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An examination of acute changes in serotonergic neurotransmission using the loudness dependence measure of auditory cortex evoked activity: effects of citalopram, escitalopram and sertraline

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Objective The underlying effect of serotonergic neurotransmission has been implicated in several psychiatric disorders. The inability to routinely and non-invasively determine the integrity of the serotonergic system *in vivo* has limited our understanding of disorders with a putative serotonergic abnormality. The loudness dependence of the auditory evoked potential (LDAEP) has been proposed as a reliable measure of central serotonin function in humans. While animal studies suggest that the LDAEP is sensitive to changes in central serotonin neurotransmission, evidence in humans has been indirect and inconsistent. The aim of this study was to assess the sensitivity of the LDAEP to acute augmentation in central serotonergic neurotransmission in humans.
Methods The study used a double-blind, placebo-controlled cross-over design, in which healthy subjects were tested under four acute treatment conditions, with pharmacologically equivalent single doses of placebo, escitalopram (10 mg), citalopram (20 mg) and sertraline (50 mg) to examine the direct effect of acute enhancement of synaptic serotonin on the LDAEP. Furthermore, the outcome of the serotonergic modulatory effects on the LDAEP was also examined using two methods (dipole source analysis (DSA) vs. scalp analysis).
Results Escitalopram, citalopram and sertraline had no effects on the LDAEP and were independent of the analysis method used.
Conclusion These findings question the sensitivity of the LDAEP to acute changes in serotonin neurotransmission and its validity as a reliable measure of central serotonin function in humans.

KEY WORDS — serotonin; selective serotonin reuptake inhibitor; loudness dependence auditory evoked potential; escitalopram; citalopram; sertraline

INTRODUCTION

Despite advances in medical research, the inability to routinely and non-invasively determine the integrity of the serotonergic system *in vivo* in humans has limited our understanding of disorders with a putative serotonergic abnormality. Current biological markers of central serotonin function are not routinely employed in clinical settings because they are either indirect (i.e. peripheral measures) or invasive (cerebrospinal fluid measurements of serotonin and its metabolites, positron emission tomography (PET) imaging with radioactive isotopes). While PET

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imaging and quantification of receptors or transporters are excellent molecular markers, they are not *functional markers* of the serotonergic system. Furthermore, the radioactive nature of PET imaging greatly limits our capacity for repeated testing.

A neurophysiological approach, the assessment of the loudness dependence of the auditory evoked potential (LDAEP), has been reported as a potential non-invasive marker of central serotonin function in humans (Hegerl and Juckel, 1993; Hegerl *et al*., 2001; Nathan *et al*., 2006). The LDAEP is thought to be a measure of auditory cortex activity, reflecting an increase or decrease in the slope of the relationship between the auditory evoked potential amplitude and tone loudness. While the exact mechanisms responsible for these effects are unknown, most evoked potentials, including the LDAEP, reflect activity of cortical pyramidal cells (Barth and Di, 1990; Mitzdorf, 1985). It is thought that while the amplitude of cortical evoked potentials may be related to phasic release of glutamate or GABA (Knight and Brailowsky, 1990; Schroeder *et al*., 1990; Zemon *et al*., 1986), the intensity dependence of the AEP amplitude may be related to neuromodulatory system of subcortical origin including the serotonergic system (Connolly, 1987; Juckel *et al*., 1997). Since the primary (A1) auditory cortex has a high density of serotonergic innervation (Wilson and Molliver, 1991), and receives dense specific thalamic sensory input, the serotonin system is well positioned to modulate cortical signal processing (Morrison *et al*., 1982) and the LDAEP (Juckel *et al*., 1997). Some animal studies have shown direct evidence for serotonergic modulation of the LDAEP. A steeper LDAEP (i.e. an increase in the slope of the minimum (N1)/maximum (P2) amplitude with increasing tone loudness relation) has been shown following local application of the 5-HT_{1A} receptor agonist 8-OH-DPAT in the dorsal raphe nucleus (DRN) (which decreases serotonin release) (Juckel *et al*., 1999) and following antagonism of postsynaptic 5-HT_{2A} receptors with ketanserin (Juckel *et al*., 1997). In contrast, a shallower LDAEP (a decrease in the slope of the N1/P2 amplitude with increasing tone loudness relation) has been found following administration of the 5-HT_{1A} receptor antagonist spiperone in the DRN (which increases serotonin release) (Juckel *et al*., 1999) and stimulation of postsynaptic 5-HT_{1A} receptors with 8-OH-DPAT (Juckel *et al*., 1997).

Evidence in humans, however, has been indirect and inconsistent. Although clinical studies have provided indirect support of the animals findings, with demonstration of a steeper LDAEP in disorders with supposed serotonergic dysfunction including depression (Buchsbaum *et al*., 1971), generalised anxiety disorder (Senkowski *et al*., 2003), MDMA users (Croft *et al*., 2001; Tuchtenhagen *et al*., 2000) and some personality traits (Hegerl *et al*., 1989; Zuckerman, 1988), a direct correlation with serotonin levels in the synapse awaits further evaluation. Furthermore, a relationship between the LDAEP and serotonin function has been inferred from indirect findings with lithium (Hegerl *et al*., 1990; Hubbard *et al*., 1979) and ethanol (Hegerl *et al*., 1996) (which are not selective serotonergic modulators) and from correlations with plasma 5-hydroxy-indole-acetyic-acid (5-HIAA) (Von Knorring and Perris, 1981) (only a weak marker of central serotonin function).

Other studies have directly examined the relationship between serotonin function and the LDAEP, but findings have been inconsistent. For example, studies examining functional polymorphism in the serotonin transporter gene (5-HTTLPR) have shown both a shallower (Gallinat *et al*., 2003) and steeper (Hensch *et al*., 2006; Strobel *et al*., 2003) LDAEP in individuals homozygous for the *l* allele (associated with higher serotonin uptake and central serotonin activity). Acute decrease of serotonin availability using tryptophan depletion has been shown to have no effect (Debener *et al*., 2002; Dierks *et al*., 1999; Massey *et al*., 2004), or a paradoxical decrease in the LDAEP (Kahkonen *et al*., 2002). Similarly, acutely enhancing serotonin availability with the selective serotonin reuptake inhibitor (SSRI) fluvoxamine was found to decrease the LDAEP in depressed patients, but not in healthy subjects (Hegerl *et al*., 1991). In contrast, we recently showed that acutely increasing serotonin with a more selective SSRI (citalopram) reduced the LDAEP, as predicted from animal findings. However more recently, this was not replicated in a study using intravenous citalopram (20 mg) (Uhl *et al*., 2006). The discrepant findings may be related to differences in potencies and selectivity of the SSRIs for inhibition of serotonin reuptake, which in turn would influence the extent of extracellular serotonin increase.

In order to further clarify the relationship between changes in serotonin neurotransmission and the LDAEP directly in humans, we examined the acute effects of enhancing synaptic serotonin using three SSRIs (i.e. escitalopram, citalopram and sertraline) in healthy participants. Escitalopram is the therapeutically active S-enantiomer of citalopram and is a highly selective and a potent inhibitor of the serotonin transporter (Sanchez *et al*., 2004; Waugh and Goa, 2003). Animal studies using a variety of *in vitro* and *in vivo* measures (i.e. reuptake inhibition, binding, behavioural models)

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suggest that escitalopram is at least twice as potent as citalopram (Sanchez *et al*., 2004). In support, clinical studies suggest superior efficacy of escitalopram in comparison to citalopram (at pharmacologically equivalent doses) (Montgomery *et al*., 2001; Sanchez *et al*., 2004). The differences in potency have been suggested be related to the ability of the R-enantiomer to inhibit the effects of the S-enantiomer on serotonin release (Mork *et al*., 2003; Sanchez *et al*., 2004). Indeed, a number of microdialysis studies have shown that escitalopram alone is more effective at increasing extracellular serotonin levels in the brain than an equivalent dose of citalopram (Cremers and Westerink, 2003; Sanchez *et al*., 2004). Amongst the SSRIs, *in vitro* and *in vivo* animal studies suggest that sertraline is less selective than escitalopram and citalopram for the serotonin system (for a review see Sanchez *et al*., 2004). Based on previous findings, we hypothesised that all three SSRIs would decrease the slope of the LDAEP. No predictions were made regarding potency as this study did not examine a dose response relationship for each SSRI.

METHODS

Participants

Sixteen non-smoking male subjects aged between 18 and 36 years (mean $\pm$ SD: 23.2 ± 5.1 years) were recruited through university advertisements. All participants had normal-to-corrected vision and no hearing impairments, had no personal or family history of neurological or psychiatric illness and were free of any prescription medications as assessed by a semi-structured clinical examination by a medical physician. All participants gave written informed consent to take part in the study, which was approved by the Swinburne Human Research Ethics Committee.

Study design

The study was conducted in a double-blind, placebo-controlled design. All participants attended four full-day testing sessions, separated by a minimum 1-week washout period. The treatments conditions included: (i) sertraline (Zoloft®, 50 mg, Pfizer, Australia); (ii) escitalopram (Lexapro®, 10 mg, Lundbeck); (iii) citalopram (Cipramil®, 20 mg, Lundbeck, Australia); (iv) placebo. For blinding purposes, all tablets were enclosed in an opaque gelatine capsule, filled with plain flour. The doses were the minimum therapeutic dosages based on prescription information for each drug. The treatment administration was randomised and was counterbalanced using a Latin Square design to ensure that an equal number of participants were tested under each acute treatment condition and participants were tested at the same time of day for each of the four testing sessions.

Procedure

The study was conducted at the Brain Sciences Institute, Swinburne University of Technology. Participants were required to abstain from alcohol or caffeinated products for 24 h prior to testing. Participants arrived at approximately 09.00am on testing days. They first completed a baseline mood questionnaire (Visual Analogue Mood Scale; VAMS). They then received either placebo, citalopram (20 mg), escitalopram (10 mg) or sertraline (50 mg). For the next 3 h, participants remained alone in a quiet room and were provided a standard selection of magazines of neutral content or allowed to rest or do personal study. Participants were then re-administered the VAMS 3 h post-drug administration, followed by electroencephalography (EEG) recording (3.5 h post-drug administration). The timing of drug administration was chosen to coincide with the peak plasma levels for each treatment condition: sertraline ($t_{max} = 3.2$–9.2 h) (DeVane *et al*., 2002), escitalopram ($t_{max} = 4$–5 h) (Waugh and Goa, 2003) and citalopram ($t_{max} = 2$–4 h) (Hyttel, 1994). An overlapping T_{max} range for the drugs allowed compliance with the double-blind design regarding the timing of drug administration and assessments.

Mood assessment

Possible mood changes were evaluated with the modified VAMS (Bond and Lader, 1974). The VAMS consists of 16, 100 mm horizontal scales such as Happy-Sad, Sociable-Withdrawn, Relaxed-Tense. Participants were asked to place a mark on each line that described their current mood state.

Data acquisition

Recordings were performed in an electrically shielded room. Participants were seated comfortably in an armchair, with their eyes 60 cm from a computer LCD monitor. Participants were instructed to avoid excessive movement during the test session. EEG was recorded from 68 scalp (Cz) sites at locations based on the International 10/20 recording system using tin electrodes inserted in a highly elastic fabric cap (Quik-Caps, Neuro Scan Inc., Sterling, VA, USA),

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referenced to an electrode midway between Cz and CPz. Five additional electrodes were employed: a bipolar montage below the right eye to record electromyographic activity, and monopolar recordings from below and above the left eye to record eye movement activity (electro-oculogram, EOG), and on the nose. EEG was recorded continuously, digitised at 500 Hz and filtered using a band-pass filter of 0.05–500 Hz. At the end of each EEG session, the electrodes locations (3D map) were digitised using a 3D sensing pen (Polhemus Inc., Colchester, VT, USA) with electrode locations digitised in relation to three anatomical landmarks (left and right preauricular points, and nasion: PAN landmarks). This process allows electrode locations to be determined relative to participant head anatomy and to match the EEG data for the dipole source analysis (DSA).

Stimuli

Stimuli were presented using the STIM Audio System and STIM software (Neuro Scan Inc., Australia), with sounds applied to the participant binaurally using E.A.R. ear inserts (Aero Company Auditory System, Indianapolis, IN, USA). Stimuli consisted of 100 ms (10 ms rise and fall time) binaural 1000 Hz tones of five intensities (60, 70, 80, 90, 100 dB, SPL) and were presented in a pseudo-randomised fashion with 1.85 ± 0.2 s SOA. This task lasted for 8 min. Participants were also presented a series of faces during the recording session (at different times to the presentation of auditory stimuli) and asked to respond to them with a button press if, and only if, the face had a nose. The face task was conducted to distract attention from the auditory stimuli, as attention has been shown to modulate the LDAEP in humans (Baribeau and Laurent, 1987; Carrillo-de-la-Pena, 1999).

Data analysis

One participant was excluded from the analysis due to recording problems for one of the testing sessions. Hence, group analysis was conducted in 15 subjects. We examined the outcome of the modulatory effects of the SSRIs on the LDAEP using two analysis methods (DSA and Cz LDAEP analysis). DSA analysis allows the separation of the auditory evoked N1/P2 component into subcomponents generated by the A1 auditory and secondary (A2) auditory cortex. While it has been suggested that the Cz LDAEP generated from the A1 auditory cortex using DSA is more sensitive to serotonin function (Hegerl *et al*., 2001), this has not been directly shown by comparing the two analyses methods within the same study.

ERP analysis. For each participant and testing session, data were EOG corrected (Croft and Barry, 2000), visually inspected to remove non-ocular artefacts, re-referenced to a common average reference, epoched −100–400 ms post-stimulus and averaged (separately for each stimulus intensity: 60, 70, 80, 90 and 100 dB). Further, to perform the DSA analysis, a number of summary averages were created from these individual ERP averages. First, a 'grand average' was created, being the average of the above ERPs across all participants, the four treatments (placebo, citalopram, escitalopram and sertraline) and the five stimulus intensities (60, 70, 80, 90 and 100 dB). Second, for each participant separately, ERPs were averaged across all treatments and stimulus intensities: 'subject average'. Finally, for each participant and treatment separately, ERPs were averaged across the five stimulus intensities: 'subject-treatment average'.

Dipole source analysis (DSA). DSA was performed with CURRY® 5.0 software (Neuro Scan Inc., Australia) on the ERP data. The boundary element model (BEM)-interpolated model (Fuchs *et al*., 1998) was used for dipole localisation consisting of 8043 nodes and 16 074 triangles overall (brain: 3858, skull: 2681 and skin: 1504 nodes) and edge lengths were: 7.5 mm (skin), 5.1 mm (skull) and 3.3 mm (brain).

The optimal location and orientation of the dipole were found by an iterative process derived from the model described by Scherg and Picton (1991). The dipoles were fitted by two stages fit procedure consisting of a '*Basic Dipole Model*' followed by an '*Individual Dipole Model*' (Hegerl *et al*., 1994). The '*Basic Dipole Model*' was performed on the '*grand average*' to provide an estimation of the centre of activity within A1 and A2 separately in each hemisphere. This procedure involved two steps:

(1) In line with the DSA model proposed by Scherg and Von Cramon (1986), four components were chosen to explain the measured data using an independent component analysis (ICA) (Hyvarinen and Oja, 2000).
(2) The locations of the four dipoles were fitted to the data (two dipoles per hemispheres, one within each of A1 and A2, Scherg and Von Cramon, 1986) using regional dipole model. These dipoles were constrained within 10 mm of A1 and A2 for each hemisphere separately; according to the centroid stereotaxic coordinates for A1 and A2

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as described by Brown *et al*., (2004) (right hemisphere A1 (41): $x = 48$, $y = -18$, $z = 10$; right hemisphere A2 (42): $x = 58$, $y = -8$, $z = 8$; left hemisphere A1 (41): $x = -42$, $y = -18$, $z = 10$; left hemisphere A2 (42): $x = -54$, $y = -14$, $z = 10$).

The '*Individual Dipole Model*' was performed in order to enable a more accurate estimate of the dipole location for each participant. It was performed on the 'subject-averaged' data, the 'subject-treatment average' and the ERP of the five intensities stimuli separately for each participant and each treatment conditions subsequently. The same fit procedure was used as that in the '*Basic Dipole Model*' with the exception that the dipole constraints were within 5 mm of A1 and A2 locations (as opposed to 10 mm) and the dipole coordinates derived from each steps for the A1 and A2 were used in place of the coordinates used by Brown *et al*., (2004).

Scalp topography method. Evoked responses were analysed in terms of peak-to-peak N1/P2 amplitude. N1 and P2 amplitudes were calculated as the N1 and P2 amplitudes (relative to baseline) in the 80–140 ms and 110–240 ms time windows, respectively, at Cz, and N1/P2 as the difference between the P2 and N1 amplitudes.

DSA and scalp LDAEP-slope estimation. For each session and subject, the DSA-slope of the dipole strength by loudness (dB level) function and the N1/P2 amplitude by loudness (dB level) function was estimated using least squares linear regression, where dipole strength and N1/P2 amplitude, respectively, was the criterion variable and loudness of the stimulus (60–100 dB) was the predictor variable. In the DSA analysis, this was performed separately for the tangential and radial dipoles, resulting in 'tang_slope' and 'rad_slope', respectively.

Behavioural findings (VAMS). Subjective mood ratings were obtained using the VAMS (Bond and Lader, 1974). The VAMS consists of 16 bipolar scales, anchored at each end of a 100 mm line. In factor analyses, these scales reduce to three subscales: alertness (nine items), contentedness (five items) and calmness (two items). The mean of each factor was computed for each participant and used in the statistical analysis.

Statistical analysis

All data were analysed using SPSS v14 (SPSS Inc., Chicago, IL).

Behavioural findings. A repeated measures ANOVA was performed to determine whether there were significant pre-existing differences in the participant's mood prior to treatment administration and to determine whether there was a drug-related change in mood. The dependent variables were the total VAMS scores for each of the three factors (i.e. alertness (factor 1), contentedness (factor 2), calmness (factor 3)) and the independent variables were Treatment (placebo, citalopram, escitalopram and sertraline) and Time (before and after treatment).

DSA slope. To determine whether there was an effect of the stimulus (i.e. loudness), intensity on the dipole (in the placebo condition), a repeated-measures linear contrast was conducted where the independent variable was the stimulus intensity (60, 70, 80, 90 and 100 dB), and the dependent variable was the mean of the tangential dipole strength (i.e. mean $T = (\mathrm{TR} + \mathrm{TL})/2$; where TR: tangential right dipole and TL: tangential left dipole).

To determine if there was an effect of the SSRIs collectively on the DSA-slope, a Wilcoxon's Signed-rank test was performed where the independent variable was Treatment (placebo; SSRIs) and the dependent variable was the average tangential DSA-slope across the three SSRI conditions (Tang_Slope). The non-parametric Wilcoxon's Signed-rank test was performed because the tangential left slope (TL) and the tangential right slope (TR) data did not have normal distributions and could not be normalised. Further, to determine if there was a difference between the three SSRIs, a Friedman test was preformed where the independent variable was SSRI (citalopram, escitalopram and sertraline) and the dependent variable was the Tang-slope. Following significant results, *post hoc* Wilcoxon's Signed-rank tests were performed.

To determine if there was a differential effect of the treatments on the two hemispheres, a Wilcoxon's Signed-rank test was performed where the independent variable was Treatment (placebo and SSRI) and the dependent variable was the average 'TangDif' across the three SSRI conditions (where 'TangDif' was the difference between the slopes of the two hemispheres). Further, in order to determine whether the three SSRIs differentially affected the hemispheres, a Friedman test was performed where the independent variable was SSRI (citalopram, escitalopram and sertraline) and the dependent variable was TangDif. Following significant results, *post hoc* Wilcoxon's Signed-rank tests were performed to determine where any differences lay.

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To determine whether any of the above effects were general, or specific to A1 (i.e. tangential dipole), equivalent analyses to the second set of DSA analyses described above were performed with the Radial dipole (Rad) slope in place of the Tangential. Again, non-parametric tests were performed because the radial left slope (RL) and the radial right (RR) slope data did not have normal distributions and could not be appropriately normalised.

Scalp LDAEP slope. To determine whether there was an effect of stimulus loudness on the Cz LDAEP-slope (in the placebo condition), a repeated-measures linear contrast was conducted where the independent variable was the stimulus intensity (60, 70, 80, 90 and 100 dB), and the dependent variable was the N1/P2 complex amplitude. To determine if there was any effect of the SSRIs on the Cz LDAEP-slope, a repeated-measures contrast was conducted, comparing the placebo condition to the mean of the three SSRI conditions (citalopram, escitalopram and sertraline), and the dependent variable was the Cz LDAEP-slope. Further, in order to investigate if there was any difference in the Cz LDAEP-slope between the three SSRI conditions, a repeated measures ANOVA was conducted, where the independent variable was Treatment (citalopram, sertraline and escitalopram), and the dependent variable was the Cz LDAEP-slope.

DSA versus scalp LDAEP slope analysis. Finally, to compare the two analyses methods, the following statistical analyses were performed. First, in order to determine whether there was a relation between the Cz LDAEP and DSA slope results, a Pearson's correlation was performed comparing the Cz LDAEP-slope and the DSA-slope. Second, in order to determine whether results derived from Cz LDAEP or DSA-slope showed a larger drug effect, the difference between the SSRIs and the placebo (i.e. Treatment_effect = Mean_SSRI − placebo) was computed for each method, and compared with a Wilcoxon's Signed-rank test, where the independent variable was Method (Cz LDAEP and DSA) and the dependent variable was Treatment effect. The Wilcoxon's Signed-rank test was employed because the data did not have a normal distribution and could not be normalised. Note that since DSA-slopes have a different magnitude to Cz LDAEP-slopes, the DSA-slope and the Cz LDAEP-slope values were converted into Z-scores before the Wilcoxon's Signed-rank test was performed.

RESULTS

Behavioural findings

The repeated measures ANOVA showed that there was no main effect of Treatment ($F_{(3,42)} = 0.79$, $p = 0.502$), no interaction of Treatment-by-Time ($F_{(3,42)} = 1.56$, $p = 0.214$), no Treatment-by-Factor ($F_{(6,84)} = 1.10$, $p = 0.371$) nor Treatment-by-Time-by-Factor ($F_{(6,84)} = 0.74$, $p = 0.619$), on VAMS. These results suggest that there was no effect of the treatment on mood measures (i.e. alertness, contentedness, calmness).

DSA slope

The two dipoles per hemisphere model explained the data well, with >93% variance explained by this model in each of the four treatment conditions. There was a linear increase in the tangential strength across the five stimulus intensities ($F_{(1,14)} = 123.28$, $p < 0.01$). Compared to placebo, there was no effect of the SSRIs collectively on the tangential DSA-slope (i.e. 'Tang Slope') ($z = -0.97$, $p = 0.334$), and that there was no difference in the 'Tang Slope' between the three SSRIs ($\chi^2_{(15)} = 0.13$, $p = 0.936$). In addition, the Wilcoxon's Signed-rank test showed no significant differences between the hemispheres for placebo relative to the three SSRIs ($z = -0.97$, $p = 0.334$), and there were no differential effects of the three SSRIs on the two hemispheres ($\chi^2(15) = 2.80$, $p = 0.247$, Figure 1). There were no effects of the SSRIs on the radial dipole slope ($z = -0.45$, $p = 0.650$, Figure 1).

Scalp LDAEP-slope

There was a linear increase in the N1/P2 amplitude across the five stimulus intensities ($F_{(1,14)} = 111.89$, $p < 0.01$, Figure 2). Compared to placebo, there was no significant effect of the SSRIs collectively on the Cz LDAEP slope ($F_{(1,14)} = 0.32$, $p = 0.586$), and there was no significant difference between the three SSRIs on the Cz LDAEP slope ($F_{(1,14)} = 0.161$, $p = 0.695$, Figure 3).

There was no correlation between the Cz LDAEP method and the DSA method ($r = 0.11$, $p = 0.343$). Furthermore, there were no differences in the findings between the DSA and Cz LDAEP analysis methods ($z = -0.85$; $p = 0.394$).

DISCUSSION

The current study examined the effects of acute augmentation of serotonergic neurotransmission using

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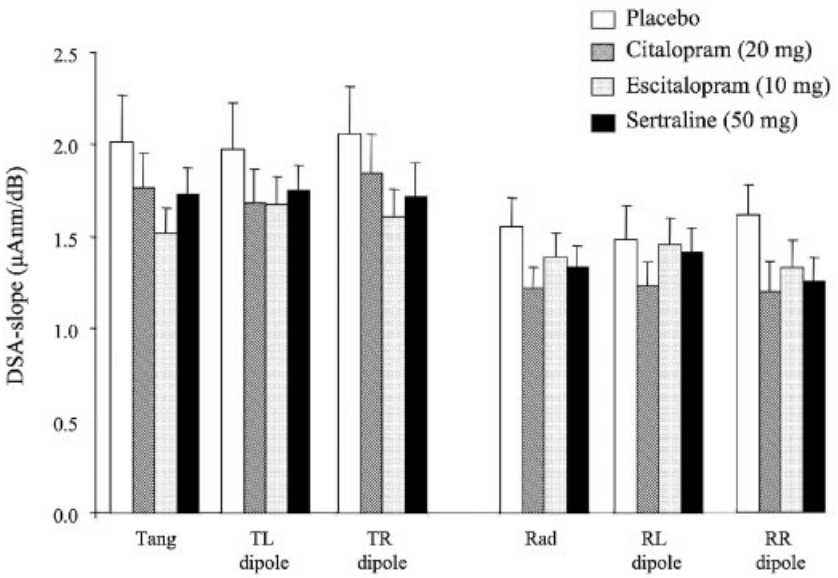

Figure 1. Group mean (±SEM) for the DSA-slope after acute administration of citalopram, escitalopram, sertraline and placebo in healthy participants ($n = 15$). TL, tangential left; TR, tangential right; Tang, mean of the tangential; RL, radial left; RR, radial right; Rad, mean of the radial

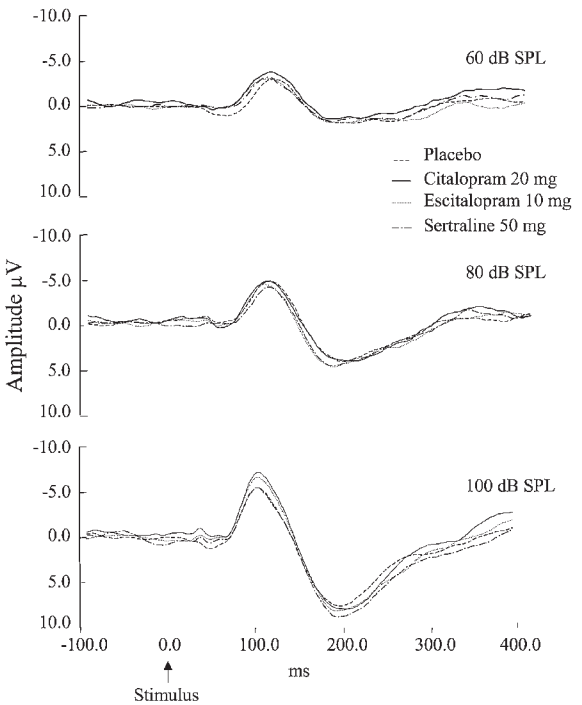

Figure 2. Grand mean ERPs at Cz of three intensities of auditory stimulus (i.e. 60, 80 and 100 dB), following treatment with citalopram, escitalopram, sertraline and placebo ($N = 15$)

three different SSRIs with varying potency and selectivity for the serotonin system, on the LDAEP. We also compared the effects of serotonergic modulation on two LDAEP analysis methods: DSA and Cz. Acutely enhancing synaptic 5-HT with the SSRIs citalopram, escitalopram and sertraline had no significant effect on the LDAEP slope relative to placebo, as determined by both the DSA and Cz methods.

While the LDAEP has been proposed as a valid marker of central serotonin function in humans (Hegerl and Juckel, 1993; Hegerl *et al*., 2001), our findings do not provide support that the LDAEP is reliably sensitive to acute changes in serotonergic neurotransmission. Our findings support a number of other studies that have shown the LDAEP to be insensitive to acute changes in serotonin levels. For example, acute serotonin depletion using tryptophan depletion and acutely enhancing serotonin neurotransmission with citalopram have been shown to have no effects on the LDAEP (Dierks *et al*., 1999; Debener *et al*., 2002; Massey *et al*., 2004; Uhl *et al*., 2006). However, other reports have noted a decrease in the LDAEP slope following acute enhancement of serotonergic neurotransmission with the SSRIs' fluvoxamine and citalopram (Hegerl *et al*., 1991; Nathan *et al*., 2006).

These discrepant findings may be explained by a number of factors. Firstly, inconsistencies could be

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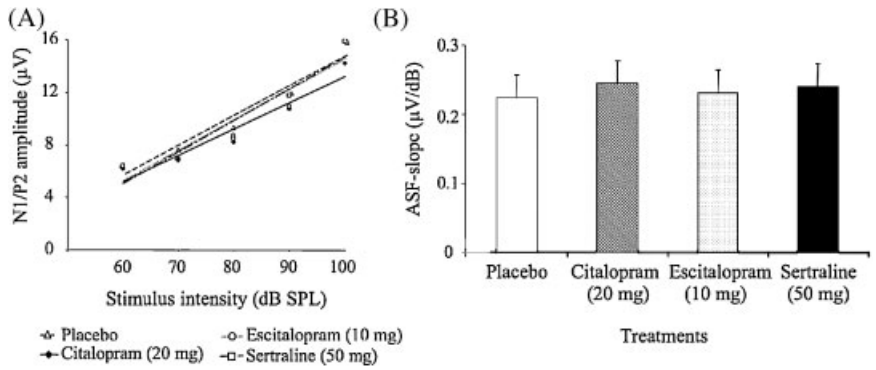

Figure 3. (A) Mean N1/P2 amplitude plotted against stimulus intensity for the four treatment conditions, (B) group mean (SEM) for the scalp LDAEP slope of citalopram, escitalopram, sertraline and placebo in healthy participants ($N = 15$)

explained by the influence of genetic variations in the serotonin transporter (5-HTT) (i.e. 5-HTT polymorphisms). A genetic influence on the LDAEP has been described in several studies (Chen *et al*., 2002; Gallinat *et al*., 2003; Strobel *et al*., 2003). For instance, l/l genotype carriers (associated with higher serotonin transporter availability) exhibited a weaker LDAEP compared to the l/s and s/s carriers (Gallinat *et al*., 2003). Given that most of the studies on the LDAEP did not investigate serotonin transporter polymorphisms, it is possible that the inconsistencies may in part be explained by genetic variations that influence serotonin neurotransmission. Secondly, the inconsistencies may relate to the time window (i.e. pharmacokinetic differences) at which electrophysiological recording was conducted. In our previous study, the LDAEP was recorded 2 h post-citalopram treatment to coincide with peak pharmacokinetic and pharmacodynamic effects of citalopram in humans (Nathan *et al*., 2006). In the present study, EEG was recorded 3.5 h post-treatment coincident with the peak plasma concentration of all SSRIs (i.e. to maximise the pharmacokinetics of all drugs). Even though electrophysiological recording took place at a time when plasma concentration of each of the SSRIs are likely to be high, one cannot rule out the possibility of selective effects at an earlier or later time point (depending on the maximum plasma concentration of each drug). Hence, it is possible that in our former study (Nathan *et al*., 2006), the citalopram concentrations may have been higher (i.e. at 2 h) compared to the current study (at 3.5 h). This is supported by the findings that the maximum effects of citalopram on plasma cortisol has been shown to vary with time (Nadeem *et al*., 2004). Finally, it is possible that the inconsistencies may be related to gender-related differences in serotonergic neurotransmission and the failure to control for this in previous studies. For example, gender-dependent differences in serotonin synthesis have been reported using PET Imaging, with the mean rate of synthesis in males found to be 52% higher than in females (Nishizawa *et al*., 1997). In addition, differences in antidepressant response rates have been reported between men and women, with women having significantly greater response to SSRIs than men (Khan *et al*., 2005) and more specifically women treated with citalopram showing a significantly greater response than men (Berlanga and Flores-Ramos, 2006). Thus, it is possible that the difference between this and our previous study (Nathan *et al*., 2006) may be because the present study exclusively tested men, whereas our previous study tested a mixture of men and women.

It is possible that the lack of effect of SSRIs on the LDAEP may in part be related to the relative effects of acute enhancement of synaptic serotonin on presynaptic activation of 5-HT_{1A} autoreceptors (and hence a reduction in serotonin neurotransmission) versus potentiation of post-synaptic serotonergic neurotransmission. A number of animal studies using microdialysis have shown the acutely administering SSRIs can increase cortical serotonin levels within 2–4 h post administration (Artigas, 1993; Cremers and Westerink, 2003; Sanchez *et al*., 2004). While it is difficult to measure serotonin release directly in humans, there is indirect evidence that acute administration of SSRIs can enhance serotonin function as demonstrated by increases in plasma and salivary cortisol release up to 4 h post-administration (Nadeem *et al*., 2004). We (Kemp et al., 2004 a, b) and others

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(Harmer *et al*., 2003) have also demonstrated enhanced processing of positive social/emotional stimuli and improved memory consolidation (Harmer *et al*., 2002) following acute administration of the SSRI, citalopram, consistent with an enhancement of serotonergic neurotransmission. Hence, it is likely that the lack of effect of the SSRIs on the LDAEP cannot be explained by a reduction in serotonin function due to 5-HT_{1A} autoreceptor activation.

It has previously been suggested that the DSA-derived LDAEP method is more sensitive to changes in 5-HT in comparison to the Cz-derived method (Hegerl *et al*., 2001). To further clarify this, we conducted additional analyses (i.e. Cz LDAEP slope vs. DSA-slope). Our findings showed no significant difference between the DSA analysis and the Cz topographic analysis of the LDAEP, at least with acute changes in serotonergic neurotransmission. The findings support a recent study which similarly found no differences between the two methods with regard to detecting changes in acute serotonin neurotransmission with citalopram (Uhl *et al*., 2006). These findings are, however, inconsistent with findings in depressive patients (Mulert *et al*., 2002) where a clear separation was observed. However, it should be highlighted that depression is associated with chronic serotonergic dysfunction, and thus the LDAEP may be modulated following chronic changes in serotonin neurotransmission and such effects may be quantified with better sensitivity using the DSA analysis.

While there are important methodological issues that need to be addressed (as discussed above), it is important to note that the LDAEP is not reliably sensitive to acute changes in serotonin neurotransmission. It is possible that it may be a better marker of chronic changes in serotonin function. Indeed studies that have investigated the effects of chronic serotonergic modulation (Simmons *et al*., 2003) or the influence of long-term serotonergic dysfunction in disease states including depression (Buchsbaum *et al*., 1971), generalised anxiety disorder (Senkowski *et al*., 2003), MDMA abuse (Croft *et al*., 2001; Tuchtenhagen *et al*., 2000) report more consistent findings with regard to the LDAEP.

In conclusion, the present study found that acutely enhancing serotonin with SSRIs with different selectivity and potency for the serotonergic system had no effect on the LDAEP. These findings question the sensitivity of the LDAEP to acute changes in serotonergic neurotransmission and its possible use as a marker of central serotonin function. Further studies are warranted to examine the sensitivity of the LDAEP to chronic changes in serotonin neurotransmission, particularly the distinction between acute and chronic changes to the serotonergic system.

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