Timing and changes of motor area excitability in beat perception

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Abstract

While listening to music, humans have an inherent ability to perceive the beat of a song and to feel its rhythm. Though the mechanism of beat perception remains elusive, changes in the activity of motor areas have shown to be involved in the process. However, the direction of this motor area modulation, as well as its timing in relation to a beat, has yet to be determined. In an attempt to shed more light onto the issue, we used transcranial magnetic stimulation (TMS) to the motor cortex and measured subsequent motor evoked potentials (MEP) as a measure of motor area excitability. Participants were stimulated with TMS while listening to sequences of tones that were either metrically simple, giving a strong sense of beat, or metrically complex, having no regular beat pattern. The TMS pulses were sent at predetermined asynchronies in relation to the beat to get a snapshot of motor area excitability leading up to the beat onset. We found that the average normalized MEP amplitudes ($n = 8$) did not differ between metricality ($p = 0.568$) or asynchrony conditions ($p = 0.837$) and that there was no significant interaction between the two factors ($p = 0.683$). However, 2 participants showed significantly higher average MEP amplitude while listening to metric simple sequences. The highest average MEP amplitude also occurred under the metric simple condition at a time before the beat onset. Therefore, the trends of our findings point towards increased motor area excitability during beat perception, with the motor areas playing an anticipatory role. Though it is uncertain how the results would translate with actual songs, a better understanding of the motor area's role in beat perception may play a part in improving the clinical applications of music.

Keywords: Beat induction, metricality, transcranial magnetic stimulation (TMS), motor evoked potentials (MEP), motor system

Introduction

Music today is an everyday part of human life, with every society having some form of it engrained in their culture. Though the melodies of music can vary greatly between and within cultures, all music shares the common characteristic of having an underlying beat. Humans have developed an inherent ability to internalize this rhythmic component of music in a process referred to as beat perception (Large et al., 2002). Examples of this phenomenon can be outwardly seen when a listener claps along to the beat of a song or a musician taps their foot to keep time. Though the ability to perceive a beat comes inherently to humans and requires very little effort, the process through which beat perception occurs is still relatively unclear.

From a physiological standpoint, investigations have looked into the possible neural systems that activate in humans during beat perception. Grahn and Brett (2007) looked for cortical regions which possibly contribute to beat perception by having participants listen to tone sequences designed to have either had a strong or weak sense of beat. Functional magnetic resonance imaging (fMRI) was used to find that parts of the motor areas- specifically the dorsal premotor area, supplementary motor area, pre-supplementary motor area, and basal ganglia- were more active while participants listened to sequences with a strong beat in comparison to when listening to those with a weak beat. Another fMRI study by Teki et al. (2011) found that the same motor areas were implicated in relative timing perception. In relative timing, participants developed an expectation of a future stimulus onset based on the timing between previous stimulus events- much like anticipating the beat of a song after a short period of listening. Similarly, Manning and Schutz (2013) asked participants to either tap along or passively listen to a sequence of woodblock notes, then to identify whether a note was on- or off-beat after a period of silence. Participants were better at identifying an off-beat note if they were physically tapping along to the sequence. This finding indicates that physical movement, and therefore activation of motor areas, leads to enhanced beat perception. Taken together, these fMRI and behavioural studies suggest that the activation of the motor areas are implicated in the processes of timing and beat perception.

One major limitation of using fMRI to investigate neural regions involved in behavioural processes is that only a correlation relationship can be drawn from changes in cortical activity of a given area and the behaviour itself (Sliwinska et al., 2014). Alternatively, causal relationships can be inferred with use of transcranial magnetic stimulation (TMS) of cortical regions during behavioural functions. TMS represents a non-invasive technique to stimulate cortical regions by creating a temporary change in the magnetic field under its coil. This change in magnetic field induces activity in neural areas that the TMS coil is placed over. In particular, triggering TMS over the motor cortex of an individual induces muscle activity in the form of motor evoked potentials (MEP). By timing the pulses with the onset of the beat, previous TMS studies recorded the amplitude of resultant MEP and used this as a measure of motor area excitability during beat perception. While holding the strength of TMS stimulations constant, an increase in the MEP amplitude suggested a greater excitability of motor areas (Rossi et al., 2009).

Studies using TMS and measured changes in MEP amplitudes during beat perception have painted a foggier picture into the excitability of motor areas during the process. For example, Stupacher et al. (2013) used TMS to investigate motor area excitability changes while listening to songs with high or low groove (the characteristic of music that makes people want to dance). The degree of changes in motor area excitability increased with higher groove, though the direction of these changes depended on the musical experience of the individual. Michaelis et al. (2014) found similarly variable results in motor area excitability using a TMS and EMG protocol. Participants were asked to spontaneously tap their fingers to obtain a measure of their preferred tempo and then listen to beat sequences of different speeds. More pronounced changes in MEP amplitudes were seen as participants listened to sequences closer to their preferred tempo. However, the direction of excitability changes was again variable between individuals as sequence tempi approached the SMT. Therefore, though it is clear that the motor areas play some role in beat internalization, the consistency and direction of the modulation in these areas is still questionable.

Cameron et al. (2012) looked to resolve the uncertainty by simply investigating changes in motor area excitability while participants listened to metric simple (strong beat) or metric complex (weak beat). For the most part, participants showed higher MEP amplitudes while listening to metric simple sequences. However, one person showed a significant decrease in MEP amplitude with metric simple sequences than while listening to metric complex sequences, further suggesting that changes in motor area excitability during beat perception are still relatively unclear and highly variable. Another interesting finding from the study was that TMS pulses sent at random points during the sequence resulted in no difference in MEP amplitudes between metric simple and complex sequences. This finding suggests that any excitability of the motor areas while perceiving a beat is not sustained throughout the entire sequence and may occur only at a point fixed in relation to the beat.

Past TMS studies involving beat perception only stimulated at fixed points in relation to the beat onset and therefore only looked at motor area excitability during that point in time. It is therefore unknown as to how motor area excitability changes in the time immediately leading up to the beat onset and if there is a consistent point at which excitability peaks. Revealing the timing of motor area excitability in relation to the beat, as well as producing a clearer picture as to how excitability changes while perceiving a beat, can serve as a first step in better understanding the role of motor areas in beat perception. Therefore, the objective of this study is to use TMS to develop further evidence of increased motor area excitability during beat perception, as well as to gain a picture of the timing in the changes of motor area excitability relative to the beat. From the results of Cameron et al., we hypothesize that the MEP amplitudes, and therefore motor area excitability, should be higher while listening to metric simple sequences in comparison to the metric complex sequences. Also, because beat perception is anticipatory in nature, we expect to find the maximal MEP amplitude at a point before the beat actually occurs.

Materials and Methods

Participants

Participants were recruited from the University of Western Ontario through the psychology research participation (SONA) pool. Participants were taken from the 18 – 50 age range and were excluded if they had claustrophobia, pacemakers, electronic implants, metal implants, were welders or soldiers, had an injury by a metallic object that was not removed, were pregnant, trying to conceive, or sexually active without the use of a contraceptive, had cerebral aneurysm clips, had a history of neurological, psychiatric, heart or lung disease, have had epileptic seizures or a family history of epilepsy, were taking psychotropic medication that cause drowsiness, or suffered from migraines/headaches. All participants provided informed consent to partake in the study.

Auditory Stimuli

The auditory sequences were composed of sinusoidal tone sounds which had consistent pitch and volume. The sequences were designed to either induce a strong sense of beat (metric simple) or a weak sense of beat (metric complex). The metric simple sequences were created so that the first count of each measure, or the beat position, must contain a tone. A count is defined as a window of time which contains either a tone sound or a period of silence, while a measure is composed of 4 counts. Having a tone played at each beat position provides a sense of regularity and gives the overall sequence a strong sense of beat (Fig. 1A). The metric complex sequences did not necessarily have a tone played at each beat position and therefore did not induce a sense of beat in the participants (Fig. 1B).

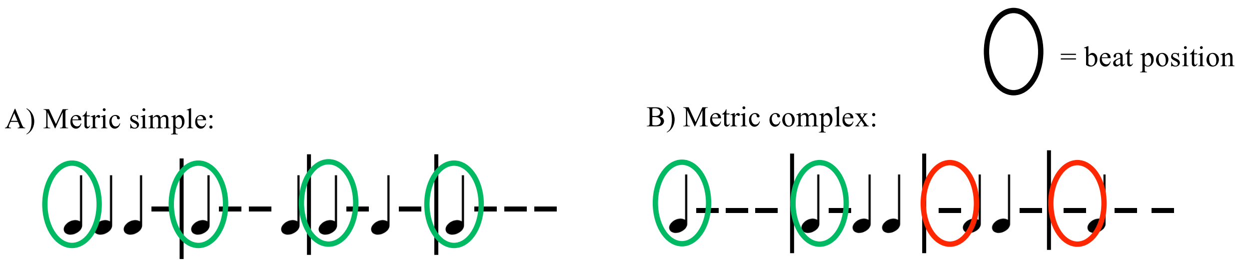

Figure 1: Excerpts from A) metric simple and B) metric complex sequences. The beat positions (indicated by the coloured ovals) always have a tone (represented by a musical note) for metric simple sequences, giving the sequence a high sense of beat. The beat positions in metric complex sequences do not necessarily contain a note and can instead have a silence (represented by the line), giving the sequence a much weaker sense of beat.

The placement of tones in metric complex sequences were not restricted except in that tones could not be played on three consecutive counts, as this pattern would create a sense of beat. The rules for metric simple and complex sequences were followed to first create 16 smaller sequences comprised of 3 measures each. The smaller sequences were then randomly selected and assembled into full sequences, approximately 35 s in length. A total of 20 unique auditory sequence patterns were made for both metricalities. All sequence patterns were then adjusted to play at three different tempos: 175, 200, and 225 ms/count. The varying tempos were created to prevent familiarity with rhythms, which may make it easier for participants to impose a beat structure onto metrical complex sequences.

TMS Stimuli

The timing of the TMS stimuli was set so that pulses would be sent at fixed asynchronies in relation to the beat. The asynchronies were measured as proportions of the time between beat positions, or the inter-beat interval (IBI), and always occurred before the onset of the beat. A total of 5 different proportions were used: 0 (right on the beat), 0.05, 0.10, 0.15, and 0.20 of the IBI. For example, if a 0.20 proportion was used for a sequence with an IBI of 800 ms, the stimulation would occur 160 ms, or 800 ms IBI × 0.20 of IBI, before the beat. To prevent overheating of the TMS machine, pulses could only be sent approximately once every four seconds. Additionally, no TMS stimulations were sent within the first four measures of the sequence in order to allow participants get a sense of the beat before data collection. Since the auditory sequences were approximately 35 s in length, 7 TMS stimulations were possible with the 175 ms and 200 ms sequences while only 6 TMS stimulations occurred in the 225 ms sequences. To make up for this disparity in stimulation events between tempi, participants listened to 6 auditory sequences of the 175 ms and 200 ms tempi and 7 auditory sequences of the 225 ms tempo for both metricalities while undergoing TMS. Therefore, participants listened to a total of 38 sequences (19 metric simple and 19 metric complex) while being stimulated 6 or 7 times per auditory sequences, giving a total of 42 stimulations per tempo and metricality. The timing of stimulations in relation to the start of the auditory sequence was determined by creating stimulation sequences. Stimulation sequences were created with 7 asynchronies per sequence, with no asynchronies occurring more than twice per sequence and with stimulations spaced about 4 s apart. The asynchrony proportions were then transformed into absolute values for each tempo, with the last stimulation being dropped for the 225 ms sequences. The sequences were then selected so that 8 – 9 asynchrony stimulations occurred per tempo and metricality.

Behavioural Tasks

Spontaneous Motor Tapping Task

Using E-Prime® 2.0 (Psychology Software Tools Inc, Sharpsburg, PA) software, the spontaneous motor tapping (SMT) rate of each participant was collected. Participants were asked to tap on the space bar of a keyboard 31 times per trial, at a pace that felt comfortable to them. Participants underwent four trials before the TMS stimulations and another four trials after stimulation. The SMT was measured by averaging the inter-tap interval (ITI) across the eight trials and used as a measure of the participant's preferred tempo. The preferred tempo of participants was collected based on the findings of Michaelis et al. (2014), who saw that the tempo of a sequence relative to an individual's SMT may influence motor area excitability during beat perception.

Beat Tapping Task

After sequences were played, participants were also given a behavioural tapping test to validate the strong and weak sense of beat in metric simple and complex sequences respectively. Participants were asked to listen to 3 metric simple and 3 metric complex sequences (one of each tempo) and tap along to what they felt was the beat of the sequence. Participants tapped using the 'm' key of the keyboard and while listening to the sequences using headphones. Data was collected on the time between taps (ITI) and the timing of the tap relative to the start of the sequence using E-Prime® 2.0. The absolute differences between tap times and the closest predicted beat position of the sequence was measured. These absolute differences were averaged and divided by the mean ITI of the sequence as a measure of tapping error. The variability of tapping was also measured using the coefficient of variation (CoV) for each sequence. The CoV was taken by dividing the standard deviation of the ITI and dividing by the mean ITI for each sequence. The CoV also serves as a validation that the participants were tapping to the underlying beat of the sequence. A beat tapping task with a CoV greater than 0.35 was considered to be invalid, as this suggests participants were attempting to tap at each tone that is played rather than to the underlying beat. The tapping error and CoV were averaged and compared between metricalities using a paired Student's T-test and SPSS software version 22 (IBM Corp, Armonk, NY).

Materials and Procedure

Single-pulse TMS, which only sends one pulse at a time, from a MagStim Super Rapid TMS machine (Magstim Company Ltd, Carmarthenshire, UK) was used in our study to stimulate the area of the primary motor cortex (M1) which controls hand movements. While the stimulations occurred, participants also listened to the audio sequences which were played from the lab computer through headphones. The synchronization of TMS triggers with the audio sequences was performed using Matlab software (Mathworks, Natick, MA) with the PsychToolBox extension (http://psychtoolbox.org) which allowed for the audio sequences to be played with minimal delay after its intended onset. The muscle activity due to TMS stimulations were collected and filtered using the Quad AC Amplifier EMG system (Grass Technologies, Warwick, WI, USA). The EMG readings were transmitted to the computer using a Micro1401-3 data acquisition unit (CED Ltd., Cambridge, England), where they were displayed and recorded using Signal software (CED Ltd., Cambridge, England).

Participants were first given a letter of information for the study and asked to sign a consent form. Participants were then asked about the number of years of musical experience and whether or not they were still practicing. Information on musical experience was collected based on the study of Stupacher et al. (2013) which found that the motor area excitability of musicians may differ from non-musicians during beat perception.

To begin the process of obtaining MEP data from the participant, an EMG recording electrode was placed on the first dorsal interossei (FDI) muscle of the right hand to measure the muscle activity created by TMS pulses. A ground electrode was also placed over the pisiform bone of the right hand to serve as a reference point. The TMS was then used to stimulate the left medial motor cortex of each participant, as the primary motor cortex controls muscle movement contralaterally. The 10-20 EEG measurement system was used to approximate the location of M1. The M1 region located approximately in the C3 or C4 locations in the 10-20 system (DaSilva et al., 2011). The coil placement was then adjusted to look for the motor hotspot area which controls the FDI muscle of the hand. Initial TMS pulses were set at 30% stimulation strength and gradually increased by 5% until the motor threshold of the FDI was reached. Location of the motor hotspot and the resting motor threshold level were determined by looking at the filtered EMG data as stimulation strength and location was adjusted. The threshold level was defined as the stimulation strength at which at least 5 of 10 consecutive stimulations created an MEP of 50 μV or above. After the hotspot was located and the threshold level was established, the TMS coil was secured on a stand and positioned over the hotspot throughout the duration of the experiment. Participants were asked to remain as still and relaxed as possible with their eyes closed.

To begin the main experiment, the TMS stimulation strength was set to 110% of the resting motor threshold. With headphones on, the auditory sequence and stimulatory sequences started simultaneously using Matlab. The auditory sequences were randomly selected by Matlab and paired with a random stimulatory sequence of the appropriate tempo. No auditory or stimulatory sequence was repeated more than once. A total of 38 auditory sequences, each approximately 35 s in length, were played for each participant. Breaks (lasting approximately 5 s) were given between each sequence. Participants were given the opportunity to withdrawal at any point and ask for longer breaks between sequences.

MEP Data Processing and Analysis

The MEP peak-to-peak amplitudes were measured by taking the difference between the maximum and minimum points on the EMG recording in a 10 – 80 ms window after the onset of the visible TMS artifact. The onset of the TMS artifact was defined as a change of slope in the MEP signal greater than 50 mV/ms. Baseline EMG levels were established by averaging the absolute EMG voltage 50 ms before the onset of the TMS artifact. The baseline level was then subtracted from the peak-to-peak amplitude to obtain a measure of the baseline-subtracted amplitude. Lastly, the TMS amplitudes were all normalized within each participant by dividing all the baseline-subtracted amplitudes by their overall average baseline-subtracted amplitude. A three-factor repeated measures analysis of variance (ANOVA) was performed using SPSS software to look for differences in the average normalized MEP amplitudes for conditions of metricality, asynchrony proportion, and tempo. The musical experience of the participants was also included as a covariate in the analysis.

In considering the effect of preferred tempo on motor area excitability, average normalized MEP amplitudes were grouped in terms of preferred and not preferred tempos. The preferred tempo of the participant was defined as the tempo that was closest to the participant's SMT rate, while the other two tempi were categorized as not preferred. Another three-factor repeated measures ANOVA was performed with factors of metricality, asynchrony proportion, and tempo preference.

Results

Participants

A total of 10 right-handed, healthy participants (7 females) were recruited for our study. The MEP data from one participant could not be obtained due to reported discomfort at TMS strengths below the motor threshold. The MEP data from another participant was excluded due to software issues which caused a delay between the expected stimulation time of the TMS and the actual stimulation time. For the 8 participants with valid MEP data, the range of musical experience was 1 – 15 years, with a mean ± SD of 3.25 ± 4.65 years. However, only one of the eight participants had musical experience greater than fours yeas and was actively practicing music during the time of participation, therefore musical experience was not considered as a covariate for subsequent analysis.

Beat Tapping Task

Data from the beat tapping task of 3 participants was discarded, as all their trials presented a CoV over the threshold of 0.35. For the valid beat tapping trials, participants tapped to metric simple sequences with less error than metric complex sequences (Fig. 2A), though this difference was not significant ($t(4) = 2.071$, $p = 0.107$). Participants also tapped more evenly to metric simple sequences than complex sequences, as seen with the lower CoV for simple sequences (Fig. 2B), with the difference was marginally significant ($t(4) = 2.708$, $p = 0.054$).

Motor Evoked Potentials

An average of 15.63 stimulations per participant was lost due to a missed trigger to the TMS, representing 6.3% of the total anticipated pulses. The three-factor repeated measures ANOVA considering metricality, asynchrony, and tempo showed no significant interaction between these factors ($F(8,56) = 1.858$, $p = 0.184$). Factors of tempo and metricality showed no

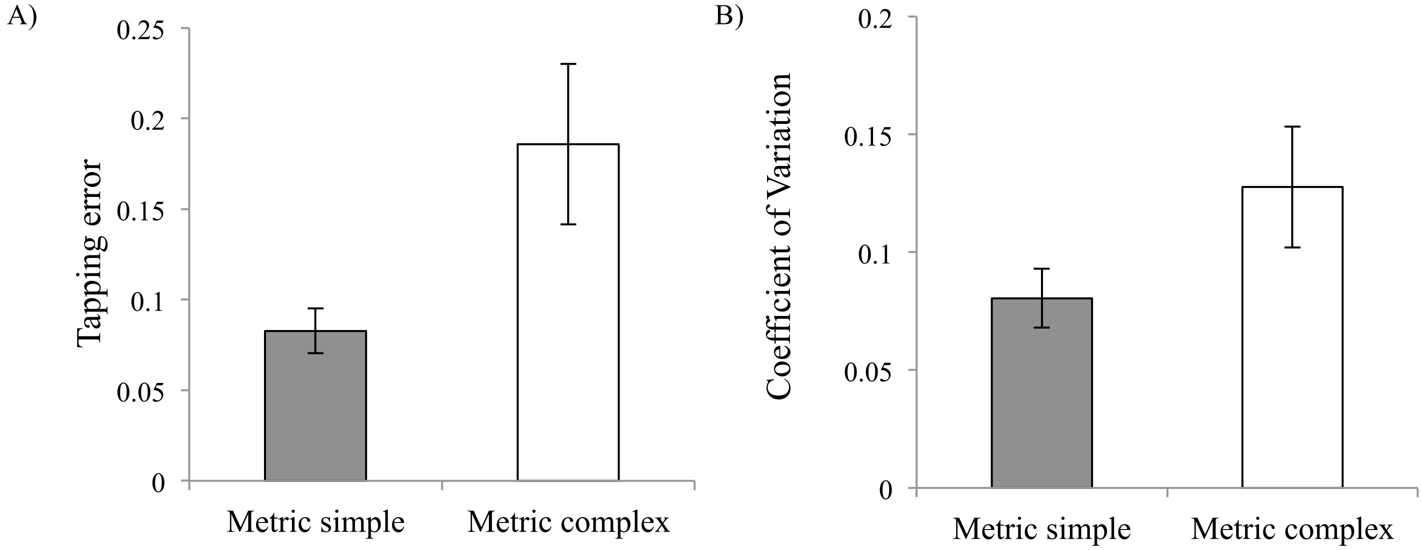

Figure 2: Performance in the beat tapping task in terms of A) tapping error and B) Coefficient of Variation (CoV) for both metric simple and complex sequences (n = 5). Each n represented data averaged over three sequences for each metricality. Data are shown as mean ± SEM. No significant differences were seen between metricalities in terms of tapping error ($p = 0.107$), though a marginally significant decrease was seen in CoV ($p = 0.056$) for metric simple sequences, which suggests more even tapping (Paired Student's t-test).

significant interaction ($F(2, 14) = 2.682$, $p = 0.103$; Fig. 3A), with an even more diminished interaction between asynchrony and metricality ($F(4, 28) = 0.576$, $p = 0.683$; Fig. 3B). However, the metric simple sequences did show a higher average normalized MEP amplitude at all 5 asynchrony conditions when collapsed across tempos, with the maximal MEP amplitude occurring at the 0.10 asynchrony value (Fig. 3B). The interaction between tempo and asynchrony was the closest to reaching significance ($F(8, 56) = 2.570$, $p = 0.090$), with the largest difference between tempos occurring at the 0.15 asynchrony proportion (Fig. 3C). The average normalized MEP amplitudes peak at the 0.10 proportion for both 175 ms and 200 ms condition (70 and 80 ms before the beat respectively), but peaks at 0.15 for the 225 ms condition (135 ms before the beat; Fig. 3C). There were no main effects for metricality ($F(1, 8) = 0.354$, $p = 0.568$), asynchrony ($F(4, 32) = 0.357$, $p = 0.837$), or tempo ($F(2, 16) = 2.779$, $p = 0.092$) conditions. Significant differences in MEP amplitudes between metricalities were seen when compared within each participant (Fig. 3D) using a paired Student's t-test. Overall, 6 of 8 participants had a higher average normalized MEP amplitude while listening to metric simple sequence, while the difference in two of those participants, participant 2 ($t(14) = 2.719$, $p = 0.017$) and 3 ($t(14) = 2.303$, $p = 0.037$), reached significance.

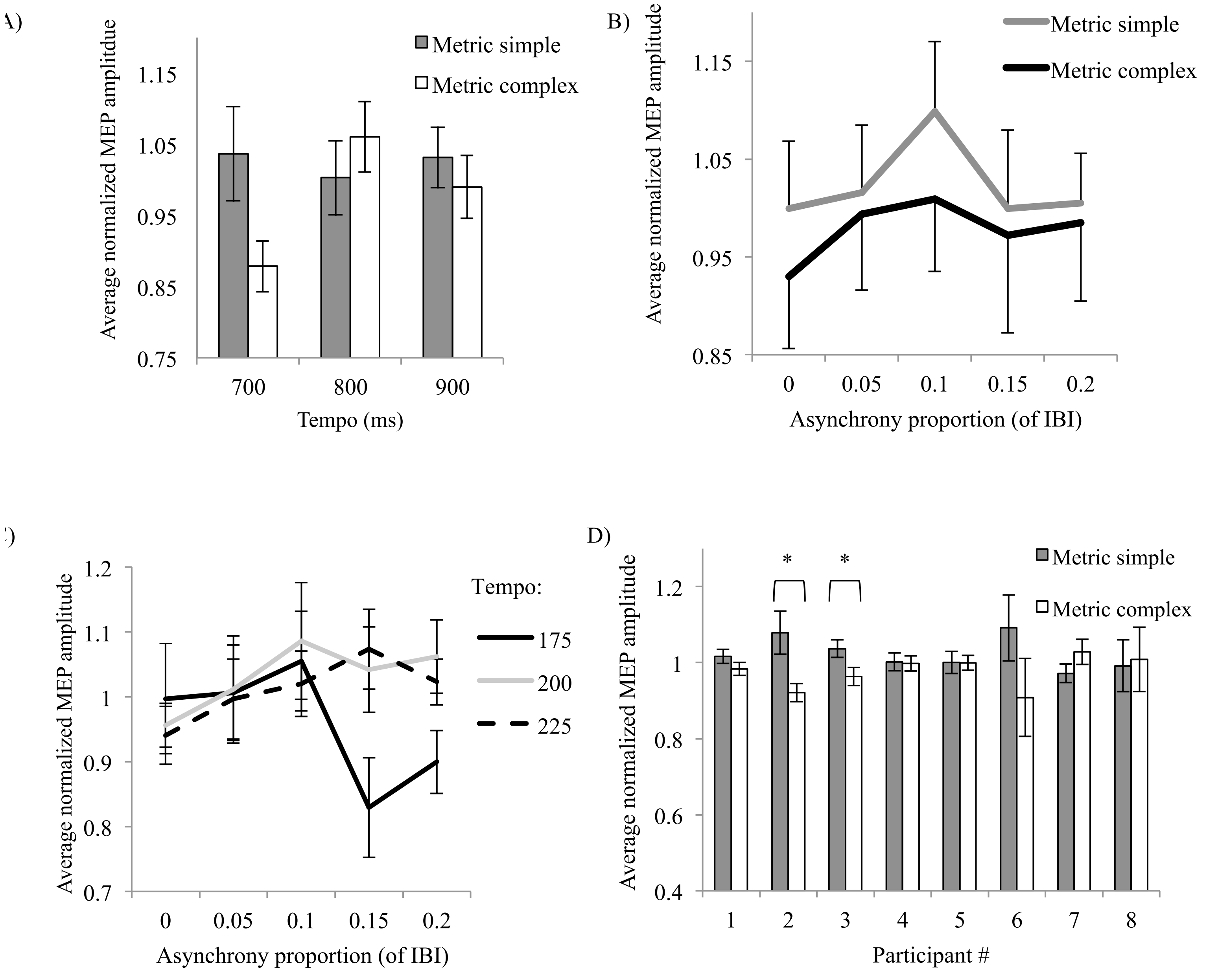

Figure 3: Average normalized MEP amplitudes grouped in terms of A) tempo conditions with metricalities shown in separate bars (n = 8); B) asynchrony proportions with simple and complex metricalities shown as separate lines (n = 8); C) asynchrony proportions with tempo conditions shown as separate lines (n = 8); and D) each participant with metricalities shown in separate bars, collapsed across tempi and asynchronies (n = 15). All data are shown as mean ± SEM. No significant interactions were seen between A) tempo and metricality ($p = 0.103$); B) asynchrony and metricality ($p = 0.683$); or C) tempo and asynchrony ($p = 0.090$) (All p values obtained from a three-factor repeated measures ANOVA). D) A significant increase in MEP amplitudes between metricalities was seen in participants 2 ($p = 0.017$) and 3 ($p = 0.037$) (paired Student's t-test, * $p < 0.05$).

Spontaneous Motor Tapping Task

The average spontaneous motor tapping rate was 631.40 ± 229.60 ms/IBI, which was below the rate of our fastest tempo (700 ms/IBI). The average normalized MEP amplitudes were categorized into preferred tempo and not preferred tempo groups, with the MEP readings from the tempo closest to their SMT being classified as the preferred tempo group. Replacing tempo with preference in the three-factor repeated measures ANOVA still revealed no significant interactions ($F(4, 28) = 1.084$, $p = 0.363$). Analysis was also performed only using data from an individual's preferred tempo. A two-way repeated measures ANOVA with factors of asynchrony and metricality within a participant's preferred tempo showed no significant interaction between factors ($F(4,32) = 0.796$, $p = 0.520$) and also revealed no main effects.

Discussion

The goal of this study was to further establish changes of motor excitability during beat perception, as well as to define the timing of this excitability in relation to the beat. Participants were asked to listen to sequences of tones organized to have a strong sense of beat (metric simple) or a weak sense of beat (metric complex) while being stimulated with TMS. The TMS pulses were sent in synchronization with the beat as well as asynchronously before the beat onset. Though the trends in our results point to increased motor area excitability during beat perception for most participants (Fig. 3D), overall we found no significant difference between average normalized MEP amplitudes (a measure of motor area excitability) when participants were listening to metric simple or metric complex sequences. The MEP amplitudes also did not differ significantly between asynchrony conditions, though trends suggest that maximal motor area excitability occurs proportionally at 0.10 of the IBI before the beat (Fig. 3B).

Unlike our results, previous studies using similar TMS protocols to assess motor areas during beat perception found more noticeable changes in excitability while listening to sequences with high sense of beat (Cameron et al., 2012; Michaelis et al., 2014; Stupacher et al., 2013). For example, our findings were less pronounced than those found by Cameron et al. (2012), who also compared MEP data from TMS pulses between metric simple and complex listening conditions. They found that two of four participants in the study had significantly higher MEP amplitudes while listening to sequences with a strong sense of beat, while one other participant showed significantly lower amplitudes in the metric simple condition. Our results were more ambiguous, showing that only two of eight participants had significantly higher amplitudes in the metric simple condition, although six of eight had higher amplitudes overall (Fig. 3D). Taken together, the findings from both studies seem to indicate a higher level of motor area excitability during beat perception. However, the inconsistency to find meaningful increases in MEP amplitudes during beat perception in our results, coupled with an individual in Cameron's study who showed significantly higher amplitude in the metric complex condition, suggests that there are likely additional factors between individuals which influence motor areas behaviour during beat perception. Therefore, the small sample sizes become an even bigger limitation when trying to infer changes of motor area excitability from the results of either study.

One factor which has been seen to affect motor area excitability in beat perception is the preferred tempo of an individual. Michaelis et al. (2014) found that although MEP amplitudes changed while listening to metric simple in comparison to metric complex sequences, the direction of the change depended on the tempo of the sequence relative to the participant's preferred tempo. Like Michaelis et al., we used the participant's SMT as a measure of their preferred tempo and grouped the MEP data according into preferred and not preferred tempi.

Analysis did not show any significant differences between the MEP amplitudes of preference groups, nor was there a difference in MEP amplitudes between metric simple and complex sequences within each participant's preferred tempo. Though our results differ from those of Michaelis et al., it is important to note that the tempi of sequences we used were also not based around the preferred tempo of the participant, which gives our design less resolution. For example, a participant who tapped at 650 ms/IBI and one who tapped at 200 ms/IBI would both have the 700 ms sequences defined as there preferred tempo. Therefore, there can be a large discrepancy between how close each participant's preferred tempo is to their actual SMT rate. Additionally, the 700 ms tempo also showed the largest difference in MEP amplitude between metricalities and the ANOVA analysis showed that tempo was the factor closest to reaching significance ($p = 0.092$). Taken together, these trends do suggest that motor area excitability can undergo a more pronounced change as participants listen to sequences closer to their preferred tempo and that this factor should be considered in future beat perception studies.

In terms of the timing of motor area excitability in relation to the beat, results showed that though the MEP amplitude was not significantly higher, the maximal amplitude averaged occurred at the 0.10 asynchrony under the metric simple condition (Fig. 2B). The MEP amplitudes were also higher while listening to metric simple sequences at every asynchrony time point, which gives further suggestion to increased motor area excitability at time points leading up to the beat during beat perception. It is not too surprising that that the maximal motor area excitability would occur at a point before the beat as beat perception has been conceived to be a predictive process (Patel and Iversen, 2014). There is also evidence to suggest that the timing of motor area excitability during this process occurs relative to the beat, rather than occurring in an absolute asynchrony (Leow and Grahn, 2014). Indeed, it has been suggested that motor areas are more involved with relative timing while deeper brainstem areas of the olivocerebellar network control absolute timing processes (Teki et al., 2011). However, it has also been proposed that the role of the motor areas during beat perception is in anticipation of some movement along to the beat (e.g. foot tapping) (Van der Steen and Keller, 2013). Therefore, it is reasonable to think that the height of motor area excitability occurs in an absolute time before the beat, according to the time it takes for the transmission of a signal from the motor cortex to a target muscle. Though the maximal excitability of each tempo did occur at different asynchrony values, two tempi had a peak average MEP amplitude occur at a 0.10 proportional asynchrony, while the overall maximum amplitude across all tempi also occurs at 0.10, with clear dips on either side (Fig. 2B). On top of this, when we converted the asynchrony values for each tempo into absolute millisecond values, a plot of the average MEP amplitudes at the time points leading up to the beat showed no clear peaks or troughs in either metricality (data not shown). Therefore, our results provide further evidence that motor area excitability occurs in a relative fashion in relation to the beat onset and suggests a 0.10 asynchrony proportion as the time of maximal motor area excitability during beat perception. However, without significant differences, neither inference can be firmly confirmed.

One possible reason that the trends we saw did not reach significance may be due to the metric strength of the tone sequences that were used. Though participants showed more accurate and less variable tapping to metric simple sequences, their performance was not significantly better than when tapping to metric complex sequences. The metric simple tone sequences were designed to create a temporal perceived accent on the beat position, which should give the overall sequence a strong sense of beat. The design was first described by Povel and Essens (1985) and has been validated through its wide use in other studies as a method of creating a strong sense of beat (Cameron et al., 2012; Gordon et al., 2014; Grahn and Brett, 2009). Therefore, though the beat tapping task results show no perceived difference in performance between the two types of sequences and limits the information we can gain from our MEP data, this is most likely due to a small sample size (n = 5) of acceptable beat tapping trials rather than the sequences not being effective in presenting a beat.

Another limitation in our design and a factor which may have influenced our results is the attention level of each participant. There has been support to show that changes in the activity of motor areas are more pronounced in beat perception if the individuals are attending to the auditory stimulus (Chapin et al., 2010). Therefore, a participant who was not actively attending to the sequences would show a less of a difference in MEP amplitudes between complex and simple sequences. Without a measure of the attention level of each participant for each sequence, we cannot be certain that this variable is not a confounding factor. However, there is also support for beat perception as a pre-attentive phenomenon, so it is still relatively unclear how attention contributes to motor excitability during the process (Ladinig et al., 2009; Geiser et al., 2010). Another limitation of our study was the approximation of the TMS stimulation site. Though the primary motor cortex was approximated using the 10-20 EEG system and was further validated by visible muscle twitches in the hands of some participants, we cannot be certain that the stimulations occurred at a point directly on the primary motor cortex. Also, although the participants were asked to remain relaxed and still, movements in the hand may have influenced MEP amplitudes and movement of the head could have changed the stimulation site between trials.

Therefore, the trends of our results point towards heightened motor area excitability in beat perception, which peaks at a 0.10 proportion before the beat onset. Though we did not find significant differences in MEP amplitude between metricality, asynchrony, or tempo conditions, this null result is more likely due to the limitations of our designs and a small sample size rather than a lack of heightened motor area excitability during beat perception. Several theories have been developed into defining the role of motor areas during the process. One suggests that even without movement, the motor planning areas communicate with the auditory regions of the brain to create an imagined movement to help better perceive the beat (Patel and Iversen, 2014). Some evolutionary models have also been formed regarding beat perception, suggesting that motor areas play a more primitive role and that the process has developed in other species as well (Large and Snyder, 2009). Therefore, though it is still unclear as to how motor areas fit into the mechanisms of beat perception, it is likely that the regions are involved in some manner and excitability does increase in the process. Our finding that maximal excitability occurs before the beat suggests that motor areas play an anticipatory role in beat perception.

There are still some questions as to how consistently these changes in excitability during beat perception translates when participants are listening to actual music (Cameron et al., 2010; Stupacher et al., 2013). However, using tone sequences to develop evidence for changes in motor area excitability and further the understanding of its timing during beat perception serves as a step towards further elucidating the role of motor areas in the process. Music therapy is already a commonly studied treatment in individuals with movement disorders (e.g. Parkinson's) and a better understanding of how motor areas are affected by beat and rhythm may be beneficial in increasing the efficacy of such treatments (Benoit et al., 2014; Nombela et al., 2014).

Overall, the trends in our results showed that the MEP amplitudes, and therefore motor area excitability, is increased during beat perception, though the differences were not significant and inconsistent between participants. The maximal amplitude of the excitability occurred at an asynchrony of 0.10 of the IBI before the beat itself, suggesting that the peak of motor area excitability occurs before the beat onset. Further testing with a larger sample size should be done to determine the relevance of our trends.

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