Br J clin Pharmac 1994; **38**: 545–555

Different sensitivity of pain-related chemosensory potentials evoked by stimulation with CO_2, tooth pulp event-related potentials, and acoustic event-related potentials to the tranquilizer diazepam

N. THÜRAUF, W. DITTERICH & G. KOBAL
Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, Krankenhausstrasse 9, 91054 Erlangen, Germany

1 The aim of this study was to investigate the sensitivity of pain-related potentials used in experimental pain models to the non-specific effects of the tranquilizer diazepam. Pain-related potentials were recorded after painful stimulation of the nasal mucosa with CO_2 and after painful stimulation of the tooth pulp. Acoustically evoked potentials were measured in order to compare their sensitivity to the tranquilizer diazepam with the sensitivity of the pain-related potentials.

2 Twenty volunteers participated in this randomised, double-blind, three-fold cross-over study. Measurements were obtained before and 20 min after the administration of the drug. Event-related potentials were recorded after painful stimulation of the nasal mucosa with CO_2 (two stimulus intensities: 60% v/v and 70% v/v CO_2), after painful stimulation of the tooth pulp (two stimulus intensities: 2.2 × and 3.3 × detection threshold), and after non-painful acoustical stimulation of the right ear. The subjects rated the perceived intensity of the painful stimuli by means of a visual analogue scale. In addition the spontaneous EEG was analysed in the frequency domain and the vigilance of the subjects was assessed in a tracking task.

3 Diazepam reduced significantly the amplitudes of the event-related potentials after painful stimulation of the tooth pulp and after acoustical stimulation. In contrast only a small, statistically non-significant reduction could be found after painful stimulation with CO_2. The pain ratings of the painful stimuli were not affected by diazepam. Diazepam reduced the performance of the tracking task. A decrease of arousal could be found in the $alpha_2$-range, whereas in the $beta_2$ and the theta-range the power density increased under diazepam.

4 We demonstrated that event-related potentials after painful stimulation of the nasal mucosa with CO_2 are less affected by the nonspecific effects of the tranquilizer diazepam than event-related potentials after painful stimulation of the tooth pulp. The effects of diazepam on the tracking task, the spontaneous EEG and the event-related potentials clearly confirm its sedative properties. Diazepam had no analgesic effect measurable by pain intensity estimates.

Keywords event-related potential electroencephalogram pain measurement benzodiazepine diazepam

Correspondence: Dr N. Thürauf, Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, Universitätsstrasse 22, 91054 Erlangen, Germany

Introduction

The measurement of pain with event-related potentials (ERPs) represents an established method in pain research used for testing and quantifying the effect of analgesic drugs in man and animals. Different techniques of inducing pain employing electrical, mechanical, cold, heat and chemical stimulation have been developed (for review see Handwerker & Kobal [1]).

In the past the reducing effect of a wide range of opioid and non-opioid analgesics on different components of the ERPs has been demonstrated in experimental pain models but the report of the reduction of auditory ERPs and tooth pulp ERPs by 33% nitrous oxide [2, 3], a drug which modifies the consciousness, weakens the assumption of specific pain-related components of the ERPs. In a study on ERPs evoked by electrical skin stimulation Miltner *et al.* [4] demonstrated the influence of attention on the ERPs. This indicates that endogenous factors such as state of the subject, meaning of the stimulus or vigilance also determine the ERPs. As a consequence of these findings independent parameters for the control of endogenous factors should be introduced in experimental pain models in order to separate specific analgesic from non-specific effects. The aim of this study was to investigate the influence of a tranquilizer on pain-related-potentials testing for specific pain-related components of the ERPs. The vigilance of the subjects was controlled by measuring the performance of a simple task on a video screen and by analysing the spontaneous EEG recorded shortly before the testing session. In addition, the effect of diazepam on acoustically evoked potentials (AEPs) was measured in order to compare the sensitivity of the AEPs to the sensitivity of the pain related potentials in this study. Diazepam was chosen because it is the classical representative of benzodiazepines.

Magnetoencephalographic studies revealed that different brain regions can be activated by different painful stimulation methods. In the case of chemical stimulation with CO_2 Huttunen *et al.* [5] succeeded in localising a generator of the late-nearfield evoked potential in or near the secondary somatosensory area (SII). It can be assumed that nociceptive afferents are primarily projected here [6]. In another study in the same laboratory the electromagnetic field patterns induced by electrical dental tooth pulp stimulation were measured [7]. The corresponding dipole was calculated to be situated at the rostral end of the Sylvian fissure a few centimetres frontal to the area activated after stimulation with CO_2. The authors of these two studies assumed that separate projection areas were activated by these two types of noxious stimuli.

The possible effects of a non-specific tranquilizer on activated brain areas responsible for the generation of the late nearfield components of pain-related-potentials can be investigated if valid information of the brain area activated after painful stimulation is available, as in the case of the electrical tooth pulp stimulation and the stimulation with CO_2. Thus, in this study we tested the effects of diazepam on ERPs induced by electrical tooth pulp stimulation and by chemical stimulation of the nasal mucosa with CO_2. Precise information on non-specific effects in this set-up helps to validate the pain model.

Methods

Subjects

Twenty young healthy volunteers (10 male and 10 female subjects, between 23 and 38 years, mean age 26.1 years) participated in the experiments. The subjects were informed about the aim of the study and all possible side effects of the drug on trial. They had given written informed consent before the beginning of the testing sessions. The Ethics Committee of the University of Erlangen-Nuremberg approved the study, which was performed in accordance with the Declaration of Helsinki/Tokyo/Venice. The subjects' health was ascertained before and after the experiments by general clinical examination.

Study design and testing procedure

A double-blind, randomised, controlled, three-fold crossover study design was chosen. In an additional training session prior to the actual experiments subjects were acquainted with the experimental conditions and procedures. In this session the subjects learned a specific breathing technique (velopharyngeal closure: Kobal & Hummel [8]) in order to avoid respiratory flow of air inside the nasal cavity during stimulation.

The subjects participated in three experiments on three different days. In each of the three experiments the medication, either placebo, 0.13 mg kg^{-1} or 0.065 mg kg^{-1} diazepam, was administered intravenously in a 100 ml 0.9% NaCl solution over 20 min. A time interval of at least 9 days between the experiments was chosen to guarantee the washing out of the administered drug. The subjects were requested to abstain from eating solid food for at least 8 h before the experiments started. Each experiment consisted of two testing sessions, the first taking place before, and the second starting 20 min after the end of the administration of the medication. The interval between the end of the first session and the beginning of the second session was 45 min. Each testing session lasted for approximately 35 min. During this time 48 painful (12 stimuli of 60% v/v CO_2, 12 stimuli of 70% v/v CO_2, 12 tooth pulp stimuli of 2.2 × detection threshold, 12 tooth pulp stimuli of 3.3 × detection threshold) were delivered pseudorandomly. The interstimulus interval was 40 s. Twenty acoustical stimuli were applied pseudorandomly during the intervals between painful stimuli.

During the entire measuring sequence subjects were comfortably seated in an airconditioned chamber. Headphones were used for acoustical stimulation and

white noise (50 dB SPL) was applied continuously in order to mask switching clicks of the stimulator.

Five minutes before the beginning of the second session, i.e. 15 min after the end of the administration of diazepam 5 ml blood were taken from a forearm vein and centrifuged (3500 rev min^{-1}; 10 min). Plasma was removed and stored in a freezer until analysed.

Painful carbon dioxide stimulation of the nasal mucosa: For chemical stimulation painful carbon dioxide stimuli were delivered to the nasal mucosa using a specially devised stimulation technique (for further details see Kobal [9, 10]). Stimulus duration was 200 ms and stimuli of two concentrations of carbon dioxide (60 and 70% v/v CO_2) were delivered to the right nasal cavity.

Tooth pulp stimulation

Prior to the beginning of each experiment a piece of stretchable rubber fabric (cofferdamm) was pulled over the upper two frontal incisors after the surface of the healthy unfilled right incisor had been cleaned with a solution of 3% H_2O_2. Then a stimulation electrode (cathode) was fixed at the upper right central incisor with Cyanoacrylat glue. The stimulation electrode developed by Raab [11] consisted of a hollow moss-gum cylinder with an open and a closed end with a length of 5.5 mm, an outer diameter of 8.5 mm and an inner diameter of 5.5 mm. To fix the electrode securely the thicker end of a silver chlorided silver wire was placed in the cavity of the cylinder and the open end of the cylinder was glued to the buccal side of the upper right central incisor. The other end of the silver wire was led through a small hole of the closed end of the moss-gum cylinder and connected to the stimulator. Electrode cream (Hellige) was injected into the cylinder in order to ensure an electrical contact between the silver chlorided silver wire and the surface of the stimulated incisor. The anodal electrode consisted of a silver-chlorided silver wire embedded in a rubber sponge (1 cm^2) and was located in the vestibulum above the stimulated tooth. The described technique guaranteed an impedance of the electrode higher than 0.7 MΩ leading to a selective stimulation of the tooth pulp. At the beginning of each experiment the resistance of the tooth pulp electrode was measured. In the case of a resistance value higher than 0.7 MΩ the experiment was continued by determining the detection threshold. In the case of lower resistances the electrode was removed, the surface of the tooth cleaned and a new stimulating electrode installed. This procedure was repeated until a resistance value higher than 0.7 MΩ was achieved. During each experiment the resistance was controlled before and after of each testing session. If the deviation of the resistance value of the control measurements from the initial measurement of the resistance at the beginning of the experiment was higher than 10% then the experiment was stopped and the acquired data were not included in the statistical analysis.

Before the beginning of the two testing sessions of each experiment the detection threshold of the subject was determined. During the determination of the detection threshold and in all of our experiments a stimulus duration of two 2 ms (Digitimer Stimulator DS7) was used.

Estimates of detection thresholds were determined by employing the method introduced by Wysocki & Beauchamp [12] (see also Eccles [13], Zatorre & Jones-Gotman [14]). At the beginning of each experiment the determination of the detection thresholds was measured three times with ascending stimulus intensity series. Two blanks and one stimulus were applied in a randomised order. Increasing steps of 0.2 μA were used during the testing series. A series ended when a clear sensation could be reported by the subject in three successive stimulus *vs* blank trials, the lowest value of the three was designated an estimate of the subject's threshold. Subsequently, the mean of the three threshold values was calculated.

The determined thresholds for electrical tooth pulp stimuli in this study were in the range from 7 μA to 30 μA. In all our experiments tooth pulp stimuli of two intensities (2.2 × detection threshold, 3.3 × detection threshold) and 2 ms stimulus duration were applied.

Acoustical stimulation

Using an acoustical stimulator (Hörniß-Zeisberg) a tone of 1000 Hz, 100 ms duration, and 10 ms rise time was presented to the right ear via headphones while white noise (50 dB SPL) was applied to the left and the right ear. The acoustical stimuli were applied pseudorandomly during the interstimulus intervals of painful tooth pulp and CO_2 stimuli.

Determination of plasma concentrations

The determination of the plasma concentration of diazepam was carried out by the Forensic Institute of the University of Erlangen-Nuremberg using a validated high performance liquid chromatographic assay as described by Cotler [15].

Estimates of painful intensities

During the experimental sessions the subjects estimated the perceived intensity of tooth pulp and CO_2-stimuli in relation to standard stimuli for the stimulation of the tooth pulp (standard stimulus: 2.2 × detection threshold, 2 ms) and the stimulation of the nasal mucosa (standard stimulus: 60% v/v CO_2, 200 ms). The standard stimuli were always presented at the beginning of the experiment. Subjects estimated the painful intensity by employing a visual analogue scale displayed on a video monitor.

Pain-related chemosensory potentials evoked by stimulation with CO_2, tooth pulp event-related potentials, and acoustic event-related potentials

Event-related potentials in the EEG were recorded from three positions of the 10/20 system (Fz, Cz, Pz) referenced to an electrode at the inion (for more

details of the recording technique and the analysis of EEG segments see [16, 18]).

Three base-to-peak amplitudes (P1, N1, P2) and two peak-to-peak amplitudes (P1/N1, N1/P2) were evaluated. The latencies of the three base-to-peak amplitudes were measured relative to stimulus onset.

The spontaneous EEG

EEG segments (n = 30) of 4096 ms allowing the calculation of reliable power spectra [17] were recorded with a sampling frequency of 125 Hz over 5 min just before the beginning of each testing session, i.e. before and 15 min after administering the medication. Data were obtained from the recording positions F3, C3, P3 and F4, C4, P4 referenced to an electrode at the inion.

After examining the segments for eye blinks and motor artifacts they were submitted to frequency analysis (Fast Fourier Transformation, FFT). The resulting power spectra were averaged and subsequently divided into seven frequency bands (delta 1–3.4 Hz, theta 3.4–7.8 Hz, $alpha_1$ 7.8–10.3 Hz, $alpha_2$ 10.3–12.7 Hz, $beta_1$ 12.7–18.1 Hz, $beta_2$ 18.1–21 Hz, $beta_3$ 21–29.8 Hz). The integrated power of these bands was used for further statistical evaluation.

Tracking performance

In order to detect changes in the state of vigilance (and/or motoric coordination), subjects were requested to perform a simple task on a video screen: they had to keep a small square, which could be controlled by a joystick, inside a larger one, which unpredictably moved around. This 'Tracking Performance' was checked by counting how often and by measuring for how long the subjects had lost track of the independently moving square [10]. This task also helped in stabilising the subjects' vigilance.

Statistical analysis

Data were submitted to the following statistical analyses:

(a) In order to compare the effects of diazepam with those of placebo, differences between data obtained after and before administration were calculated and then submitted to analysis of variance (MANOVA, repeated measurement design, medication and electrode position as 'within-subject-factor').

(b) In order to compare the different sensitivity of the different types of stimuli to diazepam a MANOVA was calculated with medication and type of stimulus as 'within subject factor'.

(c) In the case of the power spectra two additional factors of the localisation of the electrodes were added to MANOVA-analysis as 'within subject factors': left or right hemisphere (factor 'side'), frontal, central, and parietal (factor position).

SPSS PC+ programs were employed for these statistical evaluations.

Results

Plasma concentrations of diazepam

For each dose of diazepam 20 blood samples were analysed. Mean values of plasma concentration 15 min after the end of the administration diazepam were 148.1 ng ml^{-1} (s.d. 38.5, n = 20) for the lower dose of diazepam (0.065 mg kg^{-1}) and 282.4 ng ml^{-1} (s.d. 98.3, n = 20) for the higher dose of diazepam (0.13 mg kg^{-1}) indicating that shortly before the beginning of the second testing session different plasma concentrations for the two different doses of diazepam were achieved reaching clinically effective levels.

Estimates of painful intensities

No significant influence of medication (MANOVA, medication as 'within-subject-factor') on the intensity estimates could be found for both types of stimulation. A significant influence of the factor stimulus (CO_2 stimulus or tooth pulp stimulus) on the intensity estimates could be observed (MANOVA, stimulus as 'within subject factor', F = 16.84, $P < 0.001$). In the case of the stimulation with CO_2 the intensity estimates increased during the second testing session, whereas the intensity estimates of the tooth pulp stimuli decreased (Figure 1).

Tracking performance

Comparing data obtained before and after medication, it was observed that the tracking performance improved after placebo. This improvement is a phenomenon generally observed in subjects tested in subsequent sessions [10, 18] and is probably due to the subjects becoming more skilled in the course of the experiments. Analysing the differences before and after medication or placebo a significant influence of medication on the tracking performance could be observed (MANOVA, medication as 'within subject factor', F = 5.60, $P < 0.009$). In contrast to placebo the performance decreased under medication (0.065 mg kg^{-1} and 0.13 mg kg^{-1} diazepam) in a dose dependent manner indicating that the vigilance was suppressed after the administration of diazepam (Figure 2).

Frequency analysis of the spontaneous EEG

A statistically significant influence of the medication on the power density (MANOVA, medication as 'within subject factor') could be found in the $alpha_2$-range (F = 4.23, $P < 0.024$), in the $beta_2$-range (F = 4.22, $p < 0.024$) and in the theta-range (F = 3.46, $P < 0.044$; Figure. 2). The power density in the $alpha_2$-range decreased after application of diazepam (0.065 mg kg^{-1} KG and 0.13 mg kg^{-1} KG diazepam) compared with placebo in a dose dependent manner indicating a reduction of the subjects' vigilance. This effect of diazepam was more distinct at frontal and central positions (MANOVA, medication and position

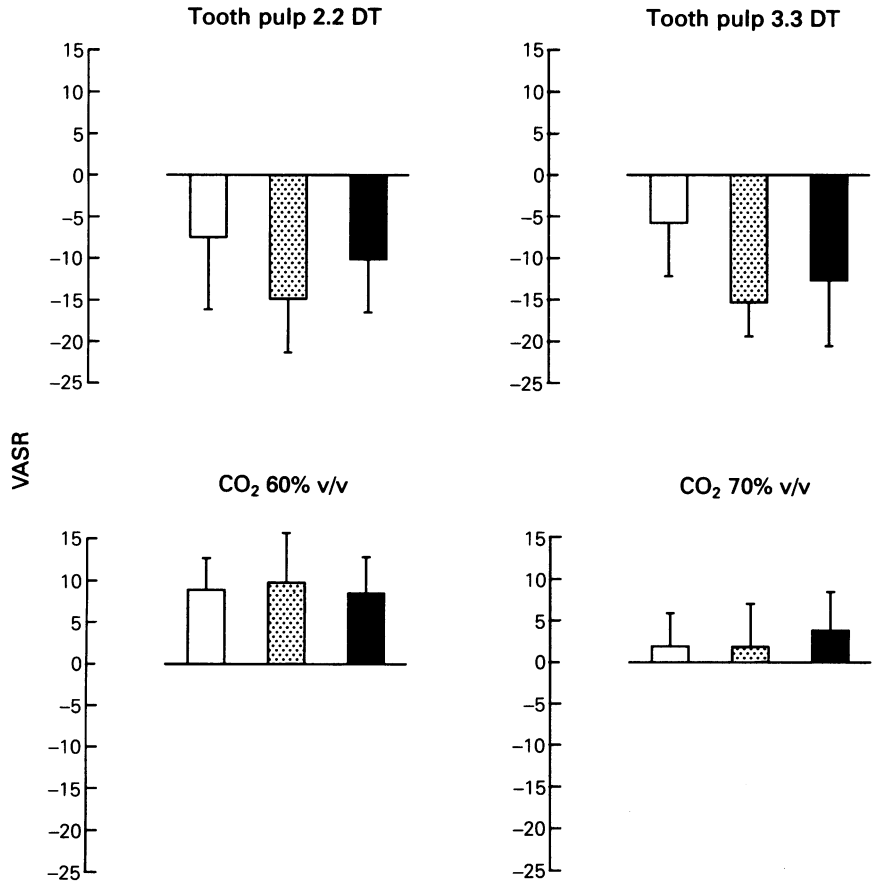

Figure 1 Mean values and standard errors of means of the intensity estimates. The results are presented as differences between data obtained after and before administration of the medication (□ placebo, ▩ 0.065 mg kg^{-1} diazepam, ■ 0.13 mg kg^{-1} diazepam), e.g. up deflection of the columns represents an increase after administration of the medication whereas down deflection represents a decrease. DT: detection threshold (details see text). VASR: visual analogue scale ratings.

as 'within subject factor', medication by position: $F = 3.27$, $P < 0.017$). The reduction of the power density in the alpha$_2$-range coincided with an increase in the beta$_2$-range after administration of the lower dose of diazepam (0.065 mg kg^{-1} diazepam) at the frontal, central and parietal positions and after administration of the higher dose (0.13 mg kg^{-1} diazepam) at the frontal and central positions (MANOVA, medication and position as 'within subject factor', medication by position: $F = 2.95$, $P < 0.027$).

In the theta-range an increase in the power density could be observed after application of placebo, whereas a small decrease of the power density in this frequency range could be found under diazepam. These effects of placebo and diazepam were more distinct at the frontal positions (MANOVA, medication and position as 'within subject factor', medication by position: $F = 8.17$, $P < 0.001$).

Tooth pulp evoked potentials (TEPs)

In contrast with the subjective intensity estimates of the tooth pulp stimuli a significant influence of medication could be found for the amplitudes P1N1 and N1P2 and as well for the separate component N1 of the TEPs in the case of the higher stimulus intensity (MANOVA, medication as 'within subject factor', $F = 9.03$, $P < 0.002$, $F = 7.95$, $P < 0.003$, $F = 3.82$, $P < 0.034$) and for the amplitudes N1P2 in the case of the lower stimulus intensity (MANOVA, medication as 'within subject factor', $F = 4.44$, $P < 0.021$). Diazepam led to a reduction of the amplitudes mentioned above (Figures 3, 4, 5). The influence of diazepam on the amplitudes of the TEPs was more distinct for the higher dose of the drug and the higher stimulus intensity at the frontal and central recording positions (MANOVA, medication by position: P1N1, $F = 4.08$, $P < 0.006$; N1P2, $F = 2.64$, $P < 0.044$; N1, $F = 3.26$, $P < 0.018$).

Chemosomatosensory evoked potentials (CSSEPs)

In contrast to the reducing effect of diazepam on the amplitudes of the TEPs and AEPs no significant influence of medication on the amplitudes and latencies of the CSSEPs could be observed (Figures 3, 4, 5). Only a tendency of the influence of medication on the latency of P1 (MANOVA, medication as 'within subject factor', $P < 0.1$) could be detected in the case of the lower stimulus intensity.

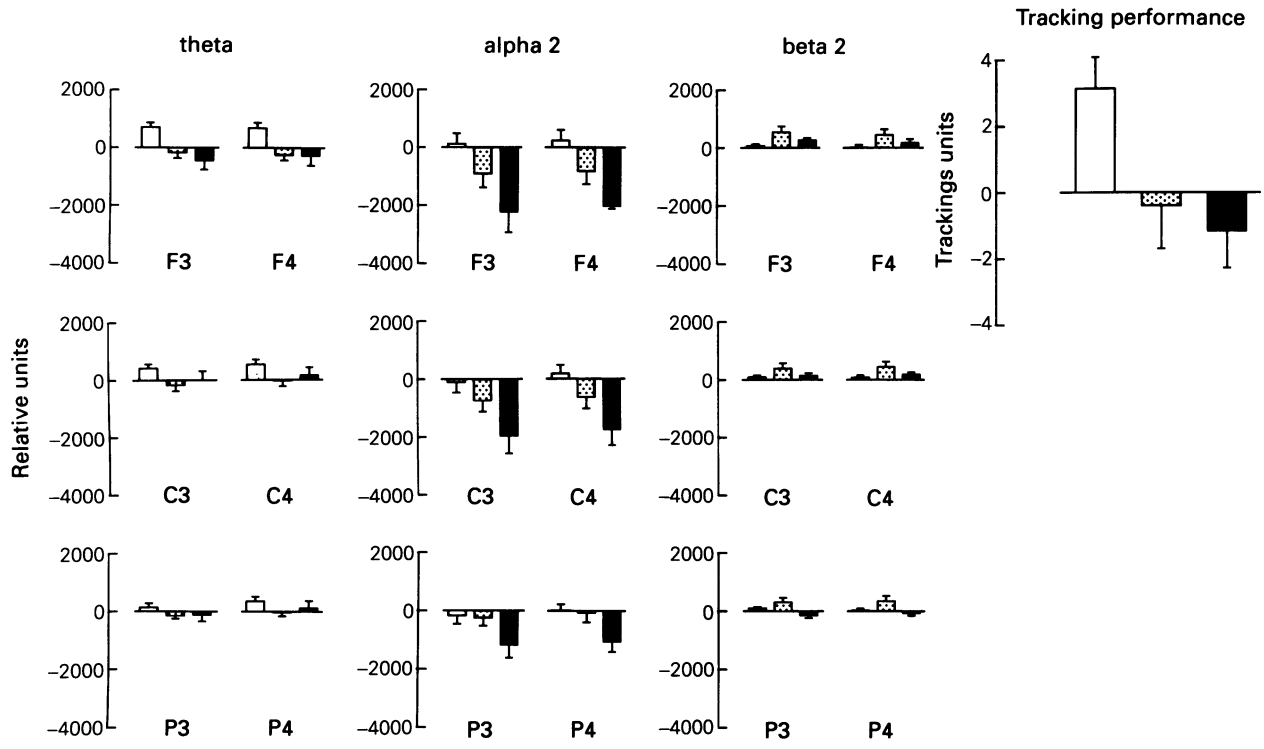

Figure 2 Mean values and standard errors of means of the tracking performance, and the frequency analyses of the spontaneous EEG at all recording positions. The results are presented as differences between data obtained after and before administration of the medication (□ placebo, ▩ 0.065 mg kg^{-1} diazepam, ■ 0.13 mg kg^{-1} diazepam), e.g. up deflection of the columns represents an increase after administration of the medication whereas down deflection represents a decrease. A significant influence of the medication on the spontaneous EEG could be found only in the frequency ranges presented in the figure.

AEPs

Similar to the effects of diazepam on the TEPs a statistically significant influence of medication (MANOVA, medication as 'within subject factor') on the AEPs could be observed for the amplitudes N1P2 ($F = 9.81$, $P < 0.002$) and P2 ($F = 5.01$, $P < 0.014$). Diazepam led to a reduction of these amplitudes in comparison with placebo (Figures 3, 4, 5). As observed for the TEPs this effect was more distinct at frontal and central recording positions (MANOVA, medication and position as 'within subject factor', medication by position: N1P2, $F = 5.06$, $P < 0.002$; P2, $F = 3.93$, $P < 0.008$).

Discussion

When event-related potentials were introduced into pain research in order to quantify the effects of analgesics, the question was raised to what extent reductions in amplitudes were dependent on a non-specific, depressant action of the drugs. So far, all publications on pain-related potentials exclusively described responses that were classified as late near-field potentials [1]. These late responses are thought to be endogenously modulated or even generated fully endogenously. By definition 'endogenous' components of event-related potentials vary with the state of the subject, the meaning of the stimulus and/or the demand of the task related to the respective stimulus. All these aspects can be influenced by opioids or other centrally acting drugs. In contrast, 'exogenous' event-related potentials are determined by the characteristics of the afferent input and hence of the eliciting stimulus. In reality, however, the two phenomena cannot always be clearly separated. Event-related potential components usually are less subject to 'endogenous' processes the earlier they occur [19]. In a study on event-related potentials evoked by electrical skin stimulation Miltner and coworkers found that N100, P200, and P300-amplitudes were larger when subjects focused their attention on the stimuli and considerably smaller when the stimuli were ignored. Independent of attention, P200s and P300s were more strongly affected by differences in the stimulus intensity than the N100s [4]. In another study this group demonstrated the interaction of the late event-related potential component N150-P260 on pain perception when it was fed back to the subjects. A decrease in subjective pain report was achieved after down-training, while an increase of the amplitude was observed after up-training [20]. Attention levels prior to the painful electrical skin stimulation were estimated by means of the alpha-power and alpha-frequency of the spontaneous EEG [4, 17, 21]. Preparation processes were estimated by means of negative shifts of the 'contingent negative variation' (CNV) [22]. In one of these studies there was no

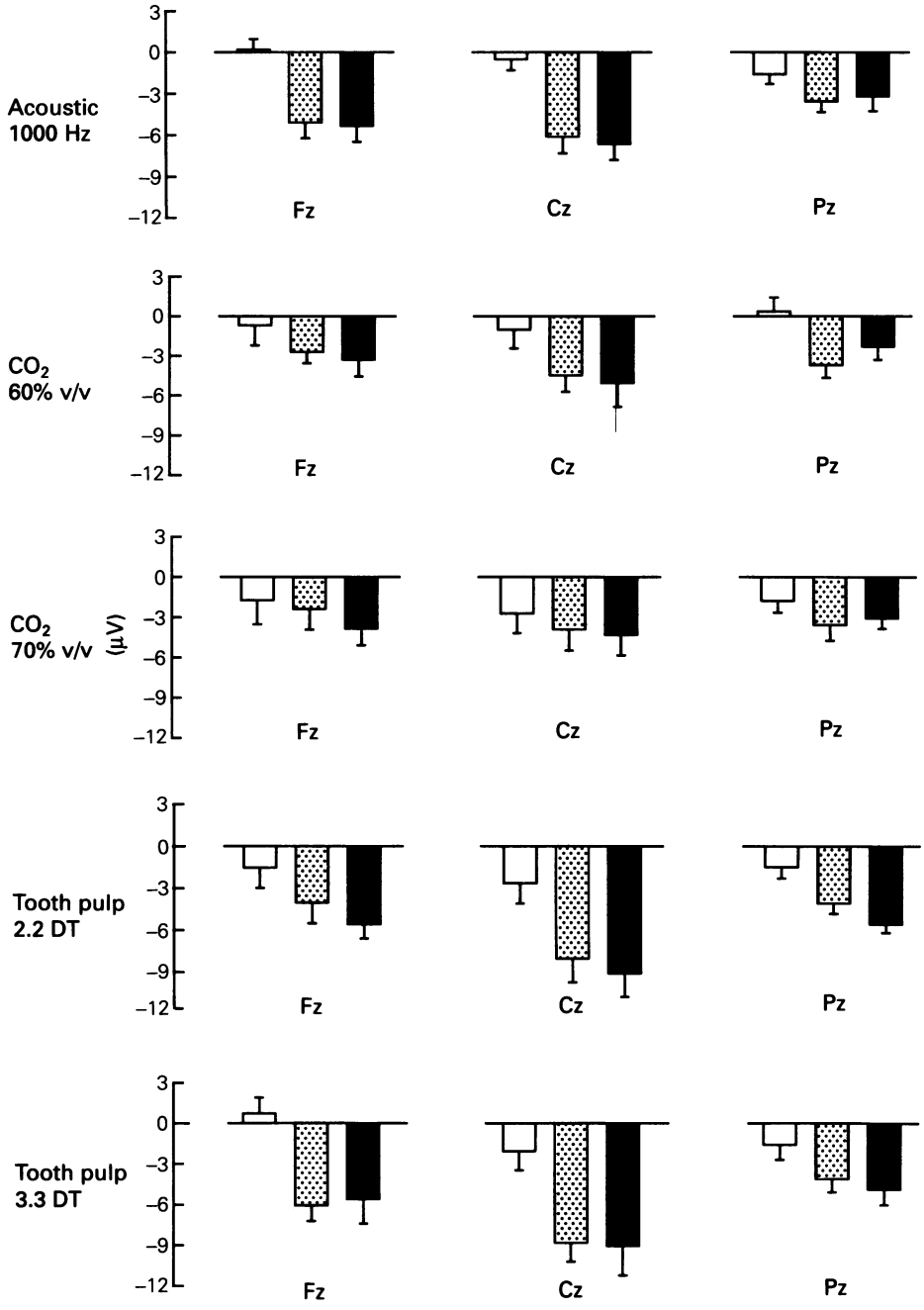

Figure 3 Mean values and standard errors of means of the peak to peak amplitude P1N1 of all subjects (n = 20). The results are presented as differences between data obtained after and before administration of the medication (□ placebo, ▩ 0.065 mg kg^{-1} diazepam, ■ 0.13 mg kg^{-1} diazepam), e.g. up deflection of the columns represents an increase after administration of the medication whereas down deflection represents a decrease. DT: detection threshold (details see text).

correlation between alpha-power, CNV, somatosensory ERP and pain-ratings. Only high negativity levels of CNV-shifts compared with low CNV-shift levels corresponded to a significant increase in the somatosensory ERP-N150-P260 size, which may be due to a simple superposition of both components [4, 21]. However, another group found a reduction in amplitudes of the somatosensory ERP when alpha-power increased [17]. These findings conclusively demonstrate that late pain related potentials are determined by more than just stimulus characteristics. Hence, it is very important to be aware of these factors and to get independent parameters of the 'endogenous' factors influencing the event-related potentials when they are used as indicators of pain sensations, e.g. when investigating the action of analgesic drugs.

On the basis of these findings it was necessary to determine to what extent event-related potentials could be influenced by drugs which have centrally depressant effect. In order to be able to test this effect separately, the benzodiazepine diazepam was chosen

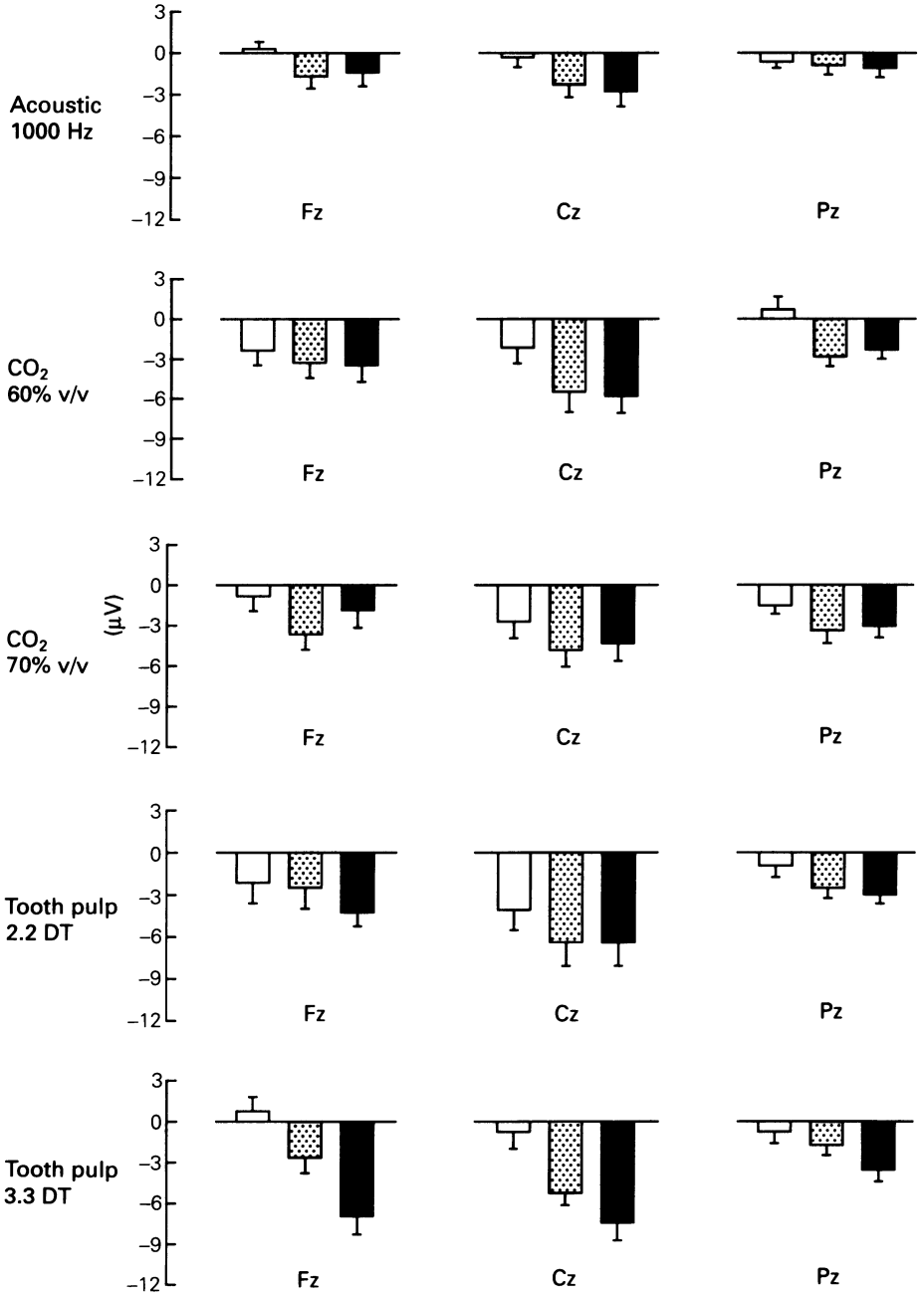

Figure 4 Mean values and standard errors of means of the peak to peak amplitude N1P2 of all subjects (n = 20). The results are presented as differences between data obtained after and before administration of the medication (□ placebo, ▨ 0.065 mg kg^{-1} diazepam, ■ 0.13 mg kg^{-1} diazepam), e.g. up deflection of the columns represents an increase after administration of the medication whereas down deflection represents a decrease. DT: detection threshold (details see text).

for this experiment, as firstly its centrally depressant effect is well known [23, 24] and secondly there is very little doubt that it has no specific analgesic action. In addition, the comparison between different types of pain stimuli, both suited for recording of event-related potentials, seemed to be of great importance.

In summary, no significant effects of diazepam could be found on the estimates of painful intensities for both stimuli, the electrical pulp and the chemical stimulation. This is in accordance with the hypothesis that benzodiazepines do not exert an analgesic action. The second hypothesis that diazepam decreases vigilance and arousal could also be confirmed by the results of this experiment.

Tracking performance did not increase when diazepam was administered as it did in the placebo condition. From many studies we know that the tracking performance improves in the placebo condition [10, 18]. This improvement is a generally observed phenomenon in subjects who are tested in subsequent sessions and is probably due to learning. Diazepam

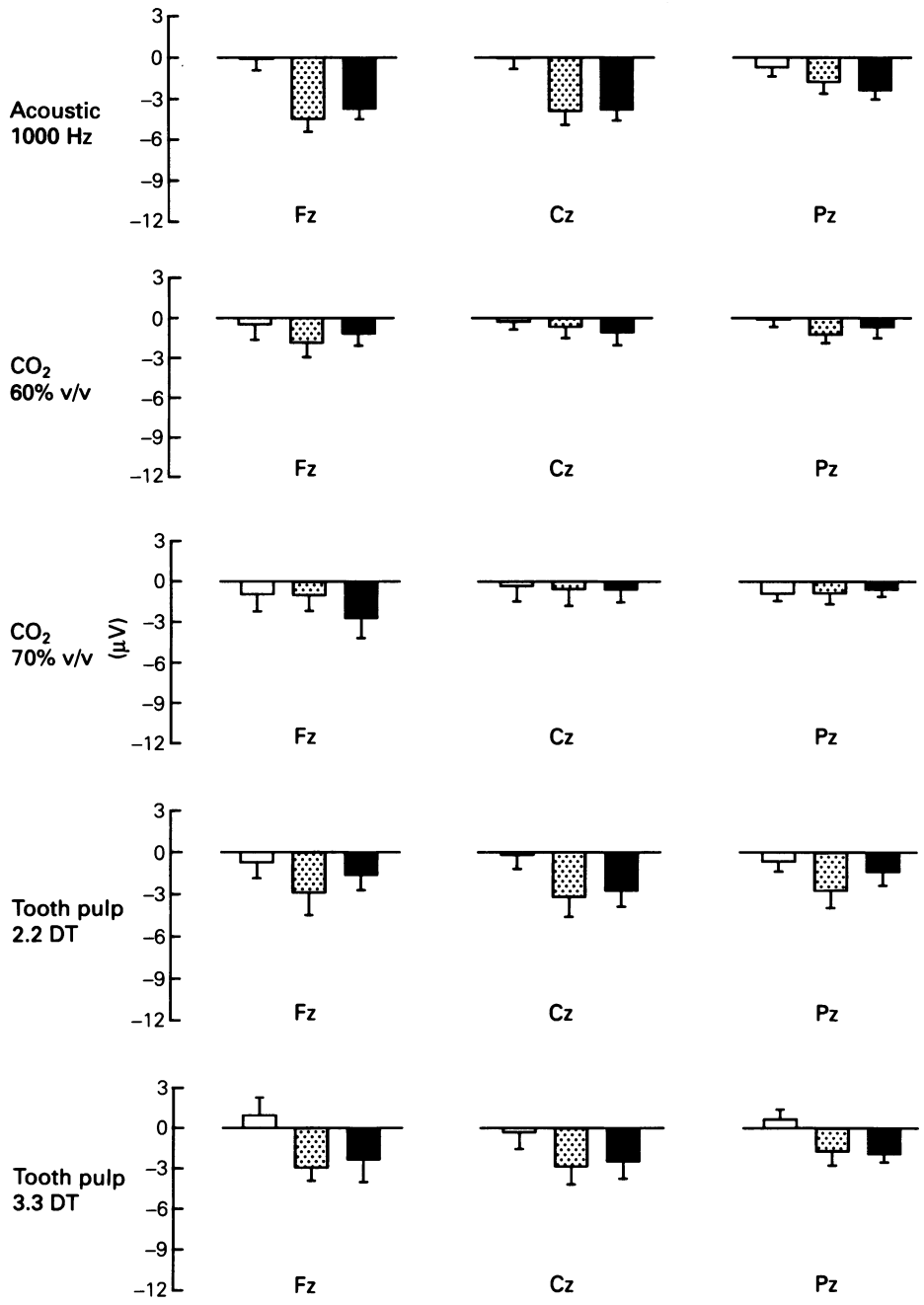

Figure 5 Mean values and standard errors of means of the base to peak amplitude P2 of all subjects (n = 20). The results are presented as differences between data obtained after and before administration of the medication (□ placebo, ▨ 0.065 mg kg^{-1} diazepam, ■ 0.13 mg kg^{-1} diazepam), e.g. up deflection of the columns represents an increase after administration of the medication whereas down deflection represents a decrease. DT: detection threshold (details see text).

suppressed this learning effect during the testing sessions in a dose dependent manner.

After administration of diazepam the spontaneous EEG changed in a way that could be expected on the basis of former results already described [23]. A decrease in the alpha-range was accompanied by an increase in the $beta_2$-range. Only the decrease in the theta-range did not precisely fit the pattern of expected changes in the spontaneous EEG. However, this phenomenon can easily be explained by the general experimental condition. Subjects were intermittently stimulated and thus activated in their state of vigilance. Hence both, the changes in the tracking performance and the changes in the frequency of the spontaneous EEG indicated a sedative action of the drug on trial.

As a result of the sedative effect of diazepam the amplitudes of the tooth pulp event-related potentials and the acoustic event-related potentials were significantly reduced. For both responses, the effect was more pronounced at the frontal leads than at the central or parietal leads. In contrast, the responses to

carbon dioxide were not affected by the benzodiazepine. It should be stated that there might have been effects if other experimental conditions had been chosen. However, these were the conditions under which the analgesic effect was measured in former studies. Given these results, the use of carbon dioxide as a specific stimulus of the nociceptive system for the registration of event-related potentials in experimental pain models is clearly recommendable. In addition, it is known from magnetoencephalographic studies that carbon dioxide stimuli very likely activate nociceptive cortical areas [5] and that the event-related potential components analysed in this study are generated at this site. Despite these results, the specific and non-specific effects of opioids have to be investigated. Only in more recent studies, the non-specific effects of centrally acting analgesics were investigated in order to differentiate between analgesic effects and those related to lowered arousal, vigilance and attention [10, 17, 25]. Event-related potentials and pain estimates may help to separate these different drug effects only when registered simultaneously with the performance of a vigilance task, the spontaneous cortical activity, and event-related potentials of another sensory channel. First attempts with this method have already demonstrated that the antidepressant imipramine, which was thought to have a specific analgesic effect, in all probability exerts its pain killing property via a central depression, reducing the attention to and the cognitive evaluation of painful stimuli [26].

This research was supported by DFG grant Ko 812/1-4. We thank Dr Elisabeth Pauli, Department of Neurology, University of Erlangen-Nuremberg, for statistical evaluation of the data.

References

1 Handwerker HO, Kobal G. Psychophysiology of experimental induced pain. *Physiological Reviews* 1993; **73**: 639–671.
2 Chapman CR, Schimek F, Gehrig JD, Gerlach R, Colpitts YH. Effects of nitrous oxide, transcutaneous electrical stimulation, and their combination on brain potentials elicited by painful stimulation. *Anaesthesiol* 1983; **58**: 250–256.
3 Harkins SW, Benedetti C, Colpitts YH, Chapman CR. Effects of nitrous oxide inhalation on brain potentials evoked by auditory and noxious dental stimulation. *Prog Neuropsychopharmacol Biol Psychiatry* 1982; **6**: 167–174.
4 Miltner W, Johnson R, Braun C, Larbig W. Somatosensory event-related potentials to painful and non-painful stimuli-effects of attention. *Pain* 1989; **38**: 303–312.
5 Huttunen J, Kobal G, Kaukorante E, Hari R. Neuromagnetic responses to painful CO_2-stimulation of nasal mucosa. *Electroenceph clin Neurophysiol* 1986; **64**: 347–349.
6 Chudler EH, Dong WK, Kawakami Y. Tooth pulp evoked potentials in the monkey: Cortical surface and intracortical distribution. *Pain* 1985; **22**: 221–223.
7 Hari R, Kaukorante E, Reinikainen K, Huopainiemi T, Mauno J. Neuromagnetic localization of cortical activity evoked by painful dental stimulation in man. *Neurosci Lett* 1983; **42**: 77–82.
8 Kobal G, Hummel T. Brain responses to chemical stimulation of trigeminal nerve in man. In *Chemical senses*, Vol. 2: Irritation, eds Green BG, Mason JR, Kare MR. New York: Marcel-Dekker 1989; 123–129.
9 Kobal G. Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. *Pain* 1985; **22**: 151–163.
10 Kobal G, Hummel C, Nuremberg B, Brune K. Effects of pentazocine and acetylsalicylic acid on pain-rating, pain-related evoked potentials and vigilance in relationship to pharmacokinetic parameters. *Agents Actions* 1990; **29**: 342–359.
11 Raab WH, Reithmayer K, Müller HF. Ein Verfahren zur Testung von Lokalanästhetika. *Dtsch Zahnärtzl Z* 1990; **45**: 629–632.
12 Wysocki CJ, Beauchamp GK. Ability to smell androstenone is genetically determined. *Proc Natl Acad Sci* 1984; **81**: 4899–4902.
13 Eccles R, Jaward MSM, Morris S. Olfactory and trigeminal thresholds and nasal resistance to airflow. *Acta Otolaryngol* 1989; **108**: 268–273.
14 Zattore RJ, Jones-Gotman M. Right-nostril advantage for discrimination of odors. *Percept Psychophys* 1990; **47**: 526–531.
15 Cotler S, Puglisi CV, Gustafson JH. Determination of diazepam and its major metabolites in man and in the cat by high-performance liquid chromatography. *J Chromatogr* 1981; **2**; 222: 95–106.
16 Picton TW, Hillyard SA. Endogenous event-related potentials. In *EEG-Handbook* (revised series, Vol. 3), ed Picton TW. Amsterdam: Elsevier 1988; 361–426.
17 Scharein E, Häger F, Bromm B. Spectral estimators for short EEG segments. In *Pain measurement in man*, ed Bromm B., Amsterdam: Elsevier 1984; 189–201.
18 Hummel T, Friedmann T, Pauli E, Niebch G, Borbe HO, Kobal G. Dose-related analgesic effects of flupirtine. *Br J clin Pharmac* 1991; **32**; 69–77.
19 Chen AC, Chapman CR. Aspirin analgesia evaluated by event-related potentials in man: possible central action in brain. *Exp Brain Res* 1980; **39**: 359–364.
20 Miltner W, Larbig W, Braun C. Biofeedback of somatosensory event related potentials: Can individual pain sensations be modified by self-control of event-related potentials. *Pain* 1988; **35**: 205–213.
21 Miltner W, Larbig W, Braun C. Attention and event related potentials elicited by intracutaneous electrical stimulation of the skin. *J Psychophysiol* 1988; **2**: 269–276.
22 Walter WG, Cooper R, Aldridge VJ, McCallum WC, Winter A. Contingent negative variation: an electric sign of sensorimotor association and expectancy in the human brain. *Nature* 1964; **203**: 380–384.
23 Sittig W, Badian M, Rupp W, Tauber K. Performance tests and pharmaco EEG after 1, 4 and 1, 5 Benzodiazepines. In *Electroencephalography in drug research*, ed Herrmann WM. Stuttgart New York: Gustav Fischer 1982; 113–129.
24 Turan MI. The significance of quantitative pharmaco-

EEG in the discovery and classification of psychotropic drugs. In *Electroencephalography in drug research*, ed Herrmann WM. Stuttgart New York: Gustav Fischer, 1982; 132–157.

25 Bromm B, Meier W, Scharein E. Pre-stimulus/post-stimulus relations in EEG spectra and their modulations by an opioid and an antidepressant. *Electroenceph clin Neurophysiol* 1989; **73**: 188–197.

26 Hummel T, Hummel C, Friedel I, Pauli E, Kobal G. Assessment of antinociceptive effects in man: Comparison of imipramine, tramadol, and anpirtoline. *Br J clin Pharmac* 1994; (in press).

(Received 13 April 1994, accepted 9 August 1994)