

Serotonin Modulates Early Cortical Auditory Processing in Healthy Subjects. Evidence from MEG with Acute Tryptophan Depletion

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We studied the effects of acute tryptophan depletion (ATD) on early cortical auditory processing. Middle-latency auditory evoked fields (MAEF) were investigated in 14 healthy subjects after 5 h of ATD or control mixture ingestion in a randomized, double-blinded, controlled crossover design study. MAEFs to monaural click stimuli (0.1-ms duration) were recorded with a 122-channel neuromagnetometer. Total plasma tryptophan (Trp), free Trp, and large neutral amino acid (LNAA) concentrations were determined by using high-performance liquid chromatography. ATD lowered the total plasma Trp levels by 75%, free Trp level by 47%, and the ratio Trp/ Σ LNAA by 92%. The control mixture increased total Trp level by 45% and free Trp by 32%, and decreased the ratio Trp/ Σ LNAA by 35%. The ratio tyrosine/ Σ LNAA did not differ

between ATD and control experiment. ATD resulted in a significant main effect on Pam latencies and a near-significant main effect on Pam amplitudes. A significant Mixture ingestion X Sex interaction on Nbm amplitude and a significant Mixture ingestion X Sex X Hemisphere interaction on Pam latency were observed. ATD did not affect the MAEF source dipoles. The Pam latencies in both hemispheres had a significant negative relationship with the extent of ATD. The results suggest that the neurotransmitter serotonin is involved in early auditory cortical processing. Further, the serotonin modulation may be different in males and females.

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Low brain serotonin (5-HT) levels and function have been implicated in various psychopathologies including depression and anxiety. Acute tryptophan depletion (ATD) is a dietary intervention that rapidly lowers plasma tryptophan (Trp) and alters 5-HT synthesis, metabolism, and release in animals and humans (Young et al. 1985; Nishizawa et al. 1997; Stancampiano et al. 1997; Carpenter et al. 1998). It involves the administration of an amino acid mixture that lacks Trp, the amino acid precursor of serotonin. The effect of ATD on 5-HT synthesis and metabolism is twofold (Young et al. 1985). First, decline in plasma Trp occurs because the Trp-deficient amino acid mixture promotes synthesis of new proteins. Trp incorporated into protein comes from blood and tissue pools, and therefore its concentration in plasma and brain falls (Biggio et al. 1974; Gessa et al.

1975). Second, all large neutral amino acids (LNAA) compete for the same carrier system that transport them across the blood-brain barrier (Oldendorf 1973). Brain Trp levels depend not only on availability of plasma Trp, but also on other LNAAs. Thus, amino acid competition for transport at the level of the blood-brain barrier plays a role in regulating brain Trp (Gessa et al. 1975). The result of ATD in rats is a decrease in brain Trp and 5-HT concentration (Moja et al. 1989) and a decrease of 5-HT and 5-hydroxyindolacetic acid (5-HIAA) concentrations in cerebrospinal fluid (Bel and Artigas 1996; Stancampiano et al. 1997).

Magnetoencephalography (MEG) is a method for the measurement of extracranial magnetic fields produced by ionic current flow generated mainly by cortical pyramidal cells. MEG is a non-invasive method for functional brain studies with temporal resolution as high as electroencephalography (EEG), but with more accurate source localization (Hämäläinen et al. 1993). Because of cortical folding in the Sylvian fissure, MEG is ideally suited for the localization and measurement of supratemporal auditory cortical activity (Hämäläinen et al. 1993).

Auditory evoked magnetic potentials (AEP) and fields (AEF), which allow the investigation underlying auditory processing, can be obtained by averaging EEG or MEG epochs time-locked to the presentation of auditory stimuli. The earliest cortical AEFs, the middle-latency AEFs (MAEFs), occur at about 20–70 ms from stimulus onset. The MAEFs are composed of several distinct deflections, with Pam peaking at approximately 30 ms and Nbm at about 45 ms.

There is evidence to suggest that serotonergic activity may modulate auditory evoked potentials (Hegerl and Juckel 1993; Juckel et al. 1997; Dierks et al. 1999) and background EEG frequencies (Knott et al. 1999). The sound intensity dependence of auditory N1/P2 amplitude has been suggested to be a specific indicator of serotonin function (Hegerl and Juckel 1993; Juckel et al. 1997). Reduced serotonin function leads to higher intensity dependence of N1/P2 amplitude and vice versa. However, Dierks et al. (1999) were not able to confirm these findings using ATD in healthy subjects. In contrast, this study showed that ATD decreases the intensity dependence of N1/P2 amplitudes. In the recent ATD study using a 52-g amino acid challenge, Hughes et al. (2000) found no changes in auditory N1, P2, or P3 amplitudes and latencies in men. Women are more susceptible to a lowering mood and to a greater memory impairment as a function of ATD (Ellenbogen et al. 1996; Riedel et al. 1999). The incidence of major depression, which is linked to serotonin deficiency, is higher in women than in men (Weissman and Olfson 1995).

We have previously shown that acetylcholine antagonist scopolamine enhances and dopamine D2-receptor

antagonist haloperidol does not affect MAEF responses (Jääskeläinen et al. 1999; Kähkönen et al. 2001). Therefore, the present study investigated auditory processing after ATD by recording the Pam and Nbm components of MAEFs in healthy volunteers. The effect of gender on MAEF was also analyzed.

METHODS

Subjects

Fourteen subjects (aged 21–30 years; mean \pm SD, 24.1 \pm 3.3; seven females) participated in the study. An informed written consent and institutional ethical committee approval were obtained. Before the inclusion the subjects underwent a medical examination and laboratory tests of blood to exclude physical or mental health problems. They were also screened for mental problems by SCL-90 (Derogatis et al. 1973; Holi et al. 1998). The subjects reported having used no medications for two weeks prior to the study. The subjects were instructed to avoid alcohol for at least 48 h, and caffeine for 12 h prior to the MEG recordings. All were non-smokers. The hearing level was confirmed by determining the auditory threshold with the auditory stimulator used for this experiment.

Design

Subjects were investigated after 5 h of ATD or control mixture ingestion by recording MAEF components Pam and Nbm in a randomized, double-blind, controlled cross-over design study. All subjects arrived at the laboratory at approximately 7:45 A.M. after an overnight fast. After a baseline heparinized blood sample was obtained for plasma total and free Trp, and for LNAA, the subject underwent either an ATD or control mixture administration. The amino-acid drink was composed of 15 amino acids with or without tryptophan, as described by Young et al. (1985). First, the subject was administered arginine, cysteine, and methionine, contained in capsules because of their very unpleasant taste. Then the subject consumed the remaining amino acids (i.e., alanine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine) as a chilled 300-cc strawberry/mint flavored drink. The capsules and the drink were consumed during a period of 20–30 min. The second blood sample for measurements of plasma Trp and LNAA was taken after 5 h of ingestion of the mixture. The sessions were separated by one week.

Stimulus Design and Data Acquisition

During MEG recording, the subject sat in a comfortable chair in a magnetically and electrically shielded room

(Euroshield, Eura, Finland). The auditory evoked fields were recorded with a 122-channel MEG device (Neuro-mag, Helsinki, Finland; Ahonen et al. 1993). Click stimuli (0.1-ms duration) were presented to the left ear at a rate of 10 Hz at 60 dB above an individually measured hearing threshold. A similar paradigm was used in our earlier psychopharmacological studies (Jääskeläinen et al. 1999; Kähkönen et al. 2001). During the stimulation, the subjects were instructed to concentrate on a video film of their own interest, and to ignore the clicks. Each two-channel sensor unit measured two independent magnetic field gradient components $\partial B_z/\partial x$ and $\partial B_z/\partial y$, the Z-axis being normal to the local helmet surface. The position of the subject's head relative to the recording instrument was determined by measuring the magnetic fields produced by marker coils in relation to cardinal points on the head (nasion, left and right pre-auricular points), determined before the experiment using an Isotrak 3D-digitizer (Polhemus, Colchester, VT) (Ahlfors and Ilmoniemi 1989). In addition, vertical and horizontal electro-oculograms (EOG) were recorded.

The recording passband was 1–250 Hz for MEG at a sampling rate of 1 kHz. Digital band-pass filtering was performed off-line at 10–120 Hz (Starr and Don 1988). The analysis period was 150 ms (including a 50-ms pre-stimulus baseline). The first few responses and all the epochs coinciding with EOG or MEG changes exceeding 150 μ V or 3000 fT/cm, respectively, were omitted from averaging. At least 1000 artifact-free responses were recorded and averaged.

Determination of Plasma L-Trp and LNAAs

Blood (10 ml) was drawn from the left ulnar vein into a vacuum tube (Venoject, Belgium). The tube was cooled immediately and centrifuged (200 rev/min) for 10 min. After centrifugation, the plasma was separated and stored at -20°C until its assay for the amino acids. A modified procedure described by Qureshi et al. (1984) was used in plasma assay for amino acids. Coefficients of variation of 1.2, 1.05, 1.11, 1.14, 5.5, and 1.0% were determined for tryptophan, tyrosine, valine, phenylalanine, isoleucine and leucine (at 150 pmol/ μ l), respectively. The detection limit was 10 pmol/ μ l.

Data Analysis

The distinct AEF peaks were obtained from latency ranges of 25 to 40 ms for Pam and 40 to 55 ms for Nbm. The AEF peak latencies and amplitudes were measured from the channel pair showing the highest-amplitude responses (the amplitude being determined as $|a| = ((\partial B_z/\partial x)^2 + (\partial B_z/\partial y)^2)^{1/2}$), separately for the left and right temporal areas. The AEF sources were located by a least-squares fit of a single equivalent current dipole (ECD) using a fixed subset of 34 channels separately

over each auditory cortex (Hämäläinen et al. 1993). Dipole fits with at most 30% residual variance were considered successful. The statistical analysis was carried out with 3-factor (Mixture X Hemisphere X Sex) repeated-measures ANOVAs. Paired and unpaired *t*-tests were used for a priori comparison when appropriate. The extent of the tryptophan depletion was correlated with MAEF responses using Spearman correlation.

RESULTS

Blood samples showed that the Trp manipulation was successful in all subjects. ATD lowered the total plasma Trp levels by 75%, free Trp level by 47%, and the ratio Trp/ Σ LNAAs by 92%. The control mixture increased total Trp level by 45% and free Trp by 32%, and decreased the ratio Trp/ Σ LNAAs by 35%. The ratio tyrosine/ Σ LNAAs did not differ between ATD and control experiment (Table 1). Click stimulation elicited prominent Pam and Nbm responses (Figure 1).

Table 2 shows the peak latencies and peak amplitudes of the MAEF components in ATD and control conditions over both measured hemispheres. ATD resulted in a significant main effect on Pam latencies and a near-significant main effect on Pam amplitudes ($F_{1,6} = 5.70$, $p = .05$; $F_{1,6} = 5.27$, $p = .06$, respectively). A significant Mixture ingestion X Sex interaction on Nbm amplitude ($F_{1,6} = 6.32$, $p = .04$) and a significant Mixture ingestion X Sex X Hemisphere interaction on Pam latency ($F_{1,6} = 7.14$, $p = .04$) were observed. The dipole moments of Pam and Nbm were not changed after ATD administration over either hemisphere. The source locations of the responses were not significantly influenced by ATD (Table 3). The latencies of Pam at the contralateral and ipsilateral hemispheres correlated negatively with Trp/ Σ LNAAs at ATD ($\sigma = -0.64$; $p = .01$, and $\sigma = -0.60$; $p = .02$, respectively).

Table 1. Amino Acid Levels (pmol/ μ l) and Uptake Ratios in Different Conditions

| Amino acids | Time (h) | Trp depletion | Control experiment |
|--------------------------|----------|-----------------|--------------------|
| Total Trp | 0 | 62.5 \pm 13.1 | 60.9 \pm 26.3 |
| | 5 | 15.6 \pm 4.2 | 100 \pm 25.1 |
| Free Trp | 0 | 5.8 \pm 2.0 | 7.0 \pm 3.2 |
| | 5 | 3.1 \pm 1.8 | 9.2 \pm 4.3 |
| Ratio Σ LNAAs/Trp | 0 | 15.6 \pm 5.3 | 12.3 \pm 2.8 |
| | 5 | 1.3 \pm 0.7 | 8.0 \pm 2.1 |
| Ratio Σ LNAAs/Tyr | 0 | 10.5 \pm 2.8 | 10.6 \pm 1.7 |
| | 5 | 4.6 \pm 1.5 | 5.0 \pm 1.8 |

Trp = Tryptophan; LNAAs = Large neutral amino acid; Tyr = Tyrosine.

Baseline Total Trp, Free Trp, Ratio Σ LNAAs/Trp, and Ratio Σ LNAAs/Tyr did not differ between Trp depletion and control experiment (paired *t*-test; $p > 0.05$).

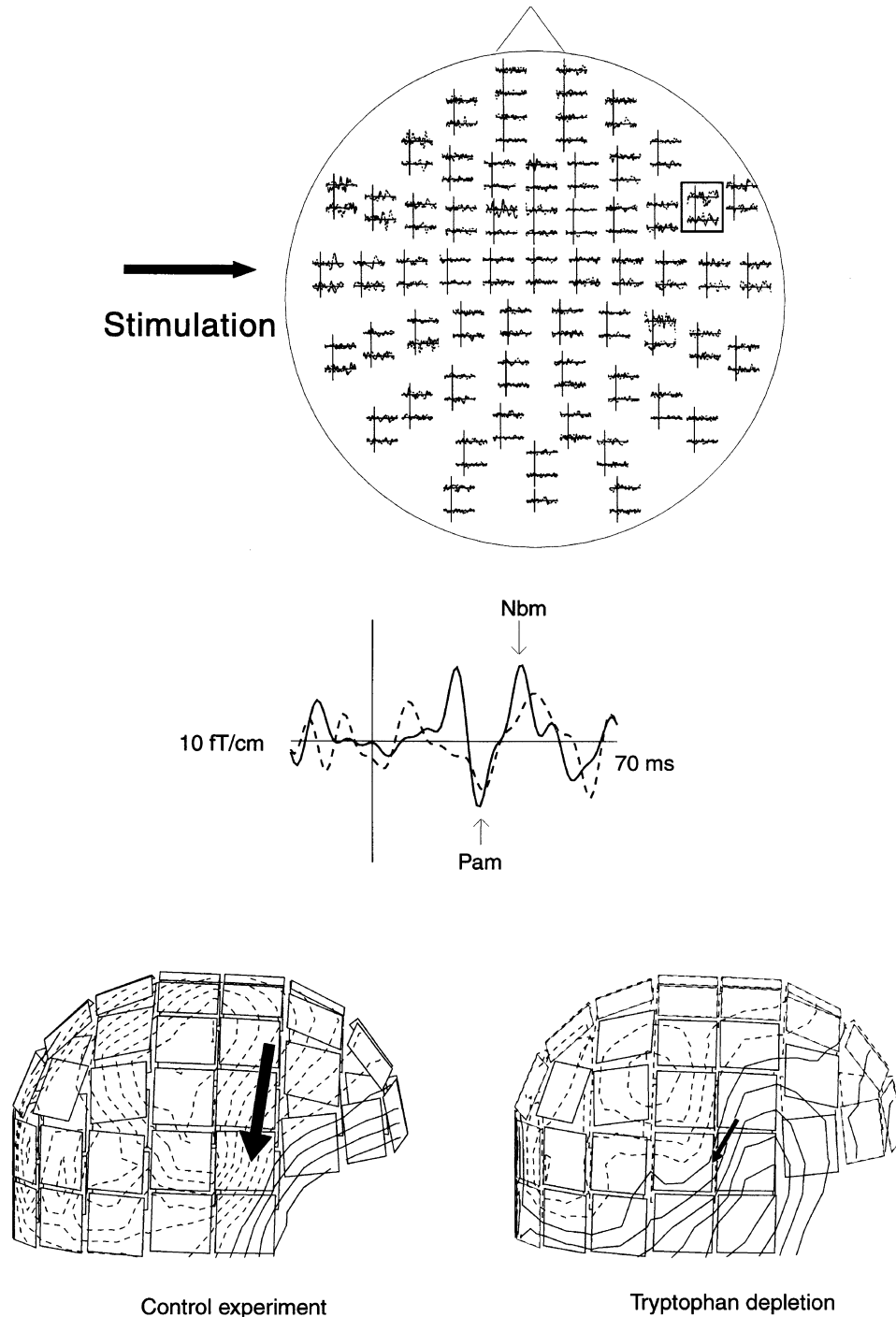


Figure 1. Upper panel. MAEF response in one subject measured with magnetometer during tryptophan depletion (dotted lines) and control experiment (solid line). The stimuli were delivered to the left ear. Middle panel. The enlarged response presents the channel showing largest responses over the right temporal areas (contralateral to the ear stimulated). Lower diagrams. Contour maps at the peak of Nbm. Solid lines indicate magnetic flux lines where it enters outside the head and dotted lines areas indicate magnetic flux lines exits within the head. Contour line separation is 3 fT. The arrows depict the strengths (proportional to arrow length) and locations of single equivalent current dipoles of Nbm applied to data.

DISCUSSION

In our experiment, ATD caused significant reduction in total and free Trp levels and in the ratio $Trp/\Sigma LNAA$.

The level of reduction of amino acids is in line with studies with ATD performed earlier. A recent positron emission tomography (PET) study indicated that ATD markedly decreases serotonin synthesis in different

Table 2. Peak Latencies and Amplitudes of MAEF Components after Tryptophan Depletion and Control Experiment

| Deflection | Hemisphere | Response | Tryptophan Depletion | Control Experiment |
|------------|---------------|-------------------|----------------------|--------------------|
| Pam | Contralateral | Latency (ms) | 34.6 ± 4.0 | 32.0 ± 3.9 |
| Pam | Ipsilateral | Latency (ms) | 32.0 ± 3.9 | 30.4 ± 3.6 |
| Pam | Contralateral | Amplitude (fT/cm) | 5.6 ± 2.3 | 5.7 ± 2.5 |
| Pam | Ipsilateral | Amplitude (fT/cm) | 5.0 ± 2.3 | 5.2 ± 3.2 |
| Nbm | Contralateral | Latency (ms) | 50.1 ± 5.8 | 47.5 ± 4.8 |
| Nbm | Ipsilateral | Latency (ms) | 49.5 ± 4.8 | 47.8 ± 3.8 |
| Nbm | Contralateral | Amplitude (fT/cm) | 5.4 ± 2.4 | 5.1 ± 2.1 |
| Nbm | Ipsilateral | Amplitude (fT/cm) | 6.0 ± 1.5 | 5.6 ± 2.0 |

brain regions (Nishizawa et al. 1997). Furthermore ATD has been shown to decrease the concentration of 5-HIAA, the principal metabolite of serotonin in cerebrospinal fluid in normal volunteers, by over 30% (Carpenter et al. 1998; Williams et al. 1999). There is evidence from animal and human studies that an amino acid drink deficient in tyrosine (Tyr) can interfere with central catecholamine function in a way similar to the effects of ATD in humans (Moja et al. 1996; Harmer et al. 2001). The ATD and control experiment influenced the ratio Tyr/ Σ LNAAs in a similar manner indicating that metabolism of dopamine and noradrenaline were not affected in our experiment. Based on these consistent findings, one may expect that ATD reduced cerebral serotonin synthesis in the present subjects as well.

Table 3. Mean \pm SD Dipole Positions in Cartesian Coordinates, Dipole Moments (Q), and Percentage of Sources Successfully Modeled in Tryptophan Depletion and Control Conditions

| ERF Deflection | | Tryptophan Depletion | Control Experiment |
|---------------------|-----------|----------------------|--------------------|
| Pam (contralateral) | x (mm) | 50 ± 18 | 53 ± 19 |
| | y (mm) | 14 ± 16 | 19 ± 15 |
| | z (mm) | 73 ± 14 | 66 ± 19 |
| | Q (nAm) | 2.8 ± 3.1 | 2.1 ± 1.5 |
| | % Modeled | 72 | 57 |
| Pam (ipsilateral) | x (mm) | -42 ± 12 | -38 ± 10 |
| | y (mm) | 9 ± 12 | 10 ± 13 |
| | z (mm) | 57 ± 10 | 64 ± 22 |
| | Q (nAm) | 4.4 ± 3.3 | 4.3 ± 1.2 |
| | % Modeled | 50 | 43 |
| Nbm (contralateral) | x (mm) | 39 ± 12 | 34 ± 36 |
| | y (mm) | 6 ± 12 | 9 ± 12 |
| | z (mm) | 64 ± 10 | 67 ± 25 |
| | Q (nAm) | 4.2 ± 1.9 | 2.7 ± 2.0 |
| | % Modeled | 58 | 58 |
| Nbm (ipsilateral) | x (mm) | -45 ± 24 | -29 ± 33 |
| | y (mm) | 7 ± 19 | 7 ± 25 |
| | z (mm) | 41 ± 32 | 61 ± 20 |
| | Q (nAm) | 3.1 ± 1.8 | 2.7 ± 1.0 |
| | % Modeled | 36 | 64 |

The x -axis runs from left pre-auricular point to the right. The y -axis runs through the nasion, and z -axis points upwards. Unpaired t -test; $p > .05$

Our results showed that ATD significantly modulated MAEF responses. Results from both MEG and intracranial recordings have suggested that MAEFs are generated in the superior aspects of the temporal lobes, in or near the primary auditory cortex (Liegeois-Chauvel et al. 1994; Scherg et al. 1989). Notably, the densest serotonergic innervation has been found in the superior temporal gyrus, including auditory cortices, with higher density in the primary than in the secondary auditory cortex (Azmitia and Gannon 1986; Lewis et al. 1986; Palmer et al. 1987). Within the primary auditory cortex, layer IV, which receives the most of specific thalamic sensory input (Zilles 1990; Pandya and Rosene 1993) appears to be also most densely innervated by serotonin (Lewis et al. 1986; Campbell et al. 1987). Serotonergic projections are suggested to modulate the early phases of sensory processing at the cortex (Morrison et al. 1982). Furthermore, ATD causes the reduction of serotonin synthesis at the temporal cortex (Nishizawa et al. 1997). It is likely that in our study the ATD caused a reduction of serotonin synthesis in the temporal cortex, which is shown to modulate the MAEF responses. Thus, our MEG results suggest that serotonin transmission modulates the early phase of cortical auditory processing in the human brain.

Several clinical and experimental studies using EEG have suggested that AEPs could be modulated by serotonin (Hegerl and Juckel 1993; Juckel et al. 1997; Dierks et al. 1999). EEG detects particularly the radial currents generated by activity in the cortical gyri, but also the tangential ones in the sulci. Middle-latency auditory responses have been shown to be generated by tangential sources in the auditory cortex, but also by radial ones (Mäkelä et al. 1994; Scherg and von Cramon 1986, 1989). Signals from deep sources, such as the postulated subcortical sources affected by ATD contributing to the MAEP (Woods et al. 1987; Nishizawa et al. 1997), can be detected by EEG. In contrast to EEG, MEG is selectively sensitive to tangential cortical but insensitive to subcortical sources of activity (Hämäläinen et al. 1993). This is due to the fact that the magnetic fields fall off rapidly with increasing distance from the source. Both in EEG and MEG, source localization requires modeling. The superiority of MEG to EEG is largely based on MEG be-

ing practically unaffected by the detailed structure and conductivity of the skull and scalp. Furthermore, MEG is selectively sensitive to tangential source currents in temporal cortex, which make the source modeling more stable than in EEG (Hämäläinen et al. 1993). Hence, it is possible that changes in electromagnetic activity following ATD administration is not detected by MEG in the gyri of the temporal cortex. In future studies, a combined use of MEG and EEG will provide a more complete view of effects of ATD in healthy volunteers and in the clinical population.

Our results are in line with the earlier findings that MAEF responses after ATD are different in women compared with men. The rate of serotonin metabolism is higher in females than in males (Young et al. 1980; Agren et al. 1986). In the PET study of Nishizawa et al. (1997), the reduction of serotonin synthesis in the temporal cortex following ATD was much higher in women than in men. The difference in the rate of serotonin synthesis after ATD may partly explain the different MAEF responses in different sexes. However, our results are based on a small number of subjects. Further studies with a larger number of subjects are needed to confirm or reject this result.

Previously, only a few attempts have been made to elucidate the transmitters involved in middle-latency evoked-response generation. Our findings (Jääskeläinen et al. 1999) and those of Buchwald et al. (1991) have suggested that brain acetylcholine plays an important role in the generation of the Pa response. In these studies, the Pa/Pam amplitude was enhanced by muscarine receptor antagonist scopolamine, and was reduced by the agonist physostigmine. We recently studied the effects of dopamine D2 receptor antagonist haloperidol on MAEF responses. No differences were observed in latencies, amplitudes, dipole moments or locations of MAEF responses between haloperidol and placebo (Kähkönen et al. 2001). The results of the later and the present study indicate that different amine neurotransmitters, serotonin and dopamine, have differential effects on MAEF generation.

In conclusion, our novel MEG results, demonstrating ATD-induced modulation of MAEF responses, suggest that serotonin modulates MAEF generation in the primary auditory cortex and thus may play a role in early cortical processing. The modulation of serotonin effects on early cortical processing may be different in females compared with males. The findings might also be associated with auditory processing deficits observed in psychiatric and neurological disorders with serotonin deficiencies.

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