばらつきを評価した．

【結果】

実験 1：従来の報告通り，増強効果を示す QPS5，抑圧性効果を示す QPS50 双方で IBI5 秒では刺激後 1 時間の皮質脊髄路興奮性増大／低下効果を認めた．しかし，IBI2.5 秒では QPS5，50 ともに有意な効果を認めなかった．3 要因の分散分析では

ISIの主効果およびISI×IBI交互作用が認められたが，TIMEの主効果，IBI，IBI×TIME，ISI×TIME，ISI×IBI×TIMEの交互作用は認められなかった．

実験2：IBI5秒では明確なMEP変化をきたしたが，IBI10秒では変化を認めなかった．IBI7.5秒ではMEPの変化が認められるが，IBI5の変化量に比して少ない効果であった．3要因の分散分析では主効果ISIに有意差あり，ISI×IBI交互作用がみられたが，TIMEの主効果，IBI×TIME，ISI×TIME，ISI×IBI×TIMEの交互作用は認められなかった．事後検定ではQPS5ではIBI5秒とIBI10秒，IBI7.5秒とIBI10秒，QPS50ではIBI5秒とIBI10秒の群間に有意差を認めた．QPS5，50ともにIBI5秒とIBI7.5秒の群間に有意差はなかった．QPS50でIBI7.5秒とIBI10秒でも差を認めなかった．この結果は，IBI7.5秒の条件では効果が安定しないことを示唆している．1要因の分散分析ではQPS5のIBI7.5秒でTIMEの主効果も認められなかった．

実験3：単相性QPSと二相性QPSのいずれもISIの違いにより双方向のMEP変化をきたしたが，二相性QPSの効果は25-30分間の持続で短く，単相性QPSが60分程度の持続をもたらした．3要因の分散分析ではISI×TYPE×TIMEの交互作用が明らかであった．単相性QPSと二相性QPSでは刺激後30分の効果がQPS5，50の双方において著しい差が認められた．二相性QPSは単相性QPSに比べて効果の持続が短く効果も弱いことが示された．

実験4：IBI5秒，刺激時間30分の条件で増強性，抑圧性QPSを施行した．被験者間には効果の個人差が大きいものの，想定される効果と反対の作用があった被験者はごく少数であった．従来の研究で示されたresponder/non-responderの判定基準を元に、QPS施行後の測定値の平均値/ベースライン値の計算式によりQPSの平均的な効果を算出した．増強性QPS5刺激終了後，平均効果は1.60であり，抑圧性QPS50刺激の効果は0.67であった．QPS5により86%，QPS50により94%の被験者がそれぞれ増強性，抑圧性効果を得た．さらにsham刺激によるMEPの変動を算出し，それに比してどの程度QPSの効果があるのかを検討した．30分間の平均化したMEPを1としたとき、sham刺激にてMEPは0.76-1.24の変動を認めた．これは従来報告されている結果に合致している．この結果によりQPS5にて1.24以上，QPS50にて0.76以下の効果がえられた被験者をresponder，0.76-1.24の被験者をnon-responder，QPS5にて0.76以下，QPS50にて1.24以上の被験者をopposite-responderと定義した．これによるとQPS5の有効率は80%，QPS50では63%と低下するものの，opposite-responderはQPS5でわずか3%であり，QPS50では皆無であった．

【考察】

本研究の結果はQPSの効果は安定的で効果のばらつきが少ないことを示している．現時点でこの研究が健康な被験者におけるQPSの効果を検討した最も大規模な研究であり，この有益な結果を論ずる前に，QPSが最も効果的であるパラメータを検討する必要があった．

[QPSのパラメータ]

実験1-3の結果は他のNIBSと同様に刺激強度，ISI，IBI，総刺激パルス数，総刺激時間，パルス波形など多数のパラメータがその効果に関与していることを示唆している．IBI2.5秒のQPSが全く効果を示さなかったことは予期しない結果であった．以前の報告で刺激時間および刺激パルス数を減らしたQPSが効果を示さなかったため，IBIを短縮して総刺激パルス数を同一に揃えたQPSは有効ではないかと予測したが実際には無効であった．IBI7.5秒のQPS5の効果がなかったことも解釈が難しい．

QPS50 では IBI7.5 秒において 50 分間の効果が認められた．この結果は ISI，IBI，刺激時間，刺激パルス数に潜在的な相互作用があり，単一の因子ではその効果を決定しえないという事実を表している．これは総刺激パルス数が同一であっても IBI が異なると可塑性誘導に大きく影響するという Huang らの報告と一致している．このような複雑な相互作用は TBS のモデリング研究でも示されている．従って NIBS の至適パラメータを決定するためには全てのパラメータの組み合わせを検討しなければならない．実際には今回の研究は各パラメータの個々の寄与を全て解明するようなデザインではなく，QPS の IBI の最適な値について確固たる結論を出すことはできない．30 分の刺激時間は可塑性誘導に必要な特異的なプロセスを始動させるのに必要かもしれないが，推論の域をでず今後の研究の蓄積が必要である．しかし今回の結果は，オリジナルの QPS パラメータが顕著な MEP 変化をもたらすことを明らかにした点で意義が大きい．実験 3 では単相性 QPS が二相性 QPS よりも効果が高いことが示された．単相性 QPS に比べて二相性 QPS では効果の持続時間がかなり短かった．硬膜外電極の研究によると単相性刺激と二相性刺激は運動皮質の異なったニューロンを刺激しており，様々な介在ニューロンを同時に刺激する二相性刺激に対して，単相性刺激は比較的均一な細胞集団を刺激している可能性がある．さらに経頭蓋反復磁気刺激(rTMS)においても，単相性刺激は二相性刺激に比して長期持続する効果が強いとされている．二相性 rTMS では様々な介在ニューロンを刺激した結果，抑制性効果と興奮性効果の打ち消し合いが生じてしまうためとされている．これらをふまえると，単相性 QPS は二相性 QPS に比して長い持続効果を有していると考えられる．

[QPS の効果の確実性、変動性]

QPS の刺激条件を単相性，刺激強度を AMT95%，刺激時間 30 分，刺激パルス数 1440，ISI5 または 50ms，IBI5 秒とし，被験者間の効果のばらつきについて検討した．これらの効果は比較的一貫しており，QPS5 では約 80%に増強効果がみられ，QPS50 では増強効果が 10%，抑圧効果が 90%であった．QPS 後の平均 MEP 振幅がベースラインに比して大か小かで被験者をグループ化した結果はやや恣意的であるため，被験者を sham 刺激によって MEP の変動性を評価したうえで，responder と non-responder，opposite responder に区分した．この区分では responder の割合は低下し QPS5 で 80%，QPS50 で 63%であった．予測された効果と反対の効果がえられた opposite responder は QPS5 で 3%，QPS50 で皆無であり顕著に少なかった．結論として，QPS の皮質脊髄路興奮性におよぼす効果は検討された条件下では比較的安定している．今回の研究ではそれぞれの被験者で各実験間での変動性は検討していないが(inter-trial variability)，近年の報告では同一被験者における効果の変動性は被験者間の差異に比して少ないとされている．従って，QPS においても各被験者の実験間での効果の差異は少ないと予想されるが，さらに研究が必要である。NIBS の効果の個人差には年令，性別，施行時間，生理学的活動性，筋活動の履歴，遺伝要因が関与しているとされている．これらの因子や，人種差，電流の向き，骨厚なども今回の研究では検討されておらず，今後検討すべきである．

【結論】

QPS の効果が乏しいか，皆無であるのは多く見積もっても 20-40%であり，刺激時間 30 分間，IBI5 秒の QPS は比較的安定した効果を得られる条件である．しかし，皮質脊髄路以外の興奮性、運動学習や臨床治療などでも安定した効果がえられるか

はまだわかっていない．今後はQPSを神経疾患の治療に適用するため，これらの条件を検討していく必要がある．

Contents

Abstract

Background:

Responses to plasticity-inducing brain stimulation protocols are highly variable. However, no data are available concerning the variability of responses to quadripulse stimulation (QPS).

Objective:

We assessed the QPS parameters of motor cortical plasticity induction in a systematic manner, and later investigated the variability of QPS using optimal parameters.

Methods:

First, two different interburst intervals (IBI) with the same total number of pulses were compared. Next we investigated three different IBIs with a different total number of pulses but with same duration of intervention. We also compared the after-effects of monophasic and biphasic QPS. Finally, variability of QPS was tested in 35 healthy subjects. Twenty motor evoked potentials (MEPs) were measured every 5 to 10 min for up to one hour after intervention.

Results:

QPS at an IBI of 5 s produced MEPs changes that are dependent on the interstimulus interval of the four magnetic pulses, consistent with previous reports. Unexpectedly, QPS at an IBI of 2.5 s did not induce any plasticity, even with the same total number of pulses, that is, 1,440. QPS at an IBI of 7.5 s produced a variable response but was likely to be comparable to conventional QPS. Biphasic QPS had shorter lasting after-effects compared with monophasic QPS. Finally, the after-effects of QPS were relatively consistent across subjects: more than 80% of subjects responded as expected in the excitatory QPS at an IBI of 5 s.

Conclusions:

The IBI, total duration of the procedure and pulse waveform strongly affected the magnitude or duration of the plasticity induced by QPS. In this cohort, 80% of subjects responded to excitatory QPS as expected.

Keywords: Quadripulse stimulation (QPS), plasticity, Motor Cortex, variability.

Highlights.

-Paradigm parameters, such as inter-burst interval (IBI), duration of the procedure and pulse wave-form have significant effects on cortical excitability changes induced by QPS.

-QPS at an IBI of 5 s, delivering 1440 monophasic pulses over 30 minutes, was found to be optimal for increasing cortical excitability changes in M1.

-The excitatory QPS 5 ms, given at an IBI of 5 s over M1, induced an expected motor cortical facilitation in 80% of subjects in this cohort.

Introduction

Non-invasive brain stimulation (NIBS) has been widely used in a variety of neuroscience fields and clinical settings. This is primarily due to its ability to induce lasting after-effects after the stimulation period. Indirect evidence in which N-Methyl-D-Aspartate (NMDA) receptor antagonist blocks at least some of the effects induced by NIBS suggests that they might represent an analog of synaptic plasticity in the human brain [1]. Thus, NIBS offers a potential means for interfering with neuronal function, as well as therapeutic applications. Nonetheless, one of the major issues of any NIBS protocols is the high variability of their effects [2]. Given the high inter-individual variability in response to other plasticity protocols such as paired associative stimulation (PAS), theta-burst stimulation (TBS), and transcranial direct current stimulation (TDCS) in which 30% to 50% of participants fail to respond in the "canonical" way [3-12], we aimed to investigate the variation in response to quadripulse stimulation (QPS), another NIBS protocol for plasticity induction [13-16].

QPS consists of bursts of four monophasic TMS pulses, separated by inter-stimulus intervals (ISI) of 1.5, 5, 10, 30, 50 or 100 ms, which are repeated with an inter-burst interval (IBI) of 5 s (i.e., 0.2 Hz) for 30 min (i.e., 1440 pulses in total). Depending on the ISI, QPS induces bidirectional changes of corticospinal excitability as indexed by the size of the motor evoked potential (MEPs); QPS at short ISIs (QPS-1.5, QPS-5, QPS-10) produces a long-lasting increase in MEP, while QPS at long ISIs (QPS-30, QPS-50, and QPS-100) induces a lasting MEP decrease for approximately one hour [14]. In the original report, the stimulus intensity and duration of QPS that is proportional to the total number of pulses are systematically investigated; there was no difference in the amount and duration of plasticity when using a QPS intensity of approximately 130% of active motor threshold (AMT) (suprathreshold) and 90% AMT (subthreshold) [13]. For the duration of QPS, 30 min (i.e. 1,440 pulses) but not 15 minutes (i.e., 720 pulses) was sufficient to induce facilitation of MEPs. However, the optimal IBI and total number of pulses (or duration of QPS) for inducing the largest after-effect remain unclear. We hypothesized that the total duration of monophasic QPS (i.e., 30 min) can be shortened to 15 min when an IBI of 2.5 s is used for monophasic QPS because the total number of pulses of this particular protocol is identical to conventional QPS (i.e., 1,440 pulses). However, this result did not support our hypothesis due to the lack of plasticity by QPS at an IBI of 2.5 s. Thus, we subsequently assessed these parameters in a systematic manner although we were not able to study them fully due to mutual interaction between certain parameters. Furthermore, we hypothesized that QPS using monophasic pulses may be more effective than conventional rTMS using biphasic pulses because monophasic repetitive TMS (rTMS) is likely to be more powerful than biphasic rTMS [17]. This hypothesis was also assessed by comparison between monophasic QPS and biphasic QPS in this investigation.

Finally, we investigated the variability of QPS using optimal parameters. We considered that this variability had practical importance in clarifying these issues, given the potential uses of QPS in clinical settings.

Materials and Methods

Subjects

Thirty-five right-handed subjects (8 females, 27 males; 20-53 years old, mean age ± SD: 37.7 ± 8.4) participated in this study. None of the participants had any contraindications to TMS, took any medication on a regular basis or had a positive history of psychiatric or neurologic diseases [18]. All subjects provided written informed consent to participate in this study. This study was approved by the Ethics Committee of Fukushima Medical University and the University of Tokyo.

Recordings

During the experiment, subjects were seated on a comfortable chair. The right first dorsal interosseus (FDI) muscle activity was recorded via Ag/AgCl cup electrodes in a belly-tendon montage. Raw signals were amplified and bandpass filtered (100 Hz to 3 kHz, Multi Amplifier 1000, DIGITEX LAB Co. Ltd., Japan). Signals were digitized at 5 kHz and data were stored on a computer for offline analysis (MultiStim tracer; Medical Try System, Japan).

Transcranial magnetic stimulation

Single-pulse TMS was performed with a figure-of-eight coil (internal wing diameter of 7 cm) connected to a Magstim 200^2 stimulator (The Magstim Co. Ltd). The hotspot was identified as the position where the largest motor evoked potential (MEPs) were elicited when applying the same intensity stimulation with the coil held 45 degrees to the midline, tangentially to the skull and the handle pointing backwards. The spot was consecutively marked on the scalp with a waterproof pen alongside to 2 additional orientation marks needed for exact repositioning of the coil. The resting motor threshold (RMT) was determined as minimum stimulator output intensity needed to achieve a minimum MEP-amplitude of 50 μV in the completely relaxed FDI-muscle in at least 5 out of 10 trials. We also assessed active motor threshold (AMT) as the lowest stimulator output intensity evoking an MEP of at least 200 μV in 5 out of 10 consecutive trials while subjects maintained 10-% of their maximum voluntary contraction (MVC) in the target muscle.

Quadripulse stimulation (QPS)

Quadripulse stimulation (QPS) was applied to the hand area of the left motor cortex using a combining module (The Magstim Co. Ltd.) connected with four stimulators (Magstim 200^2, The Magstim Co. Ltd.) as previously reported [14]. The conventional QPS protocol consisted of a burst of four TMS pulses separated by an interburst interval (IBI) of 5 s. Each burst consisted of four monophasic magnetic pulses separated by interstimulus intervals (ISI) of 5 ms (QPS5, excitatory QPS) or 50 ms (QPS50, inhibitory QPS), which can induce a potentiation and depression after-effect lasting up to 90 min [14]. The stimulus intensity of QPS was set at 95 % AMT in all experiments. For the experiment 1 (see below), we used the

octopulse stimulation (OPS) system (The Magstim Co. Ltd.). It consists of eight monophasic magnetic stimulators (Magstim 200^2, The Magstim Co. Ltd.) connected with a specially designed combining module (The Magstim Co. Ltd). This device combines the outputs from eight stimulators to allow a train of eight monophasic magnetic pulses to be delivered through a single coil. Because it takes approximately 4 s for recharging one monophasic stimulator, the conventional QPS system is not able to accomplish QPS with an IBI of 2.5 s. We used two QPS systems connecting with the same coil through an octopulse system for giving QPS with an IBI of 2.5 s. For the experiment 3, we used biphasic mode of QPS: it consists of four biphasic magnetic pulses produced by biphasic stimulators (Magstim SuperRapid, The Magstim Co. Ltd.) connected with a combining module (The Magstim Co. Ltd), which allows a train of four biphasic pulses to be delivered via a single coil.

Experimental parameters

As a measure of corticospinal excitability, we recorded twenty MEPs elicited by single pulse TMS with the intensity adjusted to evoke an approximately 0.5 mV peak-to-peak amplitude ($SI_{0.5mV}$) at rest at baseline condition. The stimulation intensity was kept constant throughout the experiment. The inter-trial interval was set at 4.5-5.5 s for the follow-up MEP measurements. Muscle activity was monitored throughout experiments using high gain audiovisual feedback (1000uV/div) on an oscilloscope, which enabled them to keep their target muscles relaxed.

Study design (Figure 1)

In all experiments, baseline corticospinal excitability measurements were followed by different QPS intervention (see below). After the application of QPS, 20 MEPs were recorded every 5 min for 30 min-, and every 10 min from 30 to 60 min after the intervention (6-9 time points, T5, T10, T15, T20, T25, T30, T40, T50, T60) (see below).

Experiment 1: This experiment was performed to compare two different IBIs, 2.5 s vs. 5 s (Fig. 1). This experiment also allowed the study of the difference between total duration of QPS, 15 min vs. 30 min with the same total number of QPS pulses (i.e., 1,440 pulses). The stimulus intensity of QPS was set at 95% AMT. Ten subjects participated in this experiment. Two IBIs were tested with excitatory QPS5 (n=5) (QPS5-IBI2.5 vs. QPS5-IBI5) and inhibitory QPS50 (n=5) (QPS50-IBI2.5 vs. QPS50-IBI5). MEP measurements were performed up to one hour after the end of QPS.

Experiment 2: We compared three different IBIs, 5, 7.5, and 10 s (Fig. 1). Because the total duration of QPS was fixed at 30 min, the total number of pulses was different among the conditions; 1,440 pulses for an IBI of 5 s, 960 pulses for an IBI of 7.5 s, and 720 pulses for an IBI of 10 s. Ten subjects participated in this experiment. Three IBIs were tested with excitatory QPS5 (n=5) (QPS5-IBI5, QPS5-IBI7.5, vs. QPS5-IBI10) and inhibitory QPS50 (n=5) (QPS50-IBI5, QPS50-IBI7.5, vs. QPS50-IBI10). MEP measurements were performed up to an hour after QPS application.

Experiment 3: Monophasic and biphasic QPSs were compared. For biphasic QPS, the coil orientation was the same as in monophasic QPS condition. Stimulus intensity was set at 95% AMT, as measured using the biphasic QPS system. Twelve subjects participated in this experiment. Two configurations were tested with excitatory QPS5 (n=6) (QPS5-Mono vs. QPS5-Bi) and inhibitory QPS50 (n=6) (QPS50-Mono vs. QPS50-Bi). MEP measurements were performed up to an hour after the end of QPS.

Experiment 4: The experiment was performed to confirm the variability of the lasting effect of QPS. In total, 35 subjects participated (18 subjects were naïve). All subjects were enrolled in the following two experiments in a randomized order. Conventional excitatory QPS5 and inhibitory QPS50 were used in this experiment: QPS5 and QPS50 consist of bursts of four stimuli (i.e. an ISI of 5 ms or 50 ms), repeated every 5 s ((i.e. an IBI of 5 s) for 30 min (total 1,440 pulses). The stimulus intensity was set at 95% AMT. MEP measurements were performed up to 60 min after QPS application.

In all of the above experiments, two consecutive experiments were separated by at least one week in the same subject.

Statistical analysis

In experiments 1 and 2, three-way repeated measures analysis of variance (ANOVA) was computed with factors ISI (QPS5 and QPS50), TIME (T5-T60) and IBI (experiment 1, 2.5 vs 5 s; experiment 2, 5 vs.7.5 vs. 10 s) using normalized MEP values after the intervention without including the baseline value (1.0). For experiment 3, three-way ANOVA using normalized MEP values after the intervention was performed with factors ISI (QPS5 and QPS50), TIME (T5-T30) and TYPE (monophasic vs. biphasic). The Greenhouse-Geisser correction was used if necessary to correct non-sphericity. For each QPS condition, one-way repeated measures ANOVA was applied with the factor TIME (baseline, T5-T60 for experiments 1 and 2; baseline, T5-T30 for experiment 4) using non-normalized MEP-values to confirm the significant changes from baseline MEP sizes. For baseline measurements data were reported as the mean value ± standard deviation (SD). Data were analyzed using SPSS-software (SPSS ver. 17.0 for Windows; SPSS Inc.).

Results

No adverse effects were reported. Baseline physiological measurements are shown in Table 1 and did not differ significantly between stimulation conditions.

Experiment 1: This experiment was performed to compare two different IBIs, 2.5 s vs. 5 s with the same total number of pulses (i.e., 1,440 pulses) (Fig. 1). Thus, the difference between QPS-IBI2.5 and QPS-IBI5 is IBI as well as the total duration of QPS, 15 min vs. 30 min. Figure 2A and 2B show the time courses of QPS in each condition (Fig.2A, excitatory QPS5; Fig.2B, inhibitory QPS50). Consistent with previous reports [14], conventional excitatory QPS5-IBI5 and inhibitory QPS50-IBI5 (i.e. an IBI of 5 s, for 1,440 pulses, Fig. 1) produced a substantial increase/decrease in corticospinal excitability for about one hour, whereas no changes were observed in corticospinal excitability after QPS5-IBI2.5 and QPS50-IBI2.5. Indeed, three-way repeated measures ANOVA on combined QPS5-IBI2.5/QPS5-IBI5/QPS50-IBI2.5/QPS50-IBI5 data revealed significant main effects of ISI, ISI x IBI interaction, but no effects of TIME, IBI, IBI x TIME, ISI x TIME, nor ISI x IBI x TIME interactions (Table 2). Post hoc analysis showed a significant difference between QPS5-IBI2.5 and QPS5-IBI5 (Bonferroni corrected, $P<0.001$), and between QPS50-IBI2.5 and QPS50-IBI5 (Bonferroni corrected, $P=0.033$). One-way ANOVA on excitatory QPS5-IBI5 and inhibitory QPS50-IBI5 data separately revealed a significant main effect of TIME, while excitatory QPS5-IBI2.5 and inhibitory QPS50-IBI2.5 did not reveal an effect (Table 3). Any interventions using IBI2.5 induced no motor cortical excitability changes.

Experiment 2: This experiment was performed to compare three different IBIs, 5 s, 7.5 s, vs. 10 s with the same total duration of QPS (i.e., 30 min), and thus the total pulses of QPS were different among the protocols; 1,440 pulses for QPS-IBI5, 960 pulses for QPS-IBI7.5, and 720 pulses for QPS-IBI10 (Fig.1). Figures 2C and 2D shows the time courses of each condition. There were clear MEP changes after QPS-IBI5, whereas no changes after QPS-IBI10. For the QPS-IBI7.5, it is likely that there are certain changes in MEP sizes, but these changes were less stable than QPS-IBI5. Three-way repeated measures ANOVA revealed significant main effects of ISI and ISI x IBI interaction, but no effects of TIME, IBI x TIME, ISI x TIME, ISI x IBI x TIME interactions (Table 2). Post hoc analysis revealed significant difference between excitatory QPS5-IBI5 and excitatory QPS5-IBI10 (Bonferroni corrected, $P=0.003$), excitatory QPS5-IBI7.5 and excitatory QPS5-IBI10 (Bonferroni corrected, $P=0.002$), inhibitory QPS50-IBI5 and inhibitory QPS50-IBI10 (Bonferroni corrected, $P=0.031$), but no difference between excitatory QPS5-IBI5 and excitatory QPS5-IBI7.5 (Bonferroni corrected, $P=1.000$), inhibitory QPS50-IBI5 and inhibitory QPS50-IBI7.5 (Bonferroni corrected, $P=0.792$), inhibitory QPS50-IBI7.5 and inhibitory QPS50-IBI10 (Bonferroni corrected, $P=0.261$), indicating a less stable effect after QPS-IBI7.5 compared with QPS-IBI5. Indeed, one-way ANOVA on each condition also revealed significant main effect of TIME in QPS5-IBI5, QPS50-IBI5, and QPS50-IBI7.5, but not in QPS5-IBI7.5, QPS5-IBI10, and QPS50-IBI10 (Table 3). Both excitatory and inhibitory QPSs using IBI5 induced significant cortical excitability changes, those using IBI7.5 variable excitability changes and those using IBI10 no changes.

Experiment 3: To assess whether monophasic magnetic pulse configuration was more effective for inducing cortical excitability changes than biphasic pulses, we performed the experiments with monophasic and biphasic QPS. Figures 2E and 2F shows the time course of each condition. Although both configuration types induced MEP changes depending on the ISI, they lasted approximately 25 to 30 min in the biphasic QPSs, which were shorter compared with monophasic QPSs. Three-way repeated measures ANOVA revealed significant ISI x TYPE x TIME interaction (Table 2), indicating that the time courses of each condition significantly differed. We found a significant difference between QPS-Mono and QPS-Bi after 30 min in both excitatory QPS5 and inhibitory QPS50 conditions (Bonferroni corrected, excitatory QPS5-Mono vs Bi; T30, P=0.038; T45, P=0.015: T60, P=0.003: inhibitory QPS50-Mono vs Bi; T45, P<0.001; T60, P=0.013). In addition, separate one-way ANOVAs in each condition revealed shorter lasting changes of MEP in biphasic QPS (Table 3). The biphasic QPS induced shallower and shorter excitability changes than monophasic QPS.

Experiment 4: In the final experiment, we investigated the variability of the QPS effect using the optimal stimulation parameters shown above for inducing plasticity. We had chosen QPS-IBI5 for 30 min for both excitatory and inhibitory QPSs, based on the findings obtained in experiments 1-3. Figures 3A and 3B plot time courses of excitatory QPS5 (Fig. 3A) and inhibitory QPS50 (Fig. 3B) in all 35 subjects. Although there was a large variation in response between individuals, it appears that only a small number of subjects responded to QPS in the opposite way. Indeed, one-way ANOVA performed separately on excitatory QPS5 and inhibitory QPS50 data revealed a main effect of TIME in both excitatory QPS5 group (F=9.417; df 6, 204; P<0.001) and inhibitory QPS50 group (F=13.261, df 6, 204; P<0.001). Two-way repeated measures ANOVA on combined QPS-5/QPS-50 data showed a significant main effect of QPS (F=78.7; df 1,68; P<0.001) and TIME x QPS interaction (F=2.7; Greenhause-Geisser corrected df 3.3, 227.4; P=0.042). Thus, on average, there was a potentiating effect of excitatory QPS5 and a depressive effect of inhibitory QPS50, consistent with original reports [13-15]. According to the criteria of responder and non-responder in previous studies [4, 12], we calculated the average effect of QPS expressed as the mean of all post-QPS measures relative to baseline. Following excitatory QPS5, the mean of the average effect was 1.60 (SD = 0.57; 95% confidence interval 1.41-1.79); inhibitory QPS-50 decreased the response by 0.67 (SD = 0.22; 95% confidence intervals 0.60-0.74). Figures 3C provides a simple summary of the response profile in this population in terms of whether the average effect at post-QPS period was greater or less than 1. Over 80% of participants increased their response after excitatory QPS-5 (86%) and decreased their response after inhibitory QPS-50 (94%). Although such a "dichotic" method to differentiate the plasticity response would be beneficial for a better understanding of the nature of NIBS plasticity, we have also attempted to evaluate the observed long-term effect based on the estimation of natural variation of MEP size. We calculated the expected variability based on MEPs after sham intervention (N=12, all naïve subjects). The mean (standard deviation) of normalized MEP sizes for 30 min was 1.00 (0.12). Thus, the range of

MEP changes under the sham condition was between 0.76 and 1.24 in normalized values. This value of normal range was nearly the same as a previously reported natural variation [12]. According to this criterion, it is possible to roughly differentiate "responders" (in whom expected responses (i.e., above 1.24 after excitatory QPS5 or below 0.76 after inhibitory QPS50) were obtained), "non-responders" (in whom MEP size lies within the above mentioned range, 0.76 to 1.24), and "opposite responders" (in whom opposite responses (i.e., below 0.76 after excitatory QPS5 or above 1.24 after inhibitory QPS50) were obtained). Figure 3D is the response profile based on this classification. Although the responder rate is reduced in both excitatory QPS5 (from 86% to 80%) and inhibitory QPS50 (from 94% to 63%) interventions and the non-responder rate increased substantially compared with the above dichotic analysis, it should be noted that opposite responder was only 3% in excitatory QPS5, and no opposite responder was found after inhibitory QPS50 (Fig. 3D).

Discussion

The present results show that the response to the QPS protocol was mostly predictable and not highly variable at least in this cohort. This value suggests that QPS should be less variable than other plasticity protocols in which approximately 50% of the participants failed to respond in the "canonical" manner [2]. To the best of our knowledge, this is the first large-scale study of the variation in after-effects of QPS protocol in healthy volunteers. Before discussing this favorable outcome, it is important to consider which parameters are critical for the QPS protocol. Thus, we investigated which parameters of QPS might substantially affected QPS-induced plasticity.

Parameters of QPS

Experiment 1 revealed that although the total number of pulses was the same (i.e. 1,440 pulses), the QPS at an IBI of 2.5 s (total duration of QPS, 15 min) did not induce any excitability changes, whereas the QPS at an IBI of 5 s induced long-lasting changes. In experiment 2, we showed that when the duration of the QPS was fixed at 30 min, plasticity was induced by QPS5/QPS50 at an IBI of 5 s (1,440 pulses in total) and QPS50 at an IBI of 7.5 s (960 pulses), but neither by QPS5 at an IBI of 7.5 s (960 pulses) nor QPS5/QPS50 at an IBI of 10 s (720 pulses). Experiment 3 revealed that biphasic QPS induced shorter-lasting cortical excitability changes (up to 30 min after intervention) compared with monophasic QPS. These data indicated that similar to other NIBS protocols, no single but multiple parameters, including stimulus intensity, ISI, IBI, total number of pulses, duration of the whole intervention and pulse configuration, engaged in plasticity induction [19, 20].

One unexpected result from experiment 1 was that the QPS at an IBI of 2.5 s did not induce any plasticity although the same total number of pulses (1,440 pulses) of QPS at an IBI of 5 s induced significant plasticity. Previously, we showed that neither 15 min of QPS at an IBI of 5 s (i.e. 720 pulses in total) nor 20 min of QPS at an IBI of 5 s (i.e. 960 pulses in total) did induce any plasticity [13, 14], although 30 min of QPS at an IBI of 5 s induced the plasticity. This finding indicated the dose dependency of QPS at an IBI of 5 s and 1,440 pulses in total was optimal to induce plasticity by QPS. Thus, we first hypothesized that the total duration of monophasic QPS (i.e., 30 min) could be shortened to 15 min when an IBI of 2.5 s was used for monophasic QPS because the total number of pulses of this particular protocol was identical to conventional QPS (i.e., 1,440 pulses). However, this finding was not observed in the present experiment: QPS at an IBI of 2.5 s for 15 min (i.e. 1,440 pulses) did not induce any plasticity. The other puzzling result was that excitatory QPS5 at an IBI of 7.5 s did not induce any plasticity, while inhibitory QPS50 at the same IBI decreased MEP sizes significantly for approximately 50 min in experiment 2. These data indicated that there is a potential interaction among ISI, IBI, total duration of pulses, and stimulation time and that no single factor could determine the induction of plasticity. This finding was consistent with the previous seminal study performed by Huang et al. (2005), showing that different IBIs profoundly influenced plasticity induction, even with the same total number of pulses [21].

Such a complicated interaction was also confirmed by the modeling study [22]. Thus, it is likely that multiple parameters mutually interacted with each other for plasticity induction. This finding also indicated that it was necessary to investigate all of the potential combinations of parameters in order to determine optimal parameters of NIBS plasticity induction protocol. Unfortunately, it is impossible to draw a firm conclusion about the optimal IBI in the QPS protocol from the results of the present experiments as the design of our experiments did not disentangle the relative contribution of all parameters individually. It could be that the total duration of the intervention (30 min) is a critical factor to determine the final outcome most likely because a defined time may be necessary to initiate specific processes of plasticity induction, but this idea remains speculative and requires further studies for confirmation.

However, our results provide practically useful information because in all of the QPS experiments using an IBI of 5 s produced significant MEP changes depending on the ISI of four magnetic pulses, which indicated that the original QPS protocol might induce robust plasticity. None of the modified versions of QPS using other IBIs were optimal for inducing plasticity, when the total duration was fixed at 30 min. In addition, even when the total duration of application time was shortened from 30 min to 15 min using an IBI of 2.5 s, this did not produce any advantage in the efficacy of plasticity induction. Thus, although all possible conditions were not tested, the present results suggest that the original QPS protocol/parameters might be, at least at the moment, the most optimal for plasticity induction.

In experiment 3, we investigated whether monophasic QPS is better than biphasic QPS. These results showed that this was the case, as biphasic QPS after-effects had a much shorter duration than monophasic QPS. However, it is uncertain which neurons in the brain are activated by magnetic stimuli, and we have no direct evidence for how monophasic and biphasic magnetic stimuli differ in the efficacy of neuronal excitation. However, according to epidural recording studies, it is likely that monophasic and biphasic pulses activate different sets of neurons in the motor cortex. A mixture of various interneurons can be activated by biphasic pulses, while monophasic pulses preferentially activate relatively homogenous population of neurons [23]. Accordingly, it has been assumed that the monophasic mode of repetitive TMS (rTMS) may have stronger after-effects than biphasic rTMS, as the activation of relatively uniform populations of interneurons by monophasic TMS would readily result in an effective summation of synaptic efficacy, while biphasic pulses may activate various interneurons, resulting in some cancellation of inhibitory and facilitatory effects with one another. Indeed, previous studies have clearly shown that monophasic rTMS is more effective than biphasic rTMS [17, 24, 25]. In keeping with these reports, we observed that monophasic QPS is capable of inducing longer-lasting after-effects than biphasic QPS. However, we did not adjust the stimulus intensity as we chose it relative to AMT, but this might be one confounding factor for interpretation.

Variability of QPS

Figure 3 shows that the response to QPS protocols is relatively consistent across subjects. We chose to examine one particular, originally reported type of QPS with canonical choices of pulse configuration (monophasic), stimulus intensity (95% AMT), duration (30 min, i.e., 1,440 pulses in total), ISI (5 or 50 ms), IBI (5 s), and target site (primary motor cortex), based on the findings obtained in experiments 1-3. These results were relatively consistent: after excitatory QPS5, approximately 80% of individuals showed facilitation, whereas after inhibitory QPS50 the proportions were approximately 10:90 (facilitation: inhibition). Grouping individuals according to whether the mean MEP amplitudes after QPS were larger or smaller than baseline was somewhat arbitrary. Due to this limitation, we also the divided individuals into "responder" (in whom expected responses (i.e. above 1.24 after excitatory QPS5 or below 0.76 after inhibitory QPS50) were obtained), "non-responder" (in whom normalized size lies within the above mentioned range, 0.76 to 1.24), and "opposite responder" (in whom opposite responses (i.e., below 0.76 after excitatory QPS5 or above 1.24 after inhibitory QPS50) were obtained) according to MEP variability evaluation after sham intervention. The responder rate was reduced in both excitatory QPS5 (80%) and inhibitory QPS50 (63%) and non-responder increased substantially compared with conventional classification. Unexpectedly, there were notably few opposite responders to excitatory QPS5 (3%) and no opposite responders to inhibitory QPS50. The main conclusion was that the after-effect of this type of QPS on corticospinal excitability was relatively consistent. Although we did not test the session-to-session variation within each person which might affect our results, recent studies have suggested that the intra-individual variability is lower than inter-individual variability [26]. Thus, it is likely that such intra-individual variability is also lower in QPS. However, this issue warrants further investigation.

Several determinants have been identified to explain the variability of NIBS plasticity protocols, such as age, gender, time of the day, physical activity, prior history of muscle activity, and genetics [11]. Furthermore, Hamada and colleagues have previously reported that the variability of recruitment of interneuron networks is another determinant for TBS and anodal TDCS [4, 12], as they may also have a functional relevance in terms of motor learning [27]. However, we did not test these factors in relationship to QPS variability as well as other potential sources of variability, such as ethnicity, direction of current flow, thickness of bone, which should be fully investigated in future studies.

Limitation

We are aware of the number of limitations in this study. Frist, the number of subjects in experiments 1-3 was small. Second, the study was performed in Japan, and thus, we cannot exclude the potential effect of ethnicity and/or genotype. Third, the duration of effects of QPS with an IBI of 7.5 ms was not explored. Fourth, another factor to be considered would be "founder" effects, in which impressive effects for other NIBS paradigms have often been reported by founder labs.

Conclusion

The effects of QPS are variable but with less than 20% to 40% at maximum of individuals having poor or absent responses. QPS at an IBI of 5 s for 30 min would be the optimal protocol for inducing relatively constant long-term effects. However, we are uncertain whether less variable responses to QPS can be obtained even with measures other than corticospinal excitability, such as motor learning and clinical outcomes. Future studies are warranted to test these parameters in order to apply QPS as a treatment options for neurological diseases.

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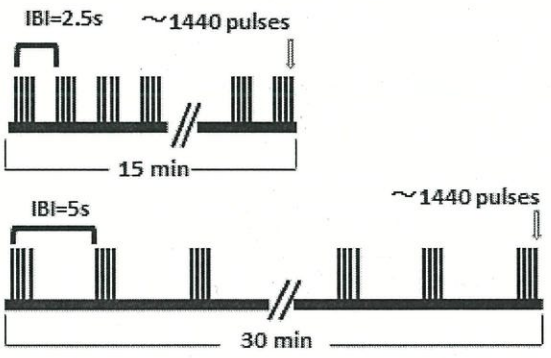
Figures and Figure legends

Experiment 1



Experiment 2

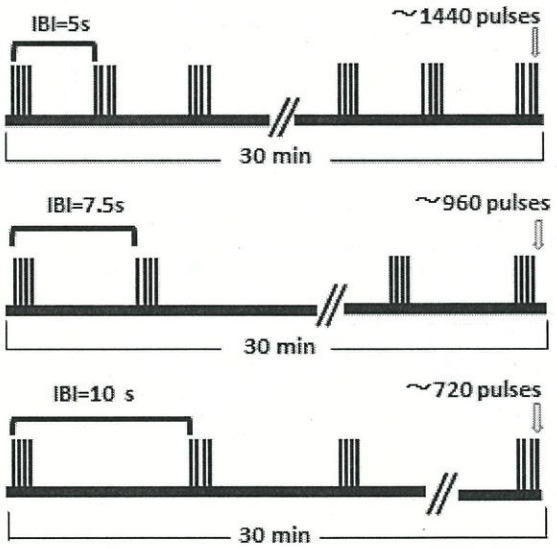


Figure 1.
Original QPS protocol consists of 360 bursts of TMS pulses for 30 min with an interburst interval (IBI) of 5 s. Each burst consists of four monophasic magnetic pulses (i.e. quadripulse stimulation: QPS) delivered at inter-stimulus intervals (ISI) of 5 or 50 ms. In experiment 1, two protocols were tested, termed QPS-IBI2.5 (top row) and QPS-IBI5 (second row). In QPS-IBI2.5, IBI was set at 2.5 s, and total duration of QPS was 15 min, thus, in total 1,440 pulses were induced. The other protocol was original QPS, which is equal to QPS-IBI5. In experiment 2, three conditions were tested, as QPS-IBI5 (third row), QPS-IBI7.5 (fourth row), and QPS-IBI10 (bottom row). As indicated, the total numbers of pulses were different because we fixed the total duration of QPS at 30 min.

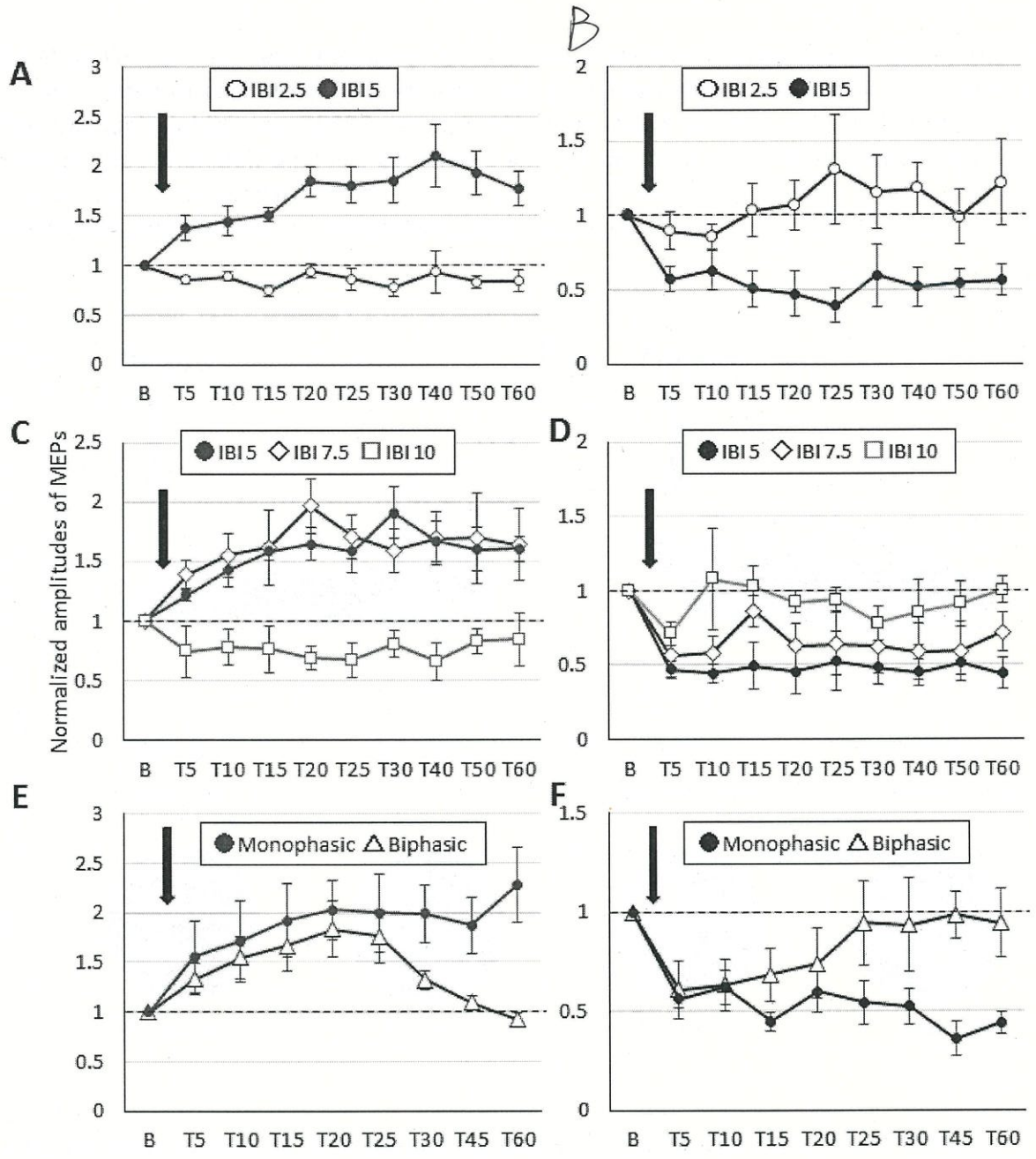


Figure 2.

A: Time courses of excitatory QPS5-IBI2.5 (red circle) and excitatory QPS5-IBI5 (filled red circle). Excitatory QPS5-IBI5 induced MEP facilitation, while no changes in MEP sizes after excitatory QPS5-IBI2.5. **B**: Time courses of inhibitory QPS50-IBI2.5 (blue circle) and inhibitory QPS50-IBI5 (filled blue circle). Inhibitory QPS50-IBI5 induced MEP suppression, while no changes in MEP sizes after inhibitory QPS50-IBI2.5. **C**: Time courses of excitatory QPS5-IBI5 (red filled circle), excitatory QPS5-IBI7.5 (red diamond), and excitatory QPS5-IBI10 (red square). Excitatory QPS5-IBI5 and excitatory QPS5-IBI7.5 had a similar time course, although variable responses were obtained by excitatory QPS5-IBI7.5. No changes were found in MEP sizes after excitatory QPS5-IBI10. **D**: Time course of inhibitory QPS50-IBI5 (blue filled circle), inhibitory QPS50-IBI7.5 (blue diamond), and inhibitory QPS5-IBI10 (blue square). Inhibitory QPS50-IBI5 produced stable decrease of MEP sizes, but much less by

inhibitory QPS50-IBI7.5 and inhibitory QPS50-IBI10. Error bars indicate the standard error. **E, F**: Time course of excitatory QPS5-monophasic (red filled circle), excitatory QPS5-biphasic (red triangle), inhibitory QPS50-monophasic (blue filled circle), and inhibitory QPS50-biphasic (blue triangle). Biphasic QPS had a shorter duration effect than monophasic QPS. Error bars indicate standard error.
In all figures, the black arrow indicates the timing of QPS.

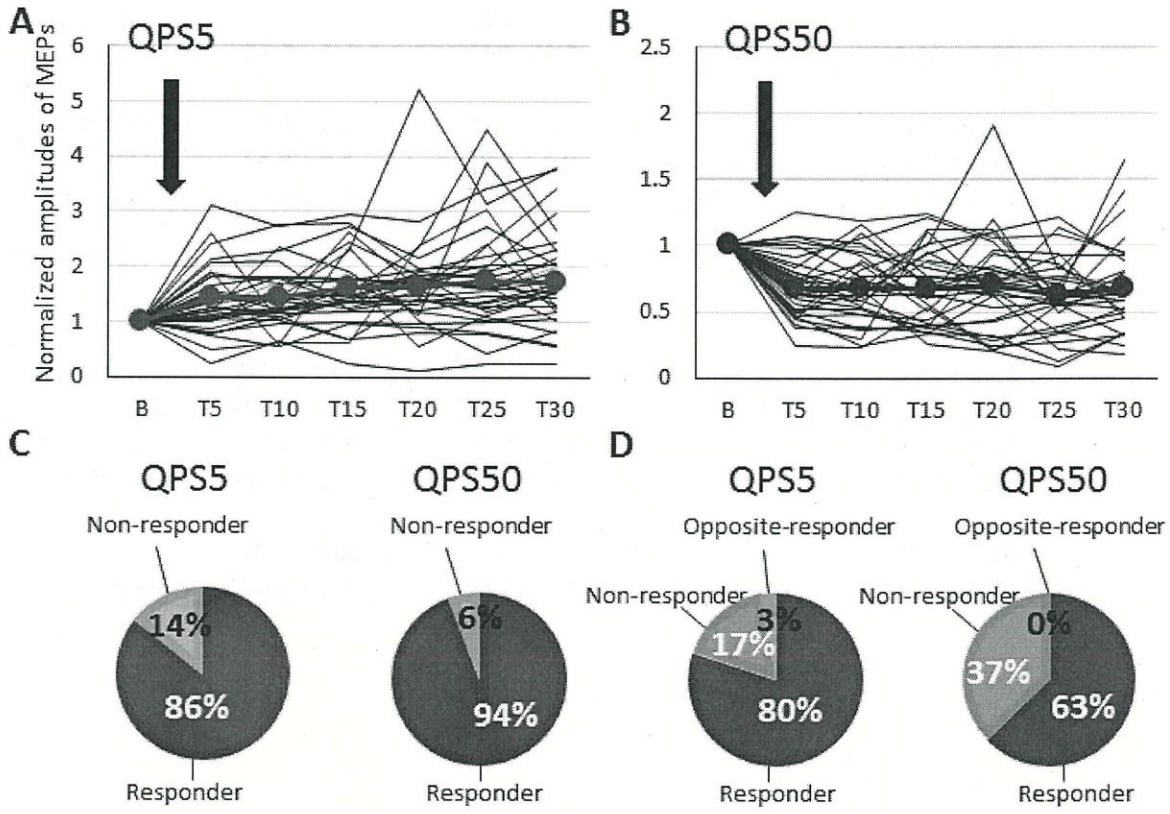


Figure 3.
Time courses of excitatory QPS5 (**A**) and inhibitory QPS50 (**B**). The x-axis shows the time points and y-axis shows the normalized amplitude of MEPs to baseline (B). The thick black line and dot indicate the mean. **C, D;** The percentage of responder, non-responder, and opposite responder is shown in each QPS session (see text).

Tables

Table 1 Baseline physiological data

	RMT	AMT	AMT biphasic	Baseline MEP sizes (mV)
Experiment1				
QPS5-IBI2.5	51.0 ± 8.5	35.8 ± 4.0	-	0.39 ± 0.23
QPS5-IBI5	51.4 ± 9.3	38.0 ± 5.7	-	0.54 ± 0.30
QPS50-IBI2.5	52.4 ± 6.2	38.2 ± 3.6	-	0.78 ± 0.26
QPS50-IBI5	51.6 ± 9.4	40.0 ± 1.6	-	0.60 ± 0.33
Experiment2				
QPS5-IBI5	54.4 ± 6.2	37.2 ± 3.3	-	0.62 ± 0.30
QPS5-IBI7.5	54.2 ± 8.1	39.2 ± 5.0	-	0.38 ± 0.12
QPS5-IBI10	53.4 ± 7.9	40.6 ± 5.0	-	0.50 ± 0.11
QPS50-IBI5	56.0 ± 3.2	38.8 ± 1.8	-	0.72 ± 0.25
QPS50-IBI7.5	52.6 ± 6.7	39.6 ± 5.2	-	0.56 ± 0.23
QPS50-IBI10	52.8 ± 8.0	38.4 ± 3.5	-	0.40 ± 0.11
Experiment3				
QPS5-Mono	60.0 ± 13.6	42.2 ± 5.2	-	0.43 ± 0.13
QPS5-Bi	58.2 ± 8.1	43.2 ± 6.6	45.2 ± 4.4	0.39 ± 0.10
QPS50-Mono	57.3 ± 9.7	42.3 ± 5.6	-	0.42 ± 0.09
QPS50-Bi	60.0 ± 13.6	42.2 ± 5.2	45.3 ± 12.3	0.43 ± 0.13
Experiment 4				
QPS5	51.2 ± 10.6	37.8 ± 7.9	-	0.58 ± 0.26
QPS50	50.7 ± 9.1	38.2 ± 7.9	-	0.54 ± 0.24

Table 2 Results of three-way repeated measures ANOVAs

Experiment 1				
Factor	df	Error	*F*	*p*
ISI	1	8	16.004	**0.004**
IBI	1	8	0.996	0.347
TIME	2.861	22.888	1.564	0.226
ISI x IBI	1	8	34.906	**<0.001**
ISI x TIME	2.971	23.768	0.686	0.568
IBI x TIME	2.861	22.888	0.232	0.865
ISI x IBI x TIME	2.971	23.768	2.293	0.104
Experiment 2				
ISI	1	12	51.099	**<0.001**
IBI	2	12	3.021	0.087
TIME	3.872	46.469	1.123	0.357
ISI x IBI	2	12	19.909	**<0.001**
ISI x TIME	3.438	41.255	1.229	0.313
IBI x TIME	3.872	46.469	0.510	0.838
ISI x IBI x TIME	6.876	41.255	0.948	0.480
Experiment 3				
ISI	1	10	24.567	**0.001**
TYPE	1	10	0.009	0.928
TIME	7	70	2.076	0.058
ISI x TYPE	1	10	2.358	0.156
ISI x TIME	7	70	3.122	**0.006**
TYPE x TIME	7	70	1.058	0.400
ISI x TYPE x TIME	7	70	4.522	**<0.001**

Table 3 Results of one-way ANOVAs for each condition

					P values compared with baseline (Dunnet's test)									
	df	Error	*F*	*p*	T5	T10	T15	T20	T25	T30	T40	T45	T50	T60
xperiment1														
QPS5-IBI2.5	9	36	0.599	0.789										
QPS5-IBI5	9	36	2.936	**0.010**	0.348	0.121	0.162	**0.008**	**0.021**	**0.010**	**0.001**	-	**0.007**	**0.025**
PS50-IBI2.5	9	36	1.016	0.444										
QPS50-IBI5	9	36	3.658	**0.002**	**0.017**	0.055	**0.001**	**0.001**	**0.001**	**0.011**	**0.003**	-	**0.004**	**0.015**
xperiment2														
QPS5-IBI5	9	36	4.766	**0.001**	0.659	**0.034**	**0.018**	**0.002**	**0.004**	**0.001**	**0.002**	-	**0.007**	**0.016**
QPS5-IBI7.5	9	36	1.381	0.233										
QPS5-IBI10	9	36	0.803	0.616										
QPS50-IBI5	9	36	3.696	**0.002**	**0.003**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	-	**0.003**	**0.002**
PS50-IBI7.5	9	36	3.060	**0.008**	**0.004**	**0.007**	0.731	**0.014**	**0.016**	**0.015**	**0.010**	-	**0.007**	0.065
QPS50-IBI10	9	36	0.597	0.790										
xperiment3											-		-	
QPS5-Mono	8	40	2.887	**0.012**	0.198	0.246	0.050	**0.017**	**0.016**	**0.015**	-	0.052	-	**0.001**
QPS5-Bi	8	40	5.951	**0.001**	0.389	0.067	**0.010**	**0.001**	**0.002**	0.483	-	0.997	-	1.000
PS50-Mono	8	40	8.068	**0.001**	**<0.001**	**0.002**	**<0.001**	**0.001**	**<0.001**	**<0.001**	-	**<0.001**	-	**<0.001**
QPS50-Bi	8	40	6.639	**0.001**	**<0.001**	**0.001**	**0.002**	**0.007**	0.783	0.573	-	0.999	-	0.765

Bold indicates p-value less than 0.05.

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