A neural basis for contagious yawning

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Summary

Contagious yawning [CY], in which yawning is triggered involuntarily when we observe another person yawn, is a common form of echophenomena -- the automatic imitation of another's words (echolalia) or actions (echopraxia) [1]. The neural basis for echophenomena is unknown, however it has been proposed that it is linked to disinhibition of the human mirror-neuron system [1-4] and hyper-excitability of cortical motor areas [1]. We investigated the neural basis for CY using transcranial magnetic stimulation [TMS]. Thirty-six adults viewed video clips that showed another individual yawning and, in separate blocks, were instructed to either resist yawning or allow themselves to yawn. Participants were videoed throughout and their yawns or stifled yawns were counted. We used TMS to quantify motor cortical excitability and physiological inhibition for each participant, and these measures were then used to predict the propensity for CY across participants. We demonstrate that instructions to resist yawning increase the urge-to-yawn and alter how yawns are expressed (i.e., full vs. stifled yawns) but do not alter the individual propensity for CY. By contrast, TMS measures of cortical excitability and physiological inhibition were significant predictors of CY and accounted for approximately 50% of the variability in CY. These data demonstrate that individual variability in the propensity for contagious yawning is determined by cortical excitability and physiological inhibition in primary motor cortex.

Key Words: Contagious yawning; Echophenomena; Transcranial magnetic stimulation [TMS]; Tourette syndrome; Motor cortex.

Results and Discussion

Contagious yawning (CY) has been demonstrated previously in humans, chimpanzees, old world monkeys, and dogs, and can be triggered by hearing or seeing another individual yawning [**5**]. Furthermore, watching or hearing another individual yawn activates a network of brain regions that are associated with motor imitation and empathy [**3,6**]. For this reason, CY has frequently been linked to the operation of the human mirror neuron system (MNS) [**3,6**]: which is thought to play a key role in action understanding, empathy, and the synchronization of group social behavior [**7**]. However, functional brain imaging studies have provided mixed evidence in support of this proposal and have reported that core regions of the human MNS are not in fact activated during CY [**3,6**). Furthermore, while the propensity for CY varies across individuals, a recent study has shown it to be stable across time (i.e., measurement sessions) and also uncorrelated with empathy scores [**8**].

Alternatively, it has been proposed that echophenomena, including CY, may be generated automatically by ethological releasing mechanisms responsible for triggering stereotyped motor acts [**9**], and that the propensity for echophenomena may be linked to individual differences in cortical motor excitability [**1**]. This proposal is consistent with the observation that echophenomena are observed within a few weeks of birth but decrease after around three years of age; consistent with the development of self-regulatory mechanisms and reduced automatic imitation of observed actions. It is also consistent with the demonstration that echophenomena are observed in a wide range of clinical conditions linked to increased cortical excitability and/or decreased physiological inhibition (e.g., epilepsy, dementia, autism, Tourette syndrome) [**1**].

In the current study we tested the hypothesis that the propensity for CY was positively associated with motor excitability. Specifically, we investigated whether individual differences in baseline measurements of motor cortical excitability and physiological inhibition were associated with the propensity for CY. Prior to commencing the CY experiment, TMS measures of cortical excitability and physiological inhibition were recorded from the left primary motor cortex (M1) for each participant and subsequently used to predict propensity for CY.

Figure 1 about here

The design of the experimental task is illustrated in **Figure 1A**. Participants viewed video clips that showed another individual yawning and, in separate blocks, were instructed to either resist yawning or allow themselves to yawn. Blocks 1 and 2 were completed without non-invasive electrical brain stimulation, but during blocks 3 and 4 transcranial electrical stimulation (tES) was delivered continuously to the supplementary motor area (SMA) region of the scalp. It should be noted however that for brevity, only data recorded from blocks 1 and 2 will be reported in this paper, and that the effects of tES on the propensity for CY will be reported elsewhere.

Participants were videoed throughout and their yawns and stifled yawns were counted. In addition, throughout the experiment the intensity of each participant's perceived urge-to-yawn was continuously recorded using a slider device that the participant operated using his or her right index finger (**Figure 1B**). This device delivered a continuous voltage signal that indexed change over time in self-estimated intensity in the perceived urge-to-yawn. Representative data from one individual are presented in **Figure 1C**.

Effects of instruction on yawning behaviour

To determine whether the instruction to resist yawning had an effect on yawning behaviour we examined the number of full and stifled yawns observed during the first two blocks of trials. Data were analysed using a two-way repeated-measures ANOVA with the factors Instruction condition (allow vs. resist yawning) and Yawn response (full vs. stifled yawns). The ANOVA revealed no significant main effects (maximum $F(1,34) = 2.22$, $p > 0.14$) but a significant Instruction x Response interaction ($F(1,34) = 54.29$, $p < 0.0001$). Relevant means are presented in **Figure 2**. The simple effects of this interaction demonstrated that whereas full yawns were substantially reduced following the instruction to resist yawning (Means: Allow condition = 5.23, Resist condition = 0.17; $t(34) = 6.31$, $p < 0.0001$; effect size [Hedges' G] = 1.46), stifled yawns were significantly increased by the instruction to resist yawning (Means: Allow condition = 0.11, Resist condition = 3.86; $t(34) = 5.51$, $p < 0.0001$; effect size [Hedges' G] = 1.28). These data confirm that the instruction to

suppress contagious yawning was only partially successful and led to a significant decrease in full yawns but an increase in the number of stifled yawns observed. (Means: full yawns = 0.17, stifled yawns = 3.86; $t(34) = -5.13$, $p < 0.0001$; effect size [Hedges' G] = -1.25).

To further determine whether the instruction to resist yawning had an effect on yawning behaviour, we examined the sum total of full and stifled yawns observed during the first two blocks of trials. This analysis revealed that the means were not significantly different from one another (Resist = 4.03, Allow = 5.34; $t(34) = -1.489$ $p > 0.05$). This finding indicates that the instruction to resist yawning significantly increases the urge-to-yawn (reported below) and alters how the yawn may be expressed (i.e., stifled yawns rather than full yawns), but it does **not** alter the individual's propensity for yawning. This is consistent with previous reports that while contagious yawning is variable across individuals, an individual's propensity for contagious yawning is nevertheless highly consistent over time. It is also consistent with our finding that the excitability of each individual's motor cortex (described below) is a significant predictor of the propensity for contagious yawning.

Figure 2 about here

Effects of instruction on self-estimates of the urge-to-yawn

We have argued elsewhere that whereas sensory signals may trigger actions outside of awareness, a distinguishing feature of urges-for-action that they are chiefly associated with actions that cannot be realized immediately and must be held in check until an appropriate time, when they can be released [**10**]. To determine whether the instruction to resist yawning led to an increase in perceived urge-to-yawn values in the current study, we compared mean self-reported urge-to-yawn values in the 'Allow' versus 'Resist' blocks of the pre-stimulation period (i.e., blocks 1 and 2). A within-subject t-test revealed that urge-to-yawn estimates increased significantly when participants were instructed to resist yawning compared to when they allowed themselves to yawn (Pre-stimulation block means: Allow = 0.15 units (0-1), Resist = 0.18 units (0-1); $t(35) = -1.85$, $p < 0.04$). These data are consistent with the proposal that awareness of urges-for-action increase in circumstances where actions are suppressed or their execution is delayed [**10**].

Effects of motor excitability and physiological inhibition on propensity for contagious yawning

It has been proposed that the propensity for echophenomena such as CY may be linked to individual variability in cortical motor excitability [**1**]. To investigate this proposal directly we used a number of single- and paired-pulse TMS protocols to measure cortical excitability and physiological inhibition within the primary motor cortex of the left hemisphere (contralateral to the dominant right hand). The measurements obtained from each participant consisted of the following: resting motor threshold (RMT); TMS recruitment curve (sometimes referred to as the Input-Output or IO curve); intracortical facilitation (ICF); short-interval intracortical inhibition (SICI); and, long-interval intracortical inhibition (LICI). These measures have been used repeatedly to characterize motor excitability and physiological inhibition [**11**]. The reader is referred to the Methods section for methodological details.

To investigate directly whether individual differences in measures of cortical motor excitability and/or physiological inhibition predicted individual variability in the propensity for CY, we conducted separate stepwise regression analyses of the total number of yawns (i.e., full + stifled) observed from each participant in the Allow and Resist conditions. The analysis confirmed that the TMS measures were not a significant predictor of the total number of yawns recorded in the Resist condition (all $p < 0.1$). By contrast, the stepwise regression analysis demonstrated that a model based upon three factors: LICI, RMT, and SICI, could significantly predict and account for close to 50% of the individual variability in the number of full yawns recorded in the Allow condition ($F=10.71$, $p < 0.001$). The order of entry into the model for these factors was as follows: LICI (coefficient = 4.15; t-statistic = 3.89; $p = 0.0005$), $F=6.81$, $p = 0.014$, Rsq = 0.18, Adj-Rsq = 0.15; RMT (coefficient = -0.38; t-statistic = -4.33; $p = 0.0002$), $F=8.65$, $p = 0.001$, Rsq = 0.36, Adj-Rsq = 0.32; SICI (coefficient = -6.78; t-statistic = -3.14; $p = 0.004$, $F=10.71$, $p < 0.001$, Rsq = 0.52, Adj-Rsq = 0.47). It should be noted that in this step-wise regression the R-squared values for RMT and SICI are calculated on the residual variance remaining after the LICI and LICI+RMT fits respectively have been accounted for.

Figure 3 about here

LICI is a paired-pulse TMS protocol in which two supra-threshold TMS pulses are delivered through a single coil with an inter-stimulus interval (ISI) of 50-200ms (see Methods section). LICI typically leads to a reduction in the size of MEPs evoked from a standard TMS pulse and is typically reported as the ratio of the conditioned over an unconditioned test MEP amplitude. LICI is taken to reflect physiological inhibition and is thought to be mediated by GABA-B receptors [**12**]. The relationship in the current study between LICI and yawning is illustrated **Figure 3A**. Inspection of this figure clearly illustrates that increased physiological inhibition (i.e., conditioned/unconditioned MEP ratio trial values less than 1) are associated with a reduction in number of yawns observed.

RMT is the amount of stimulation required (expressed as a percentage of maximum stimulator output) to reliably generate a motor-evoked potential MEP of a predefined magnitude (typically 50-100 μV) from a target muscle at rest. RMT is thought to reflect the excitability of those corticospinal neurons with the lowest excitation threshold that project to the target muscle [**13**], and the TMS-induced excitability of cortical-cortical fibre axons [**12**]; RMT is known to be highly variable between, but not within, individuals [**14**]. The relationship in the current study between RMT and the residual variance in yawning (i.e., after variance due to 100ms LICI is accounted for) is illustrated **Figure 3B**. Inspection of this figure clearly illustrates that lower motor thresholds are associated with an increased number of yawns.

SICI is a paired-pulse TMS protocol in which two TMS pulses are delivered in rapid succession (1-5ms ISI) through a single coil. However, in SICI protocols a standard supra-threshold TMS pulse is preceded by the delivery of a sub-threshold conditioning pulse. SICI typically leads to a reduction in MEP amplitudes, and is thought to reflect the operation of GABA-A mediated inhibitory interneurons acting upon corticospinal neurons [**12**]. Thus, LICI and SICI are thought to reflect quite different mechanisms of physiological inhibition. In the current study, and in contrast to the findings for LICI, we observed that increased SICI was associated with an increase in the number of yawns observed (**Figure 3C**). This

finding is consistent with the key role that GABA-A mediated inhibition is thought to play in the control of movement-related brain oscillations. Specifically, movement-related beta oscillation de-synchronization, which is linked to the initiation of movements, has been shown previously to be facilitated by increased GABA-A mediated inhibition [**15**].

Effects of motor excitability and physiological inhibition: predicting the effects of instruction

The stepwise regression analyses revealed that none of the TMS measures were statistically significant predictors of the number of stifled yawns observed in the Resist block (all $p > 0.1$). To investigate this issue further we ran a further stepwise regression in which we estimated whether the pre-stimulation TMS measures (above) predicted the *difference* in the total number of yawns (i.e., full + stifled yawns) exhibited in the Resist versus Allow conditions. The analysis revealed a marginally significant effect for RMT ($F = 3.97$, $p < 0.055$, Adj-$R^2 = 0.08$). This indicates that those individuals with a more excitable motor cortex (i.e., lower RMT values) tended to exhibit larger negative differences in the number of yawns observed in the Resist – Allow subtraction.

Effects of motor excitability and physiological inhibition on the urge-to-yawn

We conducted a stepwise regression to determine whether any single pre-stimulation TMS measure (i.e., SICI, ICF, LICI, IO Slope, or RMT), or combination of TMS measurements, was a significant predictor of the urge-to-yawn. The answer to this was that they were not (all $p > 0.05$). This suggests that while motor cortical excitability is a significant predictor of the propensity for contagious yawning, it is not a significant driver of, or associated with, the urge-to-yawn. This finding is in fact consistent with previous accounts that have proposed that the urge-for-action may be associated primarily with upstream brain areas such as the anterior insular cortex and the cingulate motor area (e.g., [**10**]).

General Discussion

We investigated the neural basis for contagious yawning (CY) -- an example of echophenomena -- using non-invasive brain stimulation (TMS) techniques. CY can be triggered by seeing another individual yawn [**5**] but the propensity for CY, while stable

over time, is known to vary across individuals [**8**]. Here we provide evidence that the propensity for contagious yawning may be triggered automatically and is strongly linked to the cortical excitability of primary motor cortex. Specifically, TMS was used to quantify baseline cortical excitability and physiological inhibition within primary motor cortex and to predict behavioural measures of CY, and we tested the hypothesis that the propensity for contagious yawning (CY) was linked to the balance of cortical excitability and physiological inhibition within the primary motor cortex [**1**].

The key findings from the study can be summarised as follows. First, the instruction to resist yawning proved to be only partially successful. While it led to a significant decrease in the number full yawns observed; there was a significant increase in the number of stifled yawns recorded. Furthermore, when the number of full and stifled yawns were combined into a single measure then the difference between the Resist and Allow condition was not statistically significant. Nonetheless, urge-to-yawn estimates increased significantly when participants were instructed to resist yawning. This is consistent with the proposal that urges-for-action are chiefly associated with actions that cannot be realized immediately and must be held in check. Together these findings demonstrate that the instruction to resist yawning significantly increases the urge-to-yawn and alters how the yawn may be expressed (i.e., stifled yawns rather than full yawns), but it does **not** alter the individual's propensity for yawning.

Second, the propensity for contagious yawning was shown to be strongly predicted by individual variability in TMS measures of cortical motor excitability and physiological inhibition recorded from the hand area of the primary motor cortex.

We suggest that these findings may be particularly important in understanding further the association between motor excitability and the occurrence of echophenomena -- which is observed in a wide range of clinical conditions, e.g., epilepsy, dementia, autism and Tourette syndrome, that have been linked to increased cortical excitability and/or decreased physiological inhibition [**1**].

Author contributions

GMJ, SRJ, BJB, DR & SK conceived and designed the study. BJB, HS, CB, JT & SK conducted the study and collected the data. BJB, SK & SRJ conducted the data analyses. BJB, SK, GMJ & SRJ wrote the paper.

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Figure captions:

Figure 1: Design of the behavioural task

A*. Participants viewed video clips that showed another individual yawning and, in four separate blocks, were instructed to either resist or permit themselves to yawn. Participants were videoed throughout and their yawns or stifled yawns were counted. During the latter two blocks (3 & 4) excitatory non-invasive electrical brain stimulation (Anodal-tDCS or tRNS) was delivered continuously to the cortical SMA region (contrasted with sham stimulation). To ensure that participants paid attention to the videos they were required to answer a question (e.g., How many people in the videos were wearing glasses?) after each block.* ***B.*** *Illustrates the slider device used to continuously record each participant's self-estimate of their current urge-to-yawn (see text for details).* ***C****. Shows a representative example of one individual's self-estimated urge-to-yawn across the four separate blocks of the behavioural task.*

Figure 2: Effect of instruction

Illustrates the effect of instructing participants to either allow themselves to yawn or resist yawning on the mean number of full and stifled yawns observed. Error bars represent the standard error of the mean (SEM).

Figure 3: Results of stepwise regression analysis

A*. Scatter plot showing the association between 100ms LICI values (x axis) and the total number of yawns (stifled + full) recorded in the Allow condition (y axis). Note a ratio value of < 1 represents an inhibitory effect of the conditioning pulse (see text for details).* ***B****. Scatter plot showing the association between resting motor threshold (RMT) (x axis) and the residual (i.e., unexplained by 100ms LICI) variance in the total number of yawns recorded in the Allow condition (y axis). Note that increased excitability is indexed by a lower RMT value. The dotted red lines represent 95% confidence intervals for the regression.* ***C****. Scatter plot showing the association between 3ms SICI values (x axis) and the residual (i.e., unexplained by 100ms LICI + RMT) variance in the total number of yawns recorded in the Allow condition (y axis). Note a ratio value of < 1 represents an inhibitory effect of the conditioning pulse (see text for details).*

STAR Methods

Experimental Model and Subject Details

Participants: Thirty-six neurologically healthy young adults aged 18-26 years (mean: 20 ±1.56 years) participated in this study. Prior to the study all participants were screened for transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES) safety and informed consent was obtained. Ethical approval was obtained from the University of Nottingham school of psychology research ethics committee. Two subjects were subsequently excluded from TMS data analysis: one due to their not tolerating the TMS procedure for long enough to collect a full data series; and the other due to their SICI response being more than 3 SD from the group mean.

Method Details

Study design: The aim of this study was to examine whether propensity for contagious yawning could be predicted by neurophysiological measures obtained from M1 using TMS. TMS measures including RMT, IO curve, SICI, ICF, LICI were obtained. This was then followed by a contagious yawning behavioural paradigm. Participants watched two blocks of video recordings featuring individuals yawning. In each block, participants were asked to either freely yawn or resist yawning. The order of instructions was counterbalanced across participants. In each block two different yawning responses (full yawn and stifle yawns) and urge to yawn were measured. Please note that this study was conducted as part of a larger study. This larger study included four blocks of yawning video viewing and tES was applied continuously during blocks 3 and 4. However, the analysis of contagious yawning in blocks 3 and 4, or the effects of tES, are not included in the current paper and will be reported elsewhere.

TMS: A Magstim Bistim^2, with a 70mm figure of eight branding iron coil, was used to administer TMS to the left M1 in an area corresponding to the first dorsal interosseous (FDI) muscle of the right hand. The motor hotspot was defined as the coil location that elicited maximal MEP responses in FDI by positioning the TMS coil over each subjects left motor cortex (M1) at approximately 45°. The coil location was continuously tracked

throughout the study, via BrainSight™ version 2.0 (Rogue Research Inc. ©, 2016) with a template brain scan. EMG responses were recorded using BrainVision system (BrainProducts GmbH, Germany) at a sampling rate of 5000 Hz and band pass filtered (10-2000 Hz). Disposable Ag-AgCl surface electrodes (diameter 24mm) were placed onto the FDI muscle in a standard 'belly-tendon' configuration.

RMT and IO curves: Following localisation of the motor hotspot, resting motor threshold (RMT) was obtained. Each subjects RMT was determined as the minimum TMS intensity needed to elicit a FDI generated MEP of at least 150–200 μV in a minimum of 5 out of 10 trials. TMS intensities administered ranged from 100% - 150% of RMT and delivered in 10% increments resulting in 6 TMS intensities with an inter-trial interval (ITI) of 5s. There were a total of 90 trials, which were split into 15 trials per TMS intensity. Trials were administered in a randomised order across the total number of trials. The IO curve measurements were estimated for each individual by calculating the median MEP amplitudes for each of the TMS intensities (i.e., 100–150% of RMT). A linear fit was then applied to the resulting values. Median values were calculated as opposed to the mean in order to limit the effect of non-standard distribution of individual data.

Paired pulse TMS (SICI, LICI, & ICF): Paired pulse TMS (ppTMS) was performed at four inter-stimulus intervals (ISIs); 1 ms, 3 ms (SICI), 12 ms (ICF) and 100 ms (LICI). For 1 and 3 ms SICI the conditioning stimulus (CS) was set as 55% of RMT, ICF at 75%, and LICI at 100% of RMT. The CS was followed by TS at the intensity yielding 1 mV (SI 1mV) (20 trials per stimulus condition). There were also 60 unconditioned stimuli (total 140 trials). All conditions were delivered in a pseudo-randomised order with an ITI of 6s. Paired pulse TMS measures were reported at a ratio to unconditioned responses (i.e. conditioned MEP/unconditioned MEP).

Behavioural task procedure: Directly following TMS procedures the participants completed the contagious yawning behavioural task. Participants were instructed to watch a 20-minute (2 blocks) video of actors yawning. In each block, participants were asked to either 'freely yawn' or 'resist yawning'. The order of instruction was counterbalanced across individuals. In both blocks participants were asked to pay close attention to the screen and

answer fours questions relating to the actors they would see such as, 'how many actors were wearing glasses'. Answers provided were later used to confirm that they were paying attention to the video clips appropriately. Each question was asked after each block and prior to the next block.

The yawning stimuli video was produced in-house and comprised four 9-minute blocks of video clips (total 52 clips) with each clip ranging from 11-20 seconds in length. Each video clip featured either a female or male actor (aged 20-28 years) spontaneously yawning. Each block of videos was also collated into 12 randomised video sets, which were then counterbalanced across all participants. All videos were shown on an Apple Macintosh desktop (screen size 22 inch) via VLC media player software. Prior to the start of each of the video blocks subjects were instructed to either *'resist the urge to yawn'* or to *'yawn freely'*. In each block both stifle and full yawns were measured.

Video clips were played continuously throughout the 9 minutes duration with no interval between each clip. However, each block was separated by a 45 second interval. At the end of each block, participants had this 45-second interval to answer the question corresponding to that particular block. For the duration that the video' recording was playing each subjects face was recorded using Open Broadcaster Software.

Each participant's face was video-recorded using the computer's built-in camera and OBS studio. They were also instructed to record their subjective urge to yawn by continuously adjusting a custom-made slider throughout the duration of each block. The length of the slider mechanism was 195mm, which was scaled to give urge readings between 0 (left end-no urge) and 1 (right end-maximum urge). The slider reading was sampled at 32Hz using Matlab 2010b (Mathworks Inc., USA).

Yawn count procedure: Two naïve raters were chosen to watch the covert video recordings and count the number of full yawns (FY) and stifled yawns (SY) displayed by the subjects during each video block. The recordings were blinded in order to prevent the display of the block condition to the raters. The two raters were also required to follow a strict yawn count protocol in order to ensure consistency and reliability.

Quantification and Statistical Analysis

The number of full yawns (FY) and stifled yawns (SY) displayed by the participants during each video block were counted using an agreed yawn count protocol. Yawn counts were collated for each instruction (allow-yawning & resist-yawning) and condition (full & stifled yawns) to allow us to examine the relationship between the TMS physiological parameters and the participants' propensity for contagious yawning. In addition, the participants' subjective urge to yawn ratings for blocks 1 and 2 were also analysed. Statistical analyses included the following; two-way repeated-measures ANOVA of behavioural data with the factors Instruction condition (allow vs. resist yawning) and Yawn response (full vs. stifled yawns); within-subject t-tests; stepwise regression analysis; and a priori planned independent-group t-tests.

Data and Software Availability

Can be obtained from the corresponding author on request.

Supplemental Material

None