Tryptophan Depletion Effects on EEG and MEG Responses Suggest Serotonergic Modulation of Auditory Involuntary Attention in Humans

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INTRODUCTION

Involuntary attention shifting enables rapid orienting to unexpected changes in the environment. Deficits in this fundamental function could result in unresponsiveness to potentially harmful events, whereas disinhibition of attention shifting may distract goal-directed behavior (Ahveninen et al., 2000). The neurochemical regulation of involuntary attention, enabling us to selectively sustain or shift the focus of attention to task-relevant aspects of information, is not yet fully known; however, monoaminergic systems innervating the forebrain have been thought to play a central role (Robbins et al., 1989).

A substantial amount of evidence associating the monoamine neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) to attention has been obtained from animal experiments and human studies. Changes in prefrontal 5-HT and dopamine utilization have been associated with a poor ability to sustain attention in rats (Puumala and Sirviö, 1998). These results have been supported by indirect evidence arising from human studies on different clinical subject groups. Attention deficits might be common in abusers of methylenedioxymethamphetamine, i.e., “ecstasy” (McCann et al., 1999), which has been shown to result in specific and irreversible deficits in 5-HT neurons in rats (Ricaurte et al., 1992). At the same time, an inability to sustain attention is often observed in depression (Liotti and Mayberg, 2001), and, notably, the prefrontal abnormalities thought to underlie such attention deficits (Dolan et al., 1994) can possibly be reversed by using serotonin uptake inhibitors such as paroxetine.
tation. N2 (MMN/N2b) is followed by the positive P3a

These overlapping components, MMN/MMNm and

behavioral distractibility, reduce the MMN and P3a

generation may be decreased by different glutamate-

ated with involuntary attention are not fully known.

Previous results, however, suggest that MMN/MMNm

change, and its MEG counterpart (MMNm), might re-

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peak latency increase after acute ethanol administra-

(Toe and Ferguson, 1986). Finally, the dopamine

D2-receptor antagonist haloperidol has been found to

crease MMN elicited by task-irrelevant deviants dur-

in a dichotic listening task, presumably reflecting im-

paired focused attention to the task-relevant channel

(Kähkönen et al., 2001). There is also evidence that

haloperidol might suppress P3a and RON (Kähkönen

et al., 2002). No evidence of noradrenergic modulation

of MMN has been found so far (Duncan and Kaye,

1987; Mervaala et al., 1993).

Despite the presumed role of 5-HT in attention def-

icits, few studies on serotonergic modulation of involun-

tary attention have been published. One of the main

lines of research on auditory ERP and the 5-HT system

has, instead, concentrated on intensity dependence of

the N1/P2 components, suggesting that low 5-HT ac-

tivity leads to a high-intensity dependence and vice

versa (Hegerl and Juckel, 1993). Several neurophar-

macological studies on P3b, the late positive ERP com-

ponent elicited by task-relevant stimuli, have been

published also, however, with hardly any reported ef-

fects by drugs affecting the 5-HT transmission, such as

methysergide or fenfluramine (Frodé-Bauch et al.,

1999; Meador et al., 1989, 1995; Pritchard et al., 1987).

Finally, to this date, few publications on 5-HT modu-

lation of auditory change detection and MMN genera-

tion have been published. This is unfortunate, because

of the configuration of the auditory-cortical 5-HT fiber

periodicities, which has been found to overlap so-called

isofrequency bands (DeFelipe et al., 1991) associated

with tonotopic organization of the auditory cortex,

which presumably underlies MMN/MMN generation

(May et al., 1999; Tiitinen et al., 1993). This configura-

tion of 5-HT fibers engenders an expectation that the

5-HT projections would also play a role in detection of

pitch changes at the auditory cortex.

The brain serotonergic function can be manipulated

by acute tryptophan depletion (ATD), a dietary inter-

vention that rapidly lowers plasma tryptophan, the

amino acid precursor of serotonin. Previous studies

indicate that ATD also rapidly decreases tryptophan

levels (Carpenter et al., 1998) and 5-HT metabolite

concentrations in cerebrospinal fluid (CSF) (Moja et

al., 1989; Bel and Artigas, 1996; Stancampiano et al.,

1997; Carpenter et al., 1998; Williams et al., 1999) and

reduces 5-HT synthesis at many cortical regions by 5 h

after ATD in humans (Nishizawa et al., 1997). Given

the lack of information on 5-HT modulation of involun-

tary attention and associated electromagnetic brain

responses, the present study aimed at elucidating the

role of the 5-HT system in involuntary attention by

combining behavioral and high-resolution EEG/MEG

measurements after ATD.

(Kennedy et al., 2001). There is also evidence that

serotonin uptake inhibitors may improve attentional

performance in healthy human subjects (Nathan et al.,

2000). However, the relationship between the level of

5-HT turnover and attention might not be linear. In

children with attention-deficit disorder, the impair-

ments in sustained attention might correlate with an

excess amount of peripheral 5-HT metabolites (Oades,

2000); moreover, a recent result suggests that experi-

mentally reduced central 5-HT levels might, in healthy

subjects, improve the ability to focus attention

(Schmitt et al., 2000).

The neural basis of involuntary attention can be

studied by combining behavioral and neurophysiologi-

cal measurements. In a recently developed paradigm

(Schröger and Wolff, 1998a,b), subjects are instructed
to discriminate between two sound durations. Occasion-

ally, the frequency of the tones changes. Increases in

reaction time (RT) and decreases in hit rate (HR)
cauased by such task-irrelevant changes provide a be-

havioral index of involuntary attention shifting, whereas

event-related potentials (ERP) and magnetic

fields, stimulus-locked and averaged epochs of electro-

encephalogram (EEG) and magnetoencephalogram

(MEG), reflect the underlying cerebral events. The

ERP component mismatch negativity (MMN) elicited

at 100–300 ms after the task-irrelevant frequency

change, and its MEG counterpart (MMNm), might re-

flect "automatic" change detection required for invol-

untary attention shifting, whereas the overlapping

N2b component may indicate "active" detection of stim-

ulus changes (Naätänen et al., 1982; Naätänen, 1992).

These overlapping components, MMN/MMNm and

N2b, are in turn often referred to as the N2 deflec-

tion. N2 (MMN/N2b) is followed by the positive P3a

component, possibly reflecting a later stage of novelty

processing, associated with the evaluation of the stim-

ulus change for subsequent behavioral action (Fried-

man et al., 2001). Finally, a slow negativity termed

reorienting negativity (RON), purported to reflect orient-

ing back to the task-relevant activity, is elicited at 400–

600 ms after stimulus onset (Schröger and Wolff, 1998a).

The neurochemical bases of brain response associ-

ated with involuntary attention are not fully known.

Previous results, however, suggest that MMN/MMNm

generation may be decreased by different glutamate-

receptor antagonists in monkeys (J avitt et al., 1996)

and by the cholinergic antagonist scopolamine in hu-

mans (Pekkonen et al., 2001). Temazepine, an agonist

of γ-aminobutyric acid subtype a (GABAa) recep-

tors, might suppress MMN (Hirvonen et al., 1998), and the

GABAa-receptor agonist lorazepam has been shown to

reduce the N2b amplitude (Berchou et al., 1996). The

inhibitory effects of acute ethanol, based on decreased

glutamatergic excitability and increased GABAa inhibi-

tion (for a review, see Harris, 1999), in turn, diminish

behavioral distractibility, reduce the MMN and P3a

amplitude, and prolong the MMN peak latency

(Jäätäinen et al., 1999; for a review, see Jäätäinen

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responses, the present study aimed at elucidating the

role of the 5-HT system in involuntary attention by

combining behavioral and high-resolution EEG/MEG

measurements after ATD.
Subjects and Design

Thirteen healthy, nonsmoking, paid volunteers (21–30 years old; 7 females) were included in the study after a medical examination, blood tests, and mental problem screening by the Symptoms Check List (SCL-90) questionnaire (Derogatis et al., 1973). The subjects reported having used no drugs during the 2 weeks preceding the study; they were instructed to avoid alcohol for at least 48 h and caffeine for 12 h before the MEG recordings. An informed written consent and institutional ethical committee approval were obtained.

The study was conducted in two sessions separated by 1 week, by using a double-blind, crossover design. The order of ATD vs control condition was randomized across the subjects. In each session, the 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 tryptophan, and for large neutral amino acids (LNAA), the subject was administered with either ATD or control mixture, each being composed of 15 amino acids with or without tryptophan, as described by Young et al. (1985). During 20–30 min, the subjects first swallowed capsules containing arginine, cysteine, and methionine (because of their very unpleasant taste) and thereafter 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.

Previous studies indicate that the brain 5-HT synthesis is significantly decreased 5 h after ATD (Nishizawa et al., 1997); therefore, the subjects were allowed to rest in a quiet room for 5 h after the ingestion of the amino acid mixture. After the 5-h resting period, the second blood sample was taken for measurements of plasma tryptophan and LNAA. Subsequently, subjective levels of anxiety, mood, and fatigue were measured with visual analogue scales. Finally, approximately 6 h after the onset of mixture ingestion, the subject was guided to the shielded room for EEG and MEG measurements.

Blood (10 ml) was drawn from the left ulnar vein into a vacuum tube (Venoject, Belgium), immediately cooled, and centrifuged (2000 revolutions/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 the plasma assay for amino acids. Coefficients of variation of 1.2, 1.01, 1.1, 1.1, 5.5, and 1.0% were determined for tryptophan, tyrosine, valine, phenylalanine, isoleucine, and leucine (at 150 pmol/μl), respectively. The detection limit was 10 pmol/μl.

Stimuli and Tasks

The subjects were presented with random sequences of pure tones (10-ms rise and fall times) that equiprobably were either 200 or 400 ms in duration with an 1110-ms offset-to-onset ISI. The tone frequency, being 700 Hz for “standard” tones (p = 0.88) and either 630 or 770 Hz for “deviant” tones (p = 0.06 for each), varied independently of the duration. The stimuli were presented at 60 dB over the individual hearing threshold (confirmed before the measurement) via a plastic tube and an ear piece to the left ear, in two blocks, with resting periods of about 60 s between the blocks.

Figure 1 illustrates the procedure of the forced-choice RT task, in which the subjects were instructed to distinguish the tone duration, varying equiprobably across all frequencies, by pressing a button as rapidly as possible after each tone. More specifically, the subjects were asked to press one button with their left-hand thumb after hearing the 200-ms tone and another button with their right-hand thumb after hearing the 400-ms tone. The subjects were warned in advance that the frequency of the tones could vary but that they were to respond only to the tone duration. The RT and HR were measured to the standards (discounting stimuli occurring immediately after deviants), to the deviants, and to the standards presented immediately after the deviants. To measure the distractibility by the task-irrelevant frequency change, the RT lag to slight deviants (RT to deviants minus RT to standards) and to standards after deviants (RT to standards after deviants minus RT to standards) were calculated.

EEG and MEG Measurements

MEG and EEG were recorded (passband 0.03–100 Hz, sampling rate 394 Hz) in a magnetically shielded...
room (Euroshield Ltd., Eura, Finland). The subjects sat in a comfortable chair with their head placed inside a helmet-shaped whole-head MEG instrument with 122 planar gradiometers (Ahonen et al., 1993) (4-D Neuroimaging Oy, Finland). Each two-channel MEG sensor unit measures two independent magnetic-field gradient components, dBx/Δx and dBz/Δy, the z axis being normal to the local helmet surface. The nose-referenced 64-channel EEG was recorded with an electrode cap with 64 Ag/AgCl electrodes (Virtanen et al., 1996) and biopotential amplifiers specifically designed for simultaneous use with MEG (Virtanen et al., 1997). Bipolar vertical and horizontal electrooculograms (EOG) were recorded (passband 0.5–30 Hz). The location of the marker coils in relation to the cardinal points of the head (nasion, left and right preauricular points) were determined with an Isotrak three-dimensional digitizer (Polhemus, Colchester, VT). The magnetic fields produced by the coils were used for determining the position of the subject’s head in relation to the MEG instrument before each stimulus block.

Specific filters targeting the frequencies that encompass the responses of interest were used. In EEG, digital band-pass filtering was performed off-line at 1–30 Hz for N1 and P2. N2 (MMN/N2b), P3a, and RON responses to deviants were band-pass filtered at 0.5–12 Hz. In MEG, N1m was filtered at 1–30 Hz and MMNm at 0.5–12 Hz. The analysis period for averaged epochs was 800 ms (including a 100-ms prestimulus baseline). The first 20 responses and all the epochs coinciding with EOG, EEG, or MEG changes exceeding 100 µV, 150 µV, or 3000 ft/cm, respectively, were omitted from averaging. At least 100 responses (25 for each deviant subset) to the deviants were averaged.

Electromagnetic brain responses were averaged separately for standard and deviant stimuli. The deviance-related responses were measured from subtraction curves (the pooled deviant-stimulus response minus standard-stimulus response). N2, P3a, and RON peak amplitudes were determined from the 64 EEG channels at the peak latency estimated at the FCz electrode location, by using signal-space projection (Uusitalo and Ilmoniemi, 1997). Specifically, the spatial distribution for each component at the peak latency was estimated from the grand-averaged ERP waveforms measured in the control condition. Subsequently, average peak amplitude of the 64 electrode locations, weighted by the grand-average spatial distribution of a given component, was calculated separately for N2, P3a, and RON. This estimate was used to dissociate the signal from the noise at the individual level, resulting in improved signal-to-noise ratio. The peak latencies and amplitudes for the ERP components to the standard tones were determined at the electrode location FCz. The deviant ERP data of three subjects were rejected because of technical reasons.

As for the MEG waveforms, the peak amplitudes and latencies of N1m and MMNm were quantified from the channel pairs showing the highest-amplitude responses over the left and right temporal areas. (Left monaural stimulation is known to elicit a MEG signal over both the right, contralateral, and the left, ipsilateral, auditory cortices.) The amplitudes were determined as a square root of sums of squares of the amplitudes at the same latency from the orthogonal sensor pair \(a = [(dBx/Δx)^2 + (dBz/Δy)^2]^{1/2}\) showing the largest amplitude response. Equivalent current dipoles (ECD) for N1m and MMNm were fitted separately for the left and right auditory cortices based on signals from a subset of 34 channels above each hemisphere. A spherical head model with the center of symmetry at \(\{x, y, z\} = \{0, 0, 45\} mm\) was utilized. In the coordinate system, the x axis points from the left to the right preauricular point, the y axis is perpendicular to the x axis and passes through the nasion, and the z axis points upward. The ECD parameters were determined to explain the measured data optimally in the least-squares sense.

One-way analysis of variance (ANOVA) was used to analyze the ATD vs control mixture differences in the ERP peak latencies and amplitudes and in the behavioral variables (Statistica 4.1 software; Stat Soft Inc., Oklahoma, USA). As for the MEG data, the N1m and MMNm peak latencies and amplitudes were tested by using a mixture (ATD vs control mixture) by hemisphere ANOVA with a priori contrasts (Winer et al., 1991). A mixture by time point (baseline vs 5 h after administration) ANOVA with contrasts was used for the plasma total and free tryptophan concentrations and for the tryptophan/LNAA and LNAA/tyrosine ratios. For these four biochemical variables, the difference between the baseline and the 5-h concentrations was calculated and correlated with EEG, MEG, and behavioral measures of involuntary attention by using the Pearson correlation coefficient.

**RESULTS**

The biochemical results are shown in Table 1. No significant differences between ATD and control conditions were observed in any of the baseline concentrations. However, there was a highly significant mixture (ATD vs control mixture) by time point (baseline vs 5 h) interaction in ANOVA (F(1,12) = 41.3, P < 0.001) for plasma total tryptophan levels, which were reduced by 75% after ATD and increased by 20% after control mixture administration. Similar mixture by time point interaction (F(1,12) = 6.8, P < 0.05) was observed for the plasma free tryptophan, indicating a 35% decrease after ATD and a 20% increase after control mixture. The tryptophan/LNAA ratio was significantly (F(1,12) = 37.1, P < 0.001) more decreased after ATD (92% decrease) than after control mixture administra-
tion (32% decrease). However, the ATD effects on LNAA/tyrosine were nonsignificant.

ATD significantly (F(1,10) = 12.2, P < 0.01) increased the depressive mood (visual analogue scale score 40 ± 21 after control mixture and 20 ± 18 after ATD) in the subjects, but the levels of anxiety or vague-ness were not significantly affected. The depressive mood did not, however, correlate with the biochemical variables (concentration difference, baseline vs 5 h after administration).

The behavioral data are presented in Table 2. There were no effects in the overall RT or HR for standards or in the RT lag or HR for deviants. However, in standards immediately after deviants, significantly increased RT lag (F(1,12) = 7.2, P < 0.05) and a trend toward HR reduction were observed after ATD (Table 2). However, the correlation of these behavioral results with the biochemical variables was not statistically significant.

The ERP results are presented in Table 3. Further, Fig. 2 indicates that the peak amplitude of N2, elicited by the task-irrelevant deviants, was significantly reduced by ATD (F(1,9) = 6.5, P < 0.05), but no significant effects were observed in the N2 peak latency or in P3a or RON. However, there was a trend of RON reduction after ATD. The P1, N1, and P2 peak amplitudes and latencies were very similar after ATD and control condition.

The two-way ANOVA indicated that MMNm peak latency, determined from the waveforms, was significantly (F(1,12) = 10.3, P < 0.01) increased after ATD (Fig. 3, Table 4). The contrasts indicated that this effect was significant over both right (F(1,12) = 6.3, P < 0.05) and left (F(1,12) = 9.1, P < 0.05) hemispheres, i.e., ipsilaterally and contralaterally to the ear stimulated. No significant effects were found in the MMNm waveform amplitude or in the ECD source locations or amplitudes for MMNm (Table 4). The N1m peak amplitudes and latencies, determined from the MEG waveforms, were very similar after ATD and control condition. Also, no significant effects were observed in the ECD source locations or dipole amplitudes of N1m (Table 5).

DISCUSSION

Electromagnetic responses elicited by task-irrelevant frequency changes were significantly affected by ATD. The N2 deflection, presumed to consist of the automatic MMN and active N2b components (Nää-tänen et al., 1982; Nää-tänen, 1992), was significantly reduced in amplitude. In MEG, which is sensitive to tangentially oriented source components (and which poorly detects the N2b component), the peak latency of MMNm was significantly increased over both hemi-spheres. Blood analyses indicated a significant reduction of plasma total and free tryptophan levels after

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**TABLE 1**

<table>
<thead>
<tr>
<th>Group Mean ± SD of Amino Acid Concentrations</th>
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<tr>
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<tr>
<td>ATD</td>
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<tr>
<td>Control</td>
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<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>ATD</td>
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<tr>
<td>Control</td>
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<tr>
<td>---------------------------------------------</td>
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<tr>
<td>Total tryptophan (µmol/L)</td>
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<tr>
<td>63.8 ± 12.7</td>
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<tr>
<td>61.9 ± 27.1 N.S.</td>
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<tr>
<td>15.9 ± 4.2</td>
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<tr>
<td>100.9 ± 25.8***</td>
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<tr>
<td>Free tryptophan (µmol/L)</td>
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<tr>
<td>5.4 ± 2.3</td>
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<tr>
<td>7.1 ± 3.3 N.S.</td>
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<tr>
<td>3.5 ± 1.7</td>
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<tr>
<td>8.5 ± 3.9**</td>
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<tr>
<td>Tryptophan/LNAA</td>
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<tr>
<td>15.6 ± 5.5</td>
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<tr>
<td>12.1 ± 28.1 N.S.</td>
</tr>
<tr>
<td>1.3 ± 0.7</td>
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<tr>
<td>8.2 ± 2.1***</td>
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<tr>
<td>LNAA/tyrosine</td>
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<tr>
<td>10.7 ± 1.7</td>
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<tr>
<td>10.6 ± 28.1 N.S.</td>
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<tr>
<td>5.1 ± 1.8</td>
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<tr>
<td>4.7 ± 1.5 N.S.</td>
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Note. LNAA, large neutral amino acids; N.S., nonsignificant in contrasts.

* P < 0.05.

**TABLE 2**

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<thead>
<tr>
<th>Mean ± SD of Reaction Time (RT) and Hit Rate (HR) Variables</th>
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<tr>
<td>ATD</td>
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<tr>
<td>Control</td>
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<td></td>
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<tr>
<td>RT (ms) to standards</td>
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<tr>
<td>602 ± 60</td>
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<tr>
<td>602 ± 43</td>
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<tr>
<td>RT lag (ms) to deviants</td>
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<tr>
<td>22 ± 24</td>
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<tr>
<td>15 ± 20</td>
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<tr>
<td>RT lag (ms) after deviants</td>
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<tr>
<td>14 ± 17</td>
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<tr>
<td>2 ± 14*</td>
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<td>HR (%) to standards</td>
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<tr>
<td>90 ± 14</td>
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<tr>
<td>95 ± 2</td>
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<tr>
<td>HR (%) to deviants</td>
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<tr>
<td>89 ± 14</td>
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<tr>
<td>95 ± 3</td>
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<tr>
<td>HR (%) after deviants</td>
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<tr>
<td>88 ± 16</td>
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<td>96 ± 3</td>
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* P < 0.05.

**TABLE 3**

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<th>Mean ± SD of the ERP Peak Amplitudes and Latencies</th>
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<td>Control</td>
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<tr>
<td></td>
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<tr>
<td>Peak amplitude (µV)</td>
</tr>
<tr>
<td>N1 -3.3 ± 2.1</td>
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<tr>
<td>-3.3 ± 1.8</td>
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<tr>
<td>98 ± 8.0</td>
</tr>
<tr>
<td>99 ± 8.8</td>
</tr>
<tr>
<td>P2 1.7 ± 1.6</td>
</tr>
<tr>
<td>1.6 ± 2.1</td>
</tr>
<tr>
<td>173 ± 11.0</td>
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<tr>
<td>174 ± 15.4</td>
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<tr>
<td>N2 -1.6 ± 1.0</td>
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<tr>
<td>-2.4 ± 0.6*</td>
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<tr>
<td>183 ± 34.9</td>
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<td>179 ± 30.1</td>
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<tr>
<td>P3a 3.9 ± 2.0</td>
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<tr>
<td>3.7 ± 2.4</td>
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<tr>
<td>328 ± 20.5</td>
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<tr>
<td>324 ± 30.8</td>
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<tr>
<td>RON -1.2 ± 2.0</td>
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<tr>
<td>-2.1 ± 1.57</td>
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<tr>
<td>510 ± 72.2</td>
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</tr>
</tbody>
</table>

* P < 0.05.
ATD in the subjects. Previous studies have shown that such a reduction of plasma tryptophan concentration rapidly decreases central 5-HT metabolite concentrations in human CSF (Carpenter et al., 1998). Positron emission tomography (PET) studies have further indicated that the brain 5-HT synthesis is significantly diminished by 5 h after ATD in humans (Nishizawa et al., 1997). Therefore, the present ERP and MEG results may be related to changes in the brain 5-HT function after ATD.

**TABLE 4**

Mean ± SD Peak Amplitudes and Latencies of N1m and MMNm, Elicited to Left-Ear Tones

<table>
<thead>
<tr>
<th></th>
<th>Peak amplitude (fT/cm)</th>
<th>Latency (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATD</td>
<td>Control</td>
</tr>
<tr>
<td>N1m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>47 ± 20</td>
<td>51 ± 14.5</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>35 ± 14</td>
<td>32 ± 16.6</td>
</tr>
<tr>
<td>MMNm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>44 ± 16</td>
<td>50 ± 15.0</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>27 ± 10</td>
<td>30 ± 11.2</td>
</tr>
</tbody>
</table>

* P < 0.05.
MMN and MMNm to Task-Irrelevant Sound Changes

Supporting previous observations (Hari et al., 1984), the MEG dipole modeling results suggested that the MMNm, supposed to be a magnetic counterpart of the supratemporal MMN subcomponent (Nääätänen, 1992), is generated at the temporal lobe, near the primary auditory cortices. PET studies have shown that ATD significantly reduces 5-HT synthesis at the vicinity of this brain region in humans (Nishizawa et al., 1997). Moreover, a large amount of 5-HT immunoreactive fibers has been found at the primate auditory cortex (Campbell et al., 1987), and cat studies suggest that there are 5-HT fibers configured into periodicities that overlap auditory-cortical isofrequency bands (DeFelipe et al., 1991). The 5-HT neurons have, furthermore, been shown to interact with GABA neurons (DeFelipe et al., 1991), crucial for frequency tuning of tonotopically organized cortical auditory neurons (Wang et al., 2000) and for the MMN generation (Javitt et al., 2000) and for the MMN generation (Javitt et al., 1991). N2 reduction could partially follow from temporarily repressed activation of the frontal MMN subcomponent. Against this theoretical background (Nääätänen, 1992), the MMNm peak latency increase suggests delayed automatic change detection in the auditory cortex by ATD. However, since the MMNm amplitude was not affected, the N2 reduction may also have been partially accounted for by effects on the frontal MMN subcomponent, which is poorly detected by MEG. Given that frontal brain lesions have been shown to reduce MMN (Alho et al., 1994), N2 reduction could partially follow from temporarily repressed activation of the frontal cortex after ATD. This theoretical perspective could also be used to explain the above-mentioned neuropsychological results of Schmitt et al. (2000): Improved performance in the Stroop and dichotic-listening tasks after ATD could as well follow from reduced distractibility task-irrelevant processing (e.g., by attenuated

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Condition</th>
<th>x (mm)</th>
<th>y (mm)</th>
<th>z (mm)</th>
<th>Q (nAm)</th>
<th>Goodness of fit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1m</td>
<td>Control</td>
<td>51 ± 8</td>
<td>7 ± 9</td>
<td>56 ± 10</td>
<td>21 ± 9</td>
<td>90 ± 8</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>49 ± 7</td>
<td>8 ± 12</td>
<td>62 ± 13</td>
<td>20 ± 9</td>
<td>88 ± 11</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral</td>
<td>-50 ± 8</td>
<td>1 ± 11</td>
<td>61 ± 17</td>
<td>14 ± 8</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>MMNm</td>
<td>Control</td>
<td>47 ± 13</td>
<td>15 ± 19</td>
<td>57 ± 15</td>
<td>27 ± 16</td>
<td>81 ± 8</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>46 ± 10</td>
<td>20 ± 14</td>
<td>62 ± 13</td>
<td>21 ± 10</td>
<td>82 ± 5</td>
</tr>
<tr>
<td></td>
<td>Ipsilateral</td>
<td>-42 ± 10</td>
<td>5 ± 19</td>
<td>58 ± 25</td>
<td>25 ± 15</td>
<td>77 ± 6</td>
</tr>
</tbody>
</table>

TABLE 5
Mean ± SD of the MEG Source Modeling Results
P3 to Task-Irrelevant Sound Changes

Supporting previous observations, a clear positive deflection with a peak latency at around 300 ms post-stimulus was elicited by the task-irrelevant frequency change (Schröger and Wolff, 1998a,b). According to the present theories (for a review, see Näätänen, 1992), this P3 to task-irrelevant stimulus is mainly dominated by the P3a component. Previous neuropharmacological studies have shown that P3a is suppressed by both ethanol (Jääskeläinen et al., 1999) and the dopamine D₂-receptor antagonist haloperidol (Kähkönen et al., 2002), whereas muscarinic agonists may enhance the P3a counterpart in monkeys (O’Neill et al., 2000). As for the 5-HT system, few prior efforts have been published, but since no significant ATD effect on the P3 to task-irrelevant tones were observed here, one might suggest that this response might not be modulated by the 5-HT system. Perhaps the role of 5-HT modulation is more prevalent in the earlier, modality-specific, phases of involuntary attention that are thought to be represented by the subcomponents of MMN (Näätänen, 1992). However, this does not mean that the MMN/N2b components should necessarily be more sensitive measures of involuntary attention than P3a per se.

It has to be noted that the task-irrelevant frequency change (70 Hz decrease or increase) was multiply repeated during the stimulus block; in other words, this change was not a “genuinely” novel stimulus. Additionally, the paradigm was actually geared not toward eliciting robust P3a responses, but rather toward minimizing the N1/N2b contamination of the MMN response. Thus, further P3a studies utilizing novel tones embedded in stimulus blocks would be informative. Another factor complicating the interpretation of the present P3a results could be related to possible overlap by other P3 subcomponents, such as P3b. This is suggested, for instance, by the relatively posterior grand-average scalp distribution of the P3 to task-irrelevant sound changes (Fig. 2) and by its peak latency, which somewhat resembles that of the P3b. However, the fact that the P3 to task-irrelevant sounds was determined from the subtraction curves reduces the possibility that such P3b overlap would be associated with the task-relevant activity. Furthermore, little evidence of 5-HT modulation of the P3b component has been found thus far (see Frodl-Bauch et al., 1999), and it is therefore improbable that the (possibly) overlapping P3b could have altered P3a results with respect to 5-HT.

MEG and EEG Responses to Standard Tones

No ATD effects were observed in N1, P2, and N1m. This is generally in line with the observation that the serotonin-depleting agents, such as methysergide (Meador et al., 1989) or fenfluramine (Meador et al., 1995), do not significantly affect N1 or P2 amplitude or peak latency. Thus, it appears that the 5-HT system does not modulate the generation of ERP and MEG responses elicited by constantly repeated standard tones at the 100- to 200-ms latency range. However, it has to be noted that there is some evidence that the intensity dependence of N1/P2 components could be regulated by 5-HT (Hegerl and Juckel, 1993).

Behavioral Effects

The behavioral distractibility, i.e., RT lag to frequency deviants, was not significantly affected by ATD. One explanation is that EEG and MEG are more sensitive in the detection of impairments in 5-HT modulation of involuntary attention. This could be based, for example, on a better signal-to-noise ratio in electrophysiological vs behavioral measures. However, it has to be noted that this interpretation might also be complicated by the adaptive changes (down-regulation and/or up-regulation) of receptors after acute drug challenge or a comparable manipulation such as ATD. A recent PET study suggests adaptive changes in 5-HT₂ receptors subtype after ADT, which was interpreted to be an adaptive change to acutely decreased 5-HT in the brain (Yatham et al., 2001). Therefore a chronic, and more severe, deficit in the brain 5-HT function (e.g., in specific patient groups) might produce more pronounced attention effects. Furthermore, despite the fact that the RT lag to task-irrelevant tones was not affected by ATD, the RT lag to standards immediately after task-irrelevant frequency change was significantly increased. There was also a trend toward impaired HR to these stimuli. Thus, it appears that ATD impaired the subjects’ ability to shift back to the relevant task after distraction. In addition, there was a trend toward reduction of RON that has been presumed to index reorienting after distraction (Schröger and Wolff, 1998a).

Possible Limitations

ATD appeared to increase the subjective feelings of depression. However, this result was observed only in the visual analogue scale and was not confirmed with more objective means of mood assessment. Furthermore, it is important to note that the present results were mainly observed in physiological measures of involuntary attention, whereas the task-relevant performance (simple RT and HR in duration discrimination) was not affected at all.

Finally, a possible limitation to the present conclusions may stem from confounding effects of other transmitter systems. For instance, based on depletion studies of other amino acids (Harmer et al., 2001), possible tyrosine deficiency might have interfered with central catecholamine function. However, the results indicated
that LNA/tyrosine ratio was not significantly affected by ATD, showing that metabolism of dopamine and noradrenaline was not affected in the present experiment.

**CONCLUSION**

The MEG and EEG results suggest that the reduction of the brain 5-HT function by ATD may delay automatic change detection at the auditory cortex and thus impair initiation of involuntary attention shifting reflected by the N2 component. This process could also have been partially overlapped by changes in 5-HT modulation of attentional resource allocation to the task-relevant activity. Future studies combining EEG, MEG, and behavioral measures could help us to enhance the understanding of the neurochemical basis of various attentional disorders.

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