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SHORT COMMUNICATION

The loudness dependence auditory evoked potential is insensitive to acute changes in serotonergic and noradrenergic neurotransmission

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Background The loudness dependence of the auditory evoked potential (LDAEP) has been proposed as an electrophysiological marker for assessing serotonergic function *in vivo* in humans, although accumulating evidence suggests that it is insensitive to acute changes in serotonergic neurotransmission. Very little is known about the sensitivity of the LDAEP to other neurotransmitter systems including the noradrenergic system. The current study examined the effects of noradrenergic modulation as well as serotonergic modulation on the LDAEP.
Methods The study utilised a double-blind placebo-controlled design in which the LDAEP in 17 healthy males and females was tested following acute administration of each of citalopram (20 mg), reboxetine (4 mg) and placebo.
Results Neither citalopram nor reboxetine modulated the LDAEP relative to placebo treatment ($p > 0.05$).
Conclusion These findings suggest that the LDAEP is insensitive to acute changes in serotonergic or noradrenergic neurotransmission and thus is a poor pharmacodynamic marker of these systems. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS — LDAEP; serotonin; noradrenaline; biological marker; electrophysiology; healthy participants

INTRODUCTION

The loudness dependence of auditory evoked potentials (LDAEPs) has been proposed as a non-invasive biological marker of *in vivo* central serotonergic function (Hegerl and Juckel, 1993). It is a measure of auditory cortex activity, reflecting an increase or decrease in the slope of auditory evoked potentials with increasing tone loudness (Hegerl and Juckel, 1993). It has been postulated that the excitability of the auditory cortex is under serotonergic control, such that an increase or decrease in serotonin function is associated with reduced or increased cortical excitability (e.g. decrease and increase in the slope of the LDAEP, respectively).

The majority of evidence in support of an association between serotonin function and the LDAEP has been based on pharmacological studies in animals (Juckel *et al.*, 1997, 1999), with additional indirect evidence from clinical disorders with a presumed serotonergic dysfunction including migraine (Wang *et al.*, 1999), borderline personality disorder (Norra *et al.*, 2003), generalised anxiety disorder (Senkowski *et al.*, 2003) and schizophrenia (Juckel *et al.*, 2003). However, human studies that have directly examined the effects of serotonergic modulation on the LDAEP have been unable to support the animal studies (Juckel *et al.*, 1997, 1999). In one human study, we showed that the slope of the LDAEP was reduced by acutely enhancing synaptic serotonin with the selective serotonin re-uptake inhibitor (SSRI) citalopram (Nathan *et al.*, 2006). However, subsequent studies, including our own, have shown insensitivity of the LDAEP to enhancement of serotonin neurotransmission following acute administration of the SSRI's citalopram (Guille

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et al., 2008; Uhl *et al.*, 2006), escitalopram or sertraline (Guille *et al.*, 2008). Similarly, depletion of serotonin with depletion of the serotonin precursor, tryptophan was also found to have no effect on the LDAEP in the majority of studies (Dierks *et al.*, 1999; Norra *et al.*, 2008; O'Neill *et al.*, 2008a) while one study reported a paradoxical decrease in the LDAEP (Kähkönen *et al.*, 2002). Overall these findings suggest that the LDAEP may be insensitive to acute changes in serotonin neurotransmission (O'Neill *et al.*, 2008b).

Relatively little is known about the impact of other neurotransmitter systems on the LDAEP. A greater understanding of the sensitivity of the LDAEP to multiple neurotransmitter systems is required because of the reported changes in the LDAEP in clinical disorders (Juckel *et al.*, 2003; Norra *et al.*, 2003; Senkowski *et al.*, 2003; Wang *et al.*, 1999) and the lack of knowledge about the underlying influence of neurochemicals other than serotonin. We have recently shown that the LDAEP is sensitive to NMDA receptor modulation (O'Neill *et al.*, 2007) but insensitive to dopamine D_1 and D_2 receptor stimulation (O'Neill *et al.*, 2006) and dopamine depletion (O'Neill *et al.*, 2008a). It is yet to be established whether the LDAEP is directly modulated by changes in noradrenergic neurotransmission in healthy subjects, although it has been recently demonstrated that the LDAEP at baseline may be a predictor of treatment response to noradrenergic antidepressants in a clinical sample of patients with depression (Juckel *et al.*, 2007; Linka *et al.*, 2009). Hence, the current study examined the acute effects of noradrenergic modulation using the noradrenaline reuptake inhibitor, reboxetine, on the LDAEP in healthy subjects. Due to inconsistent reports with SSRIs, we also further examined the effects of the SSRI citalopram, on the LDAEP.

METHODS

Participants

The sample consisted of 21 healthy, drug naive, participants aged 18–32 (mean age 22.05, SD 3.44) with no current or past history of psychiatric or neurological disorders confirmed by a semi-structured medical examination by a registered physician. All participants provided written informed consent to take part in the study, which was approved by both the Swinburne and Monash University Human Research Ethics Committees.

Design

The study utilised a double-blind, placebo-controlled within-subject design. Participants were tested under three acute treatment conditions: citalopram (Cipramil, 20 mg), reboxetine (Edronax, 4 mg) and placebo. Order of assignment to treatment groups was randomised and counterbalanced, separated by a minimum 7-day washout period. Females were tested during the follicular phase of their menstrual cycle.

Procedure

The study was conducted at the Brain Sciences Institute. On the day of testing, participants were administered citalopram, reboxetine or placebo at approximately 10 am. Event-related potential recordings were performed 2 h post-treatment to coincide with peak pharmacokinetic effects of both reboxetine (2 h; Dostert *et al.*, 1997) and citalopram (2–4 h; Hyttel, 1994) and pharmacodynamic effects of citalopram (Kemp *et al.*, 2004).

Data acquisition

Electroencephalographic (EEG) data were recorded as described in our previous studies (Nathan *et al.*, 2006; O'Neill *et al.*, 2006, 2007). This included EEG recording using tin electrodes from 61 scalp sites according to the international 10/20 system using a Quickcap as well as 9 mm diameter tin electrodes attached at the mastoids for re-referencing, and below the left eye for recording eye movement. Auditory stimuli of the loudness dependency paradigm consisted of fifty 1000 Hz tones (1000 Hz, 100 ms duration including 10 ms rise and 10 ms fall time, SOA randomised between 1600 and 2100 ms) at a range of intensities (60, 70, 80, 90 and 100 dB), presented binaurally in a pseudorandom order through single-use foam EAR inserts. Participants were also presented with a series of faces and asked to respond with a button press if the face had a nose. The purpose of this visual task was to distract attention away from the auditory stimuli as attention has been shown to modulate the LDAEP in humans (Baribeau and Laurent, 1987). Data were collected with a sampling rate of 500 Hz and a bandpass filter of 0.5–100 Hz. The data were recorded using NeuroScan software with SynAmps2^{TM} amplifiers (NeuroScan).

Data analysis

Electroencephalogram data were digitally re-referenced to the average recorded from the mastoid electrodes, low-pass filtered at 30 Hz (24 dB/oct) and epoched −100–400 ms post-auditory stimulus. Ocular artefact was corrected using the Croft and Barry (2000)

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method, whereby epochs with data exceeding ±75 μV were rejected and averages then created for each of the five intensities separately. Averages were created for each of the five intensities separately with a mean of 47.8 ± 2.2 sweeps per intensity. On the grand mean waveform, the N1 and P2 peaks were identified at 100 and 185 ms respectively at the Cz electrode. The N1 and P2 peaks were then detected from the session mean waveforms of each participant at 70–130 and 110–260 ms respectively. The latencies of the session mean peaks were then used to determine the peak detection time ranges for each intensity waveform of that session (N1: ±15 ms; P2: ±30 ms). The peaks were determined automatically at the Cz electrode and subsequently visually inspected by the investigator to ensure accuracy. N1/P2 amplitude was calculated as the differences in amplitude between P2 and N1. The slope of the N1/P2 was calculated as the least squares linear regression slope, with stimulus intensity the independent variable and N1/P2 amplitude the dependent variable.

Statistical analysis

A repeated measures linear contrast was performed to determine whether N1/P2 amplitude increased linearly with stimulus intensity in the placebo condition, and two repeated measures planned contrasts were performed to test for an effect of citalopram and reboxetine (compared to placebo) on the slope of the LDAEP. Gender was employed as a between-subjects factor to reduce error variance as gender has been found to influence visual amplitude–intensity slopes (Buchsbaum and Pfefferbaum, 1971; Camposano and Lolas, 1992). Because these two comparisons were based on a theoretically driven rationale and because there are fewer tests than degrees of freedom, each planned contrast had an alpha level set at 0.05 (Tabachnick and Fidell, 2007).

RESULTS

Four people were excluded from the analysis because recognisable ERP components (and in particular the N1/P2 complex) could not be discerned in the waveforms. The remaining samples [$n = 17$ ($M = 8$, $F = 9$)] are presented here. The N1/P2 amplitude increased linearly with increasing stimulus intensity in the placebo condition ($F_{(1,\ 16)} = 42.67$, $p < 0.0001$, partial $\eta^2 = 0.73$) (Figure 1).

The mean slopes for placebo, citalopram and reboxetine were 0.33, 0.38 and 0.30 respectively (Figure 2).

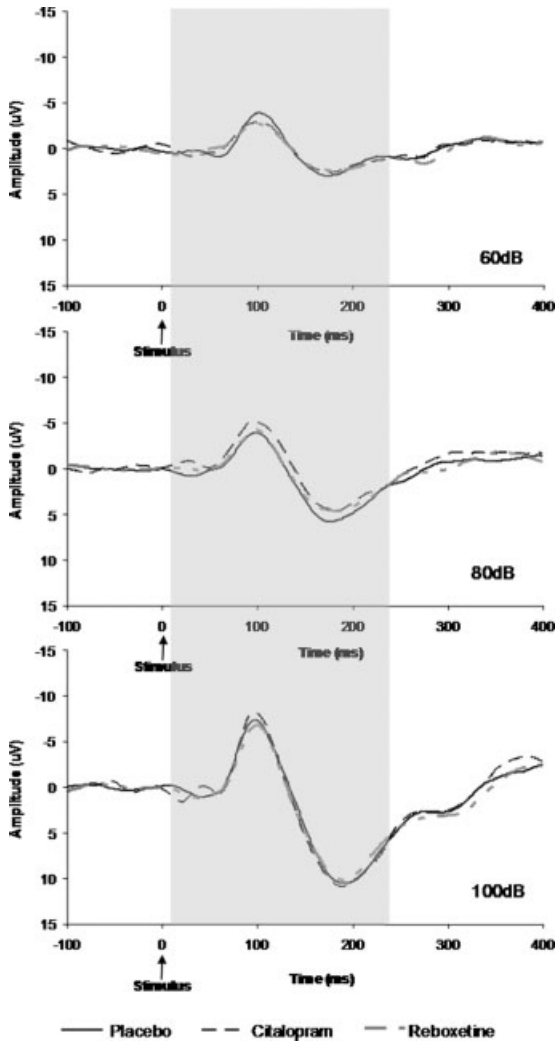

Figure 1. Grand mean auditory evoked potentials at Cz for placebo, citalopram and reboxetine treatment conditions at 60, 80 and 100 dB intensities of the auditory stimuli

Compared to placebo, there was no significant effect of citalopram ($F_{(1,15)} = 0.09$, $p > 0.05$, partial $\eta^2 = 0.006$) or reboxetine ($F_{(1,\ 15)} = 3.92$, $p > 0.05$, partial $\eta^2 = 0.207$) on the LDAEP slope (Figure 2).

DISCUSSION

Acutely enhancing serotonergic or noradrenergic neurotransmission with citalopram and reboxetine respectively did not modulate the LDAEP. These findings are consistent with the majority of studies conducted in humans that have similarly shown that acute enhancement of synaptic serotonin with the SSRI's citalopram (Guille *et al.*, 2008; Uhl *et al.*, 2006), escitalopram and sertraline (Guille *et al.*, 2008) and depletion of synaptic

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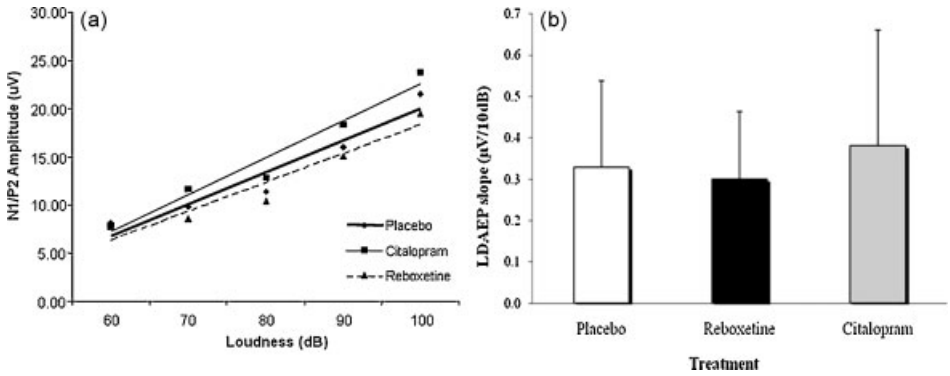

Figure 2. (a) Mean N1/P2 amplitude (μV) plotted against stimulus intensity (loudness, dB) and (b) mean values (mean ± standard deviation) of LDAEP (μV/10 dB) at Cz, for placebo, citalopram and reboxetine treatment conditions

serotonin using tryptophan depletion (O'Neill *et al.*, 2008a) has no effect on the LDAEP. The insensitivity of the LDAEP to noradrenergic modulation supports previous studies in animals and humans. For example, the α_2-receptor agonist clonidine was found to have had no effect on the LDAEP in cats (Juckel *et al.*, 1997), and in humans, a lack of association was reported between the noradrenaline metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), in CSF and the amplitude–intensity function of visual evoked potentials (Von Knorring and Perris, 1981).

Based on these studies it could be argued that the LDAEP is insensitive to acute changes in serotonergic or noradrenergic neurotransmission and hence the LDAEP may be considered a poor pharmacodynamic marker of these neurochemical systems. Although it is possible that the LDAEP may be sensitive to long-term changes in serotonergic function (for a review see O'Neill *et al.*, 2008b) and thus may potentially be a trait or biological marker, this is yet to be directly demonstrated. Together, the findings suggest that the changes in the LDAEP previously reported in clinical disorders (Juckel *et al.*, 2003; Norra *et al.*, 2003; Senkowski *et al.*, 2003; Wang *et al.*, 1999) may not be entirely attributed to a dysfunction in the serotonergic system as there is insufficient consistent data linking changes in serotonergic neurotransmission and the LDAEP and such a relationship is yet to be directly shown following long-term modulation of the serotonin neurotransmission. As we have previously shown that the LDAEP is sensitive to NMDA receptor modulation (O'Neill *et al.*, 2007), it is possible that the LDAEP changes observed in clinical disorders including schizophrenia and mood disorders may be secondary to glutamatergic dysfunction (Laruelle *et al.*, 2003; Sanacora *et al.*, 2008). Future studies focussing on the effects of long-term modulation of the serotonergic (and other systems) on the LDAEP will help clarify the neurochemical basis of the LDAEP changes observed in clinical disorders.

There are some methodological issues that warrant discussion. First, the negative findings are unlikely due to inadequate power as a statistical analysis showed that the effect sizes were small and greater than 200 subjects would be required to achieve significant effects (with a power of 0.8 at alpha 0.05). Second, this study used scalp data to determine the slope of the LDAEP. Previous studies have claimed that the dipole source localisation method is more sensitive than the scalp-derived method (Hegerl *et al.*, 1994; Hegerl and Juckel, 1993) as it can separate the tangential dipole which may be more sensitive to changes in serotonin neurotransmission (Campbell *et al.*, 1987). However, as yet, the advantage of dipole source localisation method over the scalp-derived method has not been directly demonstrated. Furthermore, we recently showed that the dipole source analysis is no more sensitive than the scalp-derived method in detecting the acute effects of citalopram on the LDAEP (Guille *et al.*, 2008). As such, it is unlikely that the negative findings in this study are a result of the data being analysed using the scalp-derived method.

In summary, acute enhancement of serotonergic or noradrenergic neurotransmission using citalopram and reboxetine respectively had no effects on the LDAEP. These findings together with previous findings in the literature suggest that the LDAEP is a poor pharmacodynamic marker of the serotonin and noradrenergic systems.

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