

Auditory Evoked Potentials Reflect Serotonergic Neuronal Activity—A Study in Behaving Cats Administered Drugs Acting on 5-HT_{1A} Autoreceptors in the Dorsal Raphe Nucleus

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A valid indicator of central serotonergic neurotransmission would be useful for various diagnostic and psychopharmacological purposes in psychiatry. However, known peripheral serotonergic measures only partially reflect serotonergic function in the brain. Previous findings suggest that the intensity dependence of auditory evoked potentials (AEPs) is closely related to central serotonergic activity. The present study examines the effects of microinjection of a 5-HT_{1A} agonist (8-OH-DPAT) and a 5-HT_{1A} antagonist (spiperone) into the dorsal raphe nucleus (DRN) on AEP recorded epidurally from the primary and secondary auditory cortex in behaving cats. We found a stronger intensity dependence only of AEP

from the primary auditory cortex after 8-OH-DPAT, which inhibits the firing rate of serotonergic DRN neurons, and a weaker intensity dependence after spiperone, which increases serotonergic cell firing, as compared to baseline measurements. These results demonstrate that the intensity dependence of AEP is inversely related to serotonergic neuronal activity and that it may be a promising tool for assessing central serotonergic function in humans (e.g., identifying patients with low serotonergic neurotransmission). [Neuropsychopharmacology 21:710–716, 1999] © 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.

KEY WORDS: Serotonin; Auditory evoked potentials; Auditory cortex; Dorsal raphe nucleus; 5-HT_{1A} receptor

Disturbed activity of the serotonergic system is assumed to play a major role in such psychiatric disorders as depression, obsessive compulsive and anxiety disorders,

as well as alcoholism and schizophrenia (Siever et al. 1991; Murphy et al. 1998). Several new antidepressants and antipsychotics are specifically designed to influence central serotonergic neurotransmission (Cowen 1991). A valid indicator of serotonergic function in the brain could be used to identify psychiatric patients with a serotonergic dysfunction in order to treat them more specifically. However, peripheral measures of serotonin levels or its metabolites only partially reflect central serotonergic activity (Murphy 1990). Several findings from basic and clinical research suggest that a strong stimulus intensity dependence of auditory evoked potentials (AEPs), that is the increase of AEP amplitudes attributable to increasing tone intensity (loudness), is related to

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low serotonergic activity, and vice versa. The intensity dependence is reduced by serotonin enhancing substances (zimelidine, lithium, ethanol, methamphetamines); this variable predicts the response to serotonin agonists and is related to serotonin-associated personality traits and behavior (sensation seeking, impulsivity, suicidality, antisocial/aggressive behavior), as well as, to 5-HIAA levels in cerebrospinal fluid (Hegerl and Juckel 1993).

Recently, we established an animal model of the intensity dependence in behaving cats (Juckel et al. 1996). We found that the intensity dependence of the cat AEP first positive component with the highest functional similarity to that of human AEP was enhanced by the systemic administration of the serotonin antagonist ketanserin and reduced by the serotonin agonist 8-OH-DPAT, which both may be a postsynaptic effects (Juckel et al. 1997). These changes were found only for the intensity dependence of the primary auditory cortex, which corresponds to the known higher innervation of the primary, rather than the secondary auditory cortex by serotonergic fibers (Lewis et al. 1986). To investigate the relationship between the intensity dependence of AEP and the (presynaptic) activity of serotonergic neurons itself, the somatodendritic 5-HT_{1A} autoreceptors of

the serotonergic neurons were influenced by local application of 8-OH-DPAT and spiperone into the dorsal raphe nucleus (DRN). The 5-HT_{1A} agonist 8-OH-DPAT strongly suppresses the firing rate of serotonergic neurons in the cat DRN, while the 5-HT_{1A} antagonist spiperone enhances the firing rate of these neurons (Fornal et al. 1994).

METHODS

The intensity dependence of AEP was recorded epidurally over the primary and secondary auditory cortex (AI, AII) in five adult male freely moving cats, weighting 2.8 to 3.5 kg. The cats were cared for and used in accordance to the Guide for the Care and Use of Laboratory Animals. As recording electrodes, 0.23 mm diameter enamel-coated stainless steel wires, etched for 1 mm at their tips, were used; a stainless steel screw above the bone of the frontal sinus served as reference electrode. The auditory stimuli were 4-kHz tones (sine wave bursts) of 5 ms duration, at four intensity levels (50, 60, 70, 80 dB peak equivalent SPL), and were presented in random order through a tiny bone conductor fixed to the animals' foreheads (randomized ISI of 1.8–2.2 s; 42 stimuli of each intensity). Electro-

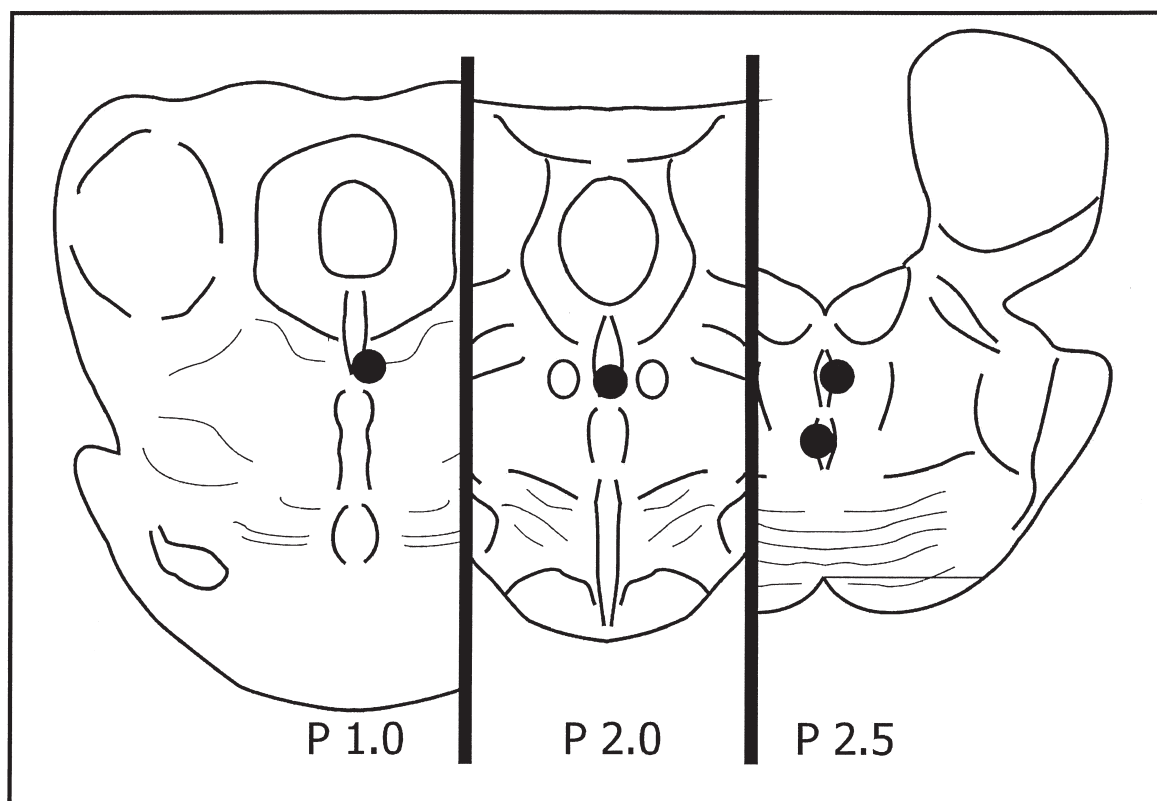


Figure 1. The localization of the injection sites in four animals studied. (P 1.0–P 2.5: the AP stereotaxic positions of the slides, according to Snider and Niemer 1961).

physiological data were collected with a sampling rate of 1 kHz in a time range of 50 ms prestimulus to 200 ms post-stimulus, and they were corrected for the prestimulus baseline, as well as for artifacts. After averaging, slopes of all possible connections between the four amplitude values of the AEP first positive components were calculated for each recording of each cat separately. The median of these slopes ($n = 8$) was taken as the intensity dependence [$\mu\text{V}/10\text{ dB}$] of the AEP first positive component, recorded from the primary and secondary auditory cortex. A detailed description of the AEP method used is given elsewhere (Juckel et al. 1996, 1997). A 22-gauge stainless steel guide cannula was implanted through the cerebellum at an angle of 40 to 50°. Through this guide cannula, a 5 μl Hamilton syringe (26-gauge) was inserted, so that its tip was in the DRN (stereotaxic coordinates: AP -0.5 , L 0.0 , V -0.5) (Jacobs et al. 1984). \pm 8-OH-DPAT HBr [$2\text{ }\mu\text{g}/1\text{ }\mu\text{l}$ saline (Sharp et al. 1989)], spiperone ($2\text{ }\mu\text{g}/1\text{ }\mu\text{l}$ saline), and saline ($1\text{ }\mu\text{l}$, 0.9%) were injected on different occasions separated by at least 48 hours. Injections were done over 1 minute, and the syringe was let in place for another minute to allow the solution to diffuse away from the site. Experiments were performed at the same time in each animal in the morning hours (8:00–10:00 A.M.). AEP record-

ings were immediately obtained before (baseline) and starting 2, 10, and 30 minutes after injection, while cats were in a quiet waking state. The quiet waking state was defined by usual criteria: cats were sitting in the experimental cage with no apparent sign of noticing their environment. Electrophysiologically, this state was characterized by a low-voltage, high-frequency cortical EEG, theta activity of the dorsal hippocampus, and frequent eye movements in the EOG. The localization of the injection sites within the DRN of four cats studied (marked by 1 μl methylblue at the end of the experiments) was verified histologically (Figure 1). In one cat, histology revealed that the tip of the syringe was located shortly behind the cerebellum in the cerebral aqueduct during drug injections. Data were statistically analyzed using analysis of variance (ANOVA) with repeated measures, followed by post hoc comparisons (Student-Newman-Keuls' test).

RESULTS

Following injection of either 8-OH-DPAT, spiperone, or saline, all cats remained in the quiet waking state throughout the experiments and showed no apparent

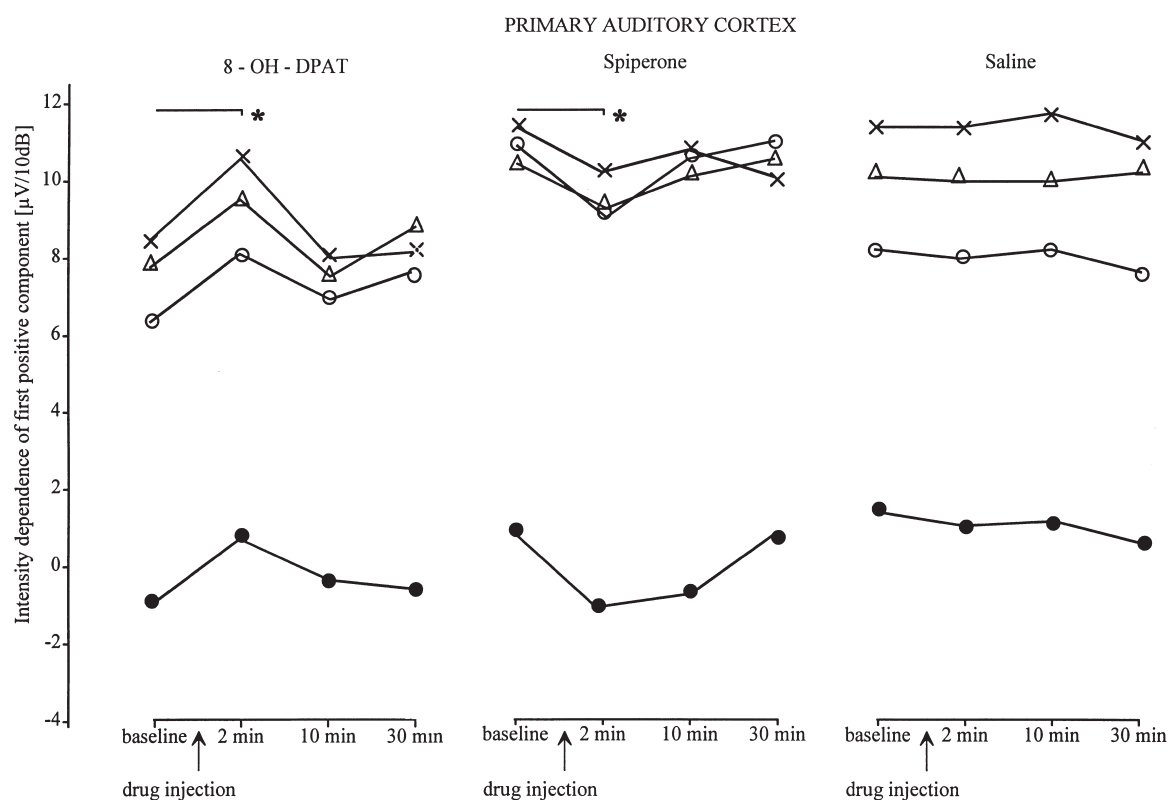


Figure 2. Intensity dependence of AEP first positive component, recorded epidurally from the primary auditory cortex, before (baseline) as well as 2, 10, and 30 minutes after injection of 8-OH-DPAT, spiperone, and saline into the DRN of the four cats. * $p < .05$, as compared to the baseline recordings; Student-Newman-Keuls' multiple comparison test.

behavioral changes. The intensity dependence of the AEP first positive component recorded from the primary auditory cortex was significantly increased 2 minutes after injection of 8-OH-DPAT into the DRN (means \pm SEM: 5.48 ± 2.21 vs. 7.15 ± 2.33 $\mu\text{V}/10$ dB, $F_{(3/9)} = 4.06$, $p < .05$; Figure 2); whereas it was significantly decreased 2 minutes after injection of spiperone (8.88 ± 3.03 vs. 7.33 ± 3.26 $\mu\text{V}/10$ dB, $F_{(3/9)} = 3.91$, $p < .05$). The intensity dependence of each cat injected into the DRN showed the same direction of change within the 15-minute pre-post period after injection of 8-OH-DPAT and of spiperone. Original AEP recordings of one cat and their changes after drug application are shown in Figure 3. In the 10 and 30 min recordings after 8-OH-DPAT and spiperone, the intensity dependence tended to return to the baseline values. Control saline injections had no effect on the intensity dependence of the AEP first positive component recorded from the primary auditory cortex ($F_{(3/9)} = 1.09$, ns). The intensity dependence of the AEP first positive component recorded from the secondary auditory cortex was not significantly altered by the injection of either 8-OH-DPAT or spiperone ($F_{(3/9)} = 0.67$ and $F_{(3/9)} = 0.93$, ns; Figure 4). In that cat, in which 8-OH-DPAT, spiperone, and saline were injected into the ventricular system, there was no significant change of the intensity dependence of the AEP first positive component recorded from the primary auditory cortex (before and after 8-OH-DPAT: 12.7, 12.0, 12.5, 12.8, 12.9 $\mu\text{V}/10$ dB; before and after spiperone: 13.5, 13.7, 13.6, 14.2, 14.0 $\mu\text{V}/10$ dB; before and after saline: 13.3, 13.0, 13.0, 13.4, 13.1 $\mu\text{V}/10$ dB).

DISCUSSION

The present study is the first to demonstrate an inverse relationship between the intensity dependence of AEP, recorded from the primary, but not of the secondary, auditory cortex, and brain serotonergic neuronal activity in behaving cats. The inactivation of serotonergic DRN neurons by local application of the 5-HT_{1A} agonist 8-OH-DPAT into the DRN increased the intensity dependence of AEP. Reciprocally, the activation of serotonergic DRN neurons by local administration of the 5-HT_{1A} antagonist spiperone decreased the intensity dependence of AEP. Studies have shown that the primary auditory cortex, particularly layer IV, which receives most of the specific thalamic sensory input, is one of the most highly innervated cortical areas by serotonergic fibers (Jacobs and Azmitia 1992; Lewis et al. 1986). Innervation of cortical layer IV is mostly derived from the serotonergic DRN neurons (Wilson and Molliver 1991). This nucleus, well known as main source of serotonergic efferents to the forebrain, is, therefore, in an excellent position to modulate neuronal activity at the initial stage of sensory processing in the primary auditory cortex. This modulation of the sensory function by serotonergic fibers from the raphe nuclei seems to be mostly inhibitory. Electrical stimulation of the raphe nuclei or microiontophoretic application of serotonin into the cortex were found to lead to a decrease of amplitudes of sensory evoked potentials as well as of evoked responses of cortical neurons (Samanin et al. 1972; Mantz et al. 1990; Waterhouse et al. 1990). The inhibitory function of

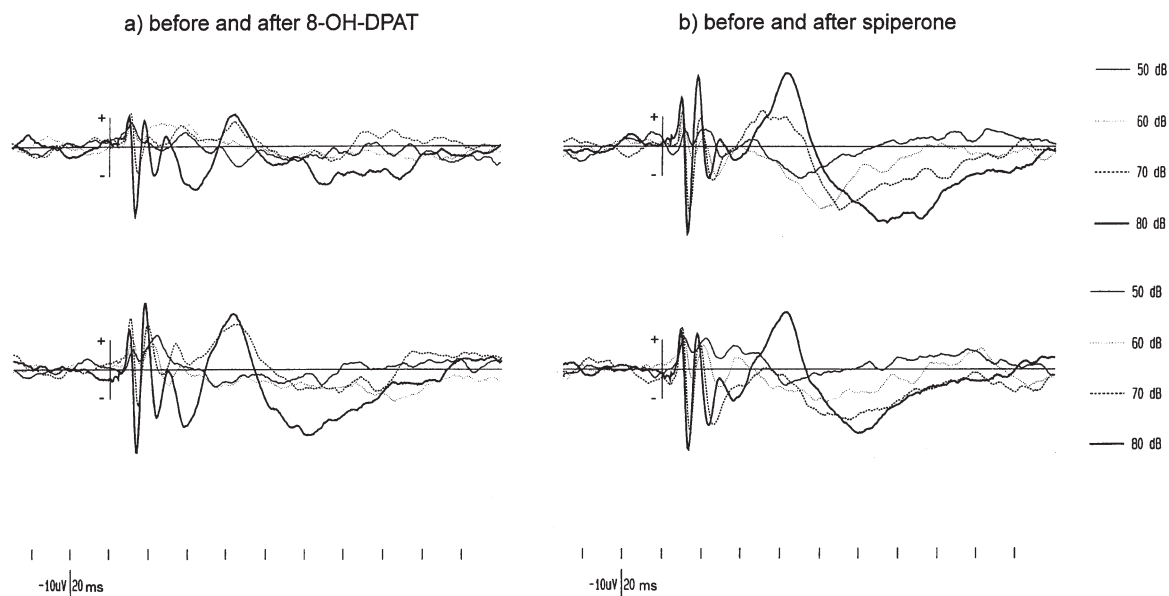


Figure 3. Original AEP recordings from the primary auditory cortex of one cat before and 2 min after injection of 8-OH-DPAT or spiperone into the DRN. The AEP first positive component is the first deflection to the bottom after auditory stimulation, which is indicated as y-axis.

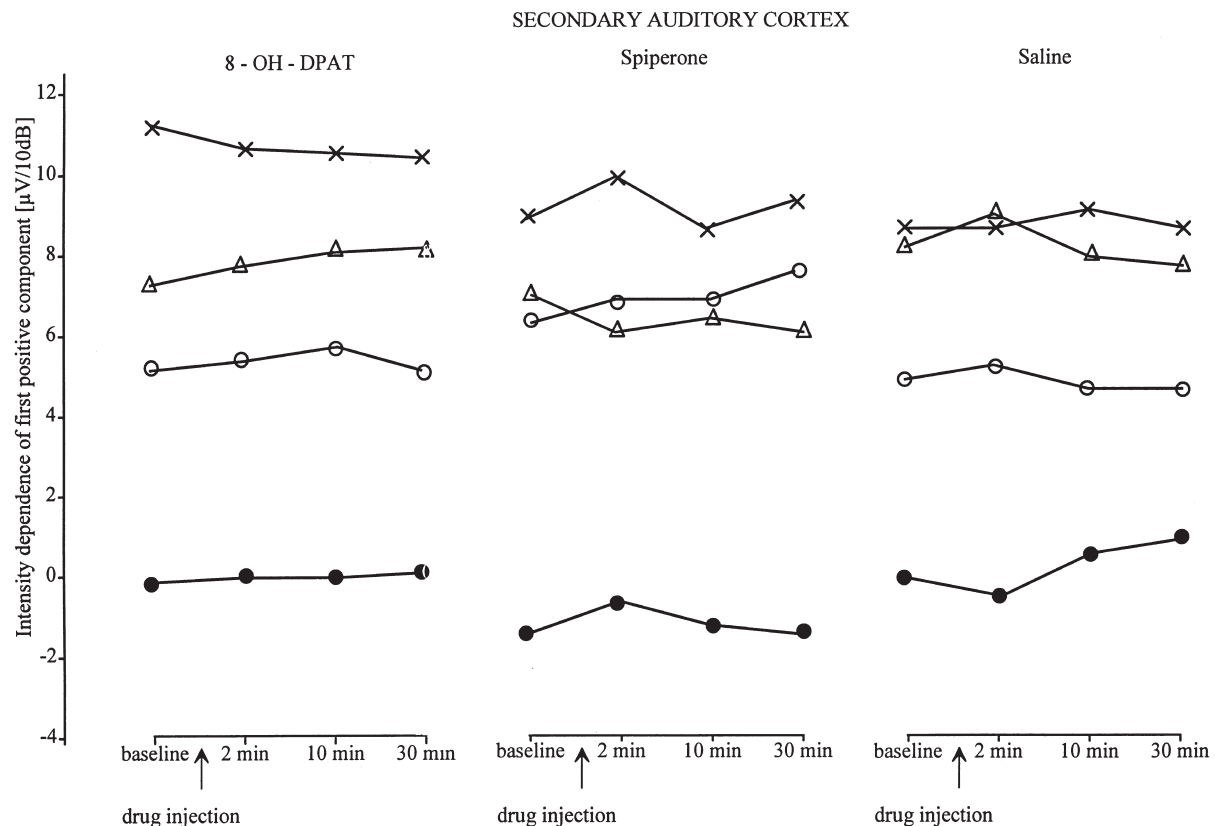


Figure 4. Intensity dependence of AEP first positive component, recorded epidurally from the secondary auditory cortex, before (baseline) as well as 2, 10, and 30 minutes after injection of 8-OH-DPAT, spiperone, and saline into the DRN of the four cats.

the serotonergic system is also supported by the findings of the present study, showing a decrease of the intensity dependence from the primary auditory cortex, as a measure of cortical reability to tones of different loudness, after enhancement of serotonergic activity. Thus, the serotonergic systems is possibly aimed to protect the cortex from sensory overstimulation.

However, three major concerns related to the results of the study has to be addressed. First is the variability of the baseline values of the intensity dependence. Such variability was observed only over a time period of several days or weeks. This variability was also seen in previous studies (Juckel et al. 1996, 1997), and could be caused by technical reasons concerning the recording electrode; for example, small changes of the electrode environment over longer time. The changes of the intensity dependence we found after drug application are very unlikely to be time effects within the experimental period of 45 minutes, because the intensity dependence of the AEP first positive component did not intraindividually change after injection of saline. In addition, the intensity dependence recorded simultaneously from the secondary auditory cortex did not change after the injection of 8-OH-DPAT, spiperone, and saline into the DRN. Thus, changes of the intensity dependence recorded from the primary auditory

cortex, found in all of the cats, may be caused by the drug effects on the firing rate of DRN serotonergic neurons.

Concerning histology, injection sites outside the DRN as well as tissue damage of the DRN could be factors influencing the results. We found, however, the injection sites of all animals showing significant changes of the intensity dependence after drug application in that area where the DRN is usually located. There was no hint for any significant damage of the DRN tissue, indicating quite normal functioning of the DRN serotonergic cells during the experiments. Given the size of the DRN with 3 to 4 mm in the rostral-caudal plane, it seems very likely that most of the drug solution had exerted its influence only within the DRN. It is also likely that the effects seen with the intensity dependence of the primary auditory cortex after drug application into the DRN were mediated by the somatodentritic 5-HT_{1A} autoreceptors within the DRN, because 5-HT_{1A} receptors are present only on the cell membranes of the DRN serotonergic neurons in this anatomic region (Sotelo et al. 1990). Because there was no significant change of the intensity dependence in the cat, in which the drugs were applied into the ventricular system, the changes of the intensity dependence from the primary auditory cortex reported here seem to be site specific for the DRN

than the result of any leakage of the injectate into the ventricular system.

Finally, the relationship between the intensity dependence and the serotonergic system seems to be not specific in an exclusive manner. Although the noradrenergic and dopaminergic neurotransmission probably have no direct influence on the intensity dependence of AEP first positive component, atropine administered intravenously in cats exhibited a clear effect on the intensity dependence of the primary auditory cortex (Juckel et al. 1997). Thus, it is assumed that the serotonergic and cholinergic system interact synergistically in the modulation of the intensity dependence, but an important part of the variance of the intensity dependence may be explained by the serotonergic neurotransmission alone.

In humans, activity from the primary and secondary auditory cortex can be separated by dipole source analysis (Scherg and Von Cramon 1990). Using this method, it was shown that only the intensity dependence of the tangential dipole, reflecting activity of the primary auditory cortex, is related to serotonergic measures (Hegerl and Juckel 1993). Although the extrapolation of the present results in cats to humans must be made cautiously, and the specificity might be limited, some evidence suggests that the intensity dependence of AEP is of clinical value in assessing central serotonergic function. For example, several studies have revealed that patients with low serotonergic activity, indicated by a strong intensity dependence before treatment, respond more favorably to drugs that increase serotonergic neurotransmission, such as fluoxetine (Paige et al. 1994), fluvoxamine (Hegerl and Juckel 1993), fenfluramine (Bruneau et al. 1989), paroxetine (Hegerl et al. 1996), or lithium (Hegerl and Juckel 1993) than patients with normal or high serotonergic activity, as indicated by a weak intensity dependence of AEP. Regarding the fact that brain serotonin cannot be measured directly and that no valid biochemical indicator of the central serotonergic function is available, the finding of a close relationship between the intensity dependence recorded from the primary auditory cortex and central serotonergic activity is of great clinical interest. Thus, the intensity dependence could, at the least, be used as a gross index of the amount of serotonin released into the synapses; this is information that cannot be provided by any other method known today.

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