

## ORIGINAL PAPER

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## Changes of the neurotransmitter serotonin but not of hormones during short time music perception

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**Abstract** We studied the impact of the perception of pleasant and unpleasant music, as rated by healthy subjects with a psychometric scale, on the hormones prolactin and ACTH. In addition, the neurotransmitter serotonin (5-HT) was studied using the platelet model for central neurotransmission of 5-HT. We did not observe any significant changes of prolactin and ACTH during the perception of different kinds of music. The 5-HT content of platelets, however, was higher during the perception of pleasant music as compared to the perception of unpleasant music indicating an increased release of 5-HT during unpleasant music (748 ng/10<sup>9</sup> platelets vs. 699 ng/10<sup>9</sup> platelets;  $p < 0.014$ ). The difference of the 5-HT level was significantly correlated to the score of unpleasantness as rated by the subjects. Our data suggest that perception of unpleasant music induces increased release and decreased peripheral and possibly also central intracellular content of 5-HT.

**Key words** Serotonin (5-HT) · Music perception · Prolactin · ACTH

### Introduction

The perception of music has been studied by several neurophysiological methods including EEG analysis (Breitling et al. 1987) and functional neuroimaging (Platel et al. 1997; Zatorre et al. 1994). To date, little attention has been focussed on the endocrine and transmitter changes during music perception and on their relation to the subjective and affective attitude towards the music presented.

Previous studies on endocrine changes during music perception examined stress hormones such as cortisol and ACTH, prolactin, catecholamines, beta-endorphin, growth hormone or atrial natriuretic peptide (Brownley et al. 1995; Gerra et al. 1998; McKinney et al. 1997; Miluk-Kolosa et al. 1994; Möckel et al. 1994; VanderArk and Ely 1992; VanderArk and Ely 1993). To summarise the results of these studies, cortisol and ACTH levels may be influenced by music perception reflecting the affective stress or relaxation response to a certain kind of music whereas prolactin is not affected by music perception in most of the studies. For all the other hormones measured, no final conclusions are possible on the basis of these studies.

Investigation of systemic hormone responses does not allow conclusions on the central neuroendocrine and neurotransmitter responses during music perception. To our knowledge, changes of neurotransmitters during music perception have not yet been studied. We, therefore, designed a study on changes of the neurotransmitter serotonin (5-HT) during music perception. ACTH, which has been shown to be influenced by music perception in several studies, and prolactin, which has been shown not to be influenced in most of the studies, were chosen as parameters for comparison of the systemic response. The central serotonergic neurotransmission is involved in affective cognitive processing during stress (Kawahara et al. 1993) and aggression (Berman et al. 1997). It is supposed that music perception is a cognitive process which should have impact on serotonergic neurotransmission.

The platelet model of 5-HT content has been shown to be a suitable model for the central serotonergic neurotransmission. The 5-HT content of platelets reflects the 5-HT content of neurons, decreased values for the intracellular content reflect increased 5-HT release with activated serotonergic neurotransmission (Pletscher 1978; Stahl 1977).

For the purpose of controlling the subject's attitude towards the music presented, a standardised scale to score the pleasantness, or unpleasantness, of the music was included in the study protocol. Previous studies on endocrine changes during music perception used the same music sequences for all subjects assuming that the same affective

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changes were induced in all subjects. In our study, both standard music and music chosen by the subjects themselves for the condition pleasant vs. unpleasant were used.

## Methods

### Subjects and procedure

We enrolled 20 healthy subjects (mean age  $27.7 \pm 4.8$  years; 11 male and 9 female). All subjects were Caucasians of German descent, a psychiatric interview excluded mental illnesses and all subjects were free of psychiatric diseases in their family. No drugs, including contraceptives, and no alcohol, nicotine or caffeine intake were allowed on the day of examination or on the previous day. The subjects included 10 musicians who had had at least three years of professional education in music and had been playing an instrument or singing for at least 10 years. The remaining 10 subjects were non-musicians without any musical training except primary school music education. All examinations were performed between 4 p.m. and 6 p.m. to avoid problems that might occur due to the presence of circadian rhythm. After giving informed consent, subjects were placed in a comfortable chair in a dark, air-conditioned and non-echoic room. They were asked to close their eyes and to rest or to listen to the music during the whole procedure. A cannula was fixed in the antecubital vein of the non-dominant arm. After 10 min of rest, two 10 ml blood samples were taken and mixed with ethylenediaminetetraacetic acid (EDTA). Then, a block of two pieces of music with a duration of 3–5 min each was presented via earphones. After that, a second blood sample was taken as described above. Finally a second block of two pieces of music was presented and at the end of this a third blood sample was taken and the cannula was removed.

The music blocks consisted of two pieces with either pleasant or unpleasant music. One piece of music was a standard which was presented to all subjects (pleasant music: Johannes Brahms, Symphony No. 3, op. 90, third movement (poco allegretto); unpleasant music: Krzysztof Penderecki, Threnos (1960, beginning) and one piece of music was individually chosen by the subjects. The blocks with the two pieces of music were presented in random order. After listening to the music, subjects rated their attitude towards every single piece of music by a German scale (so-called 'Eindrucksdifferential') for associative pleasantness or unpleasantness of emotions (Ertel 1965). The scale consists of six items with a single score between -3 (meaning a very unpleasant association) and +3 (meaning a very pleasant association), thus resulting in a total score between -18 and +18 for every single piece of music.

### Laboratory analyses

Immediately after taking, 10 ml of whole blood was centrifuged to obtain plasma. Plasma samples were coded and then frozen. Analyses for prolactin and ACTH were performed blindly by radioim-

munoassay (RIA) techniques in the endocrinological laboratory of the Medical Department at the University of Münster. The RIA normal range of prolactin is 2.8 to 15.9 ng/ml for female and 0.7 to 10.7 ng/ml for male subjects, the RIA normal range of ACTH in the evening is 5 to 45 pg/ml. The normal ranges are laboratory specific.

The other 10 ml of whole blood was centrifuged at 800 rpm in order to obtain platelet rich plasma (PRP). Platelets in PRP were counted and PRP was then centrifuged twice at 1800 rpm. The platelet pellet at the bottom of the tube was incubated with hydrochloric acid and frozen at a temperature lower than  $-20^{\circ}\text{C}$  overnight. The next day, the pellet was incubated with zincsulphate and NaOH and centrifuged at 5000 rpm. In the supernatant of this probe, 5-HT was determined by fluorescence photometry according to the method of Frattini et al. (1979). The laboratory normal range for platelet 5-HT is 413 to 1105 ng/ $10^9$  platelets (unpublished data).

### Statistical analysis

The mean values of prolactin, ACTH and 5-HT were compared by analysis of variance with repeated measures and post-hoc analysis of differences between two measures by t-test. In a second analysis of variance, the musical experience and the order of presenting the pleasant and the unpleasant music were chosen as independent factors. Correlation between the score for the subjective attitude towards the music and the different biochemical parameters was analysed by Pearson's product-moment correlation coefficient. All tests were two-tailed, the significance level was set at  $p=0.05$ .

## Results

The data of ACTH, prolactin and the platelet 5-HT content are presented in Table 1 with separate entries for the rest condition and for the two music perception conditions. Complete measures could be obtained in 19 subjects for prolactin and ACTH and in 17 subjects for 5-HT. In one subject, the plasma sample was accidentally not frozen correctly; in three subjects, the platelet pellets were spoiled mechanically during the laboratory work. Two female subjects had mildly increased prolactin levels without any clinical relevance, all other levels were within the normal ranges. Prolactin and ACTH did not show any significant differences between the conditions ( $p<0.469$  and  $p<0.120$ ; respectively). The 5-HT content of platelets was higher during pleasant music perception and lower during unpleasant music perception as compared to the rest condition. These differences of the 5-HT content were significant in a variance analysis with repeated measures ( $p<0.026$ ). However, when applying Bonferroni correc-

**Table 1** Arithmetic means (standard deviation) of platelet 5-HT content and of plasma prolactin and ACTH during rest and while listening to pleasant and unpleasant music. Statistical comparison by analysis of variance with repeated measures; ns denotes not significant

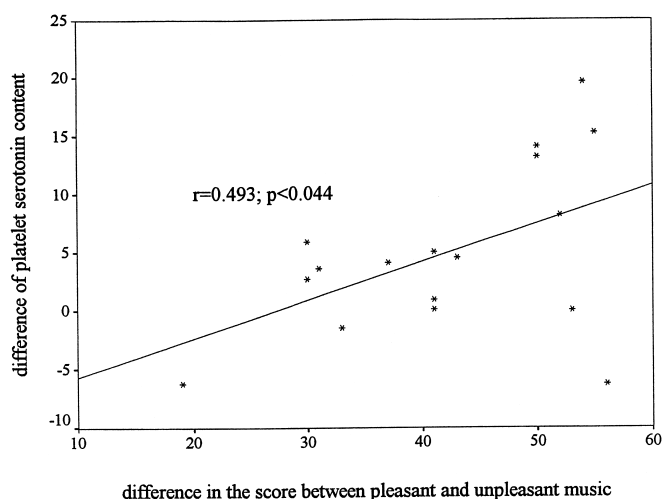
	rest	pleasant music	unpleasant music	significance
5-HT (n=17) (ng/ $10^9$ platelets)	714 (178)	748 (172)	699 (181)*	$p<0.026$ ( $F=4.69$ ; $df=15$ )
Prolactin (n=19) (ng/ml)	12.9 (11.0)	12.6 (11.1)	12.1 (10.4)	ns ( $p<0.469$ ) ( $F=0.79$ ; $df=17$ )
ACTH (n=19) (pg/ml)	9.1 (5.6)	12.2 (9.8)	11.6 (10.3)	ns ( $p<0.120$ ) ( $F=2.41$ ; $df=17$ )

\* post-hoc analysis:  $p<0.014$  for pleasant vs. unpleasant music condition ( $T=2.77$ ;  $df=16$ )

tion, the *p*-value of 0.026 cannot be considered significant with certainty. A post-hoc analysis revealed that only the difference between pleasant and unpleasant music perception contributed to the significance level of variance analysis ( $p < 0.014$ ) whereas the differences between rest and pleasant music perception and between rest and unpleasant music perception were not significant ( $p < 0.217$  and  $p < 0.348$ ; respectively).

In a second analysis of variance, the sex of the subjects, the order of presentation and the musical education (musician vs. non-musician) were chosen as independent factors. No significant influence of these factors on prolactin, ACTH or 5-HT content of platelets could be detected. The difference in the 5-HT content of platelets between pleasant and unpleasant music perception remained significant even in this analysis ( $p < 0.042$ ;  $F = 4.16$ ,  $df = 12$ ).

The two pleasant pieces of music were scored  $+13.2 \pm 4.0$  (standard) and  $+11.5 \pm 3.7$  (chosen by the subject), respectively; the two unpleasant pieces of music were scored  $-8.9 \pm 6.2$  (standard) and  $-7.8 \pm 5.4$  (chosen by the subject), respectively. The total scores for the pleasant music perception ( $+24.6 \pm 6.1$ ; range  $+7$ – $32$ ) and for the unpleasant music perception ( $-16.7 \pm 9.0$ ; range  $-30$ – $0$ ) were calculated and there was no overlap between the two total scores suggesting a very distinct differentiation of the two kinds of music by this scale. Their difference was correlated to the difference of 5-HT content of platelets between pleasant and unpleasant music perception. Calculation of Pearson's product-moment coefficient revealed a significant correlation between these two differences ( $r = 0.493$ ;  $p < 0.044$ ). The scatter plot of this correlation is presented in Fig. 1 showing increasing differences of 5-HT content (y-axis) with increasing differences between the scores for pleasant and unpleasant music (x-axis).



**Fig. 1** Scatter plot of the score difference between pleasant and unpleasant music perception (x-axis) and of the difference of the corresponding 5-HT content of platelets (y-axis). Statistical analysis by Pearson's product-moment correlation coefficient

## Discussion

The 5-HT content of platelets in human subjects has been suggested to reflect the 5-HT content of neurons and to central serotonergic transmission (Pletscher 1978; Stahl 1977). Therefore, the changes observed in our study might reflect changes in neuronal serotonergic transmission. Recent functional imaging studies of human subjects with positron emission tomography (PET) have shown that the neuroanatomical correlates of pleasant and unpleasant emotions are located in the medial prefrontal cortex, thalamus, hypothalamus and midbrain (Lane et al. 1997). Additional structures for either pleasant or unpleasant emotion, in particular the amygdala for unpleasant emotion, have also been detected but the neural substrates involved in the affective systems of pleasant vs. unpleasant share much in common or are closely inter-related. One of the most important substrates is the medial prefrontal region which is mainly activated during conscious experience of emotion (Reimann et al. 1997). In animal models, activation of this particular region, which is controlled by the amygdala, is known to increase predominantly central 5-HT metabolism with a subsequent increase of extracellular and decrease of intracellular 5-HT levels (Kawahara et al. 1993; Goldstein et al. 1996). Our results are in concordance with these findings of animal studies leading to the hypothesis that unpleasant music induces emotional stress with activation of the prefrontal cortex and subsequent 5-HT release and decreased intracellular 5-HT content of the serotonergic neurons which is reflected by 5-HT content of platelets.

As expected, we did not detect any significant differences in prolactin levels. This is in concordance with previous studies also showing that affective stimulation over a period of a maximum of 10 min does not lead to significant changes of this pituitary hormone (Richter et al. 1996). However, in concordance with the study of Möckel et al. (14), ACTH levels were not affected significantly either, although an increase of this pituitary hormone during unpleasant music perception has been suggested (Gerra et al. 1998). We noticed an insignificant increase of ACTH during both pleasant and unpleasant music perception. Possibly, the duration of music perception was too short in our study to induce significant changes. Another possible explanation could be that the power of our study was too small. The observation that ACTH was also increased during pleasant music perception is in concordance with previous findings that general arousal independent of the emotional context of the stimulation can induce increased ACTH levels (Aguilera 1994; Meyerhoff et al. 1988). However, since we could not detect significant changes in ACTH, these considerations remain highly speculative for our study.

The significant correlation between the differences in the scores for the two music blocks and the differences in 5-HT content of platelets gives evidence that the subjective attitude towards an affective stimulation is important for the measurement of physiological parameters. In contrast,

analysis of variance with repeated measures did not reveal any impact of the musical education on the changes of prolactin, ACTH or 5-HT. Most of the previous studies did not control for this factor. Our results suggest that not the musical education but the affective attitude towards the music is the more relevant factor influencing changes of neurotransmission during music perception. However, because of the weak significance levels, our results remain preliminary and should be verified in further studies with more subjects and additional endocrinological variables.

## Conclusions

Our data suggest that the 5-HT content of platelets is decreased during the perception of unpleasant music as compared to the perception of pleasant music. This decrease is significantly correlated to the subjective attitude towards the music as measured by a standardised score for the association of pleasantness and unpleasantness. Plasma prolactin and ACTH are not influenced in our design with a music perception of about 10 min duration for both the pleasant and the unpleasant music.

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