MIND GAMES:

The effects of diazepam on
Evoked Potentials

Part A: passive paradigms
Part B: EEG-EP interrelations

Marijtje Jongsma

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‘De kunstenaar is iemand,
die oplossingen in geheimen
weet te vertalen’

Karl Kraus

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To Tineke
(woppe, woppe, woppe)

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CHAPTER 1: GENERAL INTRODUCTION

1.1 The EEG

The electroencephalogram (EEG) represents the electrical activity of the brain that results from a summation of the electrical activity of a large number of individual nerve cells, or neurons [1-3]. Since the generation of the EEG from the action potentials of the neurons is beyond the scope of this thesis, further details can be found elsewhere [2, 7, 8]. Because certain groups of neurons receive input from the same underlying structures, there will be some coherence between the activity of individual cells [1]. Because of this coherence, electrical activity can be recorded at the scalp. The amplitude of the recorded activity depends on the intensity of the electrical source, on its distance from the recording electrode and on the electrical impedance of the structures between the source and the recording electrode [1, 3].

1.1.1 Recording the EEG

The amplitude of the recorded activity measured from the electrodes is very small (in the order of tens to hundreds of μV) and is therefore amplified. Normally, the EEG is also filtered. For studying the spontaneous EEG, normally the frequencies of interest are between 0-100 Hz [8]. The highest frequency of interest depends on the application for which the EEG is recorded. When the EEG is recorded for evoked potential studies, higher frequencies are of interest, therefore broader filtering is required [11]. Nowadays, the EEG signal is stored digitally. This requires sampling of the signal at the amplifier output. According to the Nyquist sampling theorem [10], the sampling rate at which the signal is recorded has to be at least twice as high as the highest frequency of the signal at interest.

1.1.2 Spectral analysis of the EEG

Spectral decomposition of the EEG by computing the Fourier transformation is by far the most used quantitative method for the analysis of EEG signals [4]. The rhythmic nature of many EEG activities lends itself naturally to this analysis. The mathematical foundation of the Fourier transformation can be found elsewhere [5, 6]. Spectral analysis is widely used to classify EEG patterns into frequency bands that have been related to different brain states, functions or pathologies [3, 4, 7, 8].

When the EEG is recorded from a normal adult subject with eyes opened, the EEG will consist of low amplitude, high frequency activity, or beta-activity (>12 Hz). Closing the eyes will result in general slowing of the EEG to frequencies around 10 Hz or the alpha rhythm (8-12 Hz). In sleeping subjects high voltage, low frequency delta (1-4 Hz)

and theta rhythms (4-8 Hz) can be observed [8]. The large amplitudes of these low frequency waves imply that large populations of neurons fire synchronously [3, 9]. In lower animals, like rats, a similar relation between the EEG and vigilance is observed as in human subjects [3]. This means that active wakefulness is accompanied by beta activity in the EEG, whereas the EEG of slow wave sleep is predominantly composed of delta waves [3, 8, 9].

1.2 Evoked Potentials

When sensory stimuli are presented to a subject, the conduction of these stimuli to the brain results in the generation of 'evoked potentials' [1]. Originally, the term 'evoked potential', or EP, was used because it was believed that these potentials reflected brain activity that was strictly evoked by the presentation of the stimulus and therefore only related to sensory processes [73]. Nowadays it is generally accepted that at least parts of these potentials are related to processes that are invoked by the stimulus paradigm [74]. It is even possible to measure such brain potentials in absence of a stimulus [72]. Therefore, in human research the more neutral term 'event related potential' is often used [74]. In this thesis however we will refer to both 'evoked potentials' as 'event-related potentials' as EPs.

The amplitude of the electrical activity elicited by sensory stimuli is very small in comparison to the amplitude of the ongoing EEG and therefore usually not visible [3, 12, 13]. The ongoing EEG is often viewed as independent of the stimulus. Therefore, averaging the responses to a large number of stimuli will result in the ongoing EEG approaches zero, while the averaged responses to the stimuli result in an evoked potential [1, 12, 14]. Thus obtained evoked potentials consist of a sequence of peaks and troughs, or components. The different peaks and troughs in the EP sequence are characterised by their polarity, amplitude and latency (e.a. the time in ms after stimulus onset) [1, 13]. The studied variables are normally the latencies (in ms) and amplitudes (in μV) of these peaks and troughs [15].

1.2.1 The averaging process

Averaging the signal after each stimulus allows canceling of activity unrelated to the stimulus and leaving activity that is consistently related to each stimulus. In the averaging process necessary to obtain an evoked potential waveform, two assumptions are made.

1. The first assumption is that the response to the sensory stimulus does not change during the recording session.
2. The second assumption is that the ongoing EEG is stimulus independent and can be regarded as a stochastic signal, not affecting the EP.

These assumptions however, may often not be valid.

With regard to the first assumption:

The responses to the sensory stimuli may change during a recording session. Habituation, for example, may occur and EPs may differ at the beginning and end of the session [16, 17]. Latency jitter of single responses, e.g. due to variability in performance during a session, causes broader and flatter waveforms [18, 19, 20]. When the sensory response is not constant during the stimulus period, the obtained average represents a mixture of responses as recorded during the stimulus period. These phenomena of habituation and latency jitter (chapter 6) will be further discussed in part A (chapters 2 to 4 and 6).

With regard to the second assumption:

Findings have indicated that the ongoing EEG and EPs are related in a fundamental manner and many investigators nowadays regard EPs as a reorganization of the spontaneous EEG [8, 21, 22]. Subtle changes in arousal or psychological state during a session, might cause changes in the ongoing EEG, which in turn affect the consecutive EP [22, 23]. These EEG-EP interrelations will be discussed further in part B (chapters 7 and 9).

1.2.2 Classification of evoked potentials

Evoked potentials can be classified in a number of ways. Commonly used classification methods are based on one or more of the following characteristics:

1. Depending on the sensory modality of used stimuli, the resulting evoked potential is classified as e.g. a somato-sensory (SSEP), visual (VEP), or auditory evoked potentials (AEP) [24]. In this thesis, we will focus mainly on the AEPs.
2. The peaks and troughs of evoked potentials can be labeled based on their latency (time of occurrence after stimulus onset). With AEPs, typically, a distinction between early-latency (0-10 ms) also known as brainstem evoked potentials, or BAEPs [11, 25] and the middle latency (10-50 ms [26, 27]), MAEPs, and late-latency (>50 ms [23]), LAEPs evoked potentials is made. The given boundaries are meant as a rough indication only; no general agreement exists on the exact values to be used. The various peaks and troughs of the AEP are named according to a generally accepted convention. In the MAEP and LAEP, also the polarity of the components is indicated. Positive peaks are labeled ‘P’ and negative peaks ‘N’.

1.2.3 Interpretation of Evoked Potentials

Interpreting evoked potential components can be approached from two angles [28, 74].

1. A neurophysiological approach, in which an EP components are defined by the neuronal structure that generates the evoked potential component. The five waves of the BAEP, for example, are generated by activity in the eighth nerve,

the cochlear nucleus, the superior olivary complex, the nucleus of the lateral lemniscus and the inferior colliculus respectively [12, 25]. These potentials arrive at the scalp through volume conduction. The proposed anatomical loci underlying the MAEP are the medial geniculate body, thalamocortical radiations and primary auditory cortex [12, 27, 29, 30, 31]. The LAEP is believed to be an exclusively cortical phenomenon [12].

2. A psychophysiological approach in which EP components are defined by the information processing demands invoked by the stimulus event. The MAEP is believed to reflect sensory aspect of information processing and is sensitive to e.g. attention and level of arousal [13, 26]. The MAEP is in general sensitive to psychoactive drugs. The LAEP is normally only seen in conjunction with cognitive tasks and is supposed to reflect those aspects of information processing that are induced by the particular paradigm. The LAEP is very susceptible to the level of arousal [12], and very sensitive to psychoactive drugs.

Based on the latter, psychophysiological approach, another commonly used classification is the distinction between exogenous and endogenous components [13, 18, 24, 29, 33]. Although no agreement on exact boundaries exists, most studies place the distinction somewhere around 50 ms after stimulus onset in humans [13, 34] and around 10 ms in rats [12]. The exogenous components vary as a function of physical stimulus parameters [18, 34] and are relatively unaffected by variations in psychophysiological state [28]. Endogenous components are predominantly modulated by aspects of information processing [18, 30] and therefore sensitive for the subjects state [28].

A major goal in psychophysiological EP research is to identify particular endogenous EP components as markers of specific aspects of information processing. This can be accomplished by determining how different EP components change in reaction to different task demands, by applying different stimulation paradigms [34].

1.3 Factors affecting the AEP

Variations in the AEP waveform may be caused by several factors. The most important ones are discussed in this section.

1.3.1 Modulation by stimulus characteristics (part A).

AEPs have been elicited using simple clicks [17], short and long tone-pips [16] or more complex stimuli like words, sentences or musical pieces [40]. Combinations of different tone-pips are often used to study several aspects of information processing [13]. First order stimulus characteristics, like frequency and amplitude of tone pips, determine for a large part the architecture of mainly early, exogenous components. Higher order

characteristics of stimuli, like their temporal pattern and their intrinsic meaning affect mainly the later, endogenous components [18].

1.3.2 Modulation by the subject's state (part B).

Changes in the level of arousal of the subject (e.g. drowsiness, sleep) greatly affect amplitudes and latencies of both MAEP and LAEP components. During waking, components in the EP are moderate in amplitude, while during slow wave sleep larger waves of especially late-latency components are visible [3, 9]. This is caused by more synchronized firing of neurons during slow-wave sleep, which results from increased hyperpolarizations.

1.3.3 Modulation by psychoactive drugs (Part A &B)

Like many other psychoactive drugs benzodiazepines are well known to affect EP components [35]. Again, primarily the cortical, endogenous MAEP and LAEP components are sensitive for these agents [35, 36, 37]. Benzodiazepines have sedating, muscle relaxant, anti-convulsant and anxiolytic effects [38]. In addition, benzodiazepines affect information processing [24, 37, 39, 75, 76]. The effects of benzodiazepines on endogenous EP components might help to interpret different EP components.

Throughout this thesis, we used examples of all three modulations mentioned above. Hence the title 'Mind Games'.

1.4 Main aim and outline part A: Passive paradigms

1.4.1 paradigms for eliciting evoked potentials

The main aim of the first part of this thesis was to develop tools to study the effects of (pharmacologically induced) sedation on information processing. By employing appropriate experimental designs, psychologists attempt to make inferences about the process that intervenes between the stimulus and response. [15, 18, 28]. Within cognitive psychophysiology these processes are studied by employing electrophysiological methods, like EP research. EPs are usually produced by sensory stimuli, and phase locked to experimental events [3, 13, 28]. Some investigators regard EPs as direct manifestations of information processing demands induced by those experimental events [28]. By applying different stimulation paradigms, different aspects of information processing can be studied. Psychophysiology has thus provided a noninvasive, reductionistic and fairly straightforward approach when studying the 'black box' [15].

Most studies that use experimental paradigms developed to investigate aspects of information processing on EPs require a specific response of the subject (e.g. button press) [21, 28]. Hence, it is difficult to determine whether changes in endogenous EP

components are due to changes in information processing demands, or are also related to the generation of a response. In passive paradigms, changes in endogenous EP components can no longer be related to the generation of a response, but only to changes in information processing demands involved in the stimulus event. A disadvantage however is that there is no control over the subject's behaviour (Coles, personal communication). However, passive paradigms may find appeal in conditions when a response is difficult to acquire e.g. in animal models, in states of lowered arousal or in certain patient groups [52, 53]. In part A of this thesis, we investigated which passive paradigms can be used to measure different aspects of information processing.

1.4.2 The passive oddball paradigm

Endogenous EP components are often elicited in an oddball paradigm [54, 55]. During the oddball task the subject is exposed to two different stimuli, one of which occurs relatively infrequently and is designated as a 'target' or 'rare'. The frequently occurring stimulus is labeled 'background' [56]. Stimulus-change and unpredictability are the main features of this paradigm [56]. In humans one of the most studied EP components elicited in the oddball paradigm is the P300, a positive peak occurring about 300 ms after stimulus onset, appearing only in reaction to 'target' stimuli. This was first reported by Sutton et all, [72] but a vast amount of work dedicated to P300 research has been undertaken since. For an overview see [73]. Most oddball studies employ an active discrimination task, e.g. by mental counting or a button press [20, 53]. Several studies however have reported similar EP results when using a passive oddball procedure in which no response to target tones is required [16, 57, 58]. In an initial experiment (chapter 2) we measured EPs elicited in a passive oddball paradigm in rats. In addition, we determined if diazepam differentially affected EPs elicited by background tones and EPs elicited by target tones. At the time this initial experiment was conducted, one of our interests was to study tolerance development by measuring the effects of chronic diazepam administration. Since the effects of chronic administration of diazepam are outside the scope of this thesis, this issue will only be discussed in chapters 2 and 7.

1.4.3 The ten-tone paradigm

By presenting two or more physically different stimuli in a passive oddball procedure, changes in EPs may not only be determined by cognitive processes, but may also be determined by the differences in the physical properties of the stimuli. Although counterbalancing could overcome this problem, another solution would be to only change the 'meaning' or higher order characteristics of single stimuli, without changes in lower order characteristics of stimuli (e.g. pitch, loudness, duration). By using such single stimulus paradigms in which only the presentation pattern of single stimuli is varied, changes in EPs can no longer be attributed to changes in stimulus characteristics, but only

be attributed to changes in aspects of information processing involved by the stimulus event.

One such passive, single-stimulus paradigm is the conditioning-testing or double click paradigm [59, 60]. This paradigm involves the presentation of pairs of stimuli. Normally, amplitude decrements of EP components in response to the second tone relative to the EP components in response to the first tone are observed. This response decrement is known as fast habituation [32, 61] or sensory gating [60].

In chapter 3 we determined whether decrements were more pronounced with short Inter-Stimulus Intervals (ISIs) than with longer ISIs. This to determine whether sensory gating could be ascribed to recovery phenomena or habituation. Instead of using the well known two-tone or double-click paradigm, we employed a ten-tone paradigm to determine whether decrement occurred fully between two tones, or developed more gradually over a train of ten tones.

In chapter 4 we studied the effect of diazepam on sensory gating in rats, by measuring the effects of diazepam on Auditory Evoked Potentials (AEPs) elicited in a ten-tone paradigm.

1.4.4 The omitted stimulus paradigm

Another passive, single-stimulus paradigm is the omitted stimulus paradigm. The omitted stimulus paradigm can be seen as a special variant of the oddball paradigm. Instead of presenting infrequently occurring target tones within a steady train of background tones, target tones are omitted [62, 63, 64]. The omitted stimulus paradigm thus provides a very direct and efficient tool to study aspects of information processing concerned with expectancy and time estimation. In chapter 5 we investigated if evoked potentials to omitted stimuli could be elicited in rats. In addition, we studied the effect of diazepam on the omission evoked potentials.

1.4.5 Addendum: a human experiment

In chapter 6 we studied the effects of diazepam on auditory evoked potentials (AEPs) elicited in a ten-tone paradigm, and on evoked potentials to omitted stimuli in humans. This was done to allow a comparison between human data and data obtained in previous experiments in rats.

1.5 Main aim and outline part B: EEG-EP interrelations

1.5.1 EEG-EP inter-relations

In the first part of this thesis we tried to develop tools to study the effects of (pharmacologically induced) sedation on information processing. We did this by measuring EPs elicited in passive paradigms. We found that diazepam affected EPs in this

situation. It is however well known that diazepam also affects the ongoing EEG [65, 66]. Findings have indicated that the ongoing EEG and EPs are related in a fundamental manner [8, 21, 22, 67]. Therefore, the main aim of the second part of this thesis was to determine if the effects of diazepam on the rat auditory EPs could be ascribed to its effects on the ongoing EEG. In other words, we determined if measuring drug effects on EPs (reflecting brain-reactivity) adds information to only measuring drug effects on the ongoing EEG (reflecting brain-activity).

1.5.2 The effect of diazepam on the ongoing EEG

Since diazepam has sedative properties, an increase in low frequencies (delta- and theta-activity) in the ongoing EEG would be expected. However, diazepam is known to increase the high frequencies (beta-activity) in the EEG, suggesting a change from a low vigilant state to a high vigilant state [65, 66]. This phenomenon is known as pharmacological dissociation [65].

In an initial experiment (chapter 7), we measured the effects of diazepam on the spectral content of the EEG in rats. Indeed, we found that (chronic) administration of diazepam caused a decrease in the power of the low frequency bands (1-8 Hz) and an increase in the power of the high frequency bands (21-40 Hz), as expected. As mentioned earlier, chronic effects of diazepam are outside the scope of this thesis and this issue will not be further discussed here.

1.5.3 The effect of changes in the ongoing EEG on the EP

In a subsequent experiment (chapter 8), we determined the influence of the ongoing EEG on auditory EPs. The ongoing EEG and EPs have been related using a variety of approaches. One such approach involves the recording of EPs during different sleep stages and wakefulness [9, 68, 69]. Similar to EEG patterns, the architecture of EPs is dependent on the state of alertness [69]. During waking, components in the EP are moderate in amplitude, while during slow wave sleep larger waves are visible [9, 70]. This is caused by more synchronized unit responses with sharper phases of excitations and inhibitions, which result from increased hyperpolarizations [3, 9].

Another approach involves recording pre- and post-stimulus EEG epochs and assessing how changes in the spectral power of the pre-stimulus EEG affects the post-stimulus EP measures [22]. The distribution of the activity in the low and high frequency bands of the EEG are considered to be an index of cortical arousal. Power in the low frequency bands (delta-activity) normally increases with a decrease in arousal and activity in the high frequency bands (beta- and gamma-activity) increases with an increase in arousal [71]. Therefore, by measuring power of the frequency bands in the ongoing EEG, the effects of more subtle variations in the level of arousal on the EP can be studied [22]. Still little is known about the relation between small pre-stimulus EEG variations and the subsequent EP. In chapter 8 we studied the EEG-EP relations in rats using this latter

approach by averaging EPs based on the relative magnitude of the frequency bands in the pre-stimulus EEG.

1.5.4 Effects of diazepam on the rat EEG-EP interrelation

In chapter 8 we found that EPs are indeed influenced by subtle changes in the ongoing EEG. Since we observed that diazepam affects both the rat EP (chapter 2, 4, 5 and 6), and the ongoing EEG (chapter 7), we investigated in our final experiment (chapter 9) if diazepam-effects on the rat auditory EPs could be ascribed to its effects on the ongoing EEG.

1.6 General methods

1.6.1 EEG recordings

EEG recordings in rats were obtained from epidurally implanted tri-polar electrodes (Plastics One, MS 333/2a) which were fixed on the skull with dental acrylic cement. All EEG recordings were obtained from freely moving rats. During recording sessions, rats were observed.

In the first experiment (chapter 2 and 8) EEG signals were measured bipolar between 1 Hz and 100 Hz and recorded digitally with a sample frequency of 512 Hz. In the subsequent experiments, EEG signals were measured between 0.1 Hz and 500 Hz and recorded digitally with a sample frequency of 1024 Hz, amplification: $(3 - 5)10^5$. All AEPs were determined by averaging EEG.

In the first experiment (chapter 2 and 8) electrodes were placed over the auditory cortex, the frontal cortex and a reference over the cerebellum (coordinates related to bregma: A -3.7, L 9.0; A 2.0, L 2.0 respectively). This electrode placement allowed us to measure AEPs [31] as well as Spike-Wave Discharges (SWDs) in the EEG [41, 42]. These SWDs in the EEG of the WAG/Rij rat provides a well known model for absence epilepsy [41, 42, 43]. Although at the time of this experiment, one of our aims was to measure the effects of diazepam on these SWDs, these results are not reported in this thesis.

Based on subsequent pilot experiments and a literature search [44, 45], we then changed our electrode placement for all the later experiments. The first active electrode was placed epidurally over the cortex (coordinates related to bregma: A -3.4, L 2.0), whereas the second active electrode and the ground electrode were placed epidurally over the cerebellum (coordinates related to lambda ca.: A –2.0, L 2.0; A –2.0, L –2.0 respectively). The main reason for this change was that these vertex recording would allow us better comparison with the results of other researchers from both our own group [46] and from other groups [44, 45].

In one experiment involving human subjects, EEG was also measured at the vertex (Cz electrode according to the 10-20 electrode system [47]). The left mastoid served as reference. A ground electrode was placed on the forehead.

1.6.2 Evoked Potential recordings

In all experiments, we elicited auditory EPs with pure tone-pip stimuli with a duration of 20 ms (rise-fall interval 5 ms). For rats, tone-frequencies of these tone-pips were always chosen between 8 kHz and 12 kHz, since the sensitivity for tones between those two frequencies is maximal in rats [48]. Loudness varied from 85 to 102 dB. White background noise of 65 dB was present in all experiments. In an experiment involving human subjects (chapter 6) tone-pips of 1000Hz were presented with a loudness of 70dB, which are common settings in human experiments [13].

All evoked potentials were determined by averaging EEG epochs between 100-50 ms before stimulus onset until 500-1000 ms after stimulus onset. A rejection program was utilized to eliminate individual trials in which the EEG exceeded 600 µV, thereby excluding trials with high EEG amplitudes due to e.g. motor artefacts.

EP components were defined by constructing a grand average EP in a control condition. Component latencies were then selected on the basis of the maximum peak amplitude. At these selected latencies amplitudes for each component and each subject were determined and were further used in the analysis. This method permitted blind scoring of component amplitudes for different experimental manipulations.

1.6.3 Diazepam administration

Throughout this thesis, we studied the effects of diazepam on EPs. Since we were not interested in dose-effect relations between diazepam and EPs, but used diazepam only as an example of a pharmacological modulation, we used only one dose of diazepam per experiment. In the first experiment (chapter 2 and 8) we used silastic tubes containing solid diazepam. The diazepam output from the implanted silastic tubes was ± 2 mg/kg per h. In subsequent experiments we administered a single dosage of 4.0 $mg*kg^{-1}$ diazepam s.c. Comparable dosages have also been used in others rat studies [49, 50]. In our human experiment (chapter 6) subjects received 10 mg diazepam p.o., a commonly used dosage in human subjects [50, 51].

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PART A

PASSIVE PARADIGMS FOR ELICITING EVOKED POTENTIALS

CHAPTER 2: TIME COURSE OF CHRONIC DIAZEPAM EFFECTS ON THE AUDITORY EVOKED POTENTIAL OF THE RAT

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Abstract: The time course of chronic diazepam effects on auditory evoked potentials was studied in rats. Auditory evoked potentials were elicited by background and target tones in a passive oddball paradigm. Diazepam was administered by slow release implants to establish constant blood concentrations. Recordings were made during 21 treatment days and 9 days after treatment ceased. Diazepam increased the amplitude of the P_{40} component and decreased the amplitude P_{72}-P_{102} components elicited by background tones. Diazepam increased the amplitude of the P_{40}-P_{48} component and decreased that of the N_{58} component elicited by target tones. These effects remained constant during treatment. Diazepam further decreased the amplitude of the P_{102} component elicited by target tones. This effect became more distinct over time. No group differences were found 9 days after treatment. The constant drug effects on middle-latency components (P_{40}-P_{48}) might reflect diazepam-induced changes in sensory information processing. The decreased long-latency component (P_{102}) might reflect a diminished attention to, or discrimination of, target tones. The time course of this effect might reflect a diazepam-enhanced habituation.

Key words: Diazepam; Tolerance; Auditory evoked potential; Habituation; Oddball paradigm; (Rat).

2.1 Introduction

Benzodiazepines have sedating, muscle relaxant, anti-convulsant and anxiolytic effects [15]. Benzodiazepines are therefore often prescribed for extensive periods of time, as long as several years or even for life [21]. However, benzodiazepines affect cognitive processes such as attention and memory [11, 20]. Tolerance develops to a number of the effects of benzodiazepines, e.g. in rats to the sedating, muscle relaxant, anti-convulsant [15, 16, 17, 27] and anxiolytic effects [17]. It is still unclear however whether the effects on cognition associated with the chronic use of benzodiazepines are persistent [28], since longitudinal studies of benzodiazepine effects on cognitive processes are [28].

Tolerance refers to the process by which the effect of the same dose of a drug decreases with repeated drug administration [25]. Whether or not signs of tolerance develop during chronic administration of benzodiazepines may depend on both the dose-regimen and the investigated variable [2, 25, 26]. Repeated doses of benzodiazepines in rats result in major fluctuations in the blood concentrations of the drug [19] due to the short half-life (± 1 h) in these animals [18]. In humans, repeated doses result in more constant blood concentrations due to the long half-life (35-100 h, [41]). Subcutaneously implanted silastic tubes containing diazepam allow for continuous release, resulting in constant blood concentrations in rats [19, 36, 39, 41]. Experiments using these silastic tube implants showed that tolerance developed to the anti-convulsant effect of diazepam [19, 36] and to its anxiolytic effect [12]. Using this method, we studied the time course of chronic diazepam effects on auditory evoked potentials in the rat.

Evoked potentials provide a sensitive method for studying the effects of drugs on sensory aspects of information processing [14, 31, 33]. Evoked potentials are discrete and minute electrical potentials that appear in the electroencephalogram (EEG). They are usually produced by, and time-locked to, sensory stimuli [9, 32]. Middle-latency components of auditory evoked potentials appearing between 10-50 ms after stimulus onset are thought to reflect sensory aspects of auditory information processing [4]. The long-latency auditory evoked potentials, occurring later than 50 ms after stimulus onset, only appear in conjunction with cognitive processes in rats [37]. Therefore, the effects of benzodiazepines on the components of the auditory evoked potential appearing >50 ms after stimulus onset might reveal insight into their effects on cognition. The effects of diazepam were studied with respect to middle- and long-latency components of auditory evoked potentials evoked by background and target tones in a passive oddball paradigm[11] [14]. An oddball paradigm is an experimental paradigm that is often used in human cognitive psychology [35]. During the oddball task the subject is exposed to two different stimuli, one of which occurs relatively infrequently and is designated as a target [5]. Stimulus-change and unpredictability are the main features of this paradigm [5].

The objective of this study was to investigate the effects of chronic administration of diazepam on the rat auditory evoked potential in order to determine whether or not tolerance would develop to these effects over a period of 21 days. Diazepam was administered by subcutaneous slow-release implants for 21 days [39]. Auditory evoked potentials were elicited by frequently occurring background tones and infrequently occurring target tones.

[1] In this paper we refer to the frequent tones as ‘backgrounds’ and the infrequent tones as ‘target’ tones. Since no response to ‘target’ tones was required, it would have been more accurate to refer to these tones as the ‘rare’ tones instead of ‘target’ tones. (Coles, personal communication).

2.2 Materials and methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethics committee for animal studies was obtained.

2.2.1 Animals

Sixteen male WAG/Rij rats (age 10 months, weight 350 ± 16 g (mean ± S.D.)) were maintained on a 12-12-h light-dark cycle with lights off at 9.00 a.m., and were singly housed with food and water ad libitum.

2.2.2 Surgical procedures

Hypnorm anesthesia (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone, 0.8 ml/kg i.m.) combined with Nembutal anesthesia (60 mg/ml sodium pentobarbital, 0.35 ml/kg i.p.) was used for implanting tri-polar electrodes (Plastics One, MS 333/2a) which were fixed on the skull with dental acrylic cement. Coordinates related to bregma were: A 2.0, L 2.0; and: A -3.7, L 9.0. respectively. The ground electrode was placed above the cerebellum. Animals were allowed to recover for four weeks before silastic tubes were implanted. The tubes were implanted, in pairs, under the skin of the back of the animals. For each experimental animal we used 8 silastic tubes of 8 cm length (Dow Corning, 0.062 inch inner diameter; 0.095 inch outer diameter), each containing 100 mg of solid diazepam without any vehicle (Roche Nederland). The diazepam output from the implanted silastic tubes was 17.6 ± 1.6 (mean ± S.D.) mg per animal per day (i.e. • 2 mg/kg per h). Control animals received 8 empty silastic tubes of the same length. Implantation and removal of the silastic tubes took place under ether anesthesia.

2.2.3 Recording procedures

EEG signals were measured between 1 Hz and 100 Hz and recorded digitally with a sample frequency of 512 Hz. EEGs were recorded during treatment on day 1, 3, 8, 14 and 21 from 14.00 h. till 15.30 h. in the afternoon. After removal of the silastic tubes all animals had two more stimulation sessions without recording on day 1 and day 4 after removal of the silastic tubes. A final recording was made 9 days after removal of the silastic tubes.

Auditory evoked potentials were evoked by two pure tone pip stimuli with a stimulus duration of 20 ms and were presented with random inter-stimulus intervals between 2.5 - 3.5 sec. Frequently occurring background tones (90% of the trials, 8 kHz, 96 dB), interspersed with infrequently occurring target tones (10% of the trials, 12 kHz, 102 dB), were presented. Per recording session a total of 1500 stimuli were presented. White background noise of 85 dB was present.

2.2.4 Data analysis

Auditory evoked potentials were determined by averaging EEG fragments recorded 100 ms before stimulus onset until 900 ms after stimulus onset. A rejection program was utilized to eliminate individual trials in which the EEG exceeded 600 µV, thereby excluding trials with high EEG amplitudes due to e.g. motor artefacts.

2.2.5 Statistical analysis

Two outliers were excluded because no clear components could be detected in the auditory evoked potential recorded 9 days after removal of the silastic tubes. Component latencies were selected on the basis of the maximum peak amplitude of the total grand average auditory evoked potential determined on the last recording day. Individual amplitudes at selected latencies were included in the analysis. Auditory evoked potentials elicited by background tones and target tones were analyzed separately.

For each component group differences in amplitude were determined as: difference = $\text{mean}_{\text{experimentals}} - \text{mean}_{\text{controls}}$, and S.E.M. = $\sqrt{((\text{S.E.M.}_{\text{controls}})^2 + (\text{S.E.M.}_{\text{experimentals}})^2)}$. Non-linear regression analysis, using the program GraphPad Prism 2.0, was performed on the data as this analysis takes into account the ratio scale of the time axis. F-tests were used to determine:

- Whether an exponential association described group differences in time significantly better than a linear regression, if not:
- Whether the linear regression differed significantly from a linear regression with slope = 0 (no time-dependent effect), and if not:
- Whether the linear regression with slope = 0 differed significantly from a linear regression with a slope = 0 and intercept = 0 (no time-dependent effect and no drug effect).

The component amplitudes measured 9 days after the end of treatment were tested by using a Student's t-test.

Figure 1a

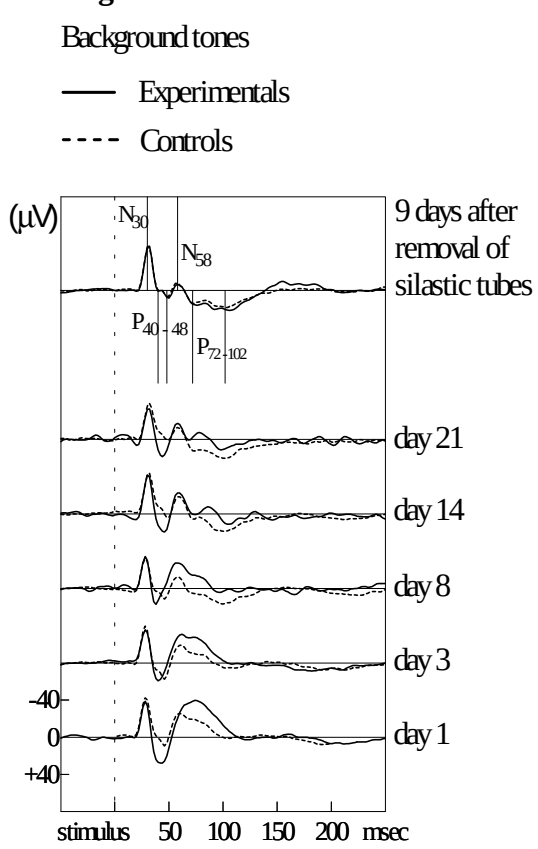

Figure 1b

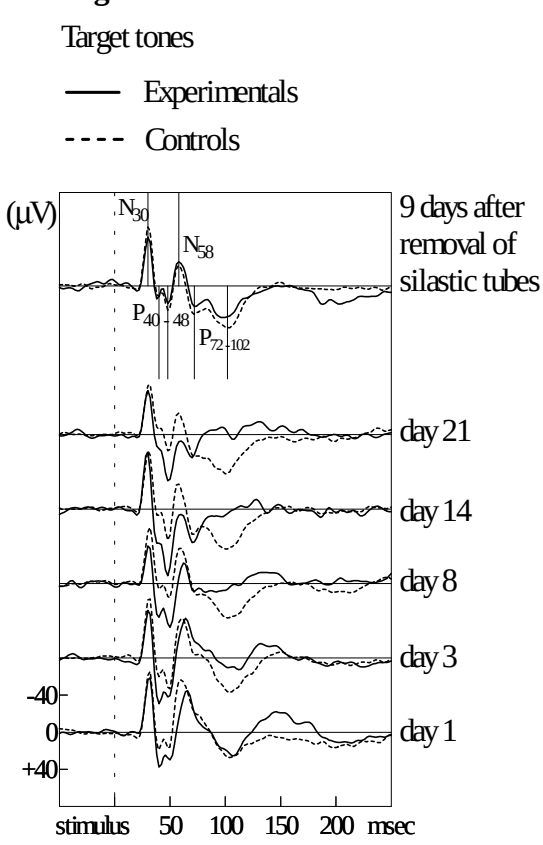

Figure 1a.

Grand average AEPs as evoked by background tones for experimental (solid lines) and control animals (dotted lines) on days 1, 3, 8, 14 and 21 during treatment and 9 days after removal of the silastic tubes. Amplitudes are given in μV (y-axes) and latencies are given in milliseconds after stimulus onset (x-axes). The N_{30}, P_{40}, P_{48}, N_{58}, P_{72} and P_{102} component are marked in the grand average AEPs as measured 9 days after removal of silastic tubes. In experimental animals the P_{40} is increased and the P_{72} and P_{102} are decreased during 21 days of treatment. No differences between experimental and control animals were found 9 days after removal of the silastic tubes.

Figure 1b.

Grand average AEPs as evoked by target tones for experimental (solid lines) and control animals (dotted lines) on days 1, 3, 8, 14 and 21 during treatment and 9 days after removal of the silastic tubes. Amplitudes are given in μV (y-axes) and latencies are given in milliseconds after stimulus onset (x-axes). The N_{30}, P_{40}, P_{48}, N_{58}, P_{72} and P_{102} component are marked in the grand average AEPs as measured 9 days after removal of silastic tubes. In experimental animals the P_{40}-P_{48} is increased and the N_{58} is decreased. The P_{102} is decreased during 21 days of treatment. This later effect becomes more distinct over time. No differences between experimental and control animals were found 9 days after removal of the silastic tubes.

2.3 Results

Grand average auditory evoked potentials over 21 treatment days and 9 days after the end of treatment for both experimental (n=6) and control animals (n=8) are shown in fig. 1a and 1b. Fig. 1a shows the grand average auditory evoked potentials evoked by background tones. Fig. 1b shows the grand average auditory evoked potentials evoked by target tones.

After determination of maximal peak values of the total grand average auditory evoked potentials recorded 9 days after tube removal, N_{30}, P_{40}, P_{48}, N_{58}, P_{72} and P_{102} components could be identified.

The difference scores for the amplitudes of the N_{30}, P_{40}, P_{48}, N_{58}, P_{72} and P_{102} components are shown in fig. 2a and 2b for background tones and target tones respectively. For each component of auditory evoked potentials, best fits are depicted. Parameter estimates and P-values are given for components for which the best fit over time differed significantly from no effect (y=ax+b, a=0, b=0).

Figure 2a

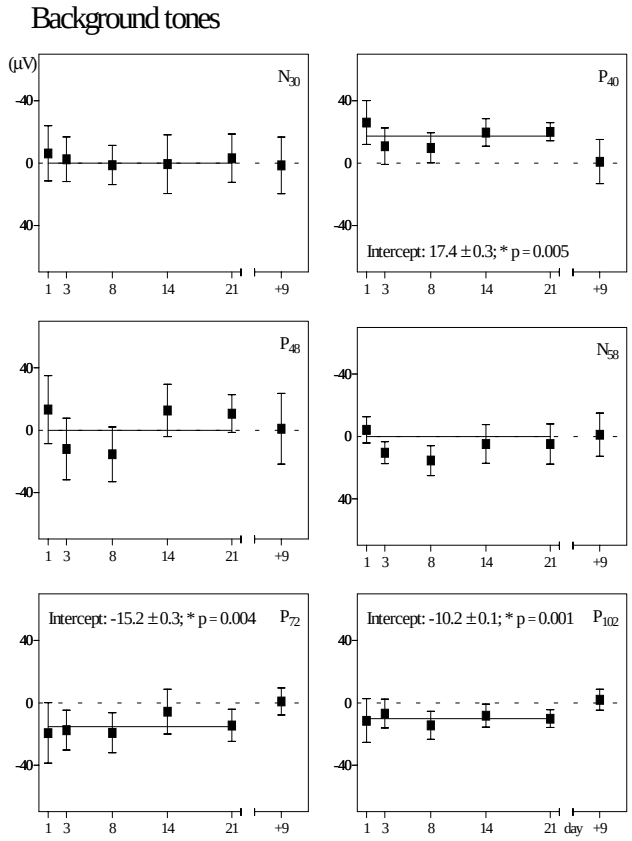

Figure 2a.

Difference scores (on y-axes in µV) between experimental and control animals on AEP components as evoked by background tones are given. Used fits over 21 treatment days (x-axes) show the time course of the diazepam effect. The P_{40} has a higher amplitude (i.e. a more positive deflection) for experimental than for control animals during 21 days of treatment. On the P_{72} and P_{102} experimental animals show a decrease in amplitude (i.e. a less positive deflection). The time course of these drug effects were best described by straight, horizontal lines and differed significantly from a constant zero effect. Intercepts and p-values are given in the panels. Differences were no longer found 9 days after removal of the silastic tubes.

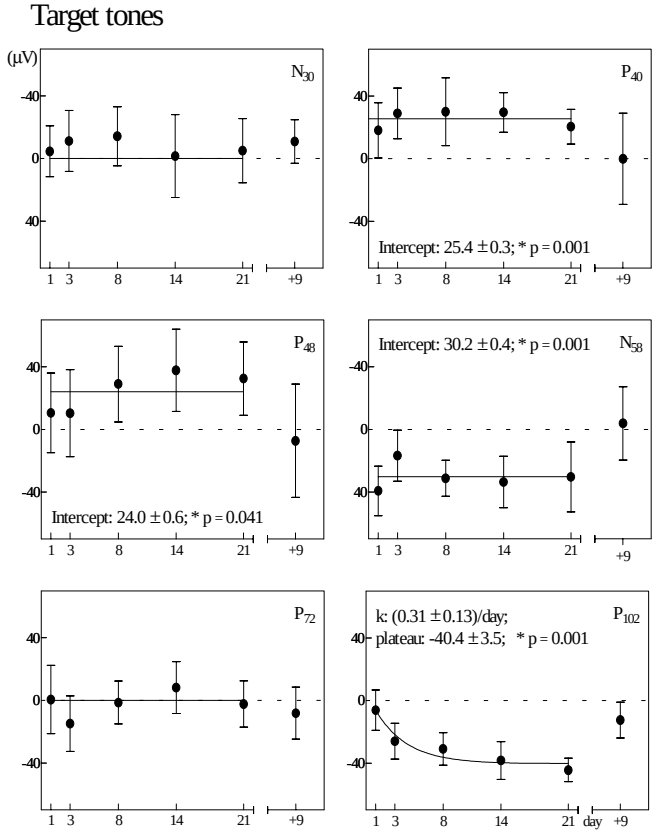

Figure 2b.
Difference scores (on y-axes in μV) between experimental and control animals on AEP components as evoked by target tones are given. Used fits over 21 treatment days (x-axes) show the time course of the diazepam effect. The P_{40} and P_{48} have a higher amplitude (i.e. a more positive deflection) for experimental than for control animals during 21 days of treatment. On the N_{58} experimental animals show a decrease in amplitude (i.e. a less negative deflection). The time course of these drug effects were best described by straight, horizontal lines and differed significantly from a constant zero effect. Intercepts and p-values are given in the panels. The P_{102} amplitude is decreased in experimental animals. The time course of this decrease was best described by an exponential decay model. Plateau, k and p-value are given in the panel. Differences were no longer found 9 days after removal of the silastic tubes.

2.3.1 Diazepam effects on auditory evoked potentials evoked by background tones

The P_{40}, component evoked by background tones (see fig. 2a) had a higher amplitude (i.e. a more positive deflection) for experimental than for control animals during the 21 days of treatment. The P_{72} and P_{102} components for experimental animals showed a decrease in amplitude (i.e. a less positive deflection) for background tones. The time course of these drug effects were best described by straight, horizontal lines (F-tests: constant drug effect (y=bx+a; b=0) differed significantly from a constant zero effect (y=bx+a; a=0, b=0). For P-values see fig. 2a). No differences between experimental and control animals were found 9 days after removal of the silastic tubes.

2.3.2 Diazepam effects on auditory evoked potentials evoked by target tones

With respect to the P_{40} and P_{48} components evoked by target tones (see fig. 2b), the amplitudes were higher (i.e. a more positive deflection) in experimental than in control animals during the 21 days of treatment. For the N_{58} component experimental animals showed a decrease in amplitude (i.e. a less negative deflection) for target tones. The time course of these drug effects were best described by straight, horizontal lines (for P-values see fig. 2b). With respect to the P_{102} component, a decreased amplitude (i.e. a less positive deflection) in experimental animals was found. This effect became more distinct

over time. The time course of this decrease was best described by an exponential decay model:
y=y(1) - y(plateau) * (exp(-kx)); with y(1) is the difference on day 1, y(plateau) and k are the parameter estimates, see fig. 1b. No differences between experimental and control animals were found 9 days after removal of the silastic tubes.

2.4 Discussion

The objective of this study was to investigate the effects of chronic administration of diazepam on the rat auditory evoked potential in order to determine whether or not tolerance would develop to these effects over a period of 21 days. In this study tolerance to diazepam did not develop for any of the changes in the rat auditory evoked potential. All diazepam effects were reversible as there were no differences between experimental and control animals 9 days after removal of the silastic tubes.

2.4.1 Diazepam effects on middle-latency components of auditory evoked potentials

An increase in the P_{40} amplitude evoked by background tones and P_{40}-P_{48} amplitude evoked by target tones was found. This effect remained stable during the 21 days of treatment. As middle-latency components of auditory evoked potentials are supposed to express aspects of sensory information processing, diazepam might have a constant effect on aspects of auditory information processing. Most studies report that diazepam decreases the amplitude of auditory evoked potentials [1, 33]. Contradictory findings however are not uncommon, as benzodiazepine's effects on the amplitude of auditory evoked potentials appear to be dose-related [3, 6]. Bringmann and Klingberg [8] reported an increased middle-latency negative peak in the rat evoked potential in states of low arousal. Increased amplitude of auditory evoked potentials due to a decrease in arousal is in agreement with the, in general, depressant effects of diazepam [15].

Furthermore, a correlation between background EEG and evoked potentials might be expected since an evoked potential is a sensory driven segment of EEG activity [8]. Background EEG activity and evoked potentials have been related by several authors [7, 29, 38]. Benzodiazepines are known to increase activity in the beta-band (12-40 Hz) of the EEG [39]. Increased beta-activity however is in conflict with a decreased arousal, because the former is normally seen under enhanced levels of arousal. Since it is known that diazepam causes both lowered arousal and increased beta-activity, this effect has been described as pharmacological dissociation [10]. Hence, both an increase in EEG beta-activity and a decrease in arousal might additionally account for the increase in the amplitude of the middle-latency components of auditory evoked potentials. Our previous study showed that the enhancement of beta-power was stable during the 21 days of treatment [39]. In the present study, the diazepam-induced increase in the middle-latency

components of auditory evoked potentials was stable during treatment and could be related to an increase in activity in the beta-band, since both these diazepam effects remain stable over time.

2.4.2 Diazepam effect on long-latency components of auditory evoked potentials evoked by background tones

We found a decrease in the P_{72} and P_{102} components elicited by background tones. A decreased amplitude of late auditory evoked potentials after acute diazepam treatment has previously been reported [1, 3, 30, 33], and may be interpreted as a decrease in the excitability of the central nervous system [33].

2.4.3 Diazepam effect on long-latency components of auditory evoked potentials evoked by target tones

The dominant component of the auditory evoked potential elicited by a target tone is a large late positivity: the P_{300} component [13, 35]. Although some studies reported a P_{300} component in the rat auditory evoked potential using a passive oddball paradigm [13, 29], others could not detect a P_{300} component using this paradigm [14]. In the present study, no P_{300} component in the auditory evoked potential elicited by the target tone was found. This could have been due to the number of trials in each session. It has been reported that the P_{300} component diminishes, and eventually disappears, in a passive oddball paradigm after more than 100 presentations of the target tone [34]. In our study about 150 target tones were presented in each session. Besides a P_{300} component, increased amplitudes at 60-80 and 120-180 ms after stimulus onset have also been found with target tones [14], and are thought to reflect cognitive processes like attention to, and discrimination of, target tones.

We found a decreased amplitude of the N_{58} component elicited by target tones after diazepam. This effect was stable over time. In addition, we found a reduction of the P_{102} component specifically with the target tones. This effect became more distinct over the 21 days of treatment such that the P_{102} component had disappeared at day 21, as can be seen in fig. 1b. To our knowledge, time-dependent effects of benzodiazepines on evoked potentials have been described only in humans. Allen et al., [1] found that alprazolam, given over 10 days, constantly decreased the amplitude of auditory evoked potentials. Higgitt et al., [23] also found that ketazolam and lorazepam decreased the amplitude of auditory evoked potentials. They also reported signs of tolerance in the last session, but only with respect to lorazepam [23]. A closer look at their results however shows that the amplitude of auditory evoked potentials in the placebo group decreased over time, whereas the lorazepam effect remained relatively stable and the ketazolam effect became more distinct over time. This last observation is in agreement with our diazepam effect with respect to the P_{102} component evoked by target tones. The different effects of

alprazolam [1], ketazolam and lorazepam [23] with time suggest that differences in drug effects over time might be attributed to the different benzodiazepines used.

In general, a decrease in a reaction to a stimulus with time reflects habituation [22]. Böker and Heinze [6] found a decrease in the P1 and N2 components of a visually evoked potential in humans. Moreover, these decreases became more distinct over trials within the session. In addition, Widgiz and Beck [40] reported that diazepam enhanced habituation of exploratory behaviour in rats over sessions. In the present study, the time course of the diazepam effect with respect to the amplitude of the P_{102} component evoked by target tones can be interpreted as an enhanced habituation elicited by diazepam.

In summary, diazepam has a constant effect on sensory aspects of information processing, as expressed in the altered amplitudes of the middle-latency components of auditory evoked potentials elicited by both the background and target tones. No tolerance developed over 21 days of treatment with respect to these effects.

Diazepam further seems to affect cognitive processes as expressed by the effect of the drug on the P_{102} component evoked specifically by target tones. It is hypothesized that during the 21 days of treatment diazepam diminished attention to, and discrimination of, target tones, as seen by a gradually disappearing P_{102} component. This effect might be related to an enhanced habituation evoked by diazepam.

The effects of benzodiazepines are reported to be either constant over time [39] or to decrease over time [25]. The time-dependency of the diazepam effect on the P_{102} component of the rat auditory evoked potential evoked by target tones adds an additional time-dependent effect, namely a more pronounced drug effect over time.

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CHAPTER 3: EFFECTS OF STIMULUS REPETITIONS WITH DIFFERENT INTER-STIMULUS INTERVALS ON THE RAT AUDITORY EVOKED POTENTIAL

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Abstract:
Background: The objective of this study was to investigate how components of the auditory evoked potential (AEP) decreased in reaction to stimulus repetition. Moreover, it was investigated whether decrements were more pronounced with short Inter-Stimulus Intervals (ISIs) than with longer ISIs.
Methods: AEPs to trains of 10 repetitive stimuli (85 dB, 8.22 kHz) were measured. Intervals in-between trains were 10 s. Over five sessions ISIs within a train were varied. ISIs of 0.3, 0.5, 1, 2 and 5 s. were used. Differences in AEP amplitudes between tone 1 and tone 2 of each train were analysed with respect to the ISI length. Additionally, differences between tone 2 and tone 10 were analysed.
Results: Between tone 1 and tone 2 an ISI-dependent decrease of the N23, P30, and P50 AEP components was found. Additionally, between tone 2 and tone 10, an ISI independent decrease of the P30 and P50 components was found, and an ISI-dependent decrease in the P150 wave.
Conclusions: It is proposed that ISI-dependent decreases in AEP component amplitudes reflect recovery phenomena underlying sensory gating, whereas ISI-independent decreases in AEP components reflect habituation to the temporal regularity of tone pips within a train.

Key words: auditory evoked potentials, sensory gating, habituation, rat.

3.1 Introduction

Auditory Evoked Potentials (AEPs) are discrete and minute electrical potentials that appear in the electroencephalogram (EEG). They are produced by and phase locked to sound stimuli [1-3]. AEPs may be regarded as direct manifestations of information processing demands induced by the stimulus paradigm [3]. Therefore, the AEP technique provides non-invasive measures for studying aspects of information processing. An often-employed paradigm for studying the effects of stimulus repetition on AEPs is the ‘conditioning-testing’, or ‘double-click’ paradigm [4-6]. This paradigm involves the presentation of pairs of stimuli. Commonly, an AEP amplitude decrement of the second AEP response relative to the first AEP response is found. This phenomenon is known as

'sensory gating', which is believed to be a complex, multi-factorial physiological process, to protect higher cortical centres from being flooded with irrelevant sensory stimuli [4]. Sensory gating has been proposed to result from recovery cycle phenomena [4-6], or from behaviourally mediated habituation [6, 7]. Several authors have investigated the effect of Inter-Stimulus Interval (ISI) length on sensory gating [5, 6, 8-12]. Others have compared AEP component amplitudes evoked by trains of stimuli with different stimulation rates [7, 9, 11].

In the present study it was determined whether amplitude reduction with stimulus repetition depended on the ISI between stimuli, thus supporting the recovery cycle theory, or occurred independently of the length of the ISI, thus favouring the habituation theories of sensory gating. Five different ISIs were used in this study. Moreover, it was determined whether amplitude reductions occurred between the first and the second stimulus within a train, or more gradually over ten tone-pip stimuli within a train. This was done in order to enhance discriminability between theories.

3.2 Materials and methods

The study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethics committee for animal studies was obtained. Sixteen male Wistar rats, with weights of 407 ± 34 g (mean ± SD) were maintained on a 12-12-h light-dark cycle with lights off at 8.00 a.m. They were singly housed with food and water ad libitum. Rats were divided in two groups (n=8) to allow counterbalancing of recording sessions. Isoflurane anaesthesia was used for implanting a tripolar electrode set (Plastics One, MS 333/2a). This set was fixed on the skull with dental acrylic cement. The coordinates of the first active electrode related to bregma were: A -3.4, L 2.0. The second active electrode and the ground electrode were placed above the cerebellum. Animals were allowed to recover for 2 weeks before recordings were made.

EEG signals were measured between 0.1 Hz and 500 Hz and recorded digitally with a sample frequency of 1024 Hz. All AEPs were determined by averaging EEG fragments recorded 100 ms before stimulus onset until 1000 ms after stimulus onset. A rejection program was used to eliminate individual trials in which the EEG exceeded 600 μV, thereby excluding trials with high EEG amplitudes e.g. due to motor artefacts. AEPs were elicited by the presentation of 150 trains of 10 repetitive tone-pip stimuli (8.22 kHz, 85 dB, stimulus duration 20 ms). Trains were followed by an intertrain interval of 10 s. The effect of stimulus repetition for several ISIs between tone-pip stimuli within a train were studied. ISIs of 0.3, 0.5, 1, 2 and 5 s were studied in five sessions. All sessions were recorded on the same day and presented in a random order for group 1 (n=8). This order was reversed for group 2 (n=8). White background noise of 65 dB was present.

A grand average AEP evoked by tone 1 of each train for all ISI conditions was constructed. Component latencies of the AEPs were selected on the basis of the maximum peak amplitude of this grand average AEP. After visual inspection of the individual AEPs one outlier was removed. Individual amplitudes at the selected latencies were determined. AEP component amplitudes evoked by tone 1, tone 2 and tone 10 within each train were further taken into analysis. For each ISI condition, differences in microvolts between AEP component amplitudes elicited by tone 1 and tone 2 within a train were calculated. A second analysis was performed on the differences between AEP component amplitudes elicited by tone 2 and tone 10 within a train. Three hypotheses were tested by nonlinear regression analysis of the AEP amplitude differences between tone 1 and tone 2, and the AEP amplitude differences between tone 2 and tone 10, over increasing ISI lengths. This was done by using the program GraphPad Prism 2.0. F-tests for goodness of fit were obtained.

3.3 Results

Figure 1 shows for all ISI conditions grand average AEPs elicited by tone 1, 2, 3, 4, 5 and 10 within each train. An N_{23}, P_{30}, N_{40}, P_{50}, and P_{150} component could be identified.

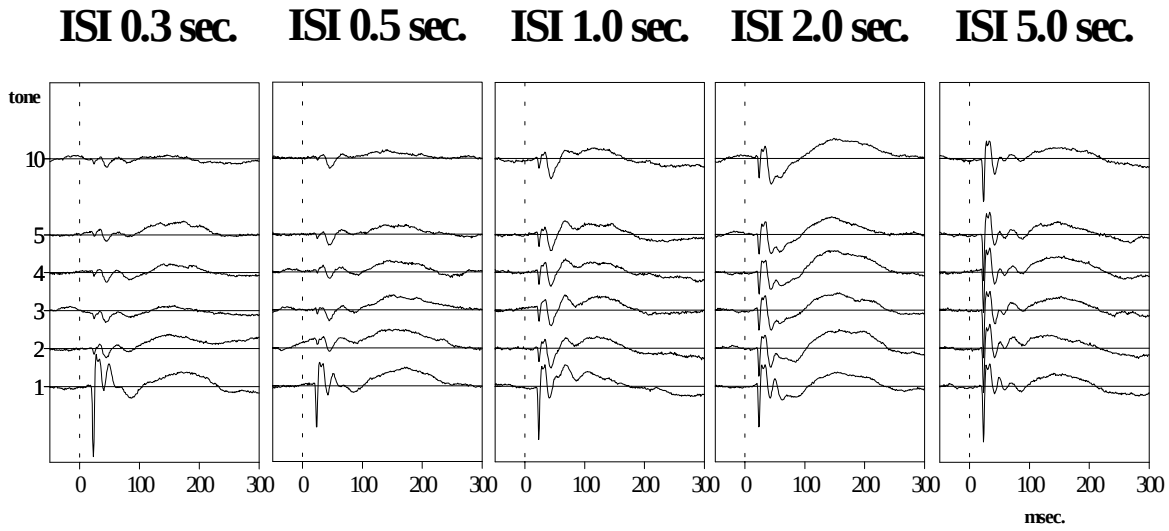

Fig. 1 shows grand average AEPs of rats (n=15) evoked by 10 consequtive tone-pips within a train (tone 1, tone 2, tone 3, tone 4, tone 5 and tone 10; y-axes, bottom to top), for all 5 ISI conditions (from left to right: 0.3-, 0.5-, 1-, 2-, and 5-s ISI conditions). Amplitudes are given in µV and latencies are given in milliseconds after stimulus onset (x-axes).

Three hypotheses were tested.

The H_0 hypothesis predicting that stimulus repetition had no effect. In this case differences in AEP component amplitudes will be best described by a straight line with a slope of 0 and an intercept of 0.

The H_1 hypothesis states that stimulus repetition will decrease the AEP component, but the amount of decrement does not depend on ISI length. The differences in AEP-component amplitudes will then best be described by a straight line with a slope of 0 and an intercept which is not 0.

Finally, the H_2 hypothesis predicts that stimulus repetition decreases the AEP component and the amount of decrement will diminish with an increase in ISI length. In the latter case the differences in AEP component amplitudes will best be described by an exponential decay of $y = y_{(max)} - y_{(min)} * \exp^{(-kx)}$; while $y_{(max)}$ is the difference in AEP component amplitude for ISI 0.3 s, whereas $y_{(min)}$ is the difference score for ISI 5s being 0 and x is the used ISI. The theoretical curves of the H_0, H_1 and H_2 hypotheses are shown in Fig. 2.

Best fits for differences between AEP component amplitudes elicited by tone 1 and tone 2 within a train, are shown in Fig. 3a. Figure 3b shows the best fits over increasing ISI lengths for differences between AEP component amplitudes elicited by tone 2 and 10 within a train.

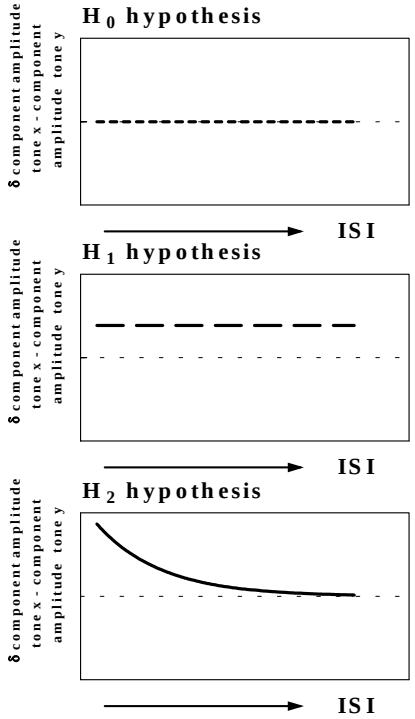

Fig. 2 shows the hypothetical curves used for anslysing difference scores of AEP component amplitudes (y-axes) by ISI (x-axes).

The H0 hypothesis showing no effects of stimulus repetition, the H1 hypothesis showing an ISI independent effects of stimulus repetition and the H2 hypothesis showing an ISI-dependent effect of stimulus repetition.

H_0 hypothesis: between tone 1 and tone 2, no decreases of the N_{40} and P_{150} component amplitudes were found. Between tone 2 and 10, no decreases of the N_{23} and N_{40} component amplitudes were found (All p-values <.05).

H_1 hypothesis: between tone 2 and tone 10 an ISI independent decrease of the P_{30}, and P_{50} component amplitudes was found (All p-values <.05).

H_2 hypothesis: between tone 1 and tone 2 an ISI dependent decrease of the N_{23}, P_{30}, and P_{50} component amplitudes was found. Between tone 2 and 10 an ISI dependent decrease of the P_{150} component amplitude was found (All p-values <.05).

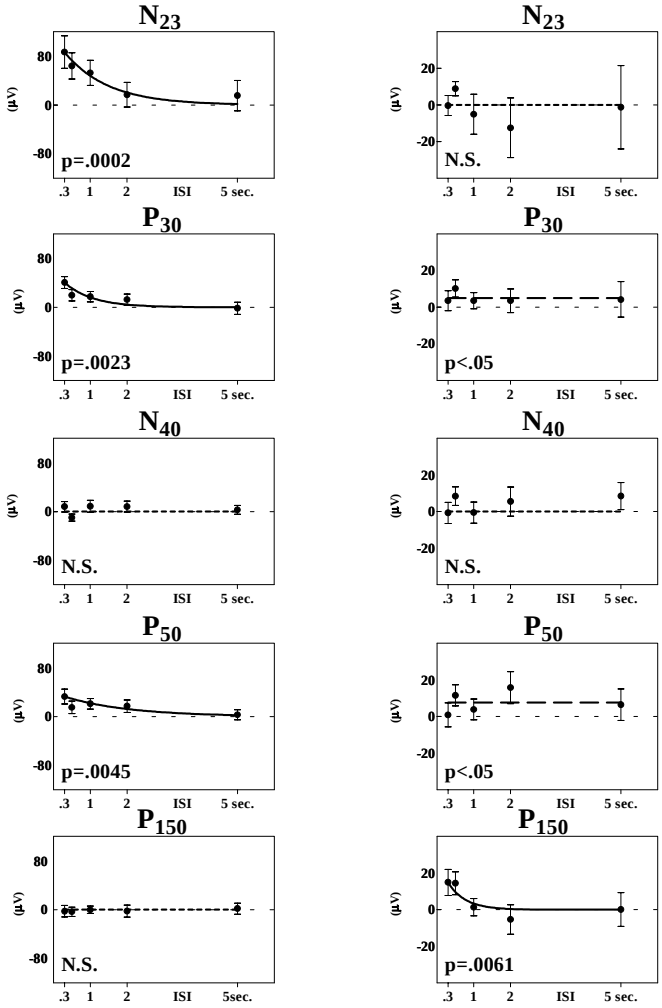

Figure 3a shows for each AEP component the difference score of each AEP component amplitudes in µV (y-axes) evoked by tone 1 and tone 2 by ISI (x-axes). Used fits show the stimulus repetition effect over ISIs. Best fits and p-values are given. With respect to decrements between tone 1 and tone 2: Decrements of the N23, P30 and P50 components could be best described by an exponential decay, thus supporting the H2 hypothesis: ISI dependent effect of stimulus repetition. The N40 and P150 components showed no stimulus repetition effects at all, thus the H0 hypothesis could not be rejected.

Figure 3b shows for each AEP component the difference score of the AEP component amplitudes in µV (y-axes) evoked by tone 2 and tone 10 by ISI (x-axes).Used fits show the stimulus repetition effect over ISIs. Best fits and p-values are given. With respect to decrements between tone 2 and 10: Decrement of the P150 component could be best described by an exponential decay, thus supporting the H2 hypothesis: ISI dependent effect of stimulus repetition. Decrement of the P30 and P50 components could be best described by a straight, horizontal line with intercept ≠ 0, thus supporting the H1 hypothesis: ISI independent stimulus repetition effect. The N23 and N40 component showed no decrease with stimulus repetition, thus the H0 hypothesis could not be rejected.

3.4 Discussion

To determine whether amplitude reductions occurred completely between the first and the second stimulus, or gradually over trains of stimuli, the decrements of AEP amplitudes between tone 2 and tone 10 with respect to different lengths of ISI were measured. It was found that the P_{30}, P_{50} and P_{150} AEP components of tone 10 were

decreased compared to tone 2. Stronger amplitude decrements with more than two stimuli have been reported earlier [8,11,15]. Of the P_{150}, an ISI-dependent decrement was found (H_2 hypothesis). Therefore, inhibition of this late-latency AEP component appears to evolve slower than inhibition of the middle-latency N_{23}, P_{30} and P_{50} AEP components. It was, however, found that the decrease in the P_{30} and P_{50} components supported the H_1 hypothesis: it was ISI-independent. This suggests that stimulus repetition alone causes these decrements, irrespective of the length of the used ISI. It is proposed that these ISI-independent decrements reflect habituation to the temporal regularity of presented stimuli. Temporal regularity of stimuli within a train is a higher order characteristic of the presented stimuli [5]. To measure aspects of temporal regularity, more than two consecutive stimuli within a train are needed. The first two stimuli determine the temporal regularity pattern within a train. Subsequent stimuli can be used to measure the processing of this pattern. It is, therefore, argued that presenting trains of stimuli adds an extra dimension to the double-click paradigm, namely that of temporal regularity of repetitive stimuli within train.

3.5 References

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CHAPTER 4: EFFECTS OF DIAZEPAM ON AUDITORY EVOKED POTENTIALS OF RATS ELICITED IN A TEN-TONE PARADIGMA

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Abstract: The effect of diazepam on sensory gating was studied in rats, by measuring diazepam effects on Auditory Evoked Potentials (AEPs) elicited in a ten-tone paradigm. Trains of 10 repetitive tone-pip stimuli were presented. Rats (n=8) received 4 mg.kg-1 diazepam s.c. or vehicle, counterbalanced over two sessions. Diazepam decreased the amplitude of the middle-latency P30 component and increased the amplitudes of the late-latency N60 and P67 components. The increase of the late-latency components might be due to a diazepam-induced decrease in arousal. Stimulus repetition decreased the amplitudes of the middle-latency N18 and P30 components in both conditions. This suggests that automated neuronal recovery functions underlying sensory gating remain intact with diazepam. In the vehicle condition the amplitude of the late-latency P67 decreased with stimulus repetition, but not in the diazepam condition. This suggests a diazepam-induced decrease of behaviourally mediated habituation.

Key words: Sensory Gating, Diazepam, Auditory Evoked Potentials, Information Processing, Habituation, Rats.

4.1 Introduction

Benzodiazepines have sedating, muscle relaxant, anti-convulsant and anxiolytic effects [1, 2]. In addition, benzodiazepines affect aspects of information processing such as attention and memory [3, 4, 5]. Evoked Potentials provide a sensitive method for studying the effects of drugs on information processing [6, 7, 8]. Therefore, we used this neurophysiological method for studying the effects of diazepam on information processing.

Evoked Potentials (EPs) are discrete and minute electrical potentials that appear in the electroencephalogram (EEG). They are usually produced by, and time-locked to, sensory stimuli [9, 10]. EP components are typically divided based on their latency into early-, middle- and late-latency components [9, 10]. Early components (appearing 0-10 ms. after stimulus onset in rats) reflect obligatory responses evoked by events outside the brain and their variance is primarily determined by the physical characteristics of the stimulus [11]. Middle-latency components (appearing 10-50 ms. after stimulus onset in rats) are assumed to be determined by sensory aspects of information processing [11, 12].

Late-latency components (appearing >50 ms. after stimulus onset in rats) are assumed to be determined by cognitive aspects of information processing [11]. Therefore, effects of diazepam on both middle- and late-latency EP components might reveal insight into its consequences on information processing [7, 13].

Many studies investigating information processing on EP components require a specific response of the subject, e.g. a button press [14, 15, 16]. Such responses might be difficult or impossible to acquire when measuring in animals, during states of (pharmacologically induced) sedation and in certain patient groups [17]. In addition, most studies that involve experimental paradigms developed to elicit EPs, present two or more physically different stimuli, e.g. the 'oddball' paradigm [15, 16, 18]. Alterations in EPs might thus be due to changes in information processing and to the physical differences of presented stimuli. By using passive, single-stimulus paradigms, changes in the EP can only be attributed to changes in information processing.

An often-employed passive, single-stimulus paradigm that studies the effects of stimulus repetition on AEPs, is the 'double-click' or 'two-tone' paradigm [8, 19, 20, 21, 22]. This paradigm involves the presentation of pairs of stimuli in a close (i.e. 500 ms) temporal relationship [20]. Commonly, an AEP amplitude decrement of the second AEP response relative to the first AEP response is found. This response suppression has been referred to as the P50 gating [23] or sensory gating [19]. Sensory gating is believed to be a complex, multifaceted physiological function protecting higher cortical centres from being flooded with incoming irrelevant sensory stimuli [19]. Sensory gating has been proposed to result both from neuronal recovery phenomena [19, 21, 22] and from behaviourally mediated habituation [8,22]. By presenting more than two auditory stimuli, more pronounced reductions have been found [21, 22, 24, 25]. The objective of this study was to investigate the effects of diazepam on sensory gating of the rat AEP as measured in a ten-tone paradigm.

4.2 Materials and methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee for animal studies has been obtained. Eight male Wistar rats, weighing 470 ± 49 grams (mean ± SD), were maintained on a 12-12 h. light-dark cycle with lights off at 8.00 a.m. They were singly housed with food and water ad libitum. Isoflurane anaesthesia was used for implanting a tripolar electrode set (Plastics One, MS 333/2a). The first active electrode was placed epidurally over the vertex. The coordinates related to bregma were: A -3.4, L 2.0 [24, 26]. The second active electrode and the ground electrode were placed epidurally over the cerebellum (coordinates related to lambda ca.: A –2.0, L 2.0; A –2.0, L –2.0 respectively). Rats (n=8) were allocated to two groups to allow counterbalancing

of vehicle and diazepam conditions. The experiment was counterbalanced with a three-day interval. Rats (n=8) received at the beginning of the experiment a subcutaneous injection (0.8 ml.kg^{-1}) with either (n=4) the vehicle Lipovenös (glycerine: 2.5%, (3-sn-phosphatidyl)choline: 1.2%, soy bean oil: 15%. Fresenius BV, 'sHertogenbosch, the Netherlands) or (n=4) Diazemuls (4.0 mg.kg-1 diazepam dissolved in lipovenös, Dumex, Hilversum, the Netherlands).

EEG recordings were obtained from freely moving rats. EEG signals were measured between 0.1 Hz and 500 Hz and recorded digitally with a sample frequency of 1024 Hz. Auditory Evoked Potentials (AEPs) were elicited by a ten-tone paradigm. Trains (n=150) of 10 repetitive tone-pip stimuli in each train (10.2 kHz, 90 dB, stimulus duration 20 ms) with a 2 s Inter-Stimulus Interval (ISI) were presented via a speaker mounted ca. 1 meter above recording cages.

We used a 2 s ISI based on a previous experiment [24]. Trains were separated by an Inter-Train Interval (ITI) of 4 s. White background noise of 65 dB was present.

Auditory Evoked Potentials were determined by averaging EEG fragments recorded 100 ms before stimulus onset until 900 ms after stimulus onset. Trials in which the EEG exceeded 600 μV were eliminated to avoid e.g. motoric artefacts. After determination of maximal peak-values of the grand average auditory evoked potentials, individual AEP amplitudes at selected latencies (18 ms, 30 ms, 60 ms 67 ms and 150 ms after stimulus onset) were further taken into analyses [13, 24]. AEP component amplitudes evoked by tone 1, tone 2, tone 3, tone 4, tone 5 and tone 10 within a train were analysed. For each component a two-within ANOVA was employed. 'Drug' being the within subjects variable and 'tone' being the repeated measure. Post-hoc one-way ANOVA analyses with repeated measures were employed for the diazepam and control conditions separately, whenever a drug * tone interaction was observed.

4.3 Results

Figure 1 shows the grand average auditory evoked potentials of eight rats in both the diazepam (solid lines) and control (dotted lines) condition, elicited by tone 1, 2, 3, 4, 5 and 10 After determination of maximal peak-values of the grand average auditory evoked potentials, an N_{18}, P_{30}, N_{60}, P_{67} and P_{150} component could be identified.

Figure 1

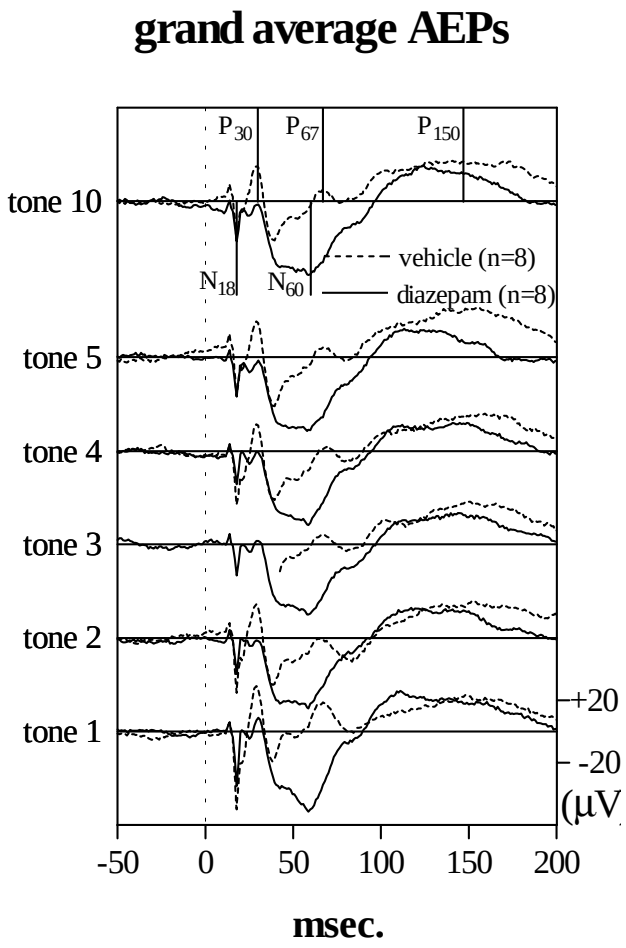

Figure 1.
Grand average AEPs (rats n=8) evoked by 10 consecutive tone pips within a train (y-axes, bottom to top: tone 1, tone 2, tone 3, tone 4, tone 5 and tone 10, each tone n=150), for both conditions (dotted lines for the control condition, solid lines for the diazepam condition). Latencies are given in milliseconds after stimulus onset (x-axes); amplitudes are given in µV (y-axes). The N_{18}, P_{30}, N_{60}, P_{67} and P_{150} components are marked in the grand average AEP elicited by tone 10.

Figure 2

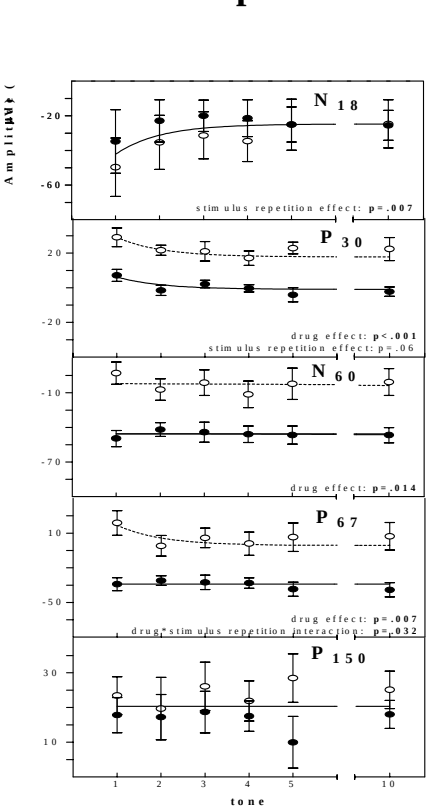

Figure 2.
From top to bottom amplitudes of the N_{18}, P_{30}, N_{60}, P_{67} and P_{150} components are depicted. AEP component amplitudes (y-axes) are given for both conditions (open circles for the control condition, solid circles for the diazepam condition), in response to tone 1, 2, 3, 4, 5 and 10 of a train (x-axes). Stimulus repetition effects are depicted for both conditions (dotted lines for the control condition, solid lines for the diazepam condition, and only one line if no drug effect was observed), with either a limited exponential growth curve (significant amplitude decrement with stimulus repetition) or a straight line, (no significant amplitude decrement with stimulus repetition). P-values of main drug effects, main tone effects and drug * tone effects are given in the panels.

Main tone effect: With respect to the amplitudes of the middle-latency N_{18} and P_{30} AEP components, decreases in amplitudes with stimulus repetition were observed (N_{18}: $F(5,35)=3.8$, $p=.007$; P30: $F(5,35)=2.4$, $p=.06$ marginally significant).

Drug * tone interaction: With respect to the amplitude of the P_{67} AEP component, a drug * tone interaction was found ($F(5,35)=14.6$, $p=.032$). Post-hoc one-way ANOVA with repeated measures revealed that the amplitude of the P_{67} AEP component decreased with stimulus repetition ($p<.05$) in the control condition, whereas in the diazepam condition no stimulus repetition effect was found. No drug, tone or interaction effects were observed with respect to the P_{150} component.

4.4 Discussion

The objective of this study was to investigate the effects of diazepam on sensory gating of AEP components in rats. In this study we found that diazepam did not alter sensory gating of the middle-latency AEP components. However, diazepam disrupted sensory gating of late-latency AEP components indicating effects on cognitive aspects of information processing. A diminished ability of sensory gating, or habituation, has been ascribed to deficits in attention [27]. Our results might thus be related to the well-known effects of benzodiazepines on attention [3-5].

4.4.1 Main drug effect.

In this study we found a decrease of the middle-latency P_{30} component due to diazepam. With respect to the late-latency components, we found an increase (more negative values) of the N_{60} and P_{67} AEP components. This is in agreement with a previous study where we found more negative values of the late-latency N_{58} and P_{72} AEP components due to diazepam in rats [13]. Increased EP peaks have commonly been reported during states of low arousal [28, 29]. Previous experiments at our department showed that total amount of sleeping time almost doubled with a comparable dosage of diazepam in rats [30]. Our finding of the increased late-latency AEP component might thus be ascribed to a decrease in arousal due to the hypnotic effects of diazepam [1].

4.4.2 Main tone effect.

We found decreased amplitudes of the middle-latency N_{18} and P_{30} AEP components with stimulus repetition in both the diazepam and the control condition. Decreased amplitudes, or sensory gating, of middle-latency AEP components with stimulus repetition are in agreement with results from studies employing the two-tone paradigm in both humans [31, 32] and rats [26, 33].

In a previous study [24] we stated that if amplitude reductions with stimulus repetition depended on the ISI length between stimuli, this would support theories of

neuronal recovery phenomena underlying sensory gating [19, 21, 22]. Alternatively, if amplitude reductions with stimulus repetitions would occur independently of the ISI length, this would suggest that behaviourally mediated habituation underlies sensory gating [8, 22].

Decrements of middle-latency AEP components appear to be ISI dependent in humans [22, 25, 34] and rats [24, 35, 36], thus supporting the neuronal recovery function explanation of sensory gating of middle-latency AEP components. In the present study, we found amplitude reductions of the middle-latency N_{18} and P_{30} AEP components in both the diazepam and control condition. Therefore, diazepam does not affect sensory gating of middle-latency components, suggesting that diazepam does neither enhance nor diminish neuronal recovery functions underlying sensory gating.

*4.4.3 Drug * tone interaction*

In this study we found that in the control condition the amplitudes of the late-latency P_{67} AEP component decreased with stimulus repetition, whereas in the diazepam condition no such decrement was found. In our previous study [24] we found that reductions of late-latency AEP components occurred independently of ISI length, thus favouring the explanation of behaviourally mediated habituation underlying sensory gating [8, 22]. Diazepam therefore seems to diminish behaviourally mediated habituation underlying sensory gating.

In all, we found that diazepam increased amplitudes of late-latency AEP components. Stimulus repetition effects with respect to middle-latency AEP components were not disrupted by diazepam, whereas stimulus repetition effects with respect to the late-latency AEP component were disrupted by diazepam. These findings suggest that highly automated neuronal recovery functions underlying sensory gating remain intact whereas behaviourally mediated habituation is diminished due to diazepam.

Acknowledgement

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CHAPTER 5: OMISSION EVOKED POTENTIALS (OEPS) IN RATS AND THE EFFECTS OF DIAZEPAM

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Abstract: Introduction: We investigated whether Evoked Potentials to omitted stimuli could be measured in rats. Such an animal model would provide a direct measure of experimental manipulations on aspects of information processing concerned with expectancy and time estimation.
Methods: In the first experiment, Omission Evoked Potentials (OEPs) were elicited in rats by omitting stimuli (10%) from a train of tone-pips with a fixed ISI (3 s) in a test session. The control session consisted of omitting stimuli (10%) from a train of tone-pips with a variable ISI (2.5-3.5 s.). In the second experiment OEPs were measured in rats that received 4 mg.kg-1 diazepam or vehicle s.c.
Results: In the test session of the first experiment half of the animals showed an OEPs which consisted of a late-latency positive wave (100-400 ms.). No OEPs were found in the control session. Animals showing an OEPs in experiment 1 were passed to experiment 2. In the vehicle condition of the second experiment all rats showed an OEPs. In the diazepam condition no OEPs were found.
Conclusions: We found that OEPs can be measured in rats. In addition, OEPs are disrupted by diazepam. We propose that OEPs might provide a direct and sensitive tool to study e.g. drug effects on aspects of information processing.

Key words: Evoked Potentials; Omitted stimuli; Diazepam; Information processing; Expectancy; Time estimation.

5.1 Introduction

EPs (Evoked Potentials) are small voltage fluctuations resulting from sensory, cognitive, or motor evoked neural activity. These electrical changes are commonly obtained by averaging EEG epochs time-locked to repetitious events. An important goal in Evoked Potential (EP) research is to examine aspects of information processing related to e.g. memory, learning, and attention [2]. Various cognitive processes that occur between stimulus and response can be studied by employing different experimental stimulation paradigms for eliciting EPs.

EPs consist of components that are typically divided based on their latency into exogenous and endogenous components [10, 26]. It is assumed that components appearing 0-10 ms. after stimulus onset, the exogenous components, are primarily

determined by the physical characteristics of the external stimulus [4, 34]. The components appearing >10 ms. after stimulus onset, the endogenous components, are assumed to be determined by cognitive aspects of information processing [4, 14, 34].

However, studying information processing by comparing endogenous EP components from different experimental designs is difficult because of the interaction between exogenous and endogenous components results in complex waveforms [14].

EPs in reaction to omitted stimuli exist entirely of endogenous components. Thus recorded EPs can be wholly attributed to aspects of information processing involved in the (internal) event of stimulus omission [4, 37]. The omitted stimulus paradigm therefore provides a gallant and straightforward tool to elicit selectively endogenous EP components.

Evoked potentials to omitted stimuli consist of a late positive wave, similar to the P300 and have been known in human subjects for many years [1, 5, 7, 8, 19, 22, 33, 36-40]. Evoked potentials to omitted stimuli are supposed to reflect expectancy and are strongly influenced by attention [3, 4, 7, 37]. Expectancy depends to a large degree upon memory and time sense [33, 37, 40].

These evoked potentials to omitted stimuli have been previously referred to as Missing Stimulus Potentials [36, 37], Omitted Stimulus Potentials [7] and Emitted Potentials [3]. In this paper we will refer to these evoked potentials as Omission Evoked Potentials, OEPs, to indicate that we are dealing with (a special kind of) Evoked Potentials (and also because we like the abbreviation so much because it sounds like ‘Oops’, there’s one missing!).

5.1.1 Objective

In the present paper, we investigated if OEPs could be measured in rats. If so, this would offer a very direct and efficient measure to analyse the influence of experimental manipulations, such as the influence of psychoactive drugs or brain lesions, on an endogenous EP component in an animal model.

In the first experiment we measured rat OEPs in a test condition by omitting 10% of the stimuli from a train of tone-pips with a fixed 3 s. inter-stimulus interval (ISI). Since OEPs are supposed to reflect the expectation of a stimulus, an obvious control condition would be to manipulate this expectation by making the prediction of the next stimulus uncertain [7]. Thus, in our control condition we measured OEPs in a session with a 2.5-3.5 s variable ISI.

In a second experiment we analysed the influence of a pharmacological manipulations on information processing by applying the omitted stimulus paradigm. We measured the effect of diazepam, a benzodiazepine, on the rat OEPs, as benzodiazepines, among other effects, are well known to affect aspects of information processing such as attention and memory [11, 16, 17]. To our knowledge, OEPs have not yet been measured

in rats nor been used to study the effects of psychoactive drugs on information processing.

5.2 Materials and methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethics committee for animal studies was obtained. Sixteen male Wistar rats, weighting 407 ± 34.4 g (mean ± S.D.) were maintained on a 12-12-h light-dark cycle with lights off at 8.00 a.m., and were singly housed with food and water ad libitum.

Isoflurane anaesthesia was used for implanting a tri-polar electrode (Plastics One, MS 333/2a) which was fixed on the skull with dental acrylic cement. Coordinates of the first active electrode related to bregma were: A -3.4, L 2.0. The second active electrode and the ground electrode were placed above the cerebellum. Animals were allowed to recover for two weeks before recordings were made.

5.2.1 recording procedures

EEG recordings were obtained from freely moving rats. EEG signals were measured between 0.1 Hz and 500 Hz and recorded digitally with a sample frequency of 1024 Hz and digitally post-filtered between 0.1 Hz and 30 Hz. Auditory Evoked Potentials (AEPs) in response to stimuli preceding and following stimulus omission and Omission Evoked Potentials (OEPs) were determined by averaging EEG fragments recorded 50 ms before stimulus (omission) onset until 500 ms after stimulus (omission) onset. A rejection program was utilised to eliminate individual trials in which the EEG exceeded 600 μV, thereby excluding trials with high EEG amplitudes due to e.g. motoric artefacts.

5.2.2 Experiment 1

In the test session, AEPs were elicited by pure tone-pip stimuli (11.0 kHz, 85 dB) with a fixed ISI of 3.0 s. OEPs were elicited by omitting 10% of the stimuli (n=150) from the background train of pure tone-pip stimuli (n=1350).

In a control session, AEPs were elicited by pure tone-pip stimuli (11.0 kHz, 85 dB) with an ISI randomly varying between 2.5 and 3.5 s. OEPs were elicited by omitting 10% of the stimuli (n=150) from the background train of pure tone-pip stimuli (n=1350).

The test and the control session were recorded on separate days. White background noise of 65 dB was present. After visual inspection of the individual OEPs, eight rats showed a late-latency positive wave in response to an omitted stimulus in the test condition, and were further taken into analysis.

5.2.3 Experiment 2

From the eight rats showing an OEPs in experiment 1, three rats lost their tri-polar electrode. The remaining five rats were used the second experiment. Rats (n=5) received 4 mg.kg-1 diazepam or vehicle s.c., counterbalanced in two sessions, 3 days apart.

AEPs were elicited by pure tone-pip stimuli (10.2 kHz, 90 dB) with a fixed ISI of 2.0 s. OEPs were elicited by omitting 10% of the stimuli (n=150) from the background train of pure tone-pip stimuli (n=1350).

5.2.4 Statistical analysis

Grand average AEPs in response to stimuli preceding and following stimulus omissions were obtained. After determination of maximal peak-values of the grand average auditory evoked potentials, individual AEP amplitudes at selected latencies (18 ms, 30 ms, 60 ms, 67 ms and 150 ms after stimulus onset) were further taken into analyses [20, 21]. AEP component amplitudes evoked by stimuli preceding and following omitted stimuli were analysed. For each component a two-within ANOVA was employed, with 'test session' and 'control session' being the first within variable and 'preceding' or 'following' omitted stimuli being the repeated measure.

Grand average OEPs (n=8) were obtained. For both the test and control session, t-profiles were constructed [22] by determining the group t-values for each sample point (50ms before till 500 ms after stimulus omission occurred). The percentages of t-values that reached significance were determined. T-values reached significance ($p \leq .05$, one-tailed, tested against 0) when $t > 1.895$ in experiment 1 (n=8), or when $t > 2.132$ in experiment 2 (n=5). An OEPs was considered to be significantly different from an averaged ongoing EEG signal, not phase-locked to stimulus omission, if more than 5% of the t-values in the t-profile exceeded the level of significance.

5.3 Results

5.3.1 Experiment 1

Figure 1 shows the grand average AEPs and OEPs of rats (n=8) in the test session (figure 1a) and the control session (figure 1c) ISI condition. No differences were found between the AEPs elicited in the test and control session. Also no differences were found AEPs preceding stimulus omission and the AEPs following stimulus omission.

Figure 1a shows the grand average AEPs and OEPs (solid line) as obtained in the test session (n=8). The reaction to an expected yet omitted stimulus, the OEPs, was a long-latency positive wave.

Figure 1b shows the constructed t-profile of the OEPs obtained in the test session. With respect to the OEPs as measured in the test session 27.0% of the t-values (clustered between 100-400ms. after stimulus omission occurred) exceeded the level of significance

and was therefore considered to be significantly different from an averaged ongoing EEG signal, not phase-locked to stimulus omission.

Figure 1c shows the grand average AEPs and OEPs (dotted line) as obtained in the control session (n=8) with the variable 2.5-3.5 ISI.

Figure 1d shows the constructed t-profile of the OEPs obtained in the control session. None of the t-values exceeded the level of significance, and the response was therefore considered to be just an averaged ongoing EEG signal, not phase-locked to stimulus omission.

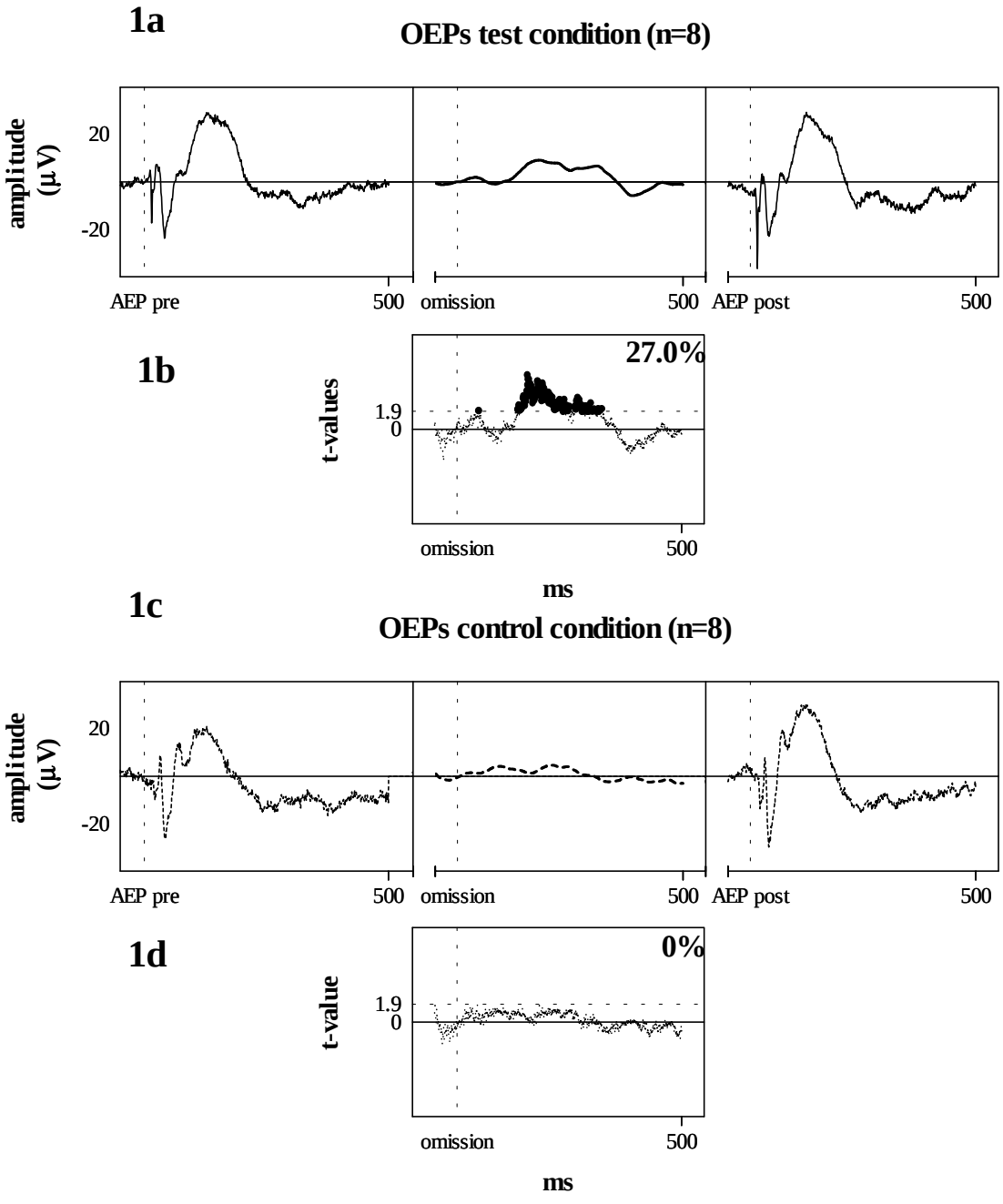

Figure 1

Figure 1a shows the grand average AEPs (n=8) elicited by tones preceding omitted stimuli (left), grand average OEPs elicited by omitted stimuli (middle) and grand average AEPs elicited by tones following omitted stimuli (right) in the test session with a 3 s. fixed ISI. Amplitudes are given in μV (y-axes) by latencies in milliseconds (x-axes). The dotted lines mark the time of stimulus (omission) onset.

Figure 1b shows the constructed t-profile for each sample point of the OEPs obtained in the test session. The dotted line on the y-axes shows the level of significance (1.895). Significant values (27.0% of total values) are depicted in solid dots, non-significant values in small points.

Figure 1c shows the grand average AEPs (n=8) elicited by tones preceding omitted stimuli (left), grand average OEPs (n=8) elicited by omitted stimuli (middle) and grand average AEPs (n=8) elicited by tones following omitted stimuli (right) in the control session with a 2.5-3.5 s. variable ISI. Amplitudes are given in μV (y-axes) by latencies in milliseconds (x-axes). The dotted lines mark the time of stimulus (omission) onset.

Figure 1d shows the constructed t-profile for each sample point of the OEPs obtained in the control session. The dotted line on the y-axes shows the level of significance (1.895). None of the values exceeded the level of significance.

5.3.2 Experiment 2

Figure 2 shows the grand average AEPs and OEPs obtained in the 'vehicle' condition (figure 2a) and the 'diazepam' condition (figure 2c).

With respect to the AEPs preceding and following stimulus omission only main drug-effects were found. The amplitude of the middle-latency P_{30} AEP component was decreased in the diazepam condition compared to the control condition ($F(1,4)=13.9$, $p=.0018$). The amplitudes of the late-latency N_{60} and P_{67} AEP components were increased, implying that they had a more negative value in the diazepam condition compared to the control condition ($F(1,4)=20.4$, $p=.0004$; $F(1,4)=23.3$, $p=.0002$ respectively). No drug effects on either the N_{18} or the P_{150} were observed.

Figure 2a shows the grand average AEPs and OEPs (solid line) as obtained in the vehicle condition (n=5). The reaction to an expected yet omitted stimulus, the OEPs, was a long-latency positive wave in the vehicle ISI condition.

Figure 2b shows the constructed t-profile of the OEPs obtained in the vehicle condition. With respect to the OEPs as measured in the vehicle condition 12.9% of the t-values (clustered between 100-400ms. after stimulus omission occurred) exceeded the level of significance and was therefore considered to be significantly different from an averaged ongoing EEG signal, not phase-locked to stimulus omission.

Figure 2c shows the grand average AEPs and OEPs (dotted line) as obtained in the diazepam condition (n=5).

Figure 2d shows the constructed t-profile of the OEPs obtained in the diazepam condition. None of the t-values exceeded the level of significance, and the response was therefore considered to be just an averaged ongoing EEG signal, not phase-locked to stimulus omission.

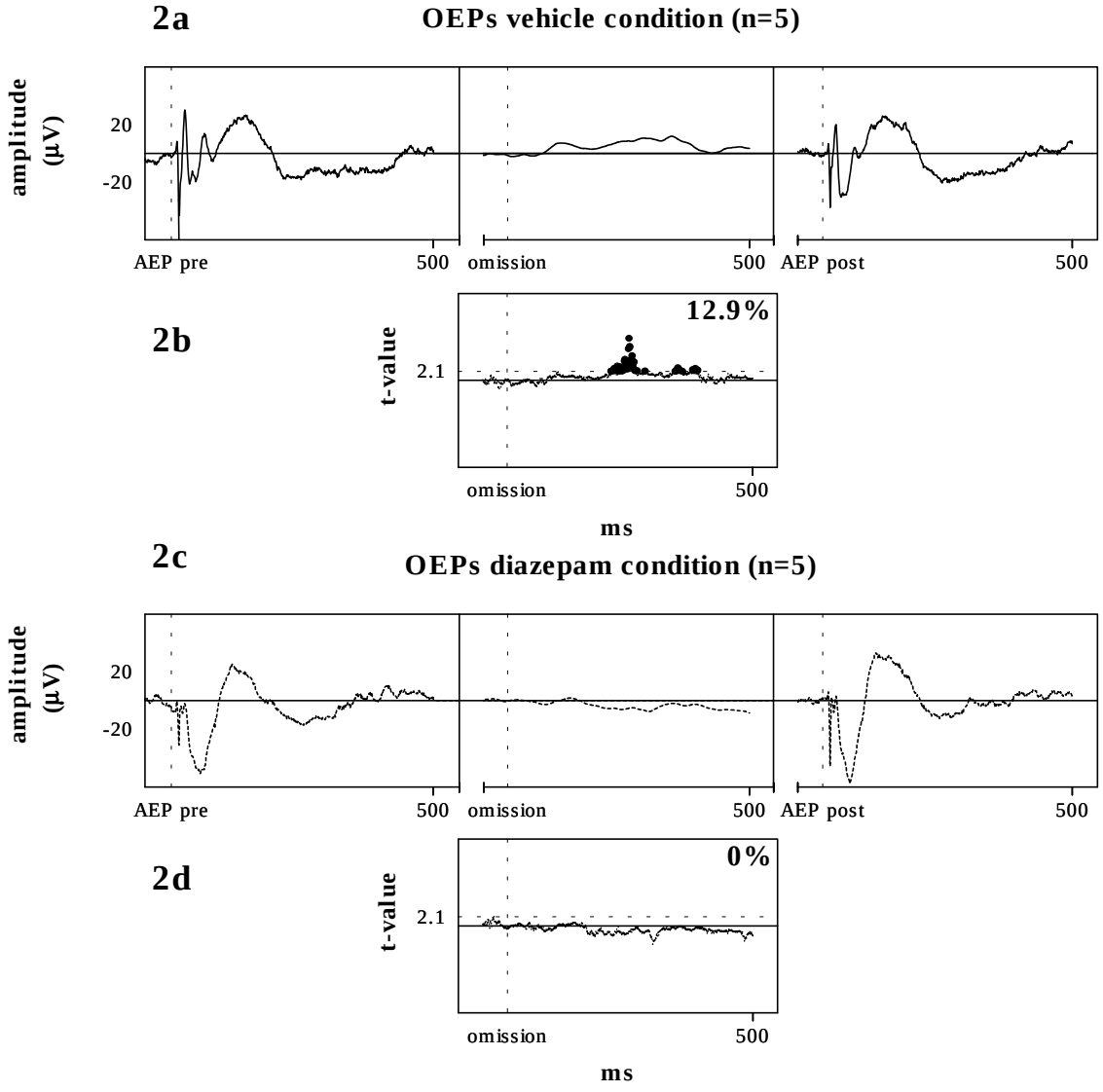

Figure 2

Figure 2a shows the grand average AEPs (n=5) elicited by tones preceding omitted stimuli (left), grand average OEPs elicited by omitted stimuli (middle) and grand average AEPs elicited by tones following omitted stimuli (right) in the vehicle condition. Amplitudes are given in µV (y-axes) by latencies in milliseconds (x-axes). The dotted lines mark the time of stimulus (omission) onset.

Figure 2b shows the constructed t-profile for each sample point of the OEPs obtained in the vehicle condition. The dotted line on the y-axes shows the level of significance (2.132). Significant values (12.9% of total values) are depicted in solid dots, non-significant values in small points.

Figure 2c shows the grand average AEPs (n=5) elicited by tones preceding omitted stimuli (left), grand average OEPs elicited by omitted stimuli (middle) and grand average AEPs elicited by tones following omitted stimuli (right) in the diazepam condition. Amplitudes are given in µV (y-axes) by latencies in milliseconds (x-axes). The dotted lines mark the time of stimulus (omission) onset.

Figure 2d shows the constructed t-profile for each sample point of the OEPs obtained in the diazepam condition. The dotted line on the y-axes shows the level of significance (2.132). None of the values exceeded the level of significance.

5.4 Discussion

5.4.1 Experiment 1

We were able to measure the rat OEPs elicited by omitting stimuli from a background train of tone-pip stimuli in a test session with a fixed ISI. The event of stimulus omission had no effect on AEPs preceding stimulus omission and the AEPs following stimulus omission.

Many studies investigating aspects of information processing on EPs require a motoric response of the subject, e.g. a button press [14]. Likewise, most studies measuring OEPs also require active participation of the subjects, by giving either a motoric response [19, 33] or by counting the omissions [1, 7, 37, 38]. Active subject participation however might be difficult to acquire e.g. in animal models.

Few studies have attempted to measure reactions to omitted stimuli in passive conditions [27, 28]. In the last study [28] only the effect of partial stimulus omissions was measured and resembled therefore more an oddball paradigm than an omitted stimuli paradigm. In the present study we measured OEPs in rats by employing a passive paradigm.

Most studies measuring OEPs use high (≥ 1Hz) stimulation rates [1, 3, 22, 27, 33, 36, 38, 39, 40].

Only few studies have measured OEPs with low (< 1Hz) stimulation rates [19, 37] or both [7]. Bullock et al. [7] described 2 types of OEPs in humans, those following fast stimulation rates (fast OEPs) and those following slow stimulation rates (slow OEPs). They found that slow OEPs consisted of a large, slow, positive wave. Like Bullock et al., [7] other studies using low stimulation rates have also reported a long-latency positive wave in response to stimulus omission [1, 7, 36, 37]. The present study shows that the rat OEPs also consist of a long-latency positive wave, occurring 100-400 ms. after the event of stimulus omission.

Bullock et al., [7] found in humans that jitter of the conditioning intervals greatly reduced the slow OEPs. This is in agreement with our results of the control session. We found that if the prediction of the omitted stimulus was made uncertain by using a variable ISI, OEPs could no longer be measured.

Although we were able to measure OEPs in rats, only eight out of sixteen rats showed a late-latency positive wave in response to omitted stimuli. This is in agreement with Alain et al., [1] who only found OEPs in half their subjects. Others also excluded subjects from their experiments who failed to show an OEPs [3, 38]. Näätänen et al., [27], also found considerable variability of OEPs between individual subjects, such that no consistent OEPs over subjects could be detected. Others have reported that training of subjects was required before an OEPs could be measured [3, 7, 33].

5.4.2 Experiment 2

In this study we found a decrease of the middle-latency P_{30} component due to diazepam. With respect to the late-latency components, we found an increase (more negative values) of the N_{60} and P_{67} AEP components. This is in agreement with a previous study where we found more negative values of the late-latency N_{58} and P_{72} AEP components due to diazepam in rats [20].

As in experiment 1 the reaction to the omission of an expected stimulus, the OEPs, was a long-latency positive wave in the vehicle condition. We found that when treated with diazepam, rats failed to produce an OEPs when stimulus omission occurred.

Expectancy and timing behaviour have also been studied in rats using measures of temporal discrimination in learning experiments [9, 15]. Lau and Heatherington [23] used a DRL (differential reinforcement of low rate) 45 s schedule in which only responses that occur after a minimum time interval (in this case 45 s.) were reinforced. Rats showed a normally distributed amount of responses, with the maximum amount of responses at 45 s. thus suggesting the ability of rats to correctly time the interval. In addition, they investigated the effects of alprazolam on timing performance using this DRL 45 s. schedule in rats [23]. Their results show that alprazolam treated rats no longer show normally distributed responses with a maximum at 45 s. but a far more flattened distribution with several peaks, thus suggesting that alprazolam diminishes the ability to time an interval. In human subjects, Ramssayer [31, 32] found that a single-dose of midazolam decreased time-estimation in the range of 1 to 2 s. Time-estimation appears to depend on memory processes [31]. Since benzodiazepines are well known to affect memory, this might explain their effect on time-estimation.

This is in agreement with the present study where we found that diazepam treated rats failed to produce an OEPs when stimulus omission occurred. Diazepam seems to affect expectancy and timing behaviour.

We found that OEPs can be measured in rats. In addition, OEPs appeared to be sensitive for a pharmacological manipulation. We propose that the rat OEPs provides a useful tool for measuring the influence of experimental manipulations, such as the influence of psychoactive drugs or brain lesions, on an endogenous EP

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CHAPTER 6: EFFECTS OF DIAZEPAM ON AUDITORY EVOKED POTENTIALS (AEPS) AND OMISSION EVOKED POTENTIALS (OEPS) IN HUMANS

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Abstract:
In this study we measured the effects of diazepam on AEPs and OEPs in humans, in order to make a comparison between human data and data obtained in previous experiments in rats.
Students received 10 mg diazepam (n=8) or placebo (n=8). Trains of 10 repetitive tone-pip stimuli (70dB, 1000Hz) evoking AEPs, followed by the omission of such a stimulus, evoking OEPs, were presented (inter-stimulus interval (ISI) 1 s; inter-train interval (ITI) 2 s).
Diazepam decreased the N180 component and increased the late-latency N370 and P420 components. The increase of the late-latency components might be due to a diazepam-induced decrease in arousal or a decrease in variability. Stimulus repetition did not affect AEP components, probably due to the small difference between the ISI (1 s) and the ITI (2 s). The reaction to the omission of an expected stimulus, the OEPs, was a long-latency positive wave in control conditions. With diazepam-treated no such deflection was found. Difference OEPs however failed to reach significance, possibly due to lack of training.
We found very similar results of diazepam on human AEPs and OEPs as on rat AEPs although latencies appeared to be much larger in humans. Since experimental control in rats is much higher they provide an excellent model to study e.g. effects of psychoactive drugs on information processing.

Key words: diazepam, auditory evoked potentials, sensory gating, habituation, omitted stimuli, time-estimation.

6.1 Introduction

Benzodiazepines have sedating, muscle relaxant, anti-convulsant and anxiolytic effects [1]. In addition, benzodiazepines affect aspects of information processing [2, 3]. Evoked Potentials (EPs) provide a sensitive method for studying the effects of drugs on aspects of information processing [4].

EPs are discrete and minute electrical potentials that appear in the EEG, produced by, and time-locked to, sensory stimuli [5]. EP components are typically divided on the basis of latency into early-, middle- and late-latency components [6]. Amplitudes and latencies of early components appearing 0-10 ms after stimulus onset [7, 8], are determined by the physical characteristics of the stimulus [5-7]. Amplitudes and latencies

of the middle- (10-100 ms) and late-latency (>100 ms) components are primarily determined by aspects of information processing involved by the stimulus event [5-8]. Therefore, the effects of diazepam on middle- and late-latency EP components might reveal insight into its effects on information processing.

Many studies investigating aspects of information processing on EPs require a motoric response of the subject (e.g. a button press) [6, 9]. However, such responses might be additionally affected by the sedative and muscle relaxant properties of diazepam [2]. In addition, by using single stimuli, changes in the middle- and late-latency EP components can not additionally be attributed to changes in the physical characteristics of the stimulus, but only be attributed to changes in aspects of information processing involved by the stimulus event.

In previous experiments we determined whether such passive single-stimulus paradigms could be used to measure the effects of diazepam on different aspects information processing in rats [10-12]. In a first experiment, we studied the effects of diazepam on sensory gating in rats, by measuring diazepam effects on Auditory Evoked Potentials (AEPs) elicited in a ten-tone paradigm [11]. In this study we found that automated neuronal recovery functions underlying sensory gating of middle-latency AEP components remained intact with diazepam. However, diazepam decreased behaviorally mediated habituation of the late-latency AEP components.

In addition, we measured the effects of diazepam on Omission Evoked Potentials (OEPs) in rats [12]. We found that OEPs could be measured in rats. However, OEPs were disrupted by diazepam suggesting that diazepam affects expectancy and time estimation. We proposed that in rats diazepam affects information processing of mainly higher order characteristics (e.g. the temporal pattern) of stimuli.

In this study we repeated these experiments by measuring the effects of diazepam on AEPs and OEPs in humans in order to make a comparison between our animal model and human data.

6.2 Materials and methods

Sixteen students (mean age 21 years) who complied with the medical exclusion criteria, received 10 mg diazepam (n=8) or placebo (n=8) p.o. All subjects signed an informed consent. EEG was registered with tin electrodes mounted in an elastic electrode cap (Electrocap International). EEG was derived from Fz, Cz, Pz, F3, C3; P3, and P4, according to the 10-20 electrode system [14]. Only Cz data will be presented in this paper. The left mastoid served as reference. A ground electrode was placed on the forehead. Electrode impedance was less than 3 kOhms. EEG signals were measured between 0.016 Hz and 30 Hz and recorded digitally with a sample frequency of 512 Hz.

Trains of 10 repetitive tone-pip stimuli (70dB, 1000Hz, ISI 1s.), evoking AEPs, followed by the omission of such a stimulus, evoking OEPs, were presented.

Data analysis: AEPs and OEPs were determined by averaging EEG fragments recorded 100 ms. before stimulus onset until 1000 ms. after stimulus onset. Besides the average of these EEG epochs, corresponding SDs with respect to tone 1 in each train were also calculated.

A grand average AEP evoked by tone 1 for the placebo condition was constructed. Component latencies of AEPs were selected based on the maximum peak amplitude of this grand average AEP. Individual amplitudes at the selected latencies were determined [9a,b]. AEP component amplitudes evoked by the consecutive tones 1, 2, 3, 4, 5 and 10 within a train were further taken into analysis. For each component a two-way ANOVA was employed. Post-hoc one-way ANOVA analyses with repeated measures were employed for the diazepam and placebo conditions separately when interaction effects were found.

Grand average OEPs were constructed for both conditions. Difference OEPs were constructed by subtracting the OEPs obtained in the diazepam condition from the OEPs obtained in the placebo condition. For these difference-OEPs a t-profile was constructed (t-values for each data point between 100 ms before omission onset and 800 ms after omission onset were calculated). T-values reached significance ($p<.05$) when $t<-2.308$ or $t>2.308$. The diazepam effect was considered significant if more than 5% of the t-values would reach significance. SDs corresponding to the AEPs evoked by tone 1 in each train were analysed this way as well.

6.3 Results

Diazepam decreased the N180 ($p<.0001$) and increased the N370 and P420 amplitudes ($p=.0006$, $p<.0001$ respectively, see fig. 1 and 2). Furthermore, lower SDs of individual AEPs due to diazepam were observed (see fig. 3). No stimulus repetition effects or interaction effects were found (see fig. 2). The reaction to the omission of an expected stimulus, the OEPs, was a long-latency positive wave (300-550msec) in control conditions. With diazepam-treated no such deflexion was found. Difference OEPs however failed to reach significance (see fig. 4a).

Figure 1
grand average AEPs: students

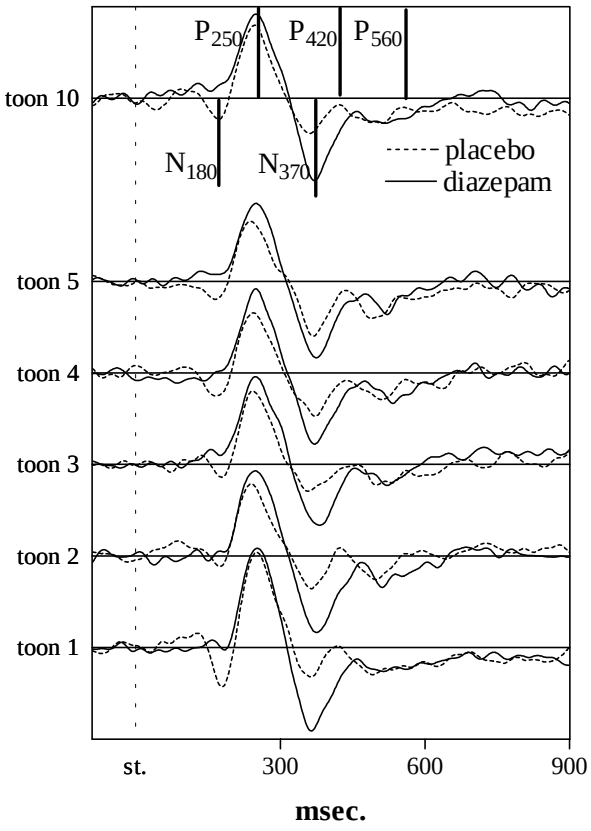

Figure 2
AEP components over 10 tones

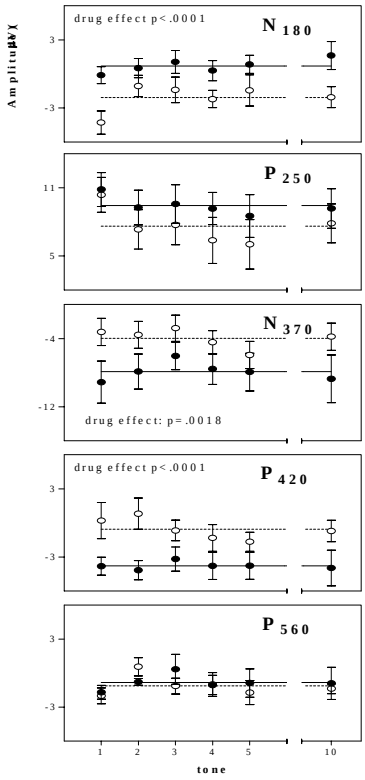

Figure 1 shows the grand average AEPs from bottom to top evoked by tone 1, 2, 3, 4, 5 and 10 within a train for both the diazepam (solid lines) and placebo condition (dotted lines).

Figure 2 shows the AEP component amplitudes with stimulus repetition for both the diazepam (black circles) and placebo condition (open circles).

Figure 3

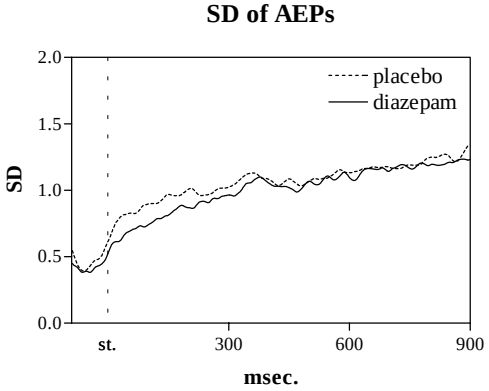

Figure 3 shows SDs corresponding to the AEPs evoked by tone 1 for both the diazepam (solid line) and placebo group (dotted line)

Figure 4a

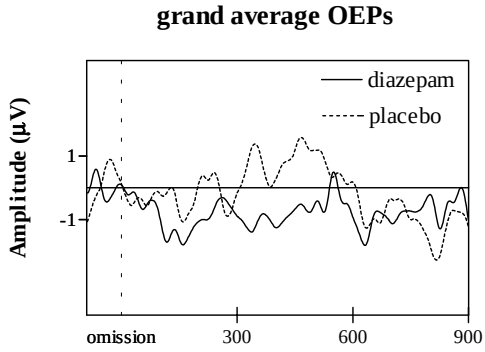

Figure 4b

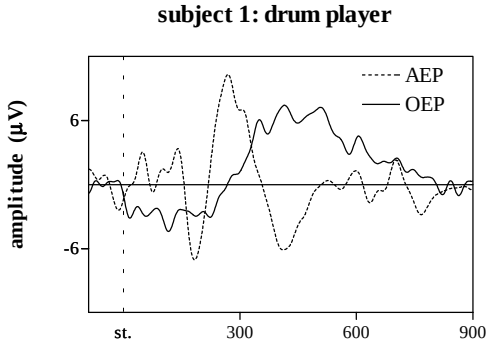

Figure 4a shows grand average OEPs for both the diazepam (solid line) and placebo condition (dotted line).
Figure 4b shows the AEP to tone 1 (dotted line) and OEP (solid line) of subject 1 (placebo condition) who appeared to be an experienced drum player.

6.4 Discussion

Diazepam decreases the amplitudes of the N180 AEP components. In our previous study [11] we found a decrease of a P30 AEP component due to diazepam in rats. This is in agreement with the in general reported decreased AEP amplitudes due to diazepam [4, 8, 15]. In addition, diazepam increased amplitudes of long-latency N370 and P420 AEP components in humans. In previous experiments, we have consistently observed increased late-latency AEP components (i. e. more negative values) in rats due to diazepam [11, 13]. Increased AEP amplitudes have also been reported during states of low arousal [16]. Our finding of increased AEP amplitudes might thus be ascribed to a decrease in arousal due to the sedative effects of diazepam [1-3, 8].

A general problem with AEP component analyses in average waveforms, is that during a session, there will be considerable variability in the state of the subject's arousal and other sources of uncontrolled variation [18, 19]. This will lead to temporal variability in the peak of the deflection (also referred to as latency jitter) in the single trials underlying the average [19]. Latency jitter will thus result in decreased amplitudes in the individually averaged AEPs [17, 19]. We observed decreased SDs when treated with diazepam. It is reasonable to assume that latency jitter is positively correlated with the SD corresponding to the averaged AEP amplitudes. We therefore propose that diazepam decreases the variability in the subject's attention, leading to more stereotypical responding and thus higher amplitudes.

In our previous study we observed stimulus repetition effects on both middle- and late-latency AEP components in rats [11]. However, no stimulus repetition effects were found in the present study, possibly due to less experimental control and higher variation between subjects. In addition, the difference in length between the ISI (1 s) and the ITI (2 s) used in this study might have been too short. In our previous rat experiment [10] we found fairly similar effects of stimulus repetition in trials where the ISI was either 1 or 2 s. Effects of stimulus repetition might thus have been masked by an insufficient recovery time between trains.

Few studies have examined the effect of (partial) stimulus omission [9, 19, 20]. It has been proposed that the response to an omitted stimulus would enable one to investigate the mechanism storing temporal information in the brain [19, 21].

Although no consistent OEPs could be measured in this study, stimulus omissions did evoke a very large P3 in subject 1 (see fig. 4b), who reported after the experiment to be a drum player, and was therefore experienced in recognizing temporal patterns. In a previous study we were able to measure OEPs in rats, although only eight out of sixteen rats showed a late-latency positive wave in response to omitted stimuli. This is in agreement with Alain et al., [23] who only found OEPs in half their subjects. Näätänen et al., [24] also found considerable variability of OEPs between individual subjects, such that no consistent OEPs over subjects could be detected. Others have reported that training of subjects was required before an OEPs could be measured [9, 25, 26]. Although the diazepam effect on OEPs was not significant, similar results were obtained in our previous rat study [12], namely a positive wave in the control condition which disappeared in the diazepam condition.

In all, we found very similar results to the effects of diazepam on AEPs and OEPs in humans compared to our previous experiments in rats [11-13]. More significant results were obtained in the rat study however, probably due to less variation between subjects and higher experimental control. Rats therefore provide an excellent model to study the effects of psychoactive drugs on information processing, as measured with EPs, in passive single-stimulus paradigms.

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PART B

EEG-EP INTERRELATIONS

CHAPTER 7: CHRONIC EFFECTS OF DIAZEPAM ON THE SPECTRAL CONTENT OF THE RAT EEG

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Abstract:
We determined the effect of long-term continuous diazepam treatment on the spectral content of the EEG in rats. Diazepam was administered for 21 days using subcutaneously implanted silastic tubes, resulting in constant blood concentrations.
Diazepam caused a decrease in the power of the low frequency bands (1-8 Hz) and an increase in the power of the high frequency bands (21-40 Hz). These changes persisted during 21 days of treatment and were no longer detected in a post drug control measurement on the 9th day after removing the tubes.
No tolerance developed to the effect of diazepam on the spectral content of the EEG. This indicates that in 21 days there is no decline in the effect of interaction of diazepam with the $GABA_A$ - benzodiazepine receptor complexes responsible for the power spectrum changes.

Key words: Benzodiazepine, Diazepam, Tolerance, Rats, EEG, Spectral Analysis.

7.1 Introduction

It is known that tolerance develops to the behavioural effects of benzodiazepines: e.g. in rats to the sedating effects, to the motor impairing effects and to the anticonvulsive activity [4,5]. A reduction in the course of time of the spectral changes in the EEG (i.e. a decrease in the power of the low frequencies and an increase in the power of the high frequencies [1,2,11]) is reported in multiple dose regime studies [12,13,17]. However, it is clinical knowledge that the EEG changes induced by benzodiazepines persist during chronic treatment (W. van Emde Boas, personal communication). Tolerance development might depend on the dose regime [4,9,19]. Repeated dosages of a benzodiazepine in rats result in major fluctuations in the concentrations of the drug [7] due to the short half life time in these animals (± 1 hour for diazepam, [6]). In humans, repeated dosages result in more constant blood concentrations due to the long half life time (± 40 hours, [10]). We investigated the effect of diazepam on the EEG of rats having constant blood concentrations during 21 days. Silastic tubes containing diazepam were subcutaneously implanted, allowing a continuous release [7,8,16,20]. During the treatment we repeatedly determined the power spectrum of the EEG.

7.2 Materials and methods

Sixteen male WAG/Rij rats were used, age 10 months and weighing 350 ± 16 grams (mean ± SD) at the start of the experiment. Animals were maintained on a 12-12 hour light-dark cycle: lights off at 9 am. Rats were single housed in standard cages with ad libitum access to standard food and water.

Three electrodes were implanted under complete Hypnorm anaesthesia (Plastic Products Company, MS 333/2A). The coordinates related to bregma were: A 2.0, L 2.0; A -3.7, L 9.0. A ground electrode was placed above the cerebellum. Animals were allowed to recover one week. EEG signals were measured between 1 Hz and 100 Hz and recorded digitally with a sample frequency of 200 Hz. Recordings took place from 11 am until 1 pm. Two baselines were recorded.

Per animal we used 8 silastic tubes of 8 cm (Dow Corning, 0.062 inch inner diameter; 0.095 inch outer diameter), each containing 100 mg of solid diazepam. Controls received empty tubes. Implantation and removal were carried out under ether anaesthesia. Experimental recording started 24 hours after tube implantation. A post-drug recording was taken 9 days after tube removal.

Total benzodiazepine activity in the blood was determined with a receptor binding assay. Blood samples of 100 μL were taken from the tail venes and hemolyzed in water. The samples were extracted in pentane/dichloormethane. Evaporated extract fractions were incubated during 90 min at 0^{o} C with rat-brain membrane preparations and 3 nM [methyl-3H]diazepam, followed by rapid filtration. Specific benzodiazepine activity was expressed relative to the activity on day 2. Statistical analysis was performed by linear regression followed by a F-test of the slope.

The spectral content of the EEG was determined by Fast Fourier Transformation for 10 periods of 3.2 sec of EEG during passive wakefulness. This state was defined as observed immobile behaviour together with low voltage and fast frequency EEG [1,2]. A mean spectrogram was constructed for each animal per recording day and expressed in standardized scores (z-scores). We determined the mean power in the delta-band (1-4 Hz), the theta-band (4-8 Hz), the alpha-band (8-12 Hz) and the beta-band (12-40 Hz) [18]. Statistical analysis was performed by ANOVA for repeated measurements.

Figure 1

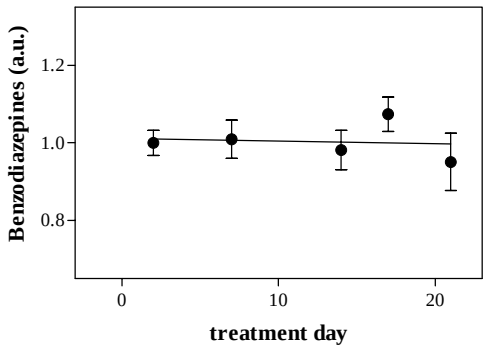

Fig. 1.
Relative blood concentrations during 21 days of diazepam treatment with subcutaneously implanted silastic tubes. Concentration values are relative the activity on day 2. Mean's and S.E.M.'s on five treatment days (2, 4, 9, 18 and 21) are given (n=8 animals with 8 tubes of 8 cm each). The slope obtained by linear regression is not significantly different from zero (F-test; p=0.6).

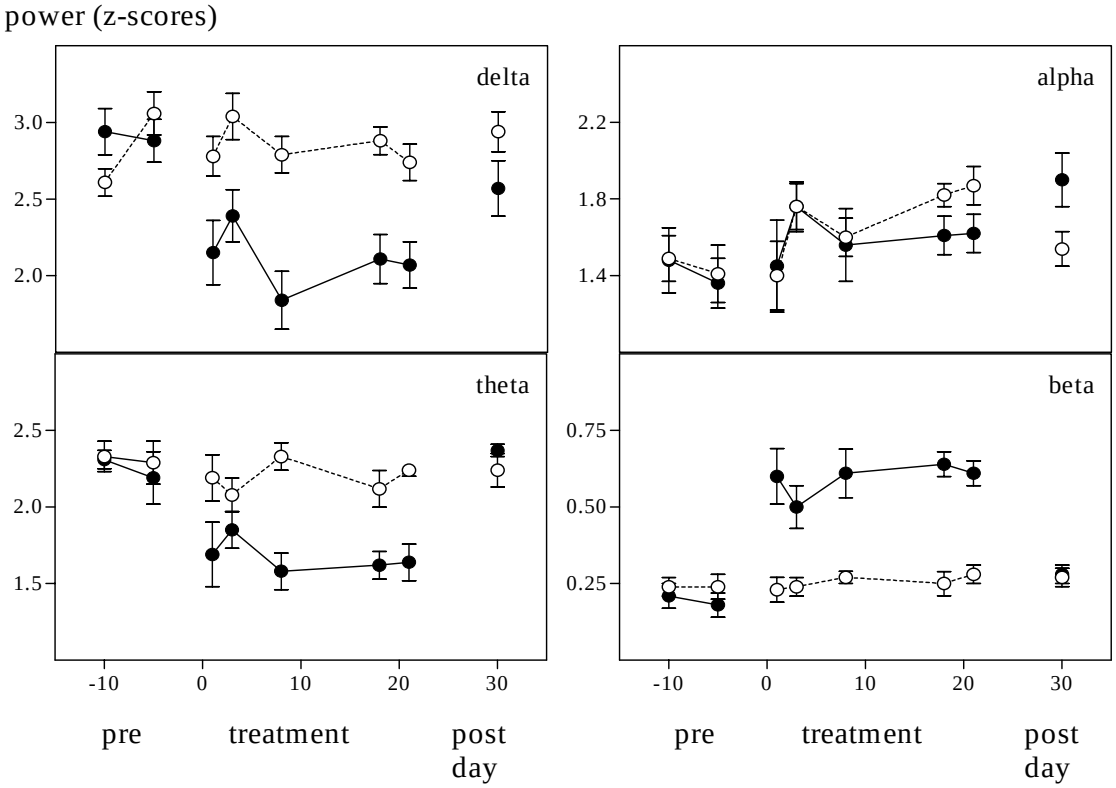

Fig. 2.
Effects of diazepam treatment during 21 days on the EEG frequency bands. Data points are given for controls (open circles, n=8) and for diazepam treated rats (filled circles, n=8).
Mean power is expressed in z-scores (mean's and S.E.M.'s) on two baseline recording days (-10 and -5), five treatment days (1, 3, 8, 18 and 21) and one post-treatment day (30).
Data are given for the delta-band (1-4 Hz), theta-band (4-8 Hz), alpha-band (8-12 Hz) and beta-band (12-40 Hz). On all treatment days diazepam induces a decrease in the delta- and the theta-band and an increase in the beta-band (ANOVA $p \leq 0.0008$). No drug-day interaction was found.

7.3 Results

The diazepam output from the implanted tubes was 17.6 ± 1.6 mg per animal per day (mean ± SD, n=8 animals with 8 tubes). The total benzodiazepine concentrations in the blood were constant during the 21 days (fig 1). A blood sample taken on day 2 analyzed by HPLC showed that the absolute concentration of diazepam was 0.7 μM (200 ng/ml) and of its main metabolite desmethyldiazepam was 1.2 μM (336 ng/ml).
The effects of these constant diazepam blood concentrations on the frequency bands of the EEG power spectra are presented in Fig. 2. During 21 days of treatment we found a decrease in the mean power of the low frequency bands (delta: $F_{1,15}=27.92$, $p<0.0001$ and theta: $F_{1,15}=17.96$, $p=0.0008$) and an increase in the high frequency band (beta: $F_{1,15}=47.15$, $p<0.0001$). No drug-day interaction was found. No differences were found on the baseline days nor on the 9th day after removal of the tubes between the control group and the experimental group.

7.4 Discussion

Blood concentrations remained constant during 21 days of implantation of silastic tubes with diazepam. This observation confirms earlier reports using this method [7].

The spectral content of the EEG was determined of EEG during passive wakefulness, a state known to be sensitive to drug effects [1]. During the entire treatment a decrease in the power of the low frequency bands and an increase in the power of the high frequency band was found. These changes are characteristic of single dose treatment with benzodiazepines [1,2,11]. During the treatment period no tolerance developed to the changes in the power spectrum. This observation confirms clinical knowledge that the EEG changes induced by benzodiazepines persist during chronic treatment. Humans, using benzodiazepines chronically, are likely to have fairly constant blood concentrations [10]. Tolerance development might be dependent on the dose regime, i.e. on changes in the concentration of the drug [4,9,19]. Sala et al. found that full tolerance developed to the increase in the beta band within 4 weeks using a single oral dose of 40 mg/kg chlordiazepoxide per day in rats [17]. We suggest that constant vs. fluctuating blood concentrations account for different results.

The changes in frequency bands of the EEG induced by diazepam reflect the interaction of the compound with the $GABA_A$ - benzodiazepine receptor complex. This was shown in a single dose study by Mandema et al. [11]. They found a perfect correlation between the benzodiazepine concentration producing half of the maximum EEG effect and the benzodiazepine affinity to the receptor site on the $GABA_A$ complex. This was determined in whole brain homogenates using tritiated flumazenil as the ligand [11]. Different $GABA_A$ - benzodiazepine receptor subtypes exist in different brain areas,

presumably serving different physiological functions [3,14]. In order to clarify the mechanisms underlying tolerance development, it is important to know whether interactions of a benzodiazepine with its effector system remain intact during tolerance development. Our data indicate that in 21 days there is no decline of the effect of interaction of benzodiazepines with those receptor complexes that are responsible for the power spectrum changes. Three weeks of continuous treatment of rats with diazepam by the method described here did not alter the total benzodiazepine binding [16] but rather decreased the coupling between the GABA site and the benzodiazepine site [8]. It was found that the γ_2 subunit mRNA levels in the cortex were decreased [20]. The presence of the γ_2 subunit in the $GABA_A$ receptor causes the typical benzodiazepine effects in vitro [15]. Could it be that the effects of benzodiazepine treatment on the spectral content of the EEG are independent of the γ_2 subunit? Indeed, most of the subunits investigated did not change during chronic treatment [20]. In agreement with a presumed difference in receptors involved in behavioural and EEG changes, benzodiazepines disrupt the regular relationship between EEG and behaviour [2]. The molecular basis underlying this pharmacological dissociation might manifest itself in chronic studies.

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CHAPTER 8: INFLUENCE OF THE POWER-SPECTRUM OF THE PRE-STIMULUS EEG ON THE CONSECUTIVE AUDITORY EVOKED POTENTIAL IN RATS

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Abstract: Evoked Potentials (EPs) are responses that appear in the EEG due to external stimulation. Findings indicate that changes in EPs can be related to changes in frequencies of the pre-stimulus EEG. Auditory EPs of rats (n=8) were measured in reaction to tone-pip stimuli (90 dB, 10.2 kHz, ISI 2s, n=1500). Trials were ranked according to the percentage of delta (1-4 Hz), theta (5-8), alpha (9-12) or beta power (13-30 Hz) in the pre-stimulus EEG. Consecutive AEPs were computed. An increase in beta and a decrease in delta resulted both in an increase in the N60 and P70 component and a decrease in the P150-200 component. These beta and delta changes have been associated with changes in arousal. The increased N60 and P70 components with increases in beta might reflect increased attention due to an increase in arousal. We found that an increase in delta activity, leads to an increase of the late-latency P150-200 component, possibly due to an increased synchronization in the EEG.

8.1 Introduction

Evoked Potentials (EPs) are discrete and minute electrical potentials that appear in the electroencephalogram (EEG). They are usually produced by, and time-locked to, external sensory stimulation [6]. Until the early 1980s, EPs were considered to be deterministic signals, whereas the background EEG was considered to be random noise [1, 2]. Findings however indicate that the background EEG and EPs are related in a fundamental manner [1, 2, 3]. Many investigators nowadays regard EPs as a reorganization of the spontaneous EEG [1, 2, 3, 14]. Background EEG activity and EPs have been related using a variety of approaches.

One such approach involves the recording of EPs during different sleep stages and wakefulness [4, 11, 12]. Similar to EEG patterns, the architecture of EPs is dependent on the state of alertness [12]. During waking, components in the EP are moderate in amplitude, while during slow wave sleep larger waves are visible [5, 11]. This is caused by more synchronized unit responses with sharper phases of excitations and inhibitions, which results from increased hyperpolarizations [6, 11].

Another approach involves recording pre- and post-stimulus EEG epochs and assessing how changes in the spectral power of the pre-stimulus EEG affects the post-stimulus EP measures [3]. The effects of more subtle variations in the level of arousal on the EP can thus be studied. Still little is known about the relation between small pre-stimulus EEG variations and the subsequent EP. We studied the EEG-EP relations in rats by averaging EPs based on the relative magnitude in the pre-stimulus EEG.

8.2 Materials and methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee for animal studies has been obtained. Male Wistar rats (n=8) weighting 470 ± 49 grams were used. Isoflurane anesthesia was used for implanting a tripolar electrode set (Plastics One, MS 333/2a). The coordinates of the first active electrode related to bregma were A-3.4, L2.0. The second active electrode and the ground electrode were placed above the cerebellum. Animals were allowed to recover for two weeks before recordings were made.

EEG signals were measured between 0.1 and 500 Hz and recorded digitally with a sample frequency of 1024 Hz. Auditory Evoked Potentials (AEPs) were elicited by 1500 tone-pip stimuli (10.2 kHz, 90 dB, stimulus duration 20 ms) with a 2 s (90%) or 4 s (10%) ISI (Inter-Stimulus Interval). White background noise of 65 dB was present.

Based on a previous studies by Başar [1] and Brandt et al. [3], we determined the spectral contents of pre-stimulus EEGs (1 s before stimulus onset) by Fast Fourier Transformations. In four separate analyses, the AEP trials were categorized in ten groups, according to their increasing percentage of delta (1-4 Hz, categories of 10%), theta (5-8 Hz, categories of 10%), alpha (9-12 Hz, categories of 2.5%) or beta (13-30 Hz, categories of 4%) of the magnitude of the total spectrum (1-30 Hz).

AEPs were constructed for each EEG band, by sub-averaging the single trials corresponding to the different categories. Individual amplitudes at selected latencies (N_{18}, P_{30}, N_{40}, N_{60}, P_{68} and $P_{150\text{-}200}$) were further taken into analysis. For each component an ANOVA with repeated measures was employed with the percentage ‘delta’, ‘theta’, ‘alpha’ or ‘beta’ being the repeated measure.

8.3 Results

Figure 1 shows the selectively averaged AEPs, belonging to increasing percentages of 'delta' (figure 1a), 'theta' (figure 1b), 'alpha' (figure 1c) and 'beta' (figure 1d) in the pre-stimulus EEG. After determination of maximal peak-values of the total grand average AEP, an N_{18}, P_{30}, N_{40}, N_{60}, P_{68} and $P_{150\text{-}200}$ component could be identified

figure 1a
delta

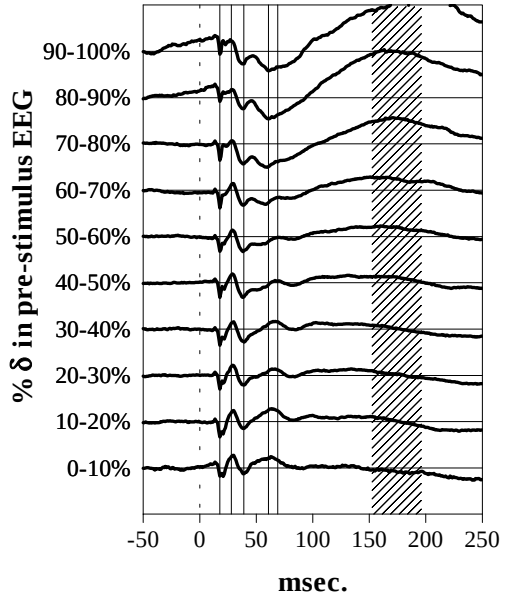


figure 1b
theta

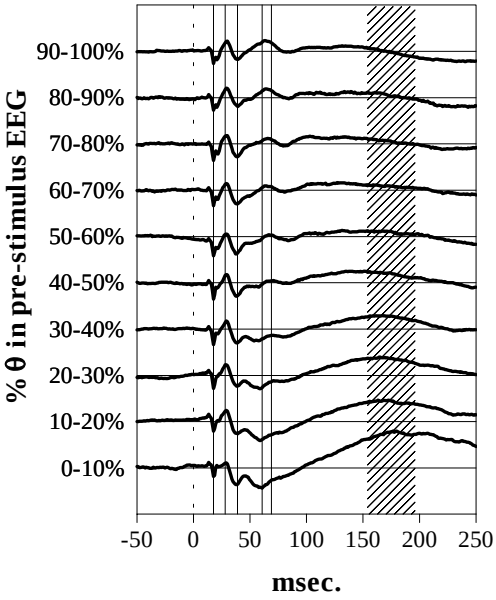


figure 1c
alpha

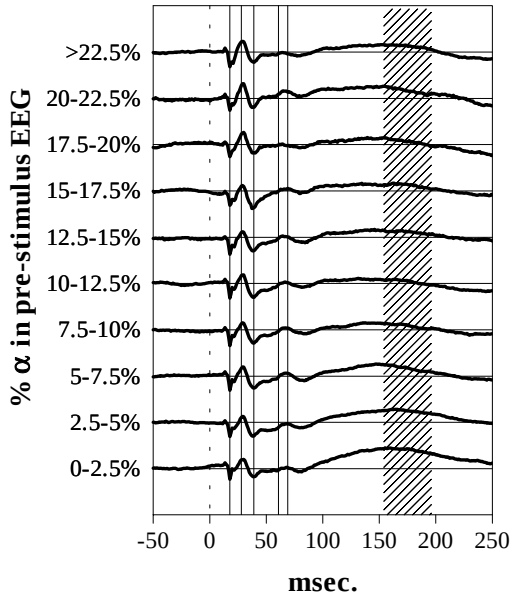


figure 1d
beta

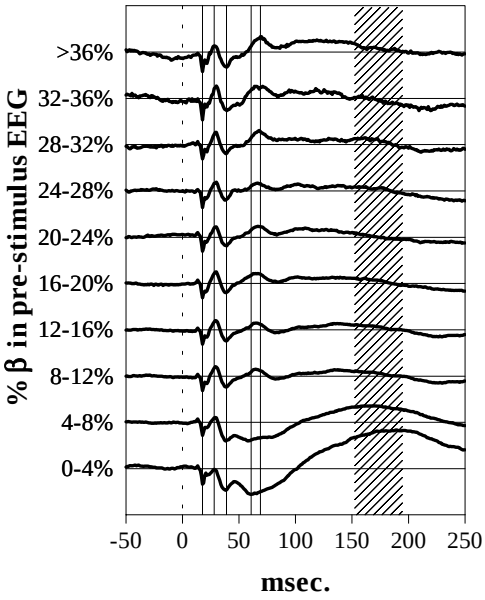
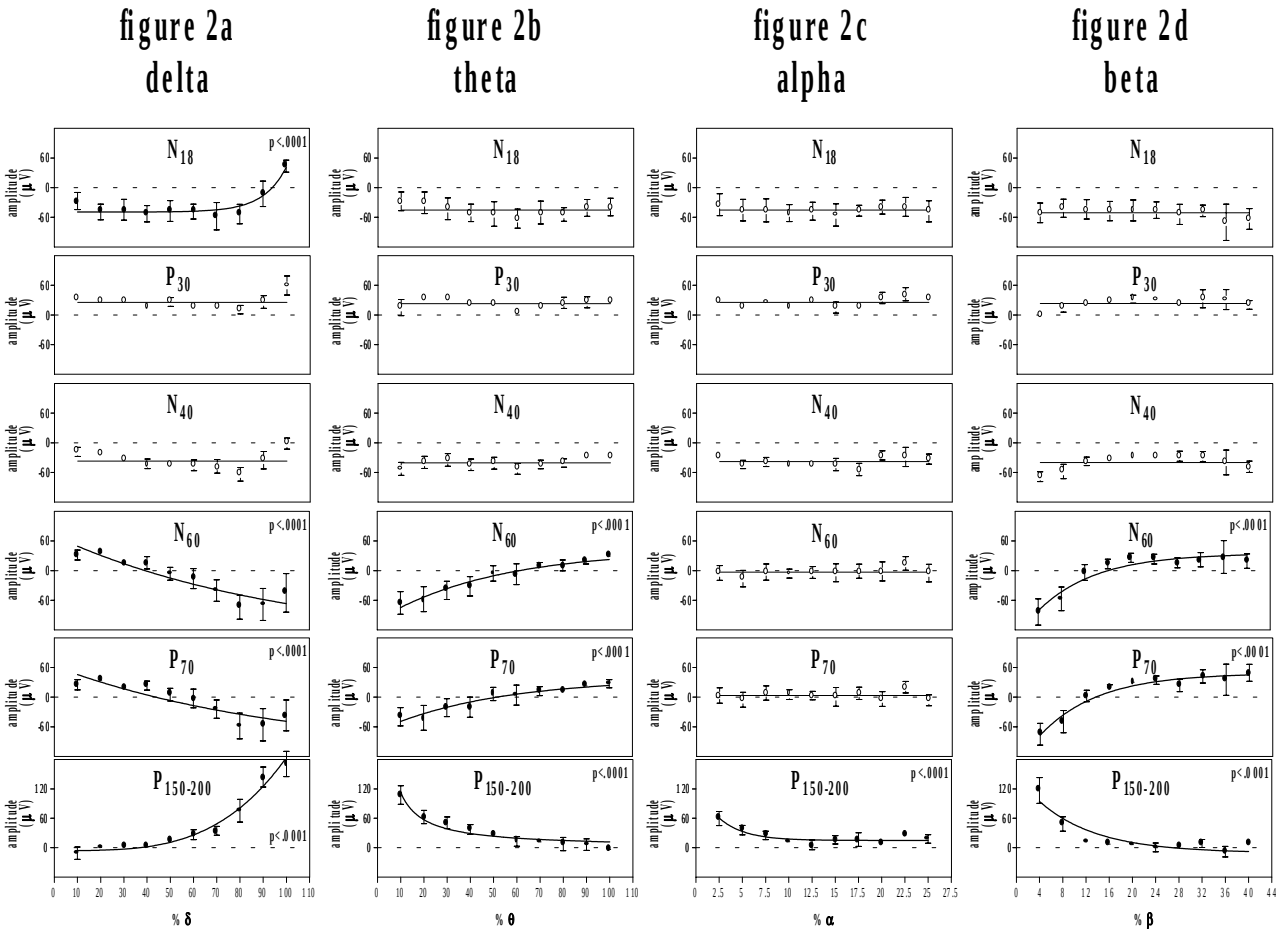


Figure 2 shows the amplitudes of the AEP components with increasing percentages of 'delta' (figure 2a), 'theta' (figure 2b), 'alpha' (figure 2c) or 'beta' (figure 2d).



Delta effect: The amplitude of the N_{18} increased with an increase in delta ($F(1,9)=4.1$; $p<.0001$). The N_{60} and P_{68} decreased and the $P_{150\text{-}200}$ increased with an increase in delta ($F(1,9)=8.7$; $p<.0001$, $F(1,9)= 5.6$; $p<.0001$ and $F(1,9)=29.4$; $p<.0001$ respectively).
Theta effect: The N_{60} and P_{68} increased and the $P_{150\text{-}200}$ decreased with an increase in theta activity ($F(1,9)=9.0$; $p<.0001$; $F(1,9)=4.8$ $p<.0001$; and $F(1,9)=12.8$ $p<.0001$).
Alpha effect: The P150-200 decreased with an increase in alpha ($F(1,9)=4.4$ $p<.0001$).
Beta effect: The N_{60} and P_{68} increased and the $P_{150\text{-}200}$ decreased with an increase in beta activity in the pre-stimulus EEG ($F(1,9)=5.7$; $p<.0001$; $F(1,9)=8.4$ $p<.0001$; and $F(1,9)=12.5$ $p<.0001$ respectively).

8.4 Discussion

In this study, decreases in ‘delta’ and increases in ‘beta’ (and to a lesser extend increases in both ‘alpha’ and ‘theta’) resulted both in increases in the N60 and P70 components and decreases in the P150-200 component. Activity in the low and high frequency bands of the EEG are considered to be an index of cortical arousal, such that power in the low frequency (delta) bands increases with a decrease in arousal and activity in the high frequency (alpha and beta) bands increases with an increase in arousal [7].

In this study we found no effect of pre-stimulus EEG on the P30 and N40 AEP components. Early detection and evaluation of auditory information seems therefore to be unaffected by the level of the subjects arousal. This is in agreement with others who found that stages of sleep have no effect on earlier EP components [8, 9].

However, we found an increase of the N60 and P68 auditory EP components with an increase in beta activity in the pre-stimulus EEG. Increases of the human N1 [3, 4, 12], P1 [9] and P2 [3, 4] components with an increase in arousal have been reported before. Others however reported a decrease of the rat N1-P2 visually EP components with an increase in arousal [10, 11]. These components have been related most commonly to attentional processes. We therefore propose that the increased N60 and P68 auditory EP components with an increase in arousal reflect an increase in attention.

In this study we also observed an increase of the P150-200 AEP component with an increase of delta activity in the pre-stimulus EEG. Previous studies have also reported an enhancement of the P2-N3 complex during slow wave sleep in both visually EPs of rats [5, 10, 11], and auditory EPs in humans [4, 9, 12, 13]. Meeren et al. [11] ascribed increased visually EP components during slow-wave sleep to the more synchronized EEG, as compared with EP components obtained during EEG-desynchronized states, such as wakefulness and REM sleep. The hypothesis underlying this approach is that EPs are due to a superposition of stimulus induced and time-locked EEG rhythms and thus reflect resonance properties of the EEG. Bearing in mind the pre-stimulus EEG can contribute to the interpretation of the components of the consecutive EP.

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CHAPTER 9: THE INFLUENCE OF DIAZEPAM ON EEG-AEP INTERRELATIONS IN RATS

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Abstract: We investigated if diazepam-effects on the rat Auditory Evoked Potential (AEP) could be ascribed to its β-increasing effect in the EEG.
Rats received vehicle or diazepam (4 mg.kg-1). AEPs were elicited by tone-pip stimuli (90dB, 10.2kHz, ISI 2 s). Trials were ranked in 10 categories according to the β-activity in 1 s pre-stimulus EEG (3% bins). AEPs were computed for each category. ANOVAs on the P14, N18, P29, N51 and P67 component were performed. In addition, Cross-correlation coefficients (CCCs) between all AEPs were determined.
In the vehicle condition the P29, N51 and P67 increased with increased β-activity and CCCs changed. In the diazepam condition AEP components and CCCs remained unchanged with increased β-activity. Diazepam affected the rat AEP, such that it resembled the AEP obtained during the lowest β-categories in the vehicle condition.
Increases in β have been associated with increases in arousal. Although being a sedative, diazepam is known to increase β-activity. We found that diazepam-effects on AEPs can not be ascribed to its β-enhancing effects on the EEG. Diazepam disrupted the normal AEP-EEG relation such that diazepam-effects on the rat AEPs seem to reflect the behaviourally sedative effects of diazepam and not its physiologically β-increasing effects.

Key words: Auditory Evoked Potentials, Diazepam, pre-stimulus EEG, ß-rhythm, Fourier analysis, cross- correlation.

9.1 Introduction

Until the early 1980s, within cognitive neuroscience, Evoked Potentials (EPs) were considered to be deterministic signals of the brain, whereas the ongoing EEG was considered to be random noise [1, 2]. Findings however indicate that the ongoing EEG and EPs are related in a fundamental manner [1, 3]. Although these EEG-EP relations have been studied during normal sleep/wake stages [4, 16], EEG-EP interrelations have not yet been determined after pharmacological induced sedation.

Benzodiazepines have, amongst other properties, sedating effects [8, 12]. Although benzodiazepines are sedatives, they are also known to increase the β-activity in the EEG [6]. Since increases in β-activity are normally understood as increases in arousal, this phenomenon is known as pharmacological dissociation [6].

Benzodiazepines also affect aspects of information processing [9, 10]. Since EPs allow objective measurement of information processing [18] they provide a sensitive method for studying the effects of psychoactive drugs on information processing [15, 17]. Indeed, benzodiazepines are known to alter endogenous EP components in both rats [11, 14] and humans [7, 19].

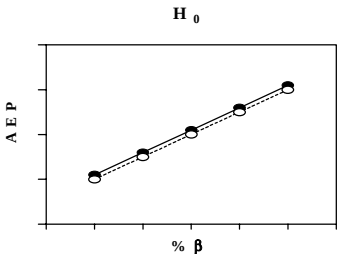
9.1.1 Objective

We investigated if the effects of diazepam on rat auditory evoked potentials (AEPs) could be ascribed to the β-increasing effects of diazepam on the EEG, by comparing AEPs of vehicle and diazepam treated rats with the same relative amount of β-activity in the pre-stimulus EEG [3].

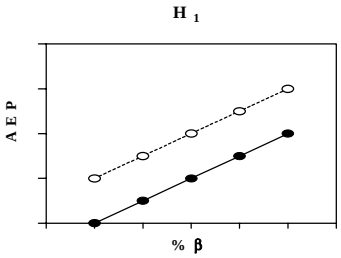
9.1.2 Hypotheses

The following three hypotheses were tested:

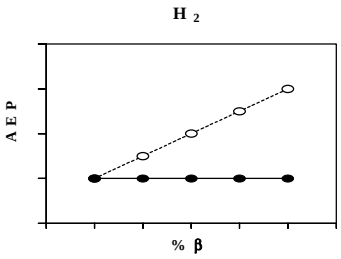
H_0: The effects of diazepam on the rat AEP can be fully ascribed to its β-increasing effect. Thus: if we compare trials of diazepam and vehicle treated rats `with the same percentage of β in the pre-stimulus EEG, drug effects on the consecutive AEPs would disappear.



H_1: The effects of diazepam on the rat AEP can be partly ascribed to its β-increasing effect. Thus: if we compare trials of diazepam and vehicle treated rats `with the same percentage of β in the pre-stimulus EEG, diazepam would affect both the EEG and AEP. However no interaction effects would be observed meaning that diazepam does not alter the EEG-AEP interrelation.



H_2: The effects of diazepam on the AEP can not be ascribed to its β-increasing effect. Thus: if we compare trials of diazepam and vehicle treated rats `with the same percentage of β in the pre-stimulus EEG, diazepam would affect both the rat EEG and AEP. In addition interaction effects would be observed meaning that diazepam does alter the EEG-AEP interrelation.



9.2 Materials and methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee for animal studies has been obtained. Eight male adult Wistar rats, (470 ± 49 grams),

were singly housed with food and water ad libitum. Isoflurane anaesthesia was used for implanting a tripolar electrode set (Plastics One, MS 333/2a). The coordinates of the first active electrode related to bregma were: A -3.4, L 2.0. The second active electrode and the ground electrode were placed above the cerebellum.

The experiment was counterbalanced with a three-day interval. Rats received either diazepam 4mg.kg-1 s.c. (Diazemuls; Dumex, Hilversum, the Netherlands) or vehicle (Lipovenös; Fresenius BV, 'sHertogenbosch, the Netherlands). EEG signals were measured between 0.1 Hz and 500 Hz and sampled with 1024 Hz. AEPs were elicited by 1350 tone-pip stimuli (10.2 kHz, 90 dB, stimulus duration 20 ms) with a 2 s (90%) or 4 s (10%) Inter-Stimulus Interval (ISI). White background noise of 65 dB was present.

The spectral content of pre-stimulus EEG was determined by FFT on 1 s EEG preceding each stimulus onset. Trials were categorised in ten groups, according to their relative magnitude in the β-band (the percentage 13-30 Hz of the total magnitude 1-100 Hz).

For each category, AEPs were determined by averaging EEG fragments recorded 100 ms before stimulus onset till 1000ms after stimulus onset. Individual amplitudes at selected latencies (P_{14}, N_{18}, P_{29}, N_{51} and P_{67}) were further taken into analysis [14]. Grand average AEPs were constructed for 10 categories of increasing β-activity (3% bin width, ranging from 0-3% β in category 1 to >27% β in category 10).

For each component, a two-way ANOVA was employed. 'Drug' being the between variable and 'β' being the within variable. Post-hoc one-way ANOVA analyses were performed whenever a 'drug'*'β' interaction effect was observed.

In addition cross-correlation coefficients (CCCs) were determined of all AEPs in the vehicle condition, in the diazepam condition, and between vehicle and diazepam conditions. We determined CCCs on the AEP endogenous components (all values in the window 10-100 ms after stimulus onset) [13]. Denoting the N samples of AEPn as AEPni (i=1..N), the zero-delay inter-AEP CCC is obtained from:

$$CCC_{n\Leftrightarrow m} = \sum_{i=1}^{N} \frac{(AEP_{ni} - \overline{AEP_n})}{N_n} \times \frac{(AEP_{mi} - \overline{AEP_m})}{N_m}$$, where the normalization factor

$N_n = \sqrt{\sum_i (AEP_{ni} - \overline{AEP_n})^2}$, and the mean AEP signal $\overline{AEP_n} = \sum_{i=1}^{N} AEP_{ni} / N$. Defined this way, CCC expresses the resemblance of the two AEPs involved: CCC=1 is obtained for identical signals, CCC=-1 for mutual inverted signals.

Figure 1

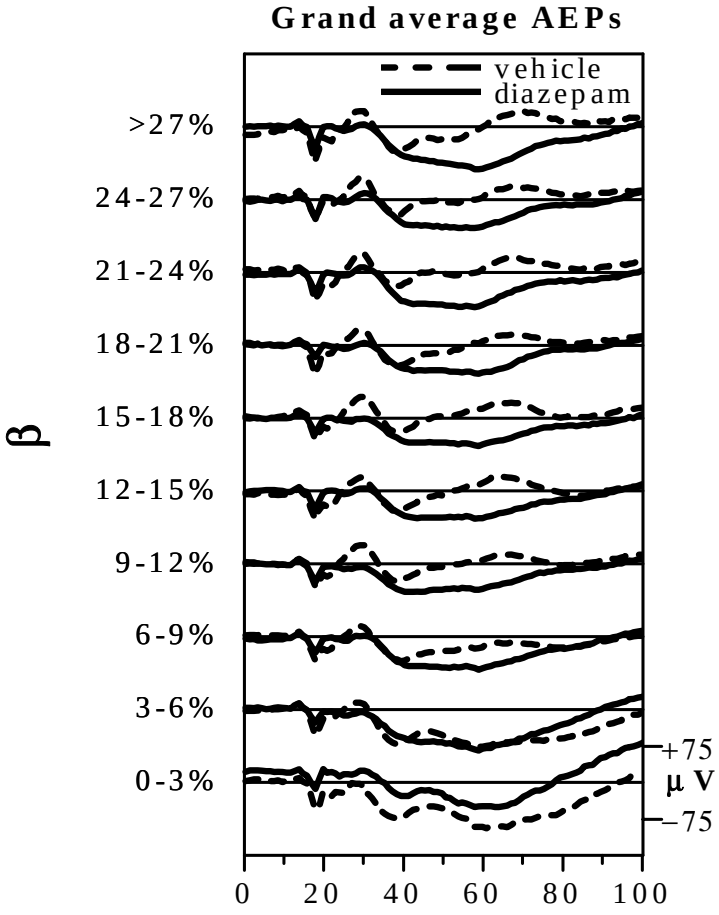


Figure 1 shows the selectively averaged AEPs (0-100 ms after stimulus onset, y-axis), belonging to increasing percentages of relative ß-activity in the pre-stimulus EEG (x-axis) for the vehicle (n=8, dotted lines) and diazepam (n=8, solid lines) condition.

9.3 Results

Diazepam increased β-activity (p=.006), mean β-activity over all trials in the vehicle condition being 14.3% β 0.46, in the diazepam condition being 18.0% β 1.04

Figure 1 shows the averaged AEPs, per β-category for both the vehicle (n=8, dotted lines) and diazepam (n=8, solid lines) condition. After determination of maximal peak-values of the total grand average AEP for the vehicle condition, a P14, N18, P29, N51 and P67 component could be identified.

Figure 2 shows the amplitudes of the AEP components with increasing percentages of β in the pre-stimulus EEG for both the vehicle (dotted lines) and diazepam (solid lines) condition.

Figure 2

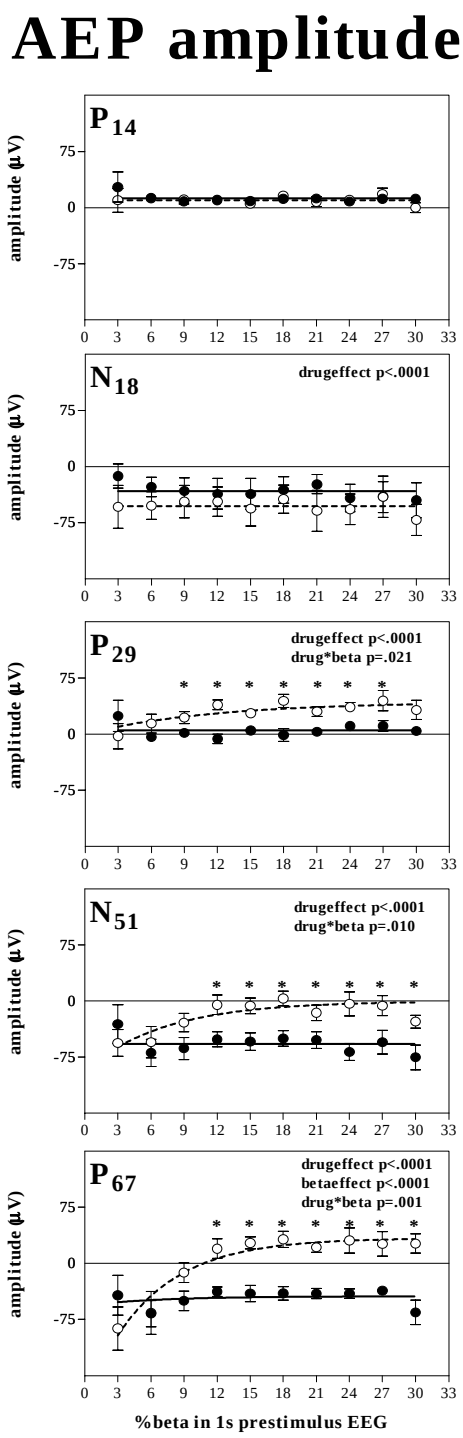


Figure 2 shows the amplitudes of the five AEP components (y-axes) with increasing percentages of β (x-axes) for both the vehicle (dotted lines) and diazepam (solid lines) condition.

9.3.1 ANOVA results

No drug- nor β-effect was found with respect to the amplitude of the P14 AEP component. The amplitudes of the N18 and P29 AEP components were decreased in the diazepam condition compared to the control condition (F=4.6; F=51, both p<.0001). The amplitude of the N51 was enhanced (e.g. more negative values, F=55.5, p<.0001) and the P67 was decreased due to diazepam (F=48.8, p<.0001).

Interactions effects with respect to the amplitudes of the P29, N51 and P67 AEP components were observed (F=3.6, p=.021; F=2.7, p=.010; and F=3.5, p=.001 respectively). Post-hoc analyses revealed that in the vehicle condition these three components showed a positive shift (e.g. more positive values) with an increase in β (all p<.05), but remained unchanged in the diazepam condition. Differences between the diazepam and vehicle condition occurred in the higher β-categories (>12%) (p<.05) whereas no differences between AEP component amplitudes were observed in the lower β-categories (<12%).

9.3.2 cross-correlation results

Table 1 shows the CCCs between AEPs (10-100 ms after stimulus onset). Within the vehicle condition (table 1a) CCCs decreased with changes in β-activity in the pre-stimulus EEG. In the diazepam condition (table 1b), all CCCs were much higher, reflecting less change of AEPs with changes in the pre-stimulus EEG.

When AEPs obtained in the vehicle condition were cross-correlated with the AEPs obtained in the diazepam conditions (table 1c) the highest CCCs were found between the AEPs in the vehicle condition with low β-activity (<12%) in the pre-stimulus EEG and AEPs in the diazepam condition of all β-categories (0->27%).

A vehicle

vehicle %β	3	6	9	12	15	18	21	24	27	30
3	1									
6	.67	1								
9	.50	.79	1							
12	.29	.60	.80	1						
15	.02	.41	.60	.84	1					
18	.19	.44	.59	.89	.85	1				
21	.09	.38	.65	.80	.81	.74	1			
24	.02	.36	.57	.76	.75	.74	.72	1		
27	.09	.41	.61	.69	.71	.61	.73	.70	1	
30	.00	.38	.59	.71	.77	.66	.79	.77	.79	1

B diazepam

vehicle %β	3	6	9	12	15	18	21	24	27	30
3										
6										
9										
12										
15										
18										
21										
24										
27										
30										

C vehicle

diazepam %β	3	6	9	12	15	18	21	24	27	30
3	.55	.42	.27	.11	.06	.02	.06	.00	.17	.08
6	.55	.43	.32	.08	.08	.01	.11	.06	.05	.07
9	.43	.55	.53	.28	.14	.17	.36	.22	.32	.34
12	.56	.47	.49	.29	.13	.22	.38	.20	.27	.25
15	.41	.59	.51	.32	.20	.19	.37	.26	.35	.36
18	.43	.51	.42	.23	.14	.14	.34	.19	.34	.27
21	.42	.52	.46	.23	.11	.11	.32	.21	.32	.28
24	.37	.53	.50	.29	.20	.17	.37	.28	.41	.40
27	.39	.56	.51	.36	.23	.19	.41	.28	.49	.43
30	.42	.52	.50	.23	.09	.08	.34	.24	.36	.32

D diazepam

diazepam %β	3	6	9	12	15	18	21	24	27	30
3	1									
6	.66	1								
9	.56	.79	1							
12	.56	.80	.82	1						
15	.55	.73	.88	.84	1					
18	.57	.70	.82	.86	.89	1				
21	.54	.76	.91	.87	.93	.89	1			
24	.48	.68	.87	.81	.90	.87	.91	1		
27	.55	.59	.74	.74	.87	.85	.80	.83	1	
30	.50	.69	.87	.83	.90	.89	.90	.91	.84	1

Legends CCC=1 N.S. *p<.05 **p<.01 ***p<.001

9.4 Discussion

Diazepam is known to increase β-activity in the EEG. When corrected for this effect by comparing AEPs subaveraged according to the amount of β-activity in 1 s pre-stimulus EEG, diazepam effects on the rat AEP were still observed. Therefore, the effects of diazepam on the rat AEP can not be ascribed to its effect on the β-band in the EEG. In addition, interaction effects were observed. We therefore accepted our H2 hypothesis, proposing that diazepam disrupts the normal EEG-AEP interrelation.

In the drug free situation, both EEG patterns and the architecture of EPs are dependent on the state of alertness Ongoing EEG activity and EPs have been related using a variety of approaches. One such approach involves the recording of EPs during different sleep stages and wakefulness (Bastuji et al., 1995; Meeren et al., 1998). During

sleep the EEG shows high voltage, low frequency activity and EP-components are large (Bringmann and Klingberg, 1995; Meeren et al., 1998). Such large EEG and EP components have been ascribed to the more synchronised EEG during sleep (Meeren et al., 1998). During waking, the EEG shows low voltage, high frequency activity (β) and EP-components are moderate in amplitude. Activity in the β-band of the EEG is considered to be an index of cortical arousal. Therefore, by assessing how changes in the β-activity of the pre-stimulus EEG affect the post-stimulus EP (Brandt et al., 1991) subtle variations in the level of arousal on the EP can be studied.

In the vehicle condition, we found increases (e.g. more positive values) of the P29, N51 and the P67 component amplitudes with an increase in β-activity in the pre-stimulus EEG. Bastuji et al., (1995) have reported an increase of the human P2 with an increase in arousal.

Diazepam affected AEP components, such that they resembled AEP components obtained during the lowest β-categories in the vehicle condition. Increments of AEP component amplitudes with an increase in β-activity were no longer observed in the diazepam condition. Therefore, diazepam effects on the rat AEP might reflect primarily the sedative properties of diazepam.

Measuring drug effects on AEPs adds information to measuring drug effects on the ongoing EEG. Moreover, diazepam effects on the rat AEPs seem to reflect the behaviourally sedative effects of diazepam and not the physiologically β-increasing effects of diazepam.

Acknowledgement

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CHAPTER 10: PROLOGUE GENERAL DISCUSSION

One of our main aims throughout this thesis was to develop tools to study the effects of (pharmacologically induced) sedation on information processing. We investigated how a single dose of diazepam affected evoked potentials elicited in different paradigms.

Over the past 20 years, many researchers have investigated the effects of benzodiazepines on evoked potentials, see table below.

Authors	Benzodiazepine	Subjects	Effect
SEP, simple stimuli			
[28] Todorova, 1993	diazepam, 0.5, 1, 5 mg*kg^{-1} i.v.	rat (n=88)	↓amplitudes ↑latencies
[29] Suzuki et al., 1991	midazolam 10 mg*kg^{-1} i.v.	rat	↓P1-N1 amplitudes
[30] Russell et a., 1995	midazolam, 0.1 mg*kg^{-1} i.v.	rat (n=36)	no effect
[31] Auguglia et al., 1996	diazepam,10 mg i.v.	Creutzfeld-Jacob patient (n=1)	↓all amplitudes
[32] Kochs et al., 1989	midazolam	human (n=12)	↓P1-N1-P2 amplitudes ↑P1-N1-P2latency
[33] Langeron et al., 1999	Midazolam, 0.3 + 0.15 mg*kg^{-1}/h	human (n=30)	no effect
VEP, simple stimuli			
[34] Sherwin, 1970	diazepam, 0.5, 1, 3, 5 mg*kg^{-1} i.v.	cat (n=16)	↓ amplitudes of all components >30 ms
[35] Santi et al., 1985	diazepam, 5-10 mg*kg^{-1} i.v.	rat	↓N1 amplitude
[36] Hudnell and Boyes, 1991	diazepam, .067, .125, .25, .5 mg*kg^{-1} i.v.	rats (n=44)	↓2F response
[36] “	diazepam, 10 mg p.o.	human (n=30)	↓2F response
[37] Bartel et al., 1988	diazepam, 10 mg p.o.	human (n=10)	no effects
[38] Boker and Heinze, 1984	diazepam, 2, 4, 8 mg p.o.	human (n=11)	↓P1-N2 amplitudes
[39] Declerck, 1985	clonazepam, 0.4-0.8 mg i.v.	epileptic patients (n=30)	↓N1-N2 amplitudes
[40] Ebe et al., 1969	diazepam, 5-6mg i.v.	epileptic patients (n=7)	↓ all components
[41] Rockstroh et al., 1991	clonazepam, 1 mg p.o.	human (n=36)	↓P1 amplitude

AEP, simple stimuli			
[42] Johnson et al., 1981	triazolam, 0.5 mg p.o.	human (n=10)	↓P1-N1 amplitude
[32]Kochs et al., 1989	midazolam	human (n=12)	↓P1-N1-P2 amplitudes ↑P1-N1-P2 latencies
[43] Schwender et al., 1993	midazolam, 0.2-0.3 mg*kg^{-1} i.v.	human (n=10)	↑P1 latency
[43] "	diazepam, 0.3-0.4 mg*kg^{-1} i.v.	human (n=10)	no effects
[43] "	flunitrazepam 0.035 mg*kg^{-1} i.v.	human (n=10)	↓Na-Pa amplitudes
[44] Brunner et al., 1999	Bolus midazolam i.v.	human (n=9)	↓Nb latency
[45]Noldy et al., 1990	diazepam, 20 mg p.o.	human (n=20)	↓N1-P2
complex stimulation paradigms			
[46] Erwin et al., 1986	diazepam, 10 mg p.o.	human (n=12)	↓N2-P2 amplitude ↑P3 latency
[47] Shinoto et al, 1989	clonazepam, 30µg*kg^{-1} p.o.	human (n=6)	↑P3 latency
[48] Pang and Fowler, 1994	triazolam, 0.25 mg p.o.	human (n=12)	↑P3 latency
[49] Engelhardt et al., 1992	midazolam, 0.1, 0.2 mg*kg^{-1} i.v.	human (n=12)	↓P3 amplitude
[50] Allen et al., 1990	alprazolam, 0.25 mg 3 x daily p.o.	human (n=12)	↓P1-N1-P2-N2 ampl.
[51] van Leeuwen et al., 1995	oxazepam, 20-40 mg p.o.	human (n=18)	↓N1-P2-N2-P3 ampl.
[52] Munte et al., 1996	alprazolam bromazepam	human	↓N1-N2-N4 ampl.
[53] Nichols et al., 1996	lorazepam	humans	↑P3 lat. and ampl.
[54] Martin et al., 1992	temazepam, 10 mg p.o.	human (n=12)	↓P3 amplitude ↑P3 latency
[55] Ray et al., 1992	diazepam, 5, 10 mg p.o.	human (n=8)	↓P3 latency
[56] Curran et al., 1998	lorazepam, 2 mg p.o.	human (n=5)	↓N1-P2-P3 amplitudes
[57] Semlitsch et al., 1995	alprazolam, 1 mg p.o.	human (n=15)	↓N1-P3 amplitudes
[58] van Leeuwen et al., 1991	bromazepam 6-12 mg p.o.	human (n=30)	↓N1-P2-N2-P3 amplitudes
[41] Rockstroh et al., 1991	clonazepam, 1 mg p.o.	human (n=36)	↓N1-P3 amplitudes and latencies ↑N2 amplitude
[59] Nakagome et al., 1998	triazolam, 0.25 mg p.o.	human (n=8)	↓N1 amplitude
[60] Reinsel et al., 1991	midazolam, 0.07 mg*kg^{-1} i.v.	human (n=10)	↓P3 amplitude ↑N3 latency
[61] Unrug et al., 1996	diazepam, 10mg p.o.	human (n=8)	↓P3 amplitude
[62] Luijtelaar et al., 1998	diazepam, 10 mg p.o.	human(n=8)	↓N1-P3 amplitudes
[63] Urata et al., 1996	triazolam, 0.125 mg p.o.	human	↓P3 amplitude ↑P3 latency

Most studies investigating the effects of benzodiazepines on Evoked Potentials use simple, sensory stimuli in the sensory [28-33], visual [34-41] or auditory [42-45] modality. Although some studies found no effects of benzodiazepines on EPs [30, 33, 37, 43], most studies have reported decreased amplitudes and increased latencies in both rats [28, 29, 35, 36], cats [34] and humans [31-33, 36, 38-41]. This is in agreement with our results with respect to the middle-latency AEP components (10-50 ms after stimulus onset), where we found a decrease of a P_{30} component (chapter 4, 5 and 9). However, in our first experiment we observed an increase of a slightly later occurring P_{40} component. This might be due to a difference in dosage [37, 38].

Others [41, 65, 66] reported the boosting of a distinct late N2 AEP component after administration of a benzodiazepine in humans [41]. In this regard, we also consistently observed increased negative values of later occurring AEP components (>50 ms), starting with an increased N_{50-60} lasting till about a P_{70-100} component. This effect has been interpreted as reflecting the sedative effect of the drug.

Though the effects of benzodiazepines on EPs elicited in more complex stimulation paradigms have been investigated [46-63], to our knowledge only human studies have been reported. Most studies found decreased amplitudes [49, 51, 54-57] and increased latencies [46-48, 54, 63] of especially the late-latency P3 component. This has been interpreted as decreases in information processing capacities. Since in humans Omission Evoked Potentials, or OEPs, have been proposed to reflect similar processes as the more conventional P3 component [27, 66], it is reasonable to expect similar diazepam-effects on the OEPs as on the P3 component. In this regard, we found the abolishment of OEPs after administration of diazepam in rats.

CHAPTER 10: GENERAL DISCUSSION

10.1 Discussion part A

10.1.1 passive paradigms for eliciting evoked potentials

The main aim of the first part of this thesis was to study how passive stimulus paradigms can be used to measure different aspects of information processing. In addition, we used these passive paradigms as a tool to study the effects of (pharmacologically induced) sedation on information processing. The results of experiments described in part A are summarised in table 10.1.1.

Table 10.1.1

Paradigm	Main effect paradigm	Main effect diazepam	Interaction effects.	Main findings
Oddball Ch. 2		Background tones: ↑P_{40}, P_{72}-P_{102} on target tones: ↑P_{40}-P_{48}, ↓N_{58}	on target tones: ↓P_{102} over sessions with diazepam, not with vehicle	- Enhanced over-session habituation due to diazepam
10-tone Ch. 3	between tone 1&2 ↓ $N_{23,}$ P_{30}, P_{50} between tone 2&10 ↓ P_{30}, P_{50} P_{150}			- ISI dependent decrements depend on recovery phenomena - ISI independent decrements depend on habituation
10-tone Ch. 4	over 10 tones ↓ $N_{18,}$ P_{30},	↓ P_{30} ↑ N_{60}-P_{67}	with vehicle: ↓P_{67} with diazepam: no effect P_{67}	- Decrease habituation due to diazepam
OEPs Ch. 5	8 out of 16 rats large positive wave	No OEPs with diazepam		- OEPs can be measured in rats - diazepam affects expectancy and time-estimation
human 10-tone Ch. 6	no effects of stimulus repetition	↓ N_{180} ↑ N_{370}- P_{420} ↓ SD		- diazepam decreases arousal - diazepam decreases response variability
human OEPS Ch. 6	only OEPs ($P_{400\text{-}600}$) in one musically trained subject			in order to elicit OEPs, training is necessary

10.1.2 The oddball paradigm

Endogenous EP components are often elicited with a two stimulus discrimination task or the oddball paradigm [1]. An oddball paradigm is an experimental paradigm that is often used in human cognitive psychology [2]. During the oddball task the subject is exposed to two different stimuli, one of which occurs relatively infrequently and is designated as a target [3]. Stimulus-change and unpredictability are the main features of this paradigm [3]. In humans, the most striking feature of EPs elicited by the infrequent target stimuli in such an oddball task is the P3, a large positive potential, maximal over the vertex with a latency around 300 to 600 ms after stimulus onset [4-6]. This component has been proposed, amongst others, to reflect working memory functions or the degree of allocated attention [1, 7]. Although so far no unitary interpretation of P3 exists, it is noteworthy that these late endogenous components are only measured in response to 'meaningful' or 'unexpected' stimuli [4]. Though less common, similar components have also been measured in animals, like monkeys [8-10] cats [11-14] and rats [15-17].

Most oddball studies employ an active discrimination task e.g. by mental counting or a button press [18, 19]. With active subject participation it is difficult to determine whether found changes in endogenous EP components are due to changes in information processing demands, or more directly related to the generation of a response. Furthermore, psychoactive substances might differentially affect the processing of target stimuli and the initiating of a response.

10.1.3 The passive oddball paradigm

Several studies however have reported similar EP results when using a passive oddball procedure [4, 20]. Therefore, in our first experiment (chapter 2) we measured EPs elicited in a passive oddball paradigm. Although we were not able to measure a P3 in this study, we did find different diazepam effects on target and background stimuli with respect to the late, endogenous components.

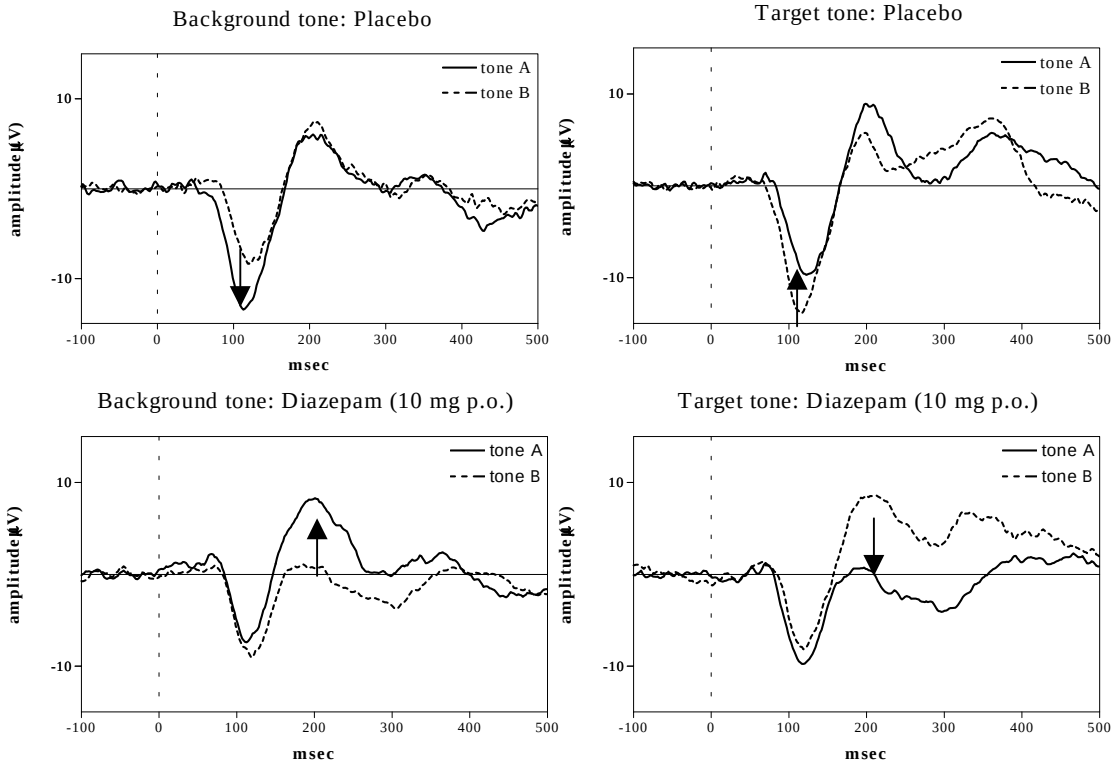
By presenting two physically different stimuli however, changes in EPs may not only be determined by cognitive processes, but may also be determined by the differences in the stimuli used. Although physical properties of the stimulus mainly shape the early, exogenous EP components, the influence of stimulus characteristics on later, exogenous EP components has recently been investigated [1, 21-23]. Both auditory tone intensity and frequency have been found to influence the human P3 as measured in an oddball paradigm [1]. Moreover, this influence appeared to be different for young subjects compared to older subjects [2]. Therefore, interaction effects between stimulus characteristics used in the oddball and the variable under investigating might occur [22].

An obvious solution would be to counterbalance background and target stimuli. In our first experiment (chapter 2) we could not counterbalance target and background tones because diazepam was administered chronically for three weeks in the experimental

group. Therefore, different effects of target and background stimuli on EPs might have been caused by differences in either first order stimulus characteristics (pitch, loudness) or second order stimulus characteristics (frequent versus infrequent occurrence). In chapter 2 we therefore did not compare AEPs elicited by target or background tones within groups.

To our knowledge, no studies so far have reported the effects of counterbalancing in an oddball procedure. However, in a recent experiment we found evidence for an interaction between diazepam effects and effects of counterbalancing on human AEPs (unpublished data, figure 10.1.3). Although thus far we have no satisfying explanation of this interaction effect, possible interaction effects should be taken into account when applying the oddball paradigm for studying the drug effects on endogenous EPs.

Figure 10.1.3: Grand average human AEPs elicited in an active oddball paradigm with counterbalancing of background and target tones.



10.1.4 single-stimulus paradigms

A more elegant solution would be to only change the 'meaning' or higher order characteristics of single stimuli, without changing the lower order characteristics of stimuli (e.g. pitch, loudness, duration). By using such single stimulus paradigms in which only the presentation pattern of single stimuli is varied, changes in EPs can no longer be attributed to changes in stimulus characteristics, but only be attributed to changes in aspects of information processing involved by the stimulus event. The major advantage of single-stimulus paradigms is that it provides a very simple method to use in clinical and animal testing situations [2, 20]. In addition, it would cancel out possible drug by tone interaction effects.

In the chapters 3 to 6, we investigated how changes in stimulus presentation alone, and not changes in stimulus characteristics, affect the middle- and late-latency components of the rat EP, by using passive, single-stimulus paradigms.

In chapter 3 we determined whether or not amplitude reductions with stimulus repetition depended on the length of the interval between stimuli (ISI). Instead of using the well known two-tone or double-click paradigm, we employed a ten-tone paradigm. We found ISI-dependent reductions of a middle-latency N_{23}. However, we found ISI-independent decreases of a later occurring P_{50} component. We proposed that this ISI-independent decrement reflected habituation to the temporal regularity of presented stimuli. Temporal regularity of stimuli within a train is a higher order characteristic of the presented stimuli [24]. In a following experiment (chapter 4), we found that diazepam did not disrupt the ISI-dependent decrease of the N_{23} component. However, the ISI-dependent decrease of a later P_{67} component was disrupted by diazepam. These findings suggest that diazepam mainly affects processing of higher-order stimulus characteristics e.g. the temporal regularity of stimuli.

To explore the issue of higher order processing of temporal patterns further, we attempted to measure EPs in reaction to omitted stimuli in chapter 5. Thus elicited omission evoked potentials, or OEPs, can be wholly attributed to aspects of information processing involved in the (internal) event of stimulus omission [25, 26] and will therefore consist entirely of endogenous components. The omitted stimulus paradigm thus provides a gallant tool to elicit selectively endogenous EP components.

Although we were able to measure OEPs in rats, we did so in only half of the rats. This is in agreement with other researchers [27] and with findings from our human experiment where we could only measure an OEPs in a subject very experienced with auditory rhythms. In addition, we found that in rats diazepam disrupted OEPs.

Because OEPs are difficult to elicit and apparently needs training of the subjects, the omitted stimuli paradigm holds mainly theoretical interests and will find little use in e.g. standard protocols for measuring drug-effects on information processing.

10.2 Conclusions part A

By employing appropriate paradigms for eliciting EPs, psychologists attempt to make inferences about processing sensory stimuli. [67-69]. One of the most important contributions of EP research to experimental psychology is the evaluation of information processing in situations in which no overt response is available [69]. An application of EPs elicited by passive paradigms could be to study information processing during general anesthesia. Although the surgical patient under general anaesthesia is assumed to be oblivious to sensory events [70, 71], there is however evidence that some degree of auditory perception may be present during general anaesthesia [72]. Measuring EPs elicited in passive paradigms might thus add to a better understanding of auditory information processing during general anesthesia. Current research is directed towards this subject.

We found that it is possible to study different aspects of information processing, like habituation (chapters 3 and 4) and time-estimation (chapter 5) by measuring EPs elicited in passive paradigms. Thus elicited EPs appeared to be sensitive to the effects of a psychoactive substance. In chapter 2, we found more pronounced drug effects on an infrequent occuring stimulus than on a frequently occurring stimulus. In chapter 4, we found that diazepam affected the processing of the temporal regularity of stimuli. This finding of differential diazepam effects higher order stimulus characteristics is in agreement with our previous findings. In chapter 5, we found that after diazepam, rats no longer showed a reaction in response to an omitted stimulus. We conclude that diazepam therefore affects mainly the processing of higher-order stimulus characteristics.

10.3 Discussion part B

10.3.1 EEG-EP interrelations

The main aim of the second part of this thesis was to study how subtle changes in the ongoing EEG affect the Evoked Potential. In an initial experiment (chapter 7), we determined the effects of diazepam on the ongoing EEG. In a following experiment (chapter 8), we determined how rat EPs changed with changes in the ongoing EEG. We found that diazepam affected the ongoing EEG. In addition, we found that EPs indeed change with changes in the ongoing EEG. Therefore, we investigated in a final experiment (chapter 9) if the effects of diazepam on EPs could be ascribed to the effects of diazepam on the ongoing EEG (as measured in chapter 8). The results of the experiments described in part B are summarised in table 10.3.1.

Table 10.3.1

Chapter	Main effect of ongoing EEG on EP	Main effect of diazepam	Main findings
Ch. 7		**On EEG** δ-activity ↓ θ-activity ↓ α-activity: no effect β activity ↑	EEG effects of diazepam on the ongoing EEG remain stable over 21 days in rats
Ch. 8	decrease in δ , θ activity and increase in β activity all lead to: ↑N_{60}, P_{70} ↓P_{150}		↑N_{60}, P_{70} reflect increase in arousal ↑P_{150} due to increased EEG synchronisation during sleep
Ch. 9	 increase in β activity: leads to: ↑ N_{51}-P_{67}	**On EPs** $N_{18,}$ P_{30} ↓, N_{51}-P_{67} more negative values increase in β activity: leads to: no changes N_{51}-P_{67}	Effects diazepam on rat AEPs reflect the sedative properties of diazepam, not the β-increasing properties

10.3.2 The EEG and EPs during sleep-wake states

There is a strong relationship between behavior and the powerspectrum of the ongoing EEG [73]. For example, during slow wave sleep large, slow delta-waves (2-4 Hz) are visible. This is caused by more synchronised unit responses with sharper phases of excitations and inhibitions, which results from increased hyperpolarizations [74, 75]. On the other hand, during active behavior low amplitude, fast beta-waves (>12 Hz) dominate the EEG. Therefore, activity in the low and high frequency bands of the EEG are considered to be an index of cortical arousal, such that power in the low frequency (delta) bands increases with a decrease in arousal and activity in the high frequency (alpha and beta) bands increases with an increase in arousal [7].

10.3.3 EPs and sleep-wake states

Similar to EEG patterns, the architecture of evoked potentials is dependent on the state of alertness [74, 75]. During waking, components in the ERP are moderate in amplitude, while during slow wave sleep larger waves are visible. Several researchers have investigated the effects of sleep on evoked potentials, see table 10.3.2

Table 10.3.2

Autor(s)	species	stimulation	effect of sleep on EP
[76] Chen and Buchwald, 1986	cat (n=8)	visual	↓P20 amplitude
[77] Bringmann and Klingberg, 1995	rat (n=30)	visual	↑N31 latency ↑N41 amplitude ↑P60-80 amplitude
[78] Knight et al., 1985	rat (n=6)	auditory	↑P23, N140 amplitudes ↓N38, N50 amplitudes
[79] Simpson and Knight, 1993	rat (n=8)	auditory	↑P25 amplitude
[75] Meeren et al., 1998	rat (n=6)	visual	↑P1, N1, P2 amplitudes ↑N3, P4 amplitudes ↑N3, P4 latencies
[80] Luijtelaar et al., 1998	rat (n=5)	auditory	↓P35 amplitude ↑N50, P75 amplitudes and latencies
[81] Webster and Colrain, 1998	human	somatosensory	Appearance ↑N300, N550 amplitudes
[82] Ujszaszi and Halasz, 1988	human	auditory	Appearance ↑N300, N550 amplitudes
[83] Erwin and Buchwald, 1986	human (n=14)	auditory	↓P1 amplitude
[84] Ogilvie et al., 1991	human (n=9)	auditory oddball paradigm	↓N1, P2 amplitudes ↑P1, N2, N3 amplitudes
[85] Harsh et al., 1994	human	auditory oddball paradigm	↓P3 amplitude ↑N3 amplitude
[86] Pratt et al., 1999	human (n=15)	auditory oddball paradigm	↑P2 amplitude
[87] Nielsen-Bohlman et al., 1991	human (n=12)	auditory passive oddball	↑N2, N3 amplitudes ↓P3 amplitudes

Some animal studies have reported decreased amplitudes of middle-latency components during sleep [76, 78, 81]. However, most animal studies found increased amplitudes and latencies of both middle-and late-latency EP components during sleep [77-79, 81, 85, 87, 88]. In line, human studies have also found decreased amplitudes of certain middle-latency EP components during sleep [84, 85]. Though more often an enhancement of middle- and late-latency components has been found [82, 83, 85-88]. These increased components have been ascribed to the in general increased ongoing EEG during slow-wave sleep. Although most commonly an enhancement of mainly the late-latency negative components is observed, studies applying an oddball paradigm have reported a decrease of the endogenous P3 component during sleep [86, 88]. This has been proposed to reflect as a decrease in information processing during sleep.

10.4 Conclusion part B

Since both the ongoing EEG and evoked potentials change with changes in the state of alertness, it is reasonable to assume that the ongoing EEG and EPs hold a strong interrelationship. Though until the 1960s EPs were considered to be independent of the ongoing EEG, experiments of Basar [88, 89] have shown that EPs are indeed highly determined by the ongoing EEG activity. The ongoing EEG can either inhibit or facilitate the genesis of EPs, and EP components can be understood as changes in amplitude and phase of conventional delta, theta alpha and beta activity. Background EEG activity and EPs have been related using a variety of approaches. Determining EPs during different sleep-wake stages is one such approach. A more direct approach involves recording pre- and post-stimulus EEG epochs and assessing how changes in the spectral power of the pre-stimulus EEG affect the post-stimulus EP measures [90]. We applied this approach in our experiments described in part B (chapter 8 and 9). By using this approach, the effects of subtle variations in the level of arousal on the EP can be studied.

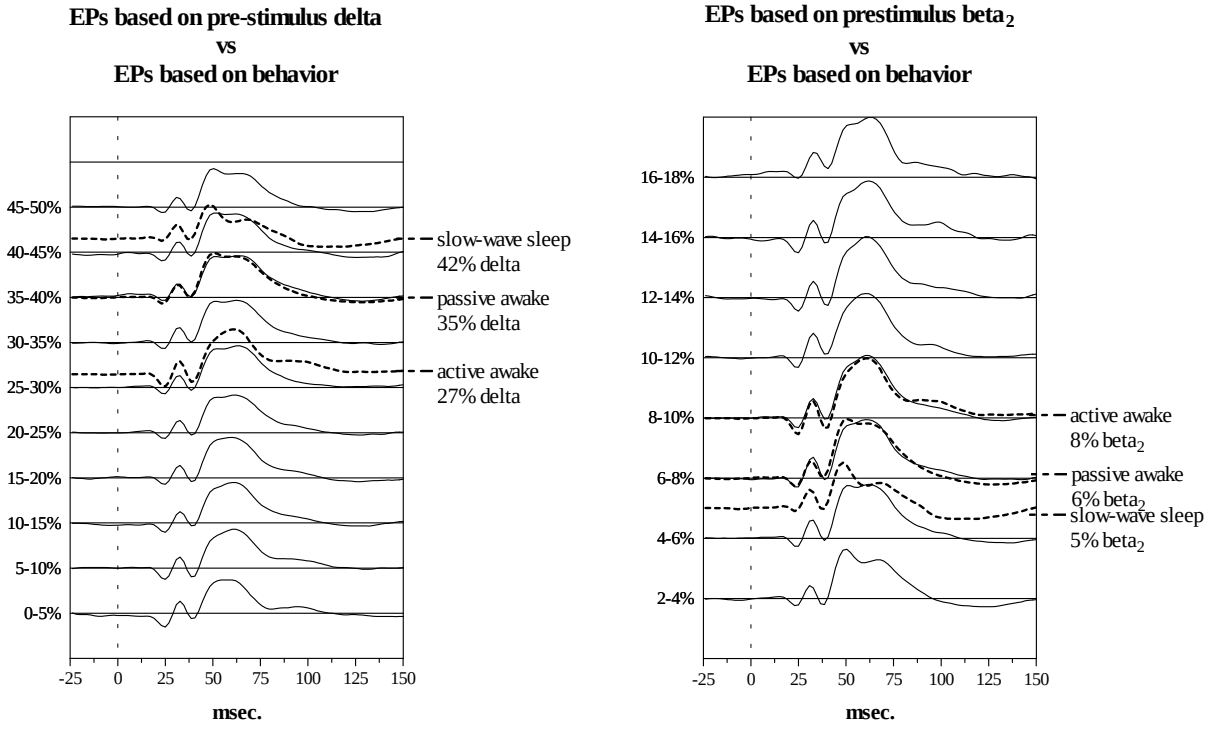
In the first EEG-EP experiment (chapter 8) we found an increase of the N_{60} and P_{68} auditory EP components with an increase in beta activity in the pre-stimulus EEG. We proposed that the architecture of EPs highly depends on the spectral content of the ongoing EEG. We therefore wondered if EPs did add information to only measuring the ongoing EEG. In a subsequent experiment (chapter 9) we then investigated if the effects of diazepam on rat EPs could be totally ascribed to its effects on the ongoing EEG.

Diazepam is known to increase beta-activity in the EEG (chapter 7). When corrected for this effect by comparing EPs subaveraged according to the amount of beta-activity in 1 s pre-stimulus EEG, diazepam effects on the rat EP were still observed. Therefore, the effects of diazepam on the rat EP could not be ascribed to its effect on the beta-band in the EEG.

Diazepam affected EP components, such that they resembled EP components obtained during the lowest beta-categories in the vehicle condition. In the vehicle condition, increments in pre-stimulus beta-activity caused increments in several EP components. These increments of EP component amplitudes with an increase in beta-activity were no longer observed in the diazepam condition. Therefore, diazepam effects on the rat EP might reflect primarily the sedative properties of diazepam. We concluded that measuring EPs adds information to measuring the ongoing EEG, especially when determining drug-effects on both the state (reflected by the ongoing EEG) and responsiveness (reflected by EPs) of the brain.

In this study we found that the diazepam-effect on rat EPs seem to reflect the behaviorally sedative effects of diazepam and not its physiologically beta-increasing effects. However, we did not verify this hypothesis by comparing our EPs based on the amount of beta-activity in the pre-stimulus EEG with EPs based on sleep-wake stages.

Figure 10.4a

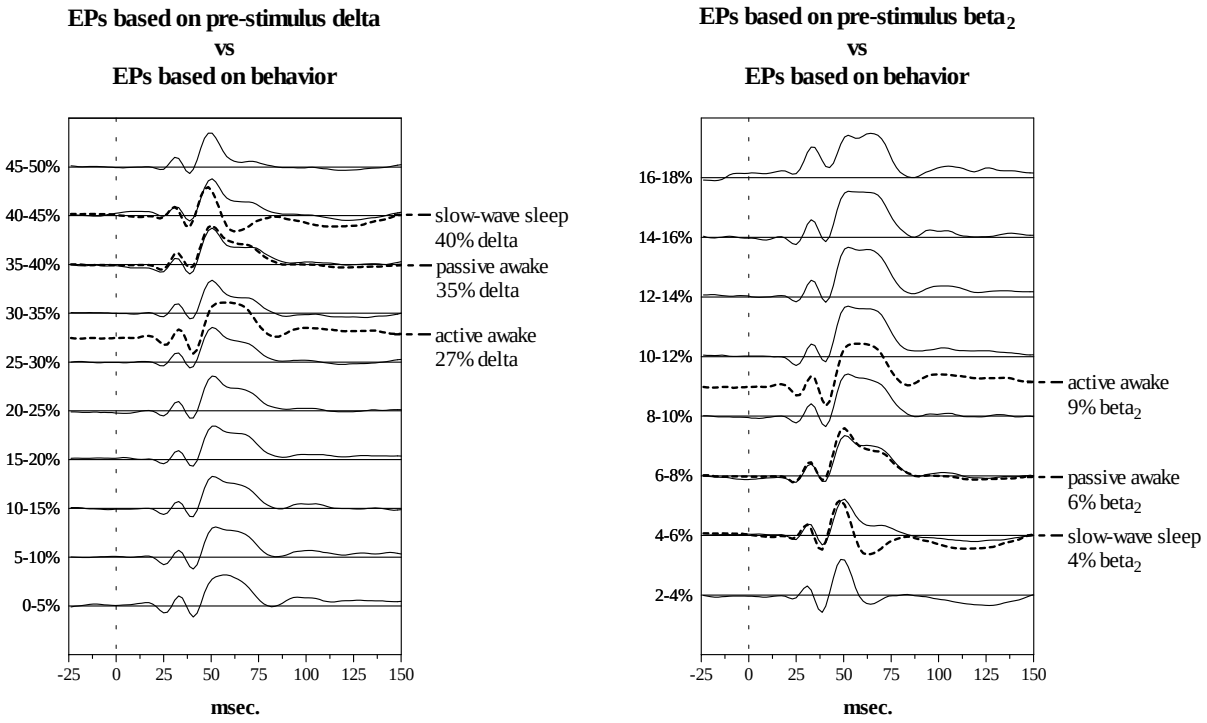


Therefore, in a recent experiment in rats (unpublished data), we did compare EPs based behavioral sleep-wake stages with EPs based on the amount of both delta (0-4 Hz) and $beta_2$ (30-60 Hz) activity in the pre-stimulus EEG. In this experiment we found indeed that delta-activity increased with a decrease in arousal and $beta_2$-activity decreased with a decrease in arousal. EPs obtained during different sleep-wake stages thus fitted in nicely with the EPs based on either delta or beta2 activity in the pre-stimulus EEG (see figure 10.4a).

In a drug-free situation EPs based on changes in the ongoing EEG are comparable with EPs based on sleep-wake stages. Advantage of the first method though is that more subtle changes in arousal can be investigated. Also, changes in EPs are thus directly related to changes in the powerspectrum of the ongoing EEG, instead of more indirectly related to the ongoing EEG via the behavioral sleep-wake stages. Since this first method can be applied fully automated, less bias by e.g. the observer will be introduced.

However, do EPs based on changes in the powerspectrum of the pre-stimulus EEG still resemble EPs based on different sleep-wake stages when diazepam is administered? We explored this question also in a recent experiment (unpublished data) by comparing EPs based behavioral sleep-wake stages with EPs based on the amount of both delta (0-4 Hz) and $beta_2$ (30-60 Hz) activity in the pre-stimulus EEG after administration of 1mg * kg^{-1} diazepam (s.c.). We found similar results as in the drug-free state (see figure 10.4b).

Figure 10.4b



We conclude that recording pre- and post-stimulus EEG epochs and assessing how changes in the spectral power of the pre-stimulus EEG affects the post-stimulus EP measures is very useful to study EEG-EP interrelations in both drug-free situations and in situations where drugs are administered.

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Summary

The electroencephalogram (EEG) represents the electrical activity of the brain. Evoked Potentials (EPs) are small voltage fluctuations in the EEG resulting from sensory, cognitive or motor evoked neural activity. Variations in the EP waveform may be caused by several factors.

1. By employing different stimulation paradigms (which is studied in Part A of this thesis).
2. By changes in the subject's state (which is studied in Part B of this thesis).
3. By psychoactive drugs (which is studied in this thesis by studying the effects of diazepam on EPs).

Part A

The main aim of the first part of this thesis was to develop tools to study the effects of (pharmacologically induced) sedation on information processing. We investigated which passive paradigms to elicit EPs can be used to measure different aspects of information processing.

EPs are often elicited in a so called 'oddball paradigm', in which a train of frequently occurring background tones is interspersed with infrequently occurring target tones. In an initial experiment, chapter 2, we measured EPs elicited in a passive oddball paradigm. In addition, we determined if diazepam differentially affected EPs elicited by background tones and EPs elicited by target tones. We found more pronounced drug effects on an infrequent stimulus than on a frequently occurring background stimulus.

By presenting two or more physically different stimuli in a passive oddball procedure, changes in EPs may not only be determined by cognitive processes, but may also be determined by the physical characteristics of the used stimuli. A solution would be to only change the 'meaning' or higher order characteristics of single stimuli, without changes in lower order characteristics of stimuli (e.g. pitch, loudness, duration). By using such single stimulus paradigms in which only the presentation pattern of single stimuli is varied, changes in EPs can no longer be attributed to changes in stimulus characteristics, but only be attributed to changes in aspects of information processing involved by the stimulus event. One such passive, single-stimulus paradigm is the conditioning-testing or double click paradigm. This paradigm involves the presentation of pairs of stimuli. Normally, amplitude decrements of EP components in response to the second tone relative to the EP components in response to the first tone are observed. This response decrement is known as sensory gating. In chapter 3 we determined whether decrements were more pronounced with short Inter-Stimulus Intervals (ISIs) than with longer ISIs. This in order to determine whether sensory gating could be ascribed to recovery phenomena or to habituation. Instead of using the well known two-tone or double-click paradigm, we employed a ten-tone paradigm to determine whether decrement occurred

fully between two tones, or developed more gradually over a train of ten tones. We found that sensory gating of certain middle-latency EP peaks could be ascribed to recovery phenomena and that sensory gating of later occurring peaks could be ascribed to habituation.

In chapter 4 we studied the effect of diazepam on sensory gating in rats, by measuring diazepam effects on auditory EPs elicited in a ten-tone paradigm. We found that diazepam affected the habituational processes underlying sensory gating of later occurring EP components but not recovery phenomena underlying sensory gating of earlier EP components.

Another passive, single-stimulus paradigm is the omitted stimulus paradigm. The omitted stimulus paradigm can be seen as a special variant of the oddball paradigm. Instead of presenting infrequently occurring target tones within a steady train of background tones, target tones are omitted. The omitted stimulus paradigm thus provides a tool to study aspects of information processing concerned with expectancy and time estimation. In chapter 5 we showed that EPs to omitted stimuli could be elicited in rats. In addition, we studied the effect of diazepam on EPs to omitted stimuli. We found that after administration of diazepam, rats no longer showed an EP in response to an omitted stimulus. Based on the experiments described in chapters 2, 4 and 5 we further concluded that diazepam mainly affects the processing of higher order stimulus characteristics.

In chapter 6 we studied in humans the effects of diazepam on auditory EPs elicited in a ten-tone paradigm, and on EPs to omitted stimuli. This to make a comparison between human data and data obtained in previous experiments in rats. The effects of diazepam on human EPs were comparable to our previous results in rats.

One of the most important contributions of EP research to experimental psychology is the evaluation of information processing in situations in which no reliable overt response is available. By employing appropriate paradigms for eliciting EPs, psychologists attempt to make inferences about processing sensory stimuli. We found that it is possible to study certain aspects of information processing by measuring EPs elicited in passive paradigms. Thus elicited EPs appear to be sensitive to the effects of psychoactive substances like diazepam.

Part B

The main aim of the second part of this thesis was to determine if the effects of diazepam on the rat auditory EPs could be ascribed to its effects on the ongoing EEG. In other words, we determined if measuring drug effects on EPs (reflecting brain-reactivity) adds information to only measuring drug effects on the ongoing EEG (reflecting brain-state).

Since diazepam has sedative properties, an increase in low frequencies (delta- and theta-activity) in the ongoing EEG would be expected. However, diazepam is known to

increase the high beta-activity in the EEG. This phenomenon is known as pharmacological dissociation.

In an initial experiment, chapter 7, we measured the effects of diazepam on the spectral content of the EEG in rats. Indeed, we found that diazepam caused an increase in the power of the high frequency bands (21-40 Hz) as expected.

Since EPs appear in the EEG, it is reasonable to assume that the ongoing EEG and EPs hold a strong interrelationship. The ongoing EEG activity and EPs have been related using a variety of approaches. Determining changes in both the ongoing EEG and EPs during different sleep-wake stages is one such approach. A more direct approach involves recording pre- and post-stimulus EEG epochs and assessing how changes in the spectral power of the pre-stimulus EEG affects the post-stimulus EP measures. By using this approach, the effects of subtle variations in the level of arousal on the EP can be studied.

In chapter 8, the initial EEG-EP experiment, we found an increase of the amplitudes of EP components with an increase in beta activity in the pre-stimulus EEG. We proposed that the architecture of EPs are highly depended on the spectral content of the ongoing EEG. We therefore wondered if EPs did add information to only measuring the ongoing EEG.

In chapter 9, our final experiment, we then investigated if the effects of diazepam on rat EPs could be totally ascribed to its effects on the ongoing EEG. Since diazepam is known to increase beta-activity in the EEG, we corrected for this beta-increasing effect by comparing EPs subaveraged according to the amount of beta-activity in 1 s pre-stimulus EEG. After correction however, diazepam effects on the rat EP were still observed. Therefore, the effects of diazepam on the rat EP could not be ascribed to its effect on the beta-band in the EEG. Diazepam affected EP components, such that they resembled EP components obtained during the lowest beta-categories in the vehicle condition. In the vehicle condition, increments in pre-stimulus beta-activity caused increments in several EP components. These increments of EP component amplitudes with an increase in beta-activity were no longer observed in the diazepam condition. Therefore, diazepam effects on the rat EP might reflect primarily the sedative properties of diazepam.

We concluded that measuring EPs adds information to measuring the ongoing EEG. This way it is possible to determine drug-effects on both the state (reflected by the ongoing EEG) and responsiveness (reflected by EPs) of the brain.

Samenvatting

Het EEG representeert de elektrische activiteit van het brein. ‘Evoked Potentials’ (EPs) zijn laag gevolteerde pieken en dalen in het EEG die het resultaat zijn van sensorisch, cognitief of motorisch geïnduceerde neurale activiteit in het brein. De vorm van de EP kan door verschillende factoren beïnvloed worden.

1. Door gebruik te maken van verschillende stimulatie paradigmata (hetgeen bestudeerd is in deel A van dit proefschrift).
2. Door veranderingen in de toestand van het subject (hetgeen bestudeerd is in deel B van dit proefschrift).
3. Door psychofarmaca (hetgeen door toediening van diazepam (valium) in dit proefschrift is bestudeerd).

Deel A

Het doel van het eerste deel van dit proefschrift was methoden te ontwikkelen om de effecten van (farmacologisch geïnduceerde) sedatie op informatieverwerking te bestuderen. We onderzochten welke EP paradigmata er gebruikt kunnen worden om verschillende aspecten van informatie verwerking te bestuderen.

EPs worden vaak gemeten in reactie op een zogenaamd ‘oddball’ paradigma. Dit paradigma bestaat uit een reeks frequente standaard-tonen, afgewisseld met infrequent voorkomende target-tonen. In ons eerste experiment, beschreven in hoofdstuk 2, hebben we EPs gemeten in reactie op een passief oddball paradigma. Daarbij bestudeerden we of diazepam een ander effect had op EPs in reactie op target-tonen dan op EPs in reactie op standaard-tonen. In dit experiment vonden we een duidelijker drug effect op EPs in reactie op target-tonen, dan op EPs in reactie op standaard-tonen.

Wanneer men echter gebruik maakt van twee verschillende tonen (standaard- en target-tonen) dan kunnen verschillen in EPs niet alleen toegeschreven worden aan verschillen in de interne cognitieve verwerking van deze tonen, maar ook veroorzaakt worden door de verschillen in de fysische karakteristieken van de gebruikte tonen. Een oplossing hiervoor kan geboden worden door van enkelvoudige tonen alleen de betekenis, ofwel de hogere orde karakteristieken, te veranderen, zonder de lagere orde karakteristieken (zoals volume, duur en toonhoogte) te veranderen. Indien men dergelijke paradigmata toepast, waarbij alleen gevarieerd wordt in de patronen van aanbieden, kunnen veranderingen in EPs niet meer worden toegeschreven aan verschillen in gebruikte tonen, maar alleen nog maar worden toegeschreven aan veranderingen in de interne cognitieve verwerking. Een voorbeeld van een passief paradigma met enkelvoudige stimuli is het dubbel-klik paradigma. In dit paradigma worden stimuli paarsgewijs aangeboden. Normaal gesproken ziet men lagere pieken in de EP in reactie op de tweede toon dan in de EP in reactie op de eerste toon. Deze afgenomen reactie staat ook wel bekend als ‘sensory gating’. In hoofdstuk 3 hebben we bepaald of deze

vermindering in reactie duidelijker te zien was wanneer de afstand tussen de toontjes heel kort was, dan waneer er een langer interval werd aangehouden. Hierdoor konden we bepalen of ‘sensory gating’ veroorzaakt wordt doordat er een bepaalde herstelperiode nodig is voor een optimale reactie, of dat ‘sensory gating’ veroorzaakt wordt door habituatie (gewenning). In plaats van het bekende dubbel-klik paradigma gebruikte we in ons experiment een 10-toon paradigma om te zien of de afname in reactie al volledig was in reactie op de tweede toon, of dat er een meer geleidelijke afname plaats zou vinden over reeksen van tien tonen. We vonden dat ‘sensory gating’ van de vroegere pieken bepaald werd door herstelfuncties van het brein, maar dat van latere pieken toe te schrijven is aan habituatie.

In hoofdstuk 4 hebben we het effect van diazepam op ‘sensory gating’ bestudeerd, door het effect van diazepam op auditieve EPs te meten zoals uitgelokt in een 10-toon paradigma. In hoofdstuk 4 zagen we dat diazepam alleen de habituatie van de latere EP pieken verstoord.

Een ander passief paradigma met enkelvoudige stimuli is het ontbrekende stimulus paradigma. Dit paradigma kan opgevat worden als een variant van het ‘oddball’ paradigma. In plaats van onverwachts target tonen aan te bieden in een reeks van standaard tonen, worden de target tonen weggelaten. Het ontbrekende stimulus paradigma biedt zodoende een methode om processen in het brein te bestuderen die betrokken zijn bij verwachting en tijdsschatting. In hoofdstuk 5 hebben we aangetoond dat EPs in reactie op ontbrekende stimuli gemeten kunnen worden in ratten. Daarnaast keken we of diazepam invloed had op dergelijke EPs. We vonden dat EPs in reactie op ontbrekende stimuli niet meer meetbaar waren na toediening van diazepam. Uit de experimenten zoals beschreven in hoofdstuk 2, 4 en 5 hebben we verder geconcludeerd dat diazepam met name de cognitieve verwerking van hogere orde karakteristieken van stimuli verstoort.

In hoofdstuk 6 bestudeerden we bij mensen het effect van diazepam op auditieve EPs in reactie op het 10-toon paradigma en EPs in reactie op ontbrekende stimuli. Hierdoor probeerden we een vergelijking te maken tussen de bevindingen uit eerder experimenten bij ratten, en de bevindingen bij mensen. De effecten van diazepam op EPs bij mensen bleken vergelijkbaar te zijn met eerdere metingen bij ratten.

Een van de belangrijkste bijdragen van het EP onderzoek aan de experimentele psychologie is het evalueren van cognitieve processen in situaties waarin geen betrouwbare overte respons meetbaar is. Door de juiste paradigmata toe te passen, proberen psychologen inzicht te krijgen in verschillende cognitieve processen. Wij concluderen dat het mogelijk is om met behulp van EPs uitgelokt in passieve paradigmata informatie verwerking te bestuderen. Dergelijke EPs blijken ook gevoelig te zijn voor de effecten van psychofarmaca zoals diazepam.

Deel B

In het tweede deel van dit proefschrift hebben we bestudeerd of het effect van diazepam op EPs veroorzaakt wordt door het effect van diazepam op het EEG. Met andere woorden: in dit deel hebben we onderzocht of de effecten van psychofarmaca op EPs (een maat voor de reactiviteit van het brein) extra informatie oplevert naast het meten van de effecten van psychofarmaca op het EEG (een maat voor de activiteit van het brein). Omdat diazepam sederende eigenschappen heeft, zou men een toename van de lage frequenties (delta- en theta-activiteit) in het EEG verwachten. Het is echter bekend dat diazepam met name de hoge frequenties (beta-activiteit) in het EEG verhoogt. Dit fenomeen staat bekend als farmacologische dissociatie.

In een eerste experiment, beschreven in hoofdstuk 7, bepaalden we het effect van diazepam op de spectraal inhoud van het EEG bij ratten. Wij vonden dat diazepam inderdaad een toename in hoge frequenties (21-40 Hz) veroorzaakte.

Omdat EPs een EEG gestuurde maat zijn, is het logisch te veronderstellen dat het EEG en EPs een duidelijke onderlinge relatie met elkaar vertonen. De relatie tussen het lopend EEG en EPs is in het verleden op verschillende manieren onderzocht. Bepalen hoe zowel het EEG als de EPs veranderen tijdens de verschillende slaap-waak stadia is één zo'n methode. Een wat directere methode omvat het meten van stukjes EEG voor en na het aanbieden van de stimulus en vervolgens bepalen hoe kleine veranderingen in de spectraal inhoud van de stukjes EEG voorafgaand aan de stimulus de EP in reactie op die stimulus beïnvloeden. Met deze methode kan zo de invloed van subtiele veranderingen in het bewustzijnsniveau op EPs bepaald worden. In hoofdstuk 8 is ons eerste EEG-EP experiment besproken. We vonden dat de amplitudes van de EP toenamen indien de hoeveelheid beta-activiteit in het pre-stimulus EEG toenam. We concludeerden dat de vorm van de EP inderdaad in sterke mate bepaald wordt door het lopend EEG.

In hoofstuk 9, waarin ons laatste experiment is beschreven, bepaalden we of de effecten van diazepam op EPs samen hangen met de effecten van diazepam op het lopend EEG. Omdat diazepam de beta-activiteit in het EEG verhoogt, corrigeerden we voor dit beta-verhogende effect door alleen EPs met elkaar te vergelijken die dezelfde hoeveelheid beta-activiteit in het pre-stimulus EEG hadden. Na deze correctie waren de effecten van diazepam op EPs nog steeds zichtbaar. Zonder diazepam namen EP componenten toe als het bewustzijnsniveau toenam. Met diazepam bleven de EPs echter onveranderlijk. Diazepam verandert EPs zodanig, dat ze het meest lijken op de EPs zonder diazepam in perioden met een laag bewustzijnsniveau. De effecten van diazepam op EPs zijn daardoor toe te schrijven aan de sederende eigenschappen van diazepam, en niet aan het beta-verhogende effect van diazepam. We concluderen dat het meten van drugeffecten op EPs informatie oplevert naast het meten van drug effecten op het lopend EEG. Op deze manier is het mogelijk om drug effecten op zowel de activiteit (zoals tot uitdrukking komt in het lopend EEG) als op de reactiviteit (zoals tot uitdrukking komt in de EPs) van het brein te bestuderen.

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- All my fellow travellers and foreign friends I met during the past few years whom I would like to see again.

CURRICULUM VITAE

Marijtje Jongsma werd op 2 augustus 1969 geboren in Zutphen. Vanaf 1981 bezocht zij het Rythoviuscollege te Eersel, waar zij in 1987 haar HAVO diploma behaalde. Aansluitend startte zij een studie aan de Docentenopleiding Dramatische Vorming te Arnhem hetgeen zij in minder dan een jaar weer voor gezien hield. In 1989 behaalde zij haar VWO diploma in het volwassenonderwijs te Arhem. Aansluitend volgde zij haar studie psychologie aan de KUN te Nijmegen. Na het behalen van haar propadeuse diploma in 1990 besloot ze een jaar vrij te nemen om een wereldreis te maken. Datzelfde jaar begon ze ook met haar werkzaamheden als vrijwilligster bij het Filmhuis Arnhem en doet dat tot op heden nog steeds met plezier. In 1991 startte zij met haar doctoraal studie in de Neuro- & Revalidatie psychologie, waarvoor ze in 1993 een klinische stage liep aan het epilepsie centrum de 'Dr Hans Berger Kliniek' te Breda. Op zoek naar een scriptiebegeleider kwam ze in contact met Tineke van Rijn en werd haar interesse voor onderzoek gewekt. In 1994 begon ze haar afstudeerstage aan de afdeling Vergelijkende & Fysiologische Psychologie aan de KUN te Nijmegen, gevolgd door een extra stage in een epilepsiecentrum in Malang, Indonesië. In januari 1996 studeerde ze af in de Neuro- & Revalidatie psychologie en begon, na diverse baantjes in de horeca (waaronder chef-kok in Thailand), aan haar eerste 'echte' baan als research-assistant bij de Cognitive Neuroscience Unit van het Westmead Hospital te Sydney, Australië. Na de moeilijke keuze tussen 'PhD student' in Australië en 'AiO' in Nijmegen besloot ze toch tot dat laatste. Van 1 januari 1997 tot 1 december 1999 had zij een 0,8 aanstelling als assistent in opleiding aan de afdeling Anesthesiologie. In deze periode deed zij onderzoek naar de effecten van diazepam op evoked potentials bij ratten in zowel het laboratorium van Anesthesiologie in het Centraal Dieren Laboratorium als op de vakgroep Vergelijkende & Fysiologische psychologie. Daarnaast assisteerde zij bij het geven van onderwijs.

Sedert 1 december 1999 heeft ze een aanstelling als post-doc aan de afdeling Anesthesiologie in het kader van het STW project getiteld: 'Peroperatieve monitoring van de anesthetische effecten op basis van nieuwe variabelen uit de chaostheorie'. De overige tijd wordt nog steeds gevuld met reizen, koken en films.

LIST OF PUBLICATIONS

Full papers:

1. Van Rijn, C.M. and Jongsma, M.L.A. (1995). Chronic effects of diazepam on the spectral content of the rat EEG. Neuroscience Research Communications. 17(2):65-69.
2. Van Den Broek, P.L.C., Van Egmond, J., Van Rijn, C.M., Jongsma, M.L.A., Coenen, A.M.L., and Dirksen, R. (1996). Benzodiazepines induce pharmacological dissociation between EEG frequencies and behaviour, but not between EEG correlation dimension and behaviour. Sleep-Wake Research in the Netherlands. 7:29-31.
3. Jongsma, M.L.A., Van Rijn, C.M., De Bruin, E.A., Dirksen, R., and Coenen, A.M.L. (1998) Time course of chronic diazepam effects on the auditory evoked potential of the rat. European Journal of Pharmacology. 341:153-160.
4. Jongsma, M.L.A., Van Rijn, C.M., Dirksen, R. and Coenen, A.M.L. (1998). Effects of stimulus repetitions with different interstimulus intervals on the rat auditory evoked potential. Excerpta Medica, ICS 1162: 249-255.
5. Jongsma, M.L.A, Van Rijn, C.M., Setz, A., Smit, A.S., Berben, I., Dirksen, R. and Coenen, A.M.L. (1998). Effects of diazepam on auditory evoked potentials (AEPs) and omission evoked potentials (OEPs) in rats and students. Sleep-Wake Research in the Netherlands. 9:65-72.
6. Jongsma, M.L.A., Quian Quiroga, R., Van Rijn, C.M., Van Schaijk, W.J., Dirksen, R., and Coenen, A.M.L. Effects of changes in pre-stimulus EEG on the consecutive auditory evoked potential in rats. in: Chaos in brain? K Lehnertz, CE Elger, J Arnhold and P Grassberger (eds.) World Scientific, Singapore. pg 309-312.
7. Jongsma, M.L.A., Van Rijn, C.M., Van Schaijk, W.J. and Coenen, A.M.L. Effects of diazepam on auditory evoked potentials of rats elicited in a ten-tone paradigm. *(Neuropsychobiology, in press).*
8. Jongsma, M.L.A., Coenen, A.M.L. and Van Rijn, C.M., Omission Evoked Potentials (OEPs) in rats and the effects of diazepam. *(Psychophysiology, submitted).*
9. Jongsma, M.L.A., Van Rijn, C.M., Van Egmond, J., Van Schaijk, W.J., Sambeth A. and Coenen, A.M.L. Diazepam effects on the relation between pre-stimulus EEG and the consecutive auditory evoked potential in rats. *(submitted as a rapid publication for Clinical Neurophysiology).*

Abstracts:

1. Jongsma, M.L.A. and Van Rijn, C.M. (1995) A study of chronic diazepam in the WAG/Rij rat on EEG and behavioural parameters. Proceedings of the 16th Low Countries Meeting *(abstract).*
2. Van Rijn, C.M. and Jongsma, M.L.A. (1995) Effects of chronic diazepam on EEG and motor behaviour of the rat. Neuroscience and Research Communications. 17(1):56 *(abstract).*
3. Van Rijn, C.M. and Jongsma, M.L.A. (1995) Effects of chronic diazepam on absence like phenomena in the EEG of the WAG/Rij rat. Neuroscience Research Communications. 17(1):57 *(abstract).*
4. Van Rijn, C.M. and Jongsma, M.L.A. (1995) The effects of diazepam on auditory evoked potentials of rats. European Journal of Neuroscience. Supple. No. 8:183 *(abstract).*
5. Van Rijn, C.M., Jongsma, M.L.A., Edelbroek, P.M. and Dirksen, R. (1996). Effects of chronic diazepam on absence-like phenomena and on the EEG on the WAG/Rij rat. Epilepsia. 37 (Supple 4):127 *(abstract).*
6. Jongsma, M.L.A., Van Rijn, C.M., Dirksen, R and Coenen, A.M.L. (1998). Two auditory stimulation paradigms to study processes of inhibition, habituation and attention in the rat auditory evoked potential (AEP) The Sixth International Evoked Potentials Symposium: Programme & Abstracts *(abstract).*
7. Jongsma, M.L.A., Van Rijn, C.M., Setz, A.C.W., Coenen, A.M.L. and Dirksen, R. (1998). Effects of diazepam on auditory evoked potentials (AEPs) and on omission evoked potentials (OEPs) in rats The Sixth International Evoked Potentials Symposium: Programme & Abstracts *(abstract).*
8. Arns, M.W., Jongsma, M.L.A. Bahramali, H. and Gordon, E. (1998). EEG and ERP interrelationships in an oddball paradigm: The effects of sequence length in healthy volunteers. Journal of Psychopharmacology 12(3) Supple A:43 *(abstract).*
9. Arns, M.W., Jongsma, M.L.A. Bahramali, H. and Gordon, E. (1998). EEG and ERP findings in a 'sequence' oddball paradigm in schizophrenic and parkinsonian patients versus controls. Journal of Psychopharmacology 12(3) Supple A:55 *(abstract).*
10. Jongsma, M.L.A., Van Rijn, C.M., Van Schaijk, W.J., Dirksen, R., and Coenen, A.M.L. (1999) Effects of diazepam on the relation between pre-stimulus EEG and the consecutive auditory evoked potential in rats. Interdiciplinary workshop chaos in the brain, Bonn, Germany *(abstract).*
11. Jongsma, M.L.A., Van Rijn, C.M., Van Schaijk, W.J. and Coenen, A.M.L. The influence of diazepam on EEG-AEP interrelations in rats. International Journal of Psychophysiology *(in press) (abstract).*