Interaction of a subanaesthetic concentration of isoflurane with midazolam: effects on responsiveness, learning and memory

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Summary

There are situations in which “light” anaesthesia combined with neuromuscular block is the only anaesthetic regimen that can be tolerated safely by the patient. Benzodiazepines have hypnotic and specific amnesic effects. Therefore, we have examined the interaction of midazolam with a subanaesthetic dose of isoflurane (0.2% end-expired concentration) in 28 healthy volunteers. Thereafter, 15 subjects received midazolam 0.03 mg kg\(^{-1}\) i.v. and 13 subjects received midazolam 0.06 mg kg\(^{-1}\) in a random, double-blind manner. Word lists were administered and response to commands was tested before and after administration of midazolam. After 1 h of recovery, memory for word lists was tested by word completion, free recall and forced choice recognition tasks. After administration of midazolam, recall and, to a lesser degree, implicit memory were absent. Recognition was also absent after administration of midazolam 0.06 mg kg\(^{-1}\) and at the 3-min and 15-min assessments after administration of midazolam 0.03 mg kg\(^{-1}\). Responsiveness was more frequent with midazolam 0.03 mg kg\(^{-1}\) than with 0.06 mg kg\(^{-1}\) and increased over time. We conclude that a larger dose of midazolam or isoflurane, or both, may be necessary to abolish responsiveness. (Br. J. Anaesth. 1998; 80: 581–587)

Keywords: anaesthetics volatile isoflurane; hypnotics benzodiazepine midazolam; memory; anaesthesia depth

There are common anaesthetic and surgical situations in which “light” anaesthesia combined with neuromuscular block may be the only anaesthetic regimen that can be tolerated safely by the patient (e.g. obstetrics, emergence from cardiopulmonary bypass, trauma surgery and patients with debilitating physical diseases). Anaesthetists are unable to determine reliably whether or not a given anaesthetized, paralysed patient is conscious during surgery. Clinical physical signs during anaesthesia are not helpful and there is no monitor that can detect awareness. Regaining consciousness during surgery and recalling this experience after operation can be a devastating experience. Benzodiazepines have hypnotic and specific amnesic effects. Their effects on the cardiovascular system are relatively mild compared with other anaesthetics and the severity of a patient’s cardiac disease does not appear to significantly influence haemodynamic responses. Therefore, it may be advisable to use a benzodiazepine whenever “light” anaesthesia is necessary, to avoid awareness and recall. There is synergism between benzodiazepines and volatile anaesthetics when used in combination. This interaction could be beneficial in reducing the required dose of benzodiazepine.

The aim of this study was to examine the interaction of two doses of midazolam with a typical subanaesthetic dose of isoflurane on responsiveness, learning and memory.

A distinction of memory, of particular interest to anaesthesia, is between explicit and implicit memory. Explicit memory refers to intentional or conscious recollection of prior experiences, as assessed by tests of recall or recognition. In contrast, implicit memory refers to changes in performance or behaviour that are produced by prior experiences on tests that do not require any intentional or conscious recollection of those experiences. The basic distinction between explicit and implicit tests involves the nature of the instructions given to the subjects. In an explicit test, subjects are asked to recall or recognize events that have been presented before. In an implicit test, the instructions make no reference to these earlier events. We assessed explicit memory by using free recall and recognition tasks and implicit memory using a word completion task. The latter task has become the prototypical task in research on priming, that is hearing or seeing the words on the list increases the likelihood of subjects responding with these words in the subsequent word completion test. This test has been used frequently to demonstrate preserved performance of amnesic patients and in drug research on memory, including anaesthetics.

Subjects and methods

We studied 28 healthy subjects. Subjects were excluded if they were suffering from any illness, were pregnant or were receiving medications, particularly centrally active agents; if they had used three or more illicit drugs; or if they were heavy users of alcohol or marijuana. Female volunteers were screened for pregnancy before the sessions. The study was approved by the local Institutional Review Board.

EXPERIMENTAL DESIGN

All subjects inhaled 0.2% end-expired concentration of isoflurane for 15 min to ensure equilibration with...
arterial blood and brain partial pressures (table 1). Thereafter, 15 subjects received midazolam 0.03 mg kg\(^{-1}\) (Versed) i.v. and 13 received midazolam 0.06 mg kg\(^{-1}\) in a random, double-blind manner. The subject and research technician administering the tests were blinded to the dose of drug administered. The word lists (see below) were counterbalanced in the order in which they were presented. Control groups of volunteers who would have received isoflurane alone and midazolam alone were not included because data are already available on their effects on responsiveness and memory.\(^{10–14}\)

### EXPERIMENTAL SECTIONS

Subjects were tested individually while lying on a reclining hospital stretcher. Isoflurane was administered via a mouth-piece attached to a semi-closed anaesthetic breathing system. A nose-clip prevented contamination of the inspired gases with room air. An end-tidal concentration of 0.2% isoflurane, as measured by mass spectroscopy, was maintained for 15 min. Arterial oxygen saturation, carbon dioxide concentration in expired air and heart rate were also monitored. Response to command and memory list presentation were then administered as a baseline assessment (“isoflurane baseline”). Thereafter, one of the two doses of midazolam was given i.v. over a 2-min period. Response to command was tested every 2 min for 46 min after administration of midazolam. Different word lists were administered at 3, 15, 30 and 45 min after administration of midazolam. Inhalation of isoflurane was stopped after testing the last response to command and, after one additional hour of rest, memory for the word lists was tested.

### TASKS

#### Response to command

At each assessment, ability to comprehend language and make a voluntary motor response to a verbal command was assessed by asking the subject to squeeze the research assistant’s fingers. Instructions to squeeze two or three times were given in random order. In all analyses of responsiveness, appropriate responses were contrasted with “unresponsiveness,” which was defined as either a lack of response or an inappropriate response (the latter were rare, constituting only 5% of all “unresponsiveness”).

#### Memory assessments

(1) Learning stage. We prepared six lists of words, each consisting of eight words. The words were selected from those used in a previous study.\(^{14}\) The lists were equated on Thorndike–Lorge word frequency and normative free recall.\(^{15–17}\) None of the words on these lists had the same initial three letters. For each word on the lists, at least 10 common words in the English language began with the same three letters.

The words on the lists were presented auditorily and visually simultaneously at a rate of one word every 5 s. For each subject, five of the six lists were used for one presentation before and four presentations after administration of midazolam. The remaining list was not presented. Words on this list served as “distracters” on the implicit memory test, that is a control condition, as described below. The assignment of specific lists to the different times of presentation and distracter condition were approximately counterbalanced over subjects receiving each dose of midazolam, using a Latin square design.

(2) Memory test stage. After 1 h of recovery from the effects of isoflurane and midazolam, implicit memory was tested by a word completion task. The subject was given a page containing the first three letters (e.g. “con”, “pen”, “man”) of the 48 words on all six lists (e.g. “concern”, “pension”, “manual”), including the “distracters” on the list that was not presented. The words from all lists were mixed together in random order. For each three-letter word beginning, the subject was asked to write the first word that came to mind beginning with those letters. The relationship between this test and the previous presentations of lists of words was not explained. The lists were equated on spontaneous frequencies of giving the words as responses to the word beginnings, based on our earlier results.\(^{14}\) The numbers of list words that were supplied by a subject on the word completion task were counted for each memory list presentation; to calculate the “priming” score (measure of implicit memory), these numbers were reduced by the number of words supplied by the subject on the word completion task for the control words that were not presented to him. Thus a priming score of zero reflected the absence of implicit memory, while a positive priming score reflected the presence of implicit memory.

After completing the word completion task, recall was tested. The subject was instructed to write the words from all previously presented lists. This was followed by a forced choice recognition test in which subjects had to choose which word had been presented previously for each of a series of word pairs.

### STATISTICAL ANALYSIS

Recognition and priming scores were submitted to repeated measures analyses of variance, with
midazolam dose (0.03 mg kg\(^{-1}\) vs 0.06 mg kg\(^{-1}\)) as a between-subjects factor and test condition (the control condition defined above, the isoflurane baseline after midazolam but before midazolam administration, and 3, 15, 30 and 45 min after administration of midazolam) as a repeated measures factor. When appropriate, Huynh-Feldt adjusted probability values were used. Pre-planned Helmert contrasts were performed to compare the control condition vs all other conditions, baseline vs all conditions after midazolam, and each condition after midazolam with midazolam subsequent to it. Corresponding analyses were neither necessary nor feasible for recall, as discussed below.

To examine if the memory test scores presumably reflecting explicit and implicit memory processes were independent, Pearson correlations of priming (reflecting implicit memory) with recall and recognition (reflecting explicit memory) were computed for the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups for each word list presentation.

Appropriate responses to command were examined with respect to dose and three time intervals after administration of midazolam bounded by the word list presentations at 15 and 30 min after midazolam. A model based on a first-order Markov process was used to relate responsiveness at each individual time of assessment within these intervals (i.e. 2–14, 16–30, and 32–46 min, after administration of midazolam) to responsiveness at the immediately preceding time. This model resulted in a 2 × 3 × 2 × 2 contingency table that was subjected to hierarchical log-linear analysis to test how responsiveness at each individual time was influenced by dose, time interval and responsiveness at the immediately preceding time.

Because some subjects seemed to drift in and out of whatever degree of consciousness was necessary to respond appropriately to commands, “fluctuations” in responsiveness, defined as occasions on which one or more consecutive appropriate responses were both preceded and followed by unresponsiveness, were compared for the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups by a \(t\) test. Pearson correlations were also computed between the number of fluctuations and free recall, priming and recognition averaged over all four word list presentations after administration of midazolam.

To determine if there was implicit memory for words presented while subjects were unresponsive, priming scores were analysed for the earliest list presentation to each subject after administration of midazolam which was both immediately preceded and immediately followed by unresponsiveness. Priming scores in the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups were compared with each other and to the chance score (zero) by \(t\) tests. Only 16 subjects (six who received midazolam 0.03 mg kg\(^{-1}\) and 10 who received midazolam 0.06 mg kg\(^{-1}\)) were included in these analyses, because no list presentation met the criterion for the remaining subjects.

The initial durations of unresponsive periods after administration of midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) were compared by life table analysis. Similar analysis methods were also applied to the maximal durations (i.e. the longest continuous sequences) of unresponsiveness and their times of onset.

All analyses were performed using the statistical computer package SAS v.6.

**Results**

Patient characteristics in the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups did not differ significantly in sex distribution, mean age, years of education, weight, height or body mass index (table 2).

**RECALL**

At the isoflurane baseline, recall did not differ significantly between the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups (mean 2.1 (SEM 0.5) words and 2.5 (0.4) words, respectively). After administration of midazolam, recall was virtually absent to a degree that made statistical analysis unnecessary. Only one subject (in the midazolam 0.03 mg kg\(^{-1}\) group) recalled any words at 15 min after administration of midazolam and four subjects (three from the midazolam 0.03 mg kg\(^{-1}\) group and one from the midazolam 0.06 mg kg\(^{-1}\) group) recalled words at 45 min after administration of midazolam. No subject recalled any words at either 3 min or 30 min after administration of midazolam.

**RECOGNITION**

Recognition scores are shown in figure 1. The repeated measures ANOVA showed significant effects of dose (\(F(1, 26) = 9.84; P = 0.0042\)) and time (\(F(5, 130) = 4.65; P = 0.0006\)), although the global dose × time interaction was not significant (\(F < 1\)). The
represents chance performance.

midazolam 0.03 mg kg\(^{-1}\) significantly exceeded chance level (i.e. performance word completion task for all subjects, whether or not pre-planned Helmert contrast comparing isoflurane baselines with the means of all times after midazolam. Data are included for all subjects, without regard to response to command. The number of words supplied by subjects on the implicit memory test for the control words that were not presented to them and which were subtracted in calculating the priming scores (see text) were, mean 0.6 (SEM 0.2) words for midazolam 0.03 mg kg\(^{-1}\) and 0.5 (0.2) words for midazolam 0.06 mg kg\(^{-1}\). The horizontal line at zero represents chance performance.

CORRELATIONS OF IMPLICIT AND EXPLICIT MEMORY

Pearson correlations of priming (reflecting implicit memory) with recall and recognition (reflecting explicit memory) were not significant at the isoflurane baseline word list presentation or at any of the word list presentations after administration of midazolam in the 0.03 mg kg\(^{-1}\) group. Only one of the 10 correlations was significant for the midazolam 0.06 mg kg\(^{-1}\) group, that is between priming and recognition at the isoflurane baseline (\(r=0.69, P=0.0086\)).

RESPONSIVENESS

The percentages of appropriate responses to command are plotted for each 2-min time interval after midazolam in figure 3 and for the time periods involved in the first-order Markov process in figure 4. The model that resulted from the hierarchical log-linear analysis indicated that appropriate responsiveness was independently influenced by dose, time period and responsiveness at the immediately preceding time (\(P<0.0001\), \(P=0.0017\) and \(P<0.0001\), respectively). Responsiveness was more frequent with midazolam 0.03 mg kg\(^{-1}\) than with 0.06 mg kg\(^{-1}\), and increased over time. The association between current and immediately preceding responsiveness indicated that, in general, when subjects were responsive they tended to remain responsive, and when they were unresponsive they tended to remain unresponsive.

INITIAL AND MAXIMAL DURATIONS OF UNRESPONSIVE PERIODS

Life table analysis indicated that initial durations of unresponsive periods were longer with midazolam 0.06 mg kg\(^{-1}\) than with 0.03 mg kg\(^{-1}\) (\(P=0.0024\), Wilcoxon test). Median durations were 30 min and 0 min, respectively. However, even midazolam 0.06 mg kg\(^{-1}\) did not produce immediate, prolonged unresponsiveness in 25% of subjects; 25% of subjects responded appropriately at 2 min or 4 min after administration of midazolam.

Maximal durations of unresponsive periods were longer with midazolam 0.06 mg kg\(^{-1}\) than with 0.03 mg kg\(^{-1}\) (\(P=0.0017\), Wilcoxon test). Median durations were 32 min and 4 min, respectively. Furthermore, the times of onset of unresponsive periods of

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**Figure 2** Mean (SEM) priming scores (reflecting implicit memory) in the word completion task for words presented at the isoflurane baseline (B) and the four memory list presentations after administration of midazolam. Data are included for all subjects, without regard to response to command. The number of words supplied by subjects on the implicit memory test for the control words that were not presented to them and which were subtracted in calculating the priming scores (see text) were, mean 0.6 (SEM 0.2) words for midazolam 0.03 mg kg\(^{-1}\) and 0.5 (0.2) words for midazolam 0.06 mg kg\(^{-1}\). The horizontal line at zero represents chance performance.

**Figure 3** Percentages of subjects showing appropriate responses to command after every 2 min for 46 min after administration of midazolam. The dotted line at the bottom illustrates the constant isoflurane concentration throughout this time.
maximal duration also differed between midazolam 0.06 mg kg\(^{-1}\) and 0.03 mg kg\(^{-1}\) (P=0.0479, Wilcoxon test); the medians were 2 min and 10 min, respectively.

**IMPLICIT MEMORY DURING UNRESPONSIVE PERIODS**

Analysis of priming scores (reflecting implicit memory) during the earliest word list presentation to each subject after midazolam which was both immediately preceded and immediately followed by unresponsiveness provided no evidence of implicit memory. The priming scores in the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups were -0.33 (0.42) words and 0.20 (0.25) words, respectively. These scores did not differ from chance level (zero) or from each other.

**FLUCTUATIONS IN RESPONSIVENESS AND THEIR RELATIONSHIP TO MEMORY**

Because some subjects seemed to drift in and out of whatever degree of consciousness was necessary to respond appropriately to commands, fluctuations in responsiveness (as defined above) were examined. The number of such fluctuations per subject did not differ significantly between the midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) groups (1.1 (0.3) and 0.8 (0.3), respectively). As presentation of word lists may have had an arousing effect, we also examined transitions from unresponsiveness to responsiveness and vice versa, excluding the times at which lists were presented. These analyses also showed no significant difference between midazolam 0.03 mg kg\(^{-1}\) and 0.06 mg kg\(^{-1}\) (1.1 (0.3) and 0.6 (0.3), respectively, for transitions from unresponsiveness to responsiveness; and 1.1 (0.3) and 0.8 (0.3) for transitions from responsiveness to unresponsiveness).

Pearson correlations were calculated to assess if memory was related to fluctuations in responsiveness. The correlation of fluctuations with recognition was significant for midazolam 0.03 mg kg\(^{-1}\) (r=0.56, P=0.0302), but not for midazolam 0.06 mg kg\(^{-1}\) (r=-0.22, P=0.48). The correlations of fluctuations with priming (reflecting implicit memory) and recall were not significant for either midazolam 0.03 mg kg\(^{-1}\) or 0.06 mg kg\(^{-1}\).

**Discussion**

We evaluated responsiveness by ability of subjects to respond to verbal instructions by squeezing the research assistant’s fingers two or three times. In the absence of other explanations for failure to respond to command, such as impaired hearing, hysteria, muscle paralysis and difficulty in differentiating between purposeful movement and reflex movements in response to surgical stimulation, such failure is currently the accepted indicator of the threshold between wakefulness or consciousness and unconsciousness.

The effects of midazolam and 0.2% isoflurane on responsiveness were somewhat variable. Four subjects (one who received 0.06 mg kg\(^{-1}\) and three who received 0.03 mg kg\(^{-1}\)) never lost consciousness. The other subjects lost responsiveness for variable periods of time. Responsiveness was more frequent with midazolam 0.03 mg kg\(^{-1}\) than with 0.06 mg kg\(^{-1}\) and increased over time. The times of onset of unresponsiveness were a function of the dose and were slower than the onset that has been reported after administration of larger doses used for induction of anaesthesia. Some subjects seemed to drift in and out of consciousness and one subject remained responsive all of the time, only to lose consciousness during the last 15 min of the session. Midazolam is used for induction of anaesthesia and decreases the anaesthetic requirements (MAC) for volatile anaesthetics. However, benzodiazepines, in clinical doses, do not reliably suppress processing of sensory and, especially, auditory stimuli. When combined with opioids (e.g. alfentanil and midazolam) most patients seem to be in an amnesic–analgesic plane, rather than truly unconscious. Our data suggest that a larger dose than 0.06 mg kg\(^{-1}\) or a higher concentration of isoflurane, or both, are necessary to abolish responsiveness.

Both doses of midazolam, when added to 0.2% isoflurane, almost completely abolished both explicit and implicit memory. The impairments extended for most of the 45-min period after administration of midazolam. These results are in agreement with our previous study which showed that midazolam impairs both types of memory. Demonstration of both explicit and implicit memory in addition to responsiveness in all subjects during administration of 0.2% isoflurane is in agreement with the studies of
Dwyer, Bennett and Eger\textsuperscript{11} and Chortkoff, Bennett and Eger,\textsuperscript{12} but different from the results of Newton and colleagues,\textsuperscript{13} who found that during administration of 0.2 MAC of isoflurane, recall and recognition were lost.

The profundity of the effect of midazolam on recall was evident, as only one subject who received the 0.03 mg kg\textsuperscript{-1} dose recalled any words that were presented between 3 and 30 min after administration of the drug. Our conclusion that midazolam abolished implicit memory must be more tentative and tempered by consideration of the study’s power to detect a small residual priming effect. Seven of the eight priming scores for words presented after midazolam administration were positive (fig. 2) and may have significantly exceeded chance level (zero) if more subjects had been tested. Calculations based on the variability of these scores indicate that mean priming scores of approximately 0.5–0.6 would have significantly exceeded chance level. The difference from chance level of the highest priming score observed for words presented after midazolam administration (0.47 score in the midazolam 0.03 mg kg\textsuperscript{-1} group for words presented at 45 min after midazolam administration) was marginally significant ($P=0.07$). None of the other priming scores for words presented after midazolam administration approached even this level, however.

An advantage of a control group would have been to allow separation of drug effects on memory from possible effects on memory of variations in the lag between presentation and testing of the word lists. For the lists presented after midazolam administration, this lag ranged from 1 h to 1 h 42 min. It is possible that the shorter lags for the lists presented 30–45 min after administration of midazolam 0.03 mg kg\textsuperscript{-1} compared with those presented earlier, could have contributed to the apparent recovery in recognition at these times. However, the finding that recognition was best for the list presented at the isoflurane baseline, which involved the longest lag between presentation and testing, suggests that changes in recognition were more related to recovery from midazolam than the passage of time.

Performance on implicit tests of memory are prone to influence by explicit memory if subjects become aware of the relationship between study and test items and exploit this knowledge.\textsuperscript{21} All but one of the 20 correlations between implicit and explicit memory examined in this study (including all but one of the four correlations examined at the isoflurane baseline) were not significant, suggesting that this was not a problem.

We looked for implicit memory when subjects were unresponsive and examined the correlation of fluctuations in responsiveness with priming, a measure of implicit memory. There was no evidence of implicit memory in unconscious subjects and there was no significant correlation between the fluctuations in responsiveness and priming. Furthermore, there was no significant correlation between fluctuations in responsiveness and recall. This agrees with results of studies using the isolated forearm technique in which patients responded to commands without explicit recall.\textsuperscript{22,24,25} Can episodes of intraoperative consciousness without subsequent recall, similar to the conditions of the present study, cause harm? There is no direct evidence for this possibility, but there are a few anecdotal reports\textsuperscript{26,27} of unfavourable comments voiced during anaesthesia and retrieved under hypnosis that caused psychological disorders. Unfortunately, case reports cannot establish a cause and effect relationship, particularly where techniques such as hypnosis, which can sometimes lead to spurious recall, have been used.

In addition, there are two discrepancies between the conditions of our report and the clinical situation. The first is that our study involved only healthy volunteers who were not subjected to surgical stimuli. It is likely that greater doses would be required to suppress responses in the presence of more powerful stimuli than those used in our study. We elected not to administer painful stimuli (e.g. tetanic peripheral nerve stimulus or immersion of a forearm in ice-cold water) to our subjects to simulate the clinical situation for two reasons. The first was ethical and concerns inflicting painful stimuli on subjects who retain responsiveness and memory, particularly during inhalation of 0.2% isoflurane. The second was practical and concerns the problem of finding a sustained painful stimulus to represent surgical arousal.\textsuperscript{13} The other discrepancy is that in some clinical situations, brain and blood concentrations of anaesthetics are not in equilibrium. A study such as ours would be difficult to conduct in patients during surgery for ethical reasons. Studying volunteers under controlled conditions provides more reliable data with assessments performed during a stable end-tidal concentration of inhaled anaesthetic after it has presumably reached equilibrium with the anaesthetic partial pressure of the brain.

In summary, both doses of midazolam (0.03 and 0.06 mg kg\textsuperscript{-1}), when combined with 0.2% isoflurane in a quiescent environment devoid of surgical stimuli, almost completely abolished both explicit and implicit memory. Impairments extended over the 45-min period after administration of midazolam. However, the effect of the two drugs on responsiveness was more variable. Administration of midazolam to patients receiving only a subanaesthetic concentration of an inhalation anaesthetic is recommended to avoid recall. A larger dose than 0.06 mg kg\textsuperscript{-1} or a higher concentration of isoflurane than 0.2% may be necessary to abolish responsiveness.

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References


