Gender differences in the neural network of facial mimicry of smiles – an rTMS study

(running title: Neural network of facial mimicry)

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Introduction

The human face is one of the most expressive channels of emotional and social communication. Accurate interpretation of clues to affective states and behavioral intentions displayed on the face are crucial abilities for smooth social interaction and successful goal pursuit in society. Indeed, impaired emotion recognition and reduced empathy are major factors leading to difficulties in social communication that characterize, for example, people with autism spectrum disorder (ASD). Tangible differences in the display and perception of emotional expressions also exist between healthy male and female individuals. It is therefore of scientific and societal interest to understand the processes and neural correlates that support emotion recognition. The present study investigated gender differences in the role of motor and somatosensory cortices in facial mimicry and emotion perception.

An influential theoretical account, which builds upon a long and prominent tradition in biology, philosophy, and psychology (Darwin, 1872; James, 1950; Lipps, 1903), suggests that emotional information is processed through somatovisceral and motoric re-experiencing (Barsalou, 2008; Iacoboni, 2009; Niedenthal, 2007). A component of this *embodied emotion theory* is the *facial feedback hypothesis*, according to which information from one's own facial expressions feeds back into the brain and triggers or colors emotional responses, and influences emotional judgments (Adelmann & Zajonc, 1989; Buck, 1980; Hatfield, Cacioppo, & Rapson, 1993; McIntosh, 1996; Strack, Martin, & Stepper, 1988). Support for this hypothesis comes

from research showing that voluntarily producing emotional facial expressions results in specific physiological activity patterns (Ekman, Levenson, & Friesen, 1983) and shapes corresponding subjective feelings. Actively facilitating or inhibiting smiling, by holding a pen either between the teeth or the lips, influences the appraisal of humorous stimuli (Soussignan, 2002; Strack et al., 1988). Similarly, recent clinical trials suggest that individuals suffering from depression may benefit from procedures leading to the paralysis of the Corrugator muscles (involved in frowning and sadness), possibly by impeding this specific facial feedback that may contribute to the build-up of negative emotions (Finzi & Rosenthal, 2014; Wollmer et al., 2012).

Another component of embodied emotion theory is the observation that people spontaneously engage in motor mimicry. The perception of a smile, for example, causes the observer to smile in return. The observer's own smile is hypothesized to facilitate the recognition of the observed expression through afferent feedback to the brain. Indeed, mimicry of happy faces increases the accuracy of judgments of smile authenticity (Korb, With, Niedenthal, Kaiser, & Grandjean, 2014; but see Hess & Blairy, 2001), and the blocking of facial mimicry reduces the speed and the accuracy of recognizing emotional facial expressions. For example, blocking facial mimicry slows the recognition of positive and negative facial expressions (Stel & van Knippenberg, 2008), impairs the distinction between true and false smiles (Maringer, Krumhuber, Fischer, & Niedenthal, 2011; Rychlowska et al., 2014), delays the perception of the offset of happy and sad facial expressions (Niedenthal, Brauer, Halberstadt, & Innes-Ker, 2001), and interferes with the recognition of happiness (Oberman, Winkielman, & Ramachandran, 2007). Furthermore, paralysis of the Corrugator muscle through injections of botulinum toxin decreases responses to angry faces in emotion centers of the brain such as the amygdala, and reduces the functional

coupling between the amygdala and brain stem regions implicated in autonomic emotional responses (Hennenlotter et al., 2009).

The hypothesis that facial mimicry occurs both spontaneously and unconsciously is supported by findings that mimicry can occur in the absence of conscious perception of the stimulus face (Dimberg, Thunberg, & Elmehed, 2000; Mathersul, McDonald, & Rushby, 2013), and that it is difficult to suppress voluntarily (Dimberg, Thunberg, & Grunedal, 2002; Korb, Grandjean, & Scherer, 2010). Facial mimicry may be crucial for the development of empathy, which requires the detection and the representation of another person's emotional state. Indeed, facial mimicry is increased in individuals high in self-reported trait empathy (Dimberg, Andréasson, & Thunberg, 2011; Sonnby-Borgstrom, 2002). However, emotion recognition can also occur without facial mimicry, for example in individuals with facial paralysis (Rives Bogart & Matsumoto, 2010), and the simulation of motor and somatosensory events linked to facial expressions can occur in the brain only, that is, in the absence of an overt peripheral response.

Which systems of the brain are responsible for the spontaneous production of facial mimicry, and which ones utilize the resulting facial feedback, or provide a visceral and somatic simulation, during the processing of facial expressions? To answer these questions we turn to the neuroscientific literature, where current models of social cognition are built upon the notion that motor, somatosensory, and emotional brain regions simulate other people's actions, sensations, and emotions, and by doing so contribute to their perception and interpretation (Iacoboni, 2009; Keysers, Kaas, & Gazzola, 2010). Studies using neuroscientific or neuropsychological methods largely suggest that perceiving another person performing a motor action, displaying a

facial expression, or being touched on their body, results in increased neural activity in the perceiver's motor, emotional, and somatosensory areas.

The mirror neuron system (MNS) provides a putative neural basis for facial mimicry. It includes the inferior frontal gyrus (IFG), the posterior parietal cortex, but also primary and secondary somatosensory cortices (S1 and S2), and the insula (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Gazzola & Keysers, 2009; Molenberghs, Cunnington, & Mattingley, 2012; Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010; Rizzolatti & Craighero, 2004; Rizzolatti, Fogassi, & Gallese, 2001). Brain imaging studies have found substantial overlap in the brain activity accompanying the production and observation of facial expressions (van der Gaag, Minderaa, & Keysers, 2007). However, only few studies have specifically investigated the neural correlates of spontaneous facial mimicry. Schilbach, Eickhoff, Mojzisch, and Vogeley (2008) reported increased brain activity, likely accompanying facial mimicry, in the face area of the left primary motor cortex (M1) and in the bilateral posterior cingulate gyrus. Likowski et al. (2012) reported significant correlations between the amplitude of facial mimicry and brain activity in various areas that belong, or are functionally connected, to the MNS, including the IFG, the SMA, the insula, the medial temporal gyrus (MTG) and the superior temporal sulcus (STS). Also of interest, the disruption of medial premotor cortices with event-related rTMS interferes with the recognition of facial expressions (Balconi & Bortolotti, 2013a, 2013b; Rochas et al., 2013), while activation of a more fronto-polar area (BA9) increases facial mimicry (Balconi & Canavesio, 2013).

If motor and premotor areas of the MNS (M1, IFG, medial premotor cortices) might constitute the “output” center of facial mimicry, somatosensory cortices (S1, S2) could be the targets of the “input” for ensuing facial feedback (be it real or

simulated). This assumption is based on several lines of empirical evidence. First, as reviewed above, these areas show increased activity during the perception of facial expressions (Molenberghs et al., 2012; van der Gaag et al., 2007). Second, as shown in a sample of 108 patients, brain lesions affecting the right somatosensory cortices are associated with performance deficits on tasks requiring the recognition of facial expressions of emotion (Adolphs, Damasio, Tranel, Cooper, & Damasio, 2000). Finally, inhibition of the right somatosensory cortex with transcranial magnetic stimulation (TMS) leads to slower responses and reduced accuracy in emotion-matching tasks (Pitcher, Garrido, Walsh, & Duchaine, 2008; Pourtois et al., 2004). Therefore, the somatosensory cortex has been suggested to simulate internally, or to be an efferent target of, tactile and proprioceptive facial feedback, which accompanies facial mimicry (Adolphs et al., 2000; Sims, Van Reekum, Johnstone, & Chakrabarti, 2012).

In summary, the neural circuitry underlying the simulation of facial expressions perceived in others (be this an overt mimicry leading to measurable changes in the facial EMG, or a “brain-only” internal simulation of motor output), and the processing of (real or simulated) somatosensory facial feedback, are not fully understood. Motor and premotor areas of the MNS (M1, IFG, medial premotor cortices) are likely substrates for the production of facial mimicry (the “output”). Somatosensory cortices (S1, S2), especially in the right hemisphere, are likely involved in the processing of facial feedback, and in the analysis of the result of facial mimicry (the “input”).

However, men and women differ in their behavioral, subjective, and neural responses to emotional stimuli. Thus, the putative neural network responsible for generating (or simulating) facial mimicry and processing facial feedback is likely to

differ by gender. Important gender differences exist in the production, perception, and regulation of facial expression. Women are more emotionally expressive and more empathic than men (Eisenberg & Lennon, 1983; Kring & Gordon, 1998); more accurate and/or efficient in processing facial expressions of emotion (Hall, 1978; Hall & Matsumoto, 2004; Hoffmann, Kessler, Eppel, Rukavina, & Traue, 2010); show more facial mimicry than men (Dimberg & Lundquist, 1990); and are more susceptible to emotional contagion, as revealed both in self-report and dyadic interaction (Doherty, Orimoto, Singelis, Hatfield, & Hebb, 1995). Gender differences have also been found in the effects of pacifier use during infancy, which arguably blocks facial mimicry, on facial mimicry recorded at age seven. Specifically, pacifier use is associated with reduced facial mimicry in boys, but not girls (Niedenthal et al., 2012). These findings suggest that men and women may utilize a different set of neural structures during the recognition and embodiment of emotional facial expressions, or at least that the degree to which elements of this network can be modulated through external influences varies by gender. The assumption that important differences may exist in the neural substrate of facial mimicry and emotion recognition seems particularly plausible if one considers that “sex influences on brain function are ubiquitous, found at every level of neuroscience from the behaving human to the ion channel” (Cahill, 2012, p. 2542). In line with this, meta-analyses of brain imaging studies have revealed that brain activation to emotional stimuli greatly differs by gender (Stevens & Hamann, 2012).

The present experiment investigated gender differences in the neural circuitry underlying the simulation/production of facial mimicry of smiles and the integration of facial feedback in the recognition process of facial expressions of happiness. In a within-subjects design, rTMS was delivered in separate sessions to inhibit the activity

of the Zygomaticus/cheek region of the right M1 or S1. Delivery of rTMS over the vertex (VTX, midline midpoint between inion and nasion) served as an active control condition. Participants then completed two tasks that involved rating the intensity of emotional facial expressions (Intensity task), and detecting the offset of a facial expression of emotion (Offset task). Both tasks were inspired by previous research reporting the occurrence of facial mimicry during the perception of movie clips of neutral-to-emotional morphs (Achaibou, Pourtois, Schwartz, & Vuilleumier, 2008), and changes in the time of detection of the offset of emotional expressions after blocking participants' facial mimicry (Niedenthal et al., 2001). Although both tasks included expressions of anger and happiness, analyses focused on trials with the latter emotion because fMRI and rTMS served to localize and subsequently target motor and somatosensory cortices innervating the cheek region (involved in smiling).

To measure facial mimicry, electromyography (EMG) was recorded over bilateral Corrugator and Zygomaticus muscles. To guarantee individual coil positioning using a frameless stereotaxic system, locations of the Zygomaticus area in M1 and of the cheek area in S1 were determined in a first session through fMRI, during which participants were asked to smile or experienced a light touch on their cheek.

Four main hypotheses were tested. We hypothesized a role of M1 in the production of mimicry of smiles, and a role of S1 in the perception of the resulting facial feedback. In other words, 1) rTMS over M1 was expected to reduce facial mimicry of smiles and to affect behavioral responses in both tasks, and 2) rTMS over S1 was expected to affect behavioral responses without reducing facial mimicry by impairing the processing and integration of facial feedback information. We expected 3) facial mimicry of smiles to be correlated with measures of mood (Moody,

McIntosh, Mann, & Weisser, 2007) and trait empathy (Dimberg et al., 2011; Sonnbly-Borgstrom, 2002). Finally, we expected 4) rTMS to have different effects on behavioral responses to and facial mimicry of smiles in male vs. female participants.

Methods

Participants

Thirty healthy participants (17 females, mean age = 22.7 years, SD = 3.4, range 19 to 31 years) were recruited through advertisements on campus at the University of Geneva. All participants reported to be right-handed and free of psychiatric disorders, and had normal or corrected-to-normal vision. They were screened to ensure they were free from neurological, psychiatric, and medical problems, as well as from contraindications for TMS and MRI. Participants gave informed consent and were paid for their participation.

Stimuli

In both the Offset and Intensity tasks stimuli consisted of short video clips showing gradual changes in emotional expressions displayed by adult faces. Stimuli were constructed in morphing software (Morpheus Photo Morpher, version 3.17) using happy, angry and neutral expressions by 14 adult faces (50% male). The same photos have been successfully used to create similar stimuli in previous studies (Halberstadt & Niedenthal, 2001; Niedenthal et al., 2001). Movie clips in the Offset task always showed a full-blown expression of happiness or anger that gradually morphed into the other expression. In the Intensity task, neutral facial expressions morphed into an expression of happiness or anger. The movie clips, which were shown at 60 frames per second, lasted five seconds in the Offset task and two seconds in the Intensity task.

Tasks

In the Offset task (Figure 1C), participants reported as quickly and accurately as possible the moment at which they perceived the offset of the initial emotional expression (e.g. anger) by pressing a button on a response box with their right index finger. Movie clips were shown for their entire length, regardless of participants' responses. Perceived offset time (reaction time, RT) was measured in milliseconds from the onset of the video clip. After each clip, if a response had been provided, RT was shown for one second on a black screen. Otherwise, the text "No response" was shown. After completion of two practice trials, 112 trials were shown in two blocks of 56 trials, with participants free to rest between blocks. The average duration of the Offset task was 20 min (SD = 1.7 min). This task was adapted from earlier studies showing that blocking of facial mimicry delays the perceived offset of an expression (Niedenthal et al., 2001).

In the Intensity task (Figure 1D), participants rated the intensity of perceived happiness and anger after each video clip by moving, with the computer mouse, a slider on a 100 point Likert scale, ranging from "very low intensity" on the far left to "very high intensity" on the far right. Separate sliders were used for happiness and anger. Each slider disappeared when the participant pressed the left mouse button. Rated intensity and response time (RT) was recorded based on slider onset. A total of 112 trials were presented in two blocks of 56 trials. The average duration of the Intensity task was 22.5 min (SD = 4.9). Two practice trials, containing faces not used in the main experiment, were shown at the beginning of the task. The Intensity task was inspired by research showing that facial mimicry can reliably be induced by dynamic expressions of happiness and anger (Achaibou et al., 2008).

----- Figure 1 about here -----

Procedure

A within-subjects design was used. The experiment was composed of four separate laboratory sessions, which were completed no fewer than five and no more than seven days apart (Figure 1A).

In the first session, participants completed two safety questionnaires to verify that MRI and TMS procedures were not contraindicated, signed informed consent, and completed structural and functional scanning in the MRI (see below). At the end of the first session, participants completed three personality and affect questionnaires: The Interpersonal Reactivity Index (IRI; Davis, 1983) and Empathy Quotient (EQ; Baron-Cohen & Wheelwright, 2004) were used to measure trait-like empathy, while the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988) measured participants' current mood. The PANAS was moreover filled out at the end of sessions two, three, and four, to control for participants' mood.

Sessions two to four were identical, except for the location of the application of TMS (M1, S1, VTX in semi-random order across participants), and in that the order of the Intensity and Offset tasks, as well as of the trials in these tasks, were different across participants and sessions (Figure 1B). At the beginning of sessions two to four participants received single pulse TMS over their right motor cortex to establish their motor threshold (MT) based on the EMG of their left thumb. The MT was defined as the minimal intensity that induced motor evoked potentials (MEPs) greater or equal to 50 μ V peak-to-peak amplitude in five out of ten trials. EMG for the MT measurement was recorded bipolarly on the left Abductor Pollicis Brevis muscle with electrodes connected to the Magstim EMG module. The stimulation intensity for the rTMS sequence (see below) was adjusted based on the measured MT. Next, facial EMG electrodes were attached (see below), and rTMS was applied. Immediately

following the rTMS sequence, participants completed the Intensity and Offset tasks, while EMG was recorded. After their last session participants were debriefed and paid.

Facial EMG recording

Facial electromyography (EMG) was bipolarly recorded from the left and right Corrugator Supercilii and Zygomaticus Major muscles, according to guidelines (Fridlund & Cacioppo, 1986). We used a Biosemi (www.biosemi.com) ActiveTwo amplifier system with Ag/AgCl active electrodes, a sampling rate of 2048 Hz and a bandwidth of DC-1600 Hz. The common mode sense and right-driven-leg electrodes (serving as ground and reference) were placed below the hairline on the center of the forehead.

rTMS stimulation

Magnetic stimulation was performed with a Rapid2 generator connected to a 70 mm figure-of-eight coil producing biphasic pulses (The Magstim Company Ltd, Whitland, Wales, UK). A modified continuous Theta burst stimulation procedure (mcTBS) was used, consisting of 200 bursts of three TMS pulses each delivered at 30 Hz, the bursts being delivered at 6 Hz. Thus, over a period of 33.3 seconds, a total of 600 pulses were administered at an intensity of 80% of the MT. This type of protocol has been employed before over M1 and the frontal eye fields, and has been shown to induce cortical inhibition lasting more than 30 min (Goldsworthy, Pitcher, & Ridding, 2012; Nyffeler et al., 2006). Precise positioning of the coil to target the right M1 or S1 cortices, or the Vertex as an active control, was achieved using a neuro-navigation system (see below) based on participant's structural MRI and functional localizer results.

Structural MRI and fMRI localizer

MRI images were acquired using a 3T whole body MRI scanner (Trio TIM, Siemens, Germany) with the product 12 channel head coil. Anatomical imaging was carried out using a T_1 weighted MPRAGE sequence ($TR/TI/TE = 1900/900/2.27$ ms, flip angle = 9° , PAT factor = 2, voxel dimensions: 1 mm isotropic, 256 x 256 x 192 voxel). A functional localizer experiment was then performed using a BOLD contrast optimized EPI sequence ($TR/TE = 2000/30$ ms, flip angle = 80° , PAT factor = 2, 64 x 64 pixel, 3.2 x 3.2 mm, 35 slices acquired in descending order, 3.2 mm slice thickness, 20% slice gap). One run comprising 250 volumes was acquired for each participant. Participants watched a back projection screen placed inside the scanner bore, in the center of which a cross or a circle was displayed. They were instructed to maintain attention to the center of the screen, to rest when a cross was displayed, to smile whenever the circle was present, and to avoid moving their head. Compliance with the motor task instruction was monitored with an MRI compatible movie clip camera (12M-i, MRC Systems GmbH, Germany). Tactile stimuli were applied to both lower cheeks of the participant with a custom built device comprising non-metallic pneumatic cylinders (TA-AC-PVC-1.0-EP, Teqcom Industries, Inc., USA) (Rieger, Domínguez-Borràs, & Vuilleumier, 2011). Tactile stimulation occurred only while the cross was shown on the screen, and so did not coincide with the motor task. The cylinders were controlled by electromagnetic valves, which were placed outside the MRI scanner room and connected to a programmable control unit. The cylinders were adjusted to be in permanent contact with the skin and traveled forward and back by five mm once per second when activated. Stimuli were timed according to a mini-block design, consisting of 53 rest periods of two to 12 seconds duration, interspersed with 26 somatosensory blocks of four or six seconds and 26 motor blocks of four or six seconds in pseudo-randomized order.

The MR data were analyzed using BrainVoyager QX (Brain Innovation, The Netherlands). The functional images were motion corrected and co-registered to the T1 image at the individual level (no spatial normalization was performed). Activation maps were calculated based on the stimulus timings convolved with a canonical HRF. The motion parameters were included as regressors of no interest.

Neuronavigation and coil placement

On each session of TMS, the coil was placed over the right M1 or S1, or over the vertex with the help of a frameless neuronavigation system based on the individual anatomical and functional MRI data. The system combines the neuronavigation module of BrainVoyagerQx software with the ultrasound CMS20 measuring system for navigation (Zebris GmbH, Tübingen, Germany). Target coordinates for TMS in the motor and somatosensory cortices were determined by locating in the functional MRI data the peak of the largest cluster of activation in the motor and somatosensory cortices, respectively. The vertex target area was defined as the midpoint betweeninion and nasion in the antero-posterior axis and on the midline with the help of the neuronavigation. The average Talairach (MNI) coordinates at which rTMS was applied were 52.2; -8; 39.8 (52.7, -10.3, 42.7) for the right M1, 55; -19.8; 39.6 (55.5, -22.4, 41.9) for the right S1 and 0; -31.9; 65.2 (0, -36.2, 69) for the Vertex (Figure 1B). On average, the target areas for M1 and S1 were thus 1.5 cm apart. The first phase of the biphasic currents flowing into the coil was oriented in an antero-medial direction for the stimulation of M1 or S1 – perpendicularly to the central sulcus. For stimulation of the Vertex, the currents were oriented forward.

Data analysis

The study was designed to investigate the role of M1 and S1 in the perception and mimicry of smiles. Therefore, data analyses focused on behavioral responses and EMG of the Zygomaticus muscle to Angry-To-Happy trials in the Offset task, and Happy trials in the Intensity task (see Supplementary Material for graphs including EMG of the Corrugator and responses to Happy-To-Angry trials). Statistical analyses were performed in SPSS (Version 20) and R (www.r-project.org). In cases of non-sphericity, corrected p values and uncorrected degrees of freedom are reported.

Questionnaires, TMS-Intensity, TMS-Time

Possible differences in mood across sessions and gender were investigated with a repeated measures analysis of variance (rmANOVA) with the between-subjects factor Gender and the within-subjects factors Valence (positive, negative mood) and TMS (M1, SI, VTX) and followed up with post hoc t-tests. Gender differences for IRI and EQ questionnaires were explored with independent-samples t-tests.

The intensity at which rTMS was applied was based on each participant's MT, and could vary between sessions of the same participant. In addition, the order of tasks was counterbalanced, and duration of experimental procedures (e.g. switching between tasks) and task durations could vary. Therefore, two rmANOVAs were computed with the between-subjects factor Gender, and the within-subjects factor TMS, to test if sessions differed in terms of TMS intensity (i.e. machine output) and TMS-Time (i.e. time in minutes passed between rTMS and the beginning of each task). Both factors were also included as covariates in analyses of covariance (ANCOVAs) of behavioral and EMG data.

Behavior

For the Offset task, trials without response ($<1\%$), or on which the perceived offset time was more than two SDs above or below the mean, were removed. On average, 4% of the trials were removed ($SD = 5.8$). Remaining Angry-To-Happy trials were analyzed in an rmANOVA with the factors TMS and Gender, and with t-tests based on hypotheses.

In the Intensity task data from one participant were lost during the VTX session due to technical problems, and the participant was excluded from analyses. The remaining 29 participants reported, on average, higher emotional intensity on the appropriate rating scale in 98.1% of the trials ($SD = 1.7$). The number of Happy trials with correct ratings (i.e. higher ratings of happiness than anger) was analyzed in a 3×2 rmANOVA with the factors TMS and Gender, as well as with planned contrasts. Trials were removed if they had incorrect ratings ($< 2\%$ of all trials across participants), or if their response time was more than two SDs over the mean of all participants ($< 7\%$). The remaining data included (across both emotions) an average of 51.9 trials ($SD = 7.4$) per condition and participant. Intensity and response times (RTs) of happiness ratings to Happy trials were analyzed with the same rmANOVA and planned contrasts as above.

In order to investigate the impact of potentially confounding factors, behavioral data from both tasks were also analyzed in ANCOVAs with the between-subjects factor Gender, the within-subjects factor TMS, and the covariates TMS-Time and TMS-Intensity.

Offset times were non-parametrically correlated (uncorrected) with the questionnaire scores to explore the effects of mood (PANAS) and trait empathy (IRI, EQ) on emotion perception (Table 1). Similarly, two-tailed non-parametric Spearman

correlations were computed between the ratings on the Intensity task and the questionnaire scores (Table 3).

EMG data

Offline, EMG data were preprocessed in Matlab (version R2012a; www.mathworks.com) partially using the EEGLAB toolbox (Delorme & Makeig, 2004). Data were submitted to bipolar montage, bandpass-filtered from 20 to 400 Hz, and segmented from one second before to five seconds (Offset task) or two seconds (Intensity task) after stimulus onset (SO). Data were then rectified and smoothed with a 40Hz low-pass filter. For each participant, we excluded trials based on behavioral analyses (see above), and trials in which the average amplitude in the baseline period (-1 sec to SO) of either muscle exceeded by more than two SDs the average amplitude over all trials' baselines for the respective muscle. For the Offset task (across all three sessions, which on total included 336 trials), an average of 23.1% of trials were excluded per participant (number of excluded trials $M = 77.5$, $SD = 17.2$). For the Intensity task, an average of 19.3% trials were excluded per participant (number of excluded trials $M = 64.8$, $SD = 10.9$). After this artifact rejection procedure, data from SO onwards were expressed as percentage of the average of the one-second long baseline (for a similar procedure see de Wied, van Boxtel, Zaalberg, Goudena, & Matthys, 2006; Korb et al., 2014). For statistics, valid EMG data were transformed to the natural logarithm and averaged over both sides of the face and over two time windows, which lasted one second for the Intensity task, and 2.5 sec for the Offset task.

To investigate the modulation of the amplitude of facial mimicry by TMS, analyses focused on Zygomaticus EMG data during the second time-window of Angry-to-Happy and Happy trials, respectively in the Offset and Intensity tasks. We

chose the Zygomaticus because inhibitory rTMS was applied over motor (M1) and somatosensory (S1) areas innervating the cheek (which comprises the Zygomaticus but not the Corrugator muscle). The second time window was chosen since it contained the apex (Intensity task) and the rise to apex (Offset task) of the happiness expressions, and therefore was most likely to reveal facial mimicry of smiles¹. These data were analyzed with rmANOVAs including the factors TMS and Gender, with hypothesis-driven t-tests and, to check for possible confounds, in mixed ANCOVAs with the factors Gender and TMS and the covariates TMS-Intensity and TMS-Time.

To test whether participants' facial mimicry was linked to their positive and negative mood (assessed after each session via the PANAS), or to their empathy traits (assessed once with the IRI and EQ), we calculated non-parametric Spearman correlations between these questionnaires and facial mimicry (defined as above).

Results

Questionnaires

An rmANOVA on the PANAS data with the factors TMS, Valence and Gender revealed a significant main effect of Valence ($F(1,28) = 261.4, p < .001, \eta_p^2 = .9$), due to higher scores on the positive than the negative mood subscale, a significant TMS X Gender interaction ($F(2,56) = 4.2, p = .023, \eta_p^2 = .13$), and a trend for Valence X Gender interaction ($F(1,28) = 3.9, p = .06, \eta_p^2 = .12$). No other effects reached significance or trend level (other F s $< 2.2, p$ s $> .14$). Post hoc t tests (uncorrected) revealed significantly higher negative mood in male (means 23.7 and 21.8) compared to female participants (means 13.2 and 14.5) in the M1 ($t(28)=2.4, p$

¹ This was confirmed through analyses including both muscles and time-windows, which are not shown due to space limits, but can be provided upon request. See also graphs in Supplementary Material.

= .02) and S1 condition ($t(28)=2.5$, $p = .02$), and in the VTX ($M=15.2$) compared to the M1 condition ($M=13.2$) in female participants ($t(16)=2.7$, $p = .02$).

The average score on the EQ was 37.6 ($SD = 7.8$, range 24 - 62). There was no difference ($t(28) = -.21$, $p = .84$) between men ($M = 37.3$, $SD = 5.6$) and women ($M = 37.9$, $SD = 9.3$). The average scores on the IRI subscales were 13.3 ($SD = 4.8$) for Perspective taking, 15.5 ($SD = 4.6$) for Fantasy, 7.7 ($SD = 3.6$) for Empathic Concern, and 8.9 ($SD = 4.6$) for Personal Distress. No differences between men and women were observed (all $t < .8$, all $p > .4$).

TMS-Intensity and TMS-Time

Mean TMS-Intensity for conditions M1, S1, and VTX were, respectively, 46.2, 45.2, 45.6 (SDs were 7.6, 8.1, 8.4) percent of the maximal TMS stimulator output. An rmANOVA with the factors Gender and TMS revealed a significant interaction ($F(2,56) = 3.34$, $p < .04$, $\eta_p^2 = .11$), which was followed up with independent-samples t-tests to compare gender groups across conditions, and with paired-samples t-tests to compare conditions amongst gender groups. In females, TMS-Intensity in the M1 condition was found ($t(16) = 2.3$, $p = .04$) to be significantly lower ($M = 44.8$, $SD = 7.6$) than in the VTX condition ($M = 45.4$, $SD = 8.5$). No other comparison reached significance or trend level (all $t < 1.77$, all $p > .1$).

An rmANOVA with factors Gender and TMS on the variable TMS-Time in the Offset task showed a trend for an effect of Gender ($F(1,28) = 3.53$, $p = .07$, $\eta_p^2 = .11$). On average, men started the Offset task 15.35 minutes after rTMS, while women started it after 20.34 minutes. The same AVOVA also resulted in a trend for an effect of Gender in the Intensity task ($F(1,28) = 2.94$, $p = .09$, $\eta_p^2 = .09$), with slightly earlier task onsets in women ($M = 15.4$ min) compared to men ($M = 20.23$ min).

Offset task

Behavior

RTs were analyzed in an rmANOVA with the factors TMS and Gender, resulting in a significant main effect of Gender ($F(1,28) = 8.4, p = .007, \eta_p^2 = .23$), due to later perceived offset in male ($M = 2610$ ms) compared to female participants ($M = 2143$ ms), and a strong trend for a TMS X Gender interaction ($F(2,56) = 2.9, p = .06, \eta_p^2 = .1$). Planned contrasts revealed that females perceived (Figure 2) offsets in Angry-To-Happy trials significantly ($t(16) = 2.2, p = .03$) later in the M1 condition ($M = 2241.1$ ms, $SD = 468$) compared to the VTX condition ($M = 2075.4$ ms, $SD = 487.7$), and to the S1 ($M = 2113.2$ ms, $SD = 499.9$) condition ($t(16) = 2.3, p = .02$). RTs for VTX and S1 did not differ significantly ($t(16) = .5, p = .6$). The same comparisons in male participants were not significant (all $t < 1.5$, all $p > .17$).

An ANCOVA with the factors Gender and TMS and the covariates TMS-Time and TMS-Intensity revealed a significant main effect of Gender ($F(1,26) = 5.97, p = .02, \eta_p^2 = .19$), such that males perceived anger offsets later ($M = 2586.7$ ms, $SD = 460.4$) than did female participants ($M = 2161.2$ ms, $SD = 456.6.7$). There was also a significant interaction of TMS X Gender ($F(2,52) = 3.97, p = .03, \eta_p^2 = .13$), characterized by increasing RTs from VTX over S1 to M1 in females, but no substantial differences in RTs between conditions in male participants. Finally, there was a trend for a TMS X TMS-Time interaction ($F(2,52) = 3.1, p = .055, \eta_p^2 = .11$). In order to further investigate the role played by TMS-Time, the RTs of each TMS condition were regressed onto their respective TMS-Time, and residuals were entered into an rmANOVA with the factors Gender and TMS. As for the ANCOVA, a significant main effect of Gender ($F(1,28) = 14.7, p = .001, \eta_p^2 = .34$) and a

significant Gender X TMS interaction ($F(2,56) = 3.52, p = .04, \eta_p^2 = .11$) were found. It can therefore be concluded that the different effect of TMS conditions in male and female participants cannot be explained by a difference in time delay between application of rTMS and beginning of the Offset task.

The influence of the personality and mood questionnaires (see Table 1) was explored with Spearman correlations. The perspective taking subscale of the IRI was significantly negatively correlated with perceived offset times in the S1 condition for all participants, and in the M1 and S1 conditions in female participants only. The empathic concern subscale of the IRI was significantly negatively correlated with perceived offset times in the S1 condition over all participants, and in males and females separately. Trends for negative correlations with empathic concern were found in the M1 condition across all participants and in females only. In female participants negative correlations with the EQ were significant in the M1 condition, and at trend level in the S1 condition.

In summary, females showed delayed detection of expression offset in Angry-To-Happy trials after TMS over M1 compared to VTX and S1. Response times for S1 went into the same direction as for M1, but did not differ significantly from VTX. No modulation of perceived offset by TMS was found in males. The perspective taking and empathic concern subscales of the IRI, and the scores of the EQ showed in females, and partially also in male participants, negative correlations with perceived offset times in the M1 and S1 conditions.

----- Table 1 about here -----

----- Figure 2 about here -----

EMG

EMG data of the Zygomaticus were analyzed in an rmANOVA with the factors TMS and Gender. This resulted in a trend for a significant interaction ($F(2,56) = 3.2, p = .07, \eta_p^2 = .1$) and non significant main effects of TMS or Gender (both $F < .5$, both $p > .5$). Based on hypotheses, effects of TMS on mimicry were also investigated with paired-samples t-tests. For female participants, we found significantly reduced ($t(16) = -2.9, p = .011$) mimicry of smiles after TMS over M1 ($M = 4.59, SD = .34$) compared to VTX ($M = 4.65, SD = .51$), and after TMS over S1 ($M = 4.62, SD = .36$) compared to VTX ($t(16) = -2.7, p = .014$). Conditions M1 and S1 did not differ from each other ($t(16) = -1.38, p = .18, ns$). The same t-tests were not significant in male participants (all $t < .8$, all $p > .4$). The ANCOVA did not reveal significant main or interaction effects, suggesting neither TMS-Intensity nor TMS-Time played a role in the modulation of mimicry by rTMS. A significant correlation (see Table 2) was found in the VTX condition between mimicry of smiles and negative affect ($\rho = .39, p = .03$).

----- Figure 3 about here -----

In summary, reduced facial mimicry of smiles was found in females after both rTMS over M1 and S1 in comparison to the VTX control condition. Male participants, on the other hand, showed no modulation of facial mimicry by rTMS. As tested with an ANCOVA, TMS-Intensity and TMS-Time played no significant roles in these results.

----- Table 2 about here -----

Intensity task

Behavior

Three separate ANOVAs with the factors TMS and Gender were carried out on the percentages of correct rating, the intensity ratings, and RTs of ratings. No main or interaction effect reached significance (all $F < 1.8$, all $p > .18$). Planned comparisons of the effects of TMS were all not significant (all $t < 1.4$, all $p > .19$). The ANCOVA on percentage of correct ratings revealed a main effect of TMS-Time ($F(1,25) = 6.5$, $p = .02$, $\eta_p^2 = .21$), and a trend for an effect of Gender ($F(1,25) = 2.9$, $p = .09$, $\eta_p^2 = .11$), but no interactions. Regressing out TMS-Time from each condition's percentages of correct ratings and running an rmANOVA with the factors Gender and TMS did not lead to any significant results (all $F < 1.4$, all $p > .25$). The ANCOVAs on perceived intensity and on RTs did not reveal any trends or significant effects (all $F < 2.3$, all $p > .14$).

A significant positive correlation was found across all participants between percentages of correct ratings and RTs in Happy trials of the VTX condition ($\rho = .69$, $p < .001$), but not of the M1 or S1 condition (both $\rho < .16$, $p > .42$). The same pattern of results was found in male and female participants separately.

Negative affect and intensity ratings were negatively correlated in the VTX condition for females, and positively correlated in the S1 condition for males (see Table 3). Empathic concern was significantly positively correlated with ratings of happiness in the M1 and S1 conditions in female participants, and at trend level across all participants. A trend for a correlation between personal distress and happiness ratings was also found for female participants in the VTX condition. Empathy measured with the EQ showed a significant correlation with happiness ratings in the M1 and S1 conditions for females, and at trend level across all participants.

In summary, no effect of rTMS on perceived emotional intensity was found in the Intensity task. Correlations of behavior and questionnaires went in the expected direction for female participants, with greater empathy and lower negative affect being associated with greater perceived happiness in happy faces, during rTMS-induced disruption of motor and somatosensory areas. An unexpected positive correlation in males was found between ratings of happiness and negative affect.

----- Table 3 about here -----

EMG

An rmANOVA with the factors TMS and Gender did not reveal any significant or trend-level effects (all $F < 1.9$, all $p > .17$). Similarly, using planned contrasts, no significant results were found in female participants (all $t < 1.2$, all $p > .28$) nor in male participants (all $t < 1.3$, all $p > .22$). The ANCOVA did not reveal any significant effects (all $F < 2.5$, all $p > .12$).

In female participants facial mimicry of smiles was significantly correlated (see Table 4) with the EQ in the M1 condition ($\rho = .57$, $p = .02$), and with perspective taking in the S1 condition ($\rho = .51$, $p = .04$). A trend for a correlation between mimicry and perspective taking was also found in the VTX condition across all participants.

----- Table 4 about here -----

In summary, the amplitude of mimicry in the Intensity task was not modulated by rTMS over M1 or S1 in either gender group. In line with hypotheses, significant

correlations were found in female participants between mimicry of smiles and empathy scales (EQ and perspective taking of the IRI).

Discussion

The aim of this experiment was to investigate the neural correlates of facial mimicry of smiles in males and females by inhibiting with rTMS, over three separate sessions, the right primary motor cortex (M1), the right somatosensory cortex (S1), or – in a control condition – the vertex (VTX). Over two tasks, facial mimicry of smiles was measured bilaterally via facial EMG. Gender differences were found for the effects of rTMS on the intensity of facial mimicry of smiles and on the perception of changes between angry and happy facial expressions. In female participants, as expected, inhibition of M1 reduced the amount of smile mimicry and delayed the perception of smiles in dynamically unfolding Angry-To-Happy morphs. Inhibition of S1, similarly to M1, significantly reduced mimicry of smiles and delayed smile recognition (the latter effect did not reach statistical significance). No effects of rTMS on mimicry or behavior were found in male participants. In the following, these results, as well as the study's limitations, will be discussed in more detail.

We tested the role of M1 in the production of facial mimicry of smiles by functionally locating with fMRI, and then inhibiting with rTMS, the specific area of the right M1 that innervates the Zygomaticus muscle and which is active during smiling. Reduced facial mimicry of happy expressions, defined as weaker Zygomaticus activation, was expected to occur in the M1 compared to the control condition, in which rTMS was applied over the vertex (VTX). In line with the hypothesis of gender differences in the neural network of facial mimicry, female but

not male participants' mimicry of smiles was significantly reduced in the Offset task after rTMS over M1, compared to VTX (Figure 3).

Since facial mimicry can influence the processing of and judgments about emotional facial expressions (Korb et al., 2014; Niedenthal et al., 2001; Rychlowska et al., 2014), inhibition of M1 was also expected to be associated with lower ratings of perceived happiness in happy faces of the Intensity task, and delayed perception of happiness in Angry-To-Happy videos in the Offset task. This hypothesis was partially confirmed, and results differed by gender. Only in female participants was the perception of change from angry to happy expressions delayed in the M1 compared to the VTX condition (Figure 2). No effects were found for either gender group in the Intensity task.

The reduction of smile mimicry and the delay in the perception of smiles, found after inhibition of the primary motor cortex in female participants, implicate M1 in the generation of spontaneous mimicry to happy expressions, strengthen the hypothesis that facial mimicry plays a role in the detection of subtle changes in facial expressions (Niedenthal et al., 2001), and suggest differences in the neural circuitry underlying facial mimicry in men and women.

The right somatosensory cortex (S1) was targeted with rTMS in order to reduce the processing of the "input" of facial mimicry, that is, the facial feedback, and/or to suppress the internal simulation of somato-visceral states typically associated with emotional expressions. We expected rTMS over S1 to affect participants' behavioral responses (which should rely on the processing of the incoming facial feedback, or on its internal simulation or representation), but to leave facial mimicry intact. For females, inhibition of S1 resulted in a significant reduction

of mimicry of smiles (Figure 3), and a non-significant delay in the perception of smile onsets. No modulation of either behavior or facial mimicry was observed for males.

The reduction of facial mimicry in the S1 condition seems intriguing at first. If S1 were only responsible for processing facial feedback, it should not be involved in the production or simulation of motor output. These findings might be explained by the fact that while S1 is not a motor output area, it receives expected sensory representations before and during movement execution (Gazzola & Keysers, 2009). These predicted sensory feedbacks play a role in the planning and carrying out of the action. Therefore, rTMS may have altered actual or expected somatosensory feedback in S1, which in turn may have reduced facial mimicry. Cortico-subcortical loops between S1 and the motor territories of the basal ganglia may also have impacted on the mimicry level.

In summary, a significant reduction of smile mimicry was found after inhibition of S1 in female participants. This result suggests that S1, as expected, plays a role in the mimicry of facial expressions. However, rather than solely being a site in which facial feedback is processed, or a somatosensory simulation is produced, S1 may interact with M1 in the production of spontaneous facial mimicry through direct cortical connections between S1 and M1 and through subcortical loops involving the basal ganglia.

The gender differences in the effects of inhibitory rTMS on M1 and S1 are unlikely to be due to differences in mood, empathy, specific TMS-Intensity (based on the individually established MT), or TMS-Time (the time delay between rTMS administration and task presentation mainly due to task counterbalancing). First, negative mood was significantly higher in males compared to females in some conditions, yet males showed a positive or no correlation with mimicry and

behavioral responses to smiles (see Table 2 and 3). Second, no gender differences were found in trait empathy, measured with the IRI and EQ questionnaires (Baron-Cohen & Wheelwright, 2004; Davis, 1983). Third, TMS-Intensity was actually lower in the M1 compared to the VTX condition in female participants, which, if anything, should have led to less cortical inhibition of M1. When included as a covariate, TMS-Intensity did not result in main or interaction effects. Fourth, non-significant trends were found for TMS-Time in men compared to women in the Offset task, and for women compared to men in the Intensity task. A trend for a TMS X TMS-Time interaction also emerged in the ANCOVA on perceived offset times, but regressing out TMS-Time did not change the main finding of a TMS X Gender interaction.

Consistent with reports of greater facial mimicry in empathic participants (Dimberg et al., 2011; Sonnyby-Borgstrom, 2002), females showed correlations between facial mimicry, perspective taking and EQ scores (Table 4). Perceived smile onsets correlated, mostly for females in the M1 and S1 conditions, with greater perspective taking, empathic accuracy, and overall empathy as measured by the EQ (Table 1). In the Intensity task (Table 3), ratings of happiness were positively correlated with empathic concern and EQ scores in the M1 and S1 conditions.

Summarizing across both tasks, behavioral measures correlated in the expected direction with empathy, especially the perspective-taking and empathic concern subscales of the IRI, and the EQ. These correlations suggest, consistent with Niedenthal et al. (2001), that participants reporting high levels of empathy perceived the changes between anger and happiness expressions more rapidly. It is interesting to note that correlations with empathy-measuring personality scales occurred exclusively in the M1 and S1 conditions. This suggests that the “advantage” that these personality traits provide for recognizing facial expressions only really comes into play when

parts of the neural infrastructure relevant for these processes are inhibited. The sparseness of the mood by behavior correlations (in females, smile mimicry in the VTX condition was inversely related to negative mood; mimicry in male participants was positively correlated with negative mood in the M1 condition) suggests that one should interpret them with caution.

The absence or weak pattern of correlations between empathy and facial mimicry (Tables 2 and 4) stands in contrast to a stronger pattern of correlations between empathy and behavioral measures of expression perception (Tables 1 and 3). This suggests that the self-report measures of empathy and mood used in the present study may be more in concordance with voluntary motor responses (button presses to indicate a consciously perceived change in emotional expression) than with spontaneous (and arguably unconscious) facial mimicry.

Conclusion

Using rTMS to induce targeted cortical inhibition, M1 and S1 were shown to play a role in the production of smile mimicry, and (in the case of M1) in the perception of angry-to-happy changes in facial expressions. Importantly, these results occurred only in female participants, pointing to potential disparities between genders in the neural circuitry underlying the perception and mimicry of facial expressions.

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Figure captions

Figure 1:

A) Description of the experimental design; B) average locations of M1, S1, and VTX where rTMS was applied (color indicates pre- and post-central gyrus); C) example of an Angry-To-Happy trial in the Offset task; D) example of a Happy trial in the Intensity task.

Figure 2:

Perceived offset times across Gender and TMS conditions. Error bars indicate SE. Female participants showed a significantly delayed perception of offset times after rTMS over M1 and S1, compared to VTX. No effect of rTMS on perceived offset times was found in male participants.

Figure 3:

EMG (log-transformed) of the Zygomaticus during the Offset task in response to Anger-To-Happy trials during the second time-window of analyses (2.5 sec to 5 sec after stimulus onset). Error bars indicate SE. Women showed reduced mimicry of smiles after TMS over M1 and S1 compared to VTX. Men showed no modulation of mimicry by rTMS.

Table 1

Table 1
Offset task – Spearman’s *rho* (and *p*) values between perceived offset times in Angry-To-Happy trials and questionnaires

		PA	NA	IRI-fan	IRI-per	IRI-emp	IRI-dis	EQ
Females (n=17)	M1	-.38 (.13)	.11 (.69)	-.16 (.54)	-.71 (.001)**	-.43 (.08)#	.04 (.89)	-.50 (.04)*
	S1	-.39 (.11)	.04 (.88)	-.16 (.58)	-.64 (.005)**	-.51 (.04)*	.16 (.53)	-.42 (.09)#
	VTX	-.19 (.45)	.22 (.39)	-.15 (.58)	-.15 (.57)	-.01 (.96)	.09 (.72)	-.09 (.74)
Males (n=13)	M1	-.36 (.23)	-.36 (.22)	-.06 (.85)	.14 (.64)	.17 (.58)	-.14 (.64)	.06 (.84)
	S1	-.21 (.48)	-.29 (.34)	-.18 (.55)	-.29 (.33)	-.57 (.04)*	-.14 (.65)	-.42 (.15)
	VTX	.07 (.83)	-.28 (.24)	-.13 (.68)	-.07 (.81)	-.17 (.57)	-.27 (.34)	-.29 (.32)
All (n=30)	M1	-.32 (.08)#	-.07 (.70)	-.16 (.39)	-.25 (.17)	-.35 (.06)#	-.07 (.72)	-.24 (.21)
	S1	-.19 (.32)	.10 (.59)	-.21 (.27)	-.44 (.01)*	-.42 (.02)*	.05 (.79)	-.33 (.07)
	VTX	-.19 (.32)	.06 (.77)	-.19 (.32)	-.20 (.28)	-.15 (.41)	-.03 (.88)	-.15 (.43)

Note: For the PANAS, which participants filled out at every session, the positive (PA) and negative (NA) affect scales of the corresponding session were used. IRI-fan = Fantasy subscale of the IRI; IRI-per = Perspective Taking subscale; IRI-emp = Empathic Concern subscale; IRI-dis = Personal Distress subscale; EQ = Empathy Quotient. ** $p < .01$; * $p < .05$; # $p < .1$ (trend)

Table 2

Offset task – Spearman's ρ (and p) values for mimicry of smiles and questionnaires

		PA	NA	IRI-fan	IRI-per	IRI-emp	IRI-dis	EQ
Females (n=17)	M1	.24 (.35)	.08 (.77)	.12 (.64)	.18 (.49)	-.12 (.64)	-.03 (.89)	.21 (.42)
	S1	.01 (.97)	.03 (.90)	.09 (.72)	.22 (.39)	-.21 (.42)	-.18 (.50)	-.09 (.72)
	VTX	-.29 (.26)	.41 (.11)	.23 (.37)	-.05 (.85)	-.27 (.29)	-.13 (.62)	-.03 (.91)
Males (n=13)	M1	-.01 (.96)	.12 (.7)	.32 (.28)	-.35 (.25)	-.09 (.75)	.21 (.49)	-.52 (.07)#
	S1	-.19 (.53)	-.12 (.7)	.43 (.14)	-.37 (.21)	-.37 (.21)	.01 (.98)	-.43 (.14)
	VTX	-.33 (.27)	.38 (.19)	-.02 (.93)	-.56 (.047)	-.45 (.12)	.14 (.65)	-.59 (.03)
All (n=30)	M1	.2 (.28)	.14 (.47)	.18 (.34)	-.05 (.79)	-.09 (.63)	.07 (.71)	-.06 (.75)
	S1	-.03 (.87)	-.04 (.82)	.24 (.21)	-.10 (.59)	-.23 (.22)	-.07 (.69)	-.19 (.32)
	VTX	-.3 (.11)	.39 (.03)*	.18 (.35)	-.23 (.21)	-.31 (.09)#	.02 (.89)	-.24 (.2)

Note: EMG of the Zygomaticus to Angry-To-Happy trials at time 2, averaged over the right and left sides, was used. PA and NA = Positive and Negative Affect subscales of the PANAS; IRI-fan = Fantasy subscale of the IRI; IRI-per = Perspective Taking subscale; IRI-emp = Empathic Concern subscale; IRI-dis = Personal Distress subscale; EQ = Empathy Quotient. ** $p < .01$; * $p < .05$; # $p < .1$ (trend)

Table 3

Table 3
Intensity task – Spearman’s *rho* (and *p*) values between ratings of Happy trials and questionnaires

		PA	NA	IRI-fan	IRI-per	IRI-emp	IRI-dis	EQ
Females (n=17)	M1	.01 (.96)	-.39 (.14)	-.01 (.98)	.21 (.42)	.69 (.003)**	.32 (.22)	.56 (.02)*
	S1	.01 (.99)	-.32 (.23)	.07 (.79)	.23 (.39)	.58 (.02)*	.37 (.15)	.57 (.02)*
	VTX	-.08 (.78)	-.63 (.01)*	.10 (.71)	.07 (.79)	.40 (.12)	.48 (.06)#	.36 (.17)
Males (n=13)	M1	.29 (.33)	.32 (.29)	.14 (.64)	.15 (.63)	-.07 (.82)	.16 (.60)	-.06 (.85)
	S1	.17 (.58)	.62 (.02)*	.12 (.70)	.11 (.72)	.01 (.96)	.29 (.33)	-.02 (.96)
	VTX	.46 (.11)	-.01 (.97)	-.27 (.38)	.25 (.40)	-.07 (.81)	-.23 (.46)	.09 (.77)
All (n=30)	M1	.14 (.46)	.09 (.65)	-.07 (.72)	.13 (.50)	.36 (.05)#	.24 (.21)	.35 (.06)#
	S1	.08 (.67)	.17 (.38)	-.03 (.88)	.10 (.61)	.31 (.09)#	.32 (.09)	.36 (.05)#
	VTX	.21 (.28)	-.23 (.24)	-.14 (.48)	.08 (.68)	.13 (.50)	.09 (.65)	.24 (.21)

Note: For the PANAS, the Positive (PA) and Negative (NA) affect scales of the corresponding session were used.
IRI-fan = Fantasy subscale of the IRI; IRI-per = Perspective Taking subscale; IRI-emp = Empathic Concern subscale; IRI-dis = Personal Distress subscale; EQ = Empathy Quotient. ** $p < .01$; * $p < .05$; # $p < .1$ (trend)

Table 4

Intensity task – Spearman's *rho* (and *p*) values for mimicry of smiles and questionnaires

		PA	NA	IRI-fan	IRI-per	IRI-emp	IRI-dis	EQ
Females (n=17)	M1	.09 (.75)	-.24 (.36)	.08 (.78)	.28 (.29)	.40 (.12)	.26 (.33)	.57 (.02)*
	S1	.03 (.90)	.17 (.54)	.03 (.92)	.51 (.04)*	.22 (.40)	-.03 (.92)	.39 (.13)
	VTX	.28 (.29)	.32 (.23)	.36 (.17)	.32 (.23)	-.17 (.52)	-.16 (.54)	.13 (.64)
Males (n=13)	M1	-.24 (.43)	-.26 (.40)	.18 (.55)	-.14 (.66)	-.08 (.79)	.04 (.89)	-.29 (.34)
	S1	-.30 (.31)	.07 (.82)	.12 (.70)	.03 (.93)	-.08 (.80)	.24 (.42)	.05 (.86)
	VTX	-.15 (.63)	.12 (.70)	.03 (.91)	.35 (.25)	-.46 (.11)	.17 (.57)	.20 (.50)
All (n=30)	M1	-.1 (.60)	-.3 (.11)	.22 (.25)	.12 (.53)	.15 (.43)	.20 (.30)	.19 (.31)
	S1	-.15 (.43)	.07 (.70)	.11 (.58)	.28 (.14)	.16 (.4)	.19 (.32)	.27 (.16)
	VTX	.09 (.65)	.07 (.73)	.17 (.37)	.34 (.07)#	-.3 (.11)	-.01 (.97)	.13 (.49)

Note: EMG of the Zygomaticus to Happy stimuli, at time 2, averaged over the right and left sides, was used. PA and NA = Positive and Negative Affect subscales of the PANAS; IRI-fan = Fantasy subscale of the IRI; IRI-per = Perspective Taking subscale; IRI-emp = Empathic Concern subscale; IRI-dis = Personal Distress subscale; EQ = Empathy Quotient. ** $p < .01$; * $p < .05$; # $p < .1$ (trend)

Figure 1

Figure 2

Figure 3