EEG AND AUDITORY EVOKED POTENTIAL P300 COMPARED WITH PSYCHOMETRIC TESTS IN ASSESSING VIGILANCE AFTER BENZODIAZEPINE SEDATION AND ANTAGONISM

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SUMMARY

We have compared the EEG and auditory evoked wave P300 with psychometric tests in assessing vigilance after flumazenil antagonism of midazolam sedation in 12 healthy volunteers. Measurements were made before and after midazolam 0.1 and 0.2 mg kg\(^{-1}\) i.v., and immediately and 30, 60, 120 and 240 min after administration of flumazenil 1 mg. The sedative effects of midazolam and antagonism by flumazenil resulted in alterations in EEG, P300 and psychometric tests (syndrome short test, letter cancellation, choice reaction and recognition). However, 60 and 120 min after flumazenil a decrease in test performance indicating rebound sedation was seen only in P300 mapping. Thus P300 mapping was a sensitive method of detecting subtle differences in vigilance. Rebound sedation occurred even when midazolam 0.2 mg kg\(^{-1}\) was antagonized with an adequate dose of flumazenil. We suggest that it is advisable to supervise patients for at least 240 min after flumazenil antagonism of midazolam 0.2 mg kg\(^{-1}\).

KEY WORDS


Assessing vigilance remains a problem and there is no single, objective method available. Neurophysiological tests such as the raw or processed EEG and various modes of evoked potentials (EP), especially somatosensory and middle-latency auditory EP, are thought to assess a patient's consciousness [1]. P300 is an endogenous potential which may or may not follow the stimulus, depending on the context [2,3]. It correlates with cognition and depends on the vigilance and attention of the subject [1]. Endogenous potentials have a relatively long latency and usually follow exogenous potentials, for example brainstem evoked potentials. The potential is termed P300 because it is positive in direction and peaks typically after 300 ms (range 250–500 ms). P300 has been shown to be stable with time in normal adults [4]. As the standard of estimation of vigilance, we have used a battery of psychometric tests for comparison. We chose widely adopted tests that allow assessment of concentration, stimulus recognition and processing, visual perception, purposeful action and reaction time.

We studied benzodiazepine sedation and antagonism because antagonism is specific and the duration of action of midazolam [5–7] is greater than that of flumazenil [8]. Thus rebound sedation has been described in several [9–13], but not all studies of flumazenil antagonism of midazolam sedation [14–18].

The purpose of this study was to determine if topographic mapping of the EEG and P300 is superior to psychometric tests in assessing different stages of altered vigilance caused by sedatives.

SUBJECTS AND METHODS

With the approval of the Ethics Committee of the University, we studied 12 healthy volunteers (six female) (mean age 24.3 yr (range 21–30 yr); body weight 64.2 (SD 7.7) kg). Written, informed consent was obtained from all volunteers, who were allowed to withdraw from the study at any time. Exclusion criteria included central nervous, cardiac or pulmonary disease, regular medication of any type, allergies and pregnancy. All volunteers fasted overnight until 1 h after administration of the benzodiazepine antagonist, when water or juice was offered orally. Room temperature was kept constant at 25 °C during the study and volunteers wore normal clothes.

Repetitive psychometric, EEG and P300 testing was made at the following times: baseline measurement before drug administration; after i.v. administration of midazolam 0.1 mg kg\(^{-1}\); after administration of a total dose of midazolam 0.2 mg kg\(^{-1}\); immediately after administration of flumazenil 1 mg; 30, 60, 120 and 240 min after flumazenil.

Midazolam was added to 250 ml of electrolyte solution and 0.1 mg kg\(^{-1}\) was infused within 5 min. After measurements, another midazolam 0.1 mg kg\(^{-1}\) was infused. Flumazenil was titrated using an initial dose of 0.2 mg administered within 15 s and additional doses of 0.1 mg every 60 s until subjective consciousness was the same as the baseline state [19].
This “wake-up” dose was noted and, in order to exclude underestimation of the appropriate dose, a total dose of 1 mg was administered.

The volunteers breathed room air spontaneously throughout. Monitoring consisted of ECG, pulse oximetry and arterial pressure.

EEG and evoked potential testing

The EEG and the acoustically evoked P300 were recorded via a 20-channel electrode montage according to the international 10/20 system referenced to linked mastoids. Electrode impedances were less than 5 kΩ. All neurophysiological variables were monitored with a Brain Atlas III (Bio-Logic, Mundelein, IL/U.S.A.). The EEG was band-pass filtered (1–30 Hz) before digitizing at a sampling rate of 128 Hz and stored on hard-disk for off-line processing. At least 3 min of EEG traces were collected at every time. Artefact-free epochs of a total duration of 20–30 s were selected visually and a Fast Fourier Transformation (FFT) performed. Mean value maps and SD maps were computed from the EEG maps of all subjects at each particular time, for the following frequency ranges: delta 0.5–3.5 Hz; theta 4–7.5 Hz; alpha 8–11.5 Hz; beta, 12–15.5 Hz; beta, 16–19.5 Hz; beta, 20–23.5 Hz.

Comparisons were made between the EEG maps at each measurement in all specified frequency bands using analysis of variance (ANOVA) including Greenhouse–Geisser–Epsilon correction for repeated measurements. If the null hypothesis (that there is no difference between the amplitudes under comparison) was rejected by the ANOVA, a t test for dependent groups was performed. The level of significance was 0.05 in all statistical tests. A common problem of quantitative multichannel-EEG is that multiple comparisons are necessary with the possibility of erroneous significances.

P300

In order to demonstrate that the peripheral auditory pathways were intact, binaural auditory evoked potentials (1000 condensation clicks monaurally, 75 dB, white masking of the non-stimulated ear, filters 30 and 1000 Hz) were measured in all subjects before any drugs were administered. The auditory evoked potential P300 was stimulated by binaural tonebursts (90 dB, 10 ms rise/fall time, 50 ms duration, Telephonics headphones) of two frequencies. Approximately 30 target tones with a frequency of 2000 Hz were counted, while the 1000 Hz non-target tones were ignored. The frequency relation was 5:1 non-target: target tones. The analysis time was 1024 ms, beginning 220 ms before the stimulus. Filters were set to 0.3 and 70 Hz; a notch filter was used. If the test subject counted the target tones, a positive potential of high amplitude arose after 250–500 ms [3]—the P300. Averages of both stimuli were stored and analysed off-line.

The latency of P300 was calculated as maximum of the global field power [21], which is spatial variance over time and excludes any subjective estimation of the peak latency by the researcher. At this latency, the corresponding amplitude is the difference between the maximum and minimum value of any of the recording channels [22]. The localization of these maxima and minima and the number of incorrect counts of the target tone (in %) were recorded.

Psychometric tests

A standardized verbal intelligence test (Mehrfach-Wortschatz-Intelligenztest MWT-B [23]) was performed once.

All psychometric tests were practised extensively before the beginning of the study, to avoid learning effects.

Subtests 4 and 5 of the syndrome short test (SST) [24] were performed. Test 4: 10 two-digit numbers must be placed in a mathematically correct, vertical row; test 5: the numbers must be placed back to their original fields as indicated on the table. The time was measured in both subtests. Mistakes had to be corrected, thus adding to the amount of time needed for the task, maximum time allowed being 60 s.

Letter-cancellation test. Two rows of the letters d and p in combination with from one to four commas were presented, where only the combination of d and two commas was required to be cancelled [25]. Time and number of mistakes were compared.

Choice reaction time and recognition. A 3-mm sized letter was displayed randomly on a screen for 50 ms (Commodore C64, program G. Gunreben, MD, Neurological Clinic of the University) at irregular intervals. The task was to press a button as fast as possible after seeing this letter on the screen and then also to name it. Mean reaction time and number of mistakes in 20 samples were noted.

Rating of vigilance. The volunteers’ vigilance was rated clinically according to the following vigilance scoring system (VSS): 1 = awake; 2 = tired, retardation; 3 = sedated, blurred speech; 4 = asleep, easily arousable by command; 5 = sleeping, difficult to arouse; 6 = not arousable.

Statistics

From the pharmacokinetics of midazolam and flumazenil [5–8], we anticipated that rebound sedation would occur 60 or 120 min after flumazenil antagonism of benzodiazepine sedation. Therefore, we compared the results of P300 and all psychometric tests immediately after administration of flumazenil, with the worst performance at either 60 or 120 min after flumazenil using the Wilcoxon matched pairs signed ranks test (SPSS Inc. Chicago). For P300 statistics, the greater latency either at 60 or 120 min after administration of flumazenil was selected for comparison together with the corresponding amplitude and mistake rate at the same time.

Clinical monitoring variables (heart rate, arterial pressure and oxygen saturation) were compared at all times using ANOVA with Greenhouse–Geisser–Epsilon correction. After rejection of the null hypothesis (that there is no difference between all variables), t tests for paired samples were applied between the different times.
Heart rate of 42 beat min\(^{-1}\) occurred shortly after the single value for oxygen saturation was 92\%.

1 and immediately and 60 and midazolam (ns) and decreased after flumazenil to 60

Side effects

The intelligence quotient was 123.3 (8.3), indicating that subjects were able to execute the tasks. All latencies of the brainstem evoked potentials were from 95 (2)\% (after both midazolam doses) to 97 (1)\%.

Clinical vigilance rating and monitoring parameters

After infusion of midazolam 0.1 mg kg\(^{-1}\), the state of nine volunteers varied from sedation to light sleep (VSS grade 3-4). Two subjects were only tired and one was not arousable. Mean wake-up dose of flumazenil was 0.55 (0.16) mg. As flumazenil 1 mg

RESULTS

The intelligence quotient was 123.3 (8.3), indicating that subjects were able to execute the tasks. All latencies of the brainstem evoked potentials were from 95 (2)\% (after both midazolam doses) to 97 (1)\%. Differences between measurements after midazolam 0.2 mg kg\(^{-1}\) and immediately and 60 and 120 min after flumazenil were significant (P < 0.01), but were too small to require treatment. The smallest single value for oxygen saturation was 92\%.

Side effects

In one subject a nodal rhythm with a minimum heart rate of 42 beat min\(^{-1}\) occurred shortly after the start of midazolam infusion. Sinus rhythm reappeared after approximately 1 min without treat-

moment. Two subjects complained of diplopia or blurred vision during midazolam sedation.

EEG

Maximal and minimal mean amplitudes (n = 12) in all frequency bands found in any of the recording electrodes are presented in table I. Comparing EEG maps before (baseline) and after midazolam 0.1 mg kg\(^{-1}\), the amplitude in the delta band increased over the whole scalp (P < 0.05) and occipital alpha amplitude predominance disappeared (P < 0.01); beta increased in all EEG channels (P < 0.01), beta, and beta, centrally and frontally (P < 0.05). There were no further changes after an additional dose of midazolam 0.1 mg kg\(^{-1}\).

Flumazenil antagonism (midazolam 0.2 mg kg\(^{-1}\) vs immediately after flumazenil) was characterized by opposite EEG effects: a decrease in delta and beta, amplitude (P < 0.05) at all recording sites, a frontal and central decrease of beta, and beta, amplitudes. The occipital alpha amplitude predominance was re-established by flumazenil (P < 0.05).

When baseline EEG maps and those immediately after flumazenil were compared, a decrease was seen in delta (P < 0.01) and theta activity (P < 0.05) in all channels and fronto-central decrease in alpha amplitude (P < 0.05). Baseline values for all frequency bands were reached 30 min after administration of flumazenil. There were no significant changes later.

P300

After midazolam 0.1 mg kg\(^{-1}\) only five subjects were able to count. The mean latency (fig. 1) and the frequency of incorrect counts of the target tone (fig. 2) at this time were clearly increased (n = 5), while the amplitude (fig. 3) decreased. P300 was absent in the seven subjects who were unable to count. After midazolam 0.2 mg kg\(^{-1}\), P300 could not be evoked in any subject.

Disappearance of P300 in seven of 12 volunteers after midazolam 0.1 mg kg\(^{-1}\) makes a statistical comparison of pre- and post-midazolam test performance inappropriate.

<table>
<thead>
<tr>
<th>Delta (0.5-3.5 Hz)</th>
<th>Theta (4.0-7.5 Hz)</th>
<th>Alpha (8.0-11.5 Hz)</th>
<th>Beta1 (12.0-15.5 Hz)</th>
<th>Beta2 (16.0-19.5 Hz)</th>
<th>Beta3 (20.0-23.5 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.4 (3.4)</td>
<td>8.8 (2.9)</td>
<td>11.0 (4.9)</td>
<td>5.9 (1.9)</td>
<td>5.5 (1.9)</td>
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<tr>
<td>2</td>
<td>25.2 (3.9)</td>
<td>18.7 (7.0)</td>
<td>31.2 (17.5)</td>
<td>10.0 (4.6)</td>
<td>9.1 (4.0)</td>
</tr>
<tr>
<td>3</td>
<td>18.5 (4.3)</td>
<td>7.8 (2.3)</td>
<td>9.6 (3.9)</td>
<td>13.3 (3.6)</td>
<td>7.9 (3.6)</td>
</tr>
<tr>
<td>4</td>
<td>34.5 (4.6)</td>
<td>18.6 (5.7)</td>
<td>22.1 (9.5)</td>
<td>33.1 (10.7)</td>
<td>14.9 (7.9)</td>
</tr>
<tr>
<td>5</td>
<td>23.4 (10.5)</td>
<td>10.2 (6.3)</td>
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<td>20.1 (11.0)</td>
<td>30.1 (17.9)</td>
<td>48.0 (11.9)</td>
<td>23.0 (19.0)</td>
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<tr>
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<td>7.0 (2.1)</td>
<td>9.8 (4.0)</td>
<td>6.1 (2.2)</td>
<td>6.1 (3.1)</td>
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<tr>
<td>8</td>
<td>22.1 (3.6)</td>
<td>13.8 (3.6)</td>
<td>32.4 (17.7)</td>
<td>11.1 (4.5)</td>
<td>10.8 (6.7)</td>
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<td>9</td>
<td>14.7 (6.0)</td>
<td>8.4 (5.5)</td>
<td>10.7 (8.3)</td>
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<td>7.3 (5.2)</td>
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<tr>
<td>10</td>
<td>27.2 (11.3)</td>
<td>17.7 (8.6)</td>
<td>34.3 (29.3)</td>
<td>13.5 (9.9)</td>
<td>13.6 (10.5)</td>
</tr>
<tr>
<td>11</td>
<td>14.9 (5.0)</td>
<td>8.6 (4.3)</td>
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<td>7.8 (3.9)</td>
<td>8.0 (5.9)</td>
</tr>
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<td>12</td>
<td>26.2 (10.6)</td>
<td>18.8 (11.3)</td>
<td>38.6 (41.8)</td>
<td>13.6 (7.8)</td>
<td>14.4 (10.0)</td>
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<tr>
<td>13</td>
<td>16.0 (5.1)</td>
<td>9.1 (4.2)</td>
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<td>7.3 (2.8)</td>
<td>6.7 (4.0)</td>
</tr>
<tr>
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<td>21.4 (12.4)</td>
<td>27.6 (20.5)</td>
<td>13.9 (7.0)</td>
<td>12.5 (6.6)</td>
</tr>
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<td>15</td>
<td>13.6 (2.4)</td>
<td>7.8 (2.2)</td>
<td>9.2 (3.8)</td>
<td>6.1 (2.2)</td>
<td>6.4 (3.8)</td>
</tr>
<tr>
<td>16</td>
<td>24.4 (5.7)</td>
<td>16.7 (5.8)</td>
<td>27.8 (15.4)</td>
<td>11.3 (5.0)</td>
<td>11.2 (8.3)</td>
</tr>
</tbody>
</table>
FIG. 1. P300 latency (mean, SD). 1 = Baseline measurement before administration of drug; 2 = after midazolam 0.1 mg kg\(^{-1}\); 3 = after a total dose of midazolam 0.2 mg kg\(^{-1}\); 4 = immediately after flumazenil 1 mg; 5 = 30 min, 6 = 60 min, 7 = 120 min, 8 = 240 min after flumazenil. \(n = 12\) except for measurement 2, when \(n = 5\).

FIG. 2. Mistakes in the P300 task (counting the target tone) (mean, SD). Measurement times as in figure 1. \(n = 12\) except for measurement 2, when \(n = 5\).

TABLE II. Comparison of the test performance immediately after flumazenil and the worst result at either 60 or 120 min after flumazenil (mean (SD)). **Significant difference \((P < 0.01; \text{Wilcoxon test})\)

<table>
<thead>
<tr>
<th>Test</th>
<th>Immediate</th>
<th>+60 or 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>P300 latency (ms)</td>
<td>331.7 (25.3)</td>
<td>380.7 (31.1)**</td>
</tr>
<tr>
<td>P300 amplitude ((\mu V))</td>
<td>18.1 (6.3)</td>
<td>16.0 (5.9)</td>
</tr>
<tr>
<td>P300 mistake rate (%)</td>
<td>3.0 (5.0)</td>
<td>29.3 (29.6)**</td>
</tr>
<tr>
<td>SST4 (s)</td>
<td>18.2 (2.5)</td>
<td>17.0 (1.7)</td>
</tr>
<tr>
<td>SST5 (s)</td>
<td>13.3 (1.3)</td>
<td>13.8 (1.7)</td>
</tr>
<tr>
<td>Reaction time (ms)</td>
<td>310.8 (51.9)</td>
<td>374.7 (153.2)</td>
</tr>
<tr>
<td>Recognition (mistakes)</td>
<td>1.5 (1.3)</td>
<td>2.2 (1.5)</td>
</tr>
<tr>
<td>Letter cancellation (s)</td>
<td>60.0 (7.7)</td>
<td>56.2 (6.9)</td>
</tr>
<tr>
<td>Letter cancel (mistakes)</td>
<td>2.0 (2.5)</td>
<td>1.9 (2.1)</td>
</tr>
</tbody>
</table>

After benzodiazepine antagonism, P300 reappeared in all subjects. Flumazenil restored the baseline latency and mistake rate in counting the target tone; also the amplitude was increased, but was still less than baseline (ns).

Comparing with values immediately after flumazenil, the P300 latency increased significantly 60 or 120 min later \((P < 0.01)\) (table II). A parallel increase in incorrect counts occurred at the same times \((P < 0.01)\) (table II), whereas P300 amplitude did not differ significantly between these times. All P300 parameters were the same as baseline 240 min after flumazenil.

The maximum amplitude of P300 was localized in the midline parietal region at all times, except after midazolam 0.1 mg kg\(^{-1}\) when it shifted to the midline central region. The minimum amplitude appeared in the frontopolar midline at all times except after midazolam 0.1 mg kg\(^{-1}\), when it was in the middle central region.

**Psychometric tests**

Eleven of the 12 subjects were able to perform the tasks after midazolam 0.1 mg kg\(^{-1}\). The time required for SST-4 and 5 and for the letter cancellation increased. After a dose of midazolam 0.2 mg kg\(^{-1}\), only four subjects were still able to fulfill the tasks and the time required increased even more. Figure 4 presents SST-5 as a typical example; the results of SST-4 and letter cancellation time show an identical time course.

The number of mistakes in the letter cancellation test increased after the full dose of midazolam. Reaction time doubled after midazolam 0.1 mg kg\(^{-1}\) and increased four-fold after midazolam 0.2 mg kg\(^{-1}\). After the 0.2-mg kg\(^{-1}\) dose, no letters could be recognized by any of the four subjects attempting to fulfill the task.

Flumazenil restored performance to baseline level in all psychometric tests. There were no significant or even measurable differences between baseline and any of the psychometric tests after flumazenil. When comparing the test performance immediately after administration of flumazenil with the worst result...
either 60 or 120 min later, contrary to the P300 results, there were no significant differences in any of the psychometric tests (table II).

**DISCUSSION**

The changes during deep benzodiazepine sedation, and especially the comparatively slight decrease in vigilance when the action of flumazenil was terminating, was well reflected by the latency of the auditory evoked P300, and by the number of mistakes in the P300 task (table II).

In contrast with P300, none of the psychometric tests revealed any changes after administration of flumazenil but all clearly documented the sedative effect of the benzodiazepine.

A previous study found a good correlation \((r = 0.81, P < 0.001)\) between blood concentrations of midazolam and P300 amplitude during midazolam sedation alone [26]. Baseline values for the P300 amplitude were reached 6 h after infusion of midazolam 0.3 mg kg\(^{-1}\). In contrast with our findings, there was no difference in discrimination between recordings of the four central channels \((Fz, Cz, Pz, Oz)\) of P300 and psychometric tests (choice reaction time and critical flicker fusion frequency). We suggest that the enhanced power of multichannel mapping is responsible for our different results. The increase in P300 latency and amplitude after midazolam agrees with a similar effect after sedation with lorazepam, another benzodiazepine [27].

The EEG revealed significant changes between baseline and both recordings after midazolam administration—mainly a general increase in the delta and all beta ranges, and a loss of the occipital alpha predominance. All changes were reversed by flumazenil to a degree that there was a general delta, theta and a fronto-central alpha amplitude reduction compared with baseline data, but there were no further changes later after administration of flumazenil. This clearly demonstrates that the EEG and the psychometric tests used in this study were less sensitive than the auditory evoked potential P300 in assessing vigilance after benzodiazepine antagonism.

We conclude that, because of its high sensitivity, P300 mapping is a suitable tool to measure slight degrees of reduced vigilance. The P300 task requires the subject to count one of two randomly presented tones. While this task alone appears to be clinically feasible and a sensitive test of vigilance, P300 mapping is too cumbersome and technically demanding for clinical use, but it may be useful in studies of new psychopharmacological drugs or the effects of non-specific antagonists given to antagonize anaesthetic drug action. If considerable degrees of impairment of vigilance are to be quantified, psychometric tests are preferable, as they are more robust—that is, they may still be performed when the P300 task can no longer be fulfilled, as was the case after midazolam 0.2 mg kg\(^{-1}\).

We found a wake-up dose of flumazenil 0.55 (0.16) mg, in accordance with previous results [13]. In a recent study, a total dose of 0.5 mg was administered and antagonism was incomplete [28]. In order to avoid inadequate dosage, we administered flumazenil 1 mg to all subjects. Nevertheless, using the more sensitive mapping of auditory evoked P300, we demonstrated a considerable increase in latency 60 and 120 min after flumazenil reversal of midazolam sedation, indicating rebound of benzodiazepine sedation. Although this rebound might be anticipated from the pharmacokinetics of midazolam and flumazenil, it has been shown only in studies comparing midazolam–flumazenil with midazolam–placebo [9–13, 29]. In these studies, a significant difference in vigilance or test performance between flumazenil and placebo groups was found only shortly after flumazenil or placebo, but not after 1 h. This lack of difference could be accounted for by rebound sedation in the flumazenil group, by a decreasing degree of sedation in the placebo group or, most likely, by a combination of both. In our study, we demonstrated that rebound sedation also occurred after flumazenil antagonism of a single dose of midazolam 0.2 mg kg\(^{-1}\). This finding may have clinical consequences, especially as flumazenil is used in outpatients undergoing gastro-, colo- or laparoscopy [15,17,18,29] and dental procedures [30]. In some patients, supervision for less than 4 h after flumazenil antagonism of midazolam may be unsafe.

We confirmed a large inter-individual variation in response to benzodiazepine [31–33]. A striking example of this variation is that one male subject was too sedated to perform any task after midazolam 0.1 mg kg\(^{-1}\) while one female subject was only slightly sedated after midazolam 0.1 mg kg\(^{-1}\) and still easily arousable after 0.2 mg kg\(^{-1}\).

In summary, we found that mapping of the auditory evoked potential P300 was superior in assessing vigilance after benzodiazepine antagonism than the EEG or psychometric tests. Using P300 mapping, rebound sedation was observed 60 and 120 min after flumazenil 1 mg antagonism of midazolam 0.2 mg kg\(^{-1}\).

**REFERENCES**


