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ORIGINAL INVESTIGATION

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Differential effects of alprazolam on the baseline and fear-potentiated startle reflex in humans: a dose-response study

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Abstract *Rationale:* The "fear-potentiated startle" paradigm has been extensively used in animal studies, and more recently in human experimental psychopharmacology to evaluate the effects of anxiogenic and anxiety-relieving drugs. Previous human studies have shown that both the baseline and the fear-potentiated responses can be inhibited by anxiety-relieving drugs, suggesting drug activity on two different emotional states, the former reflecting a resting condition and the latter more akin to pathological anxiety. *Objectives:* To examine to which extent the reductions induced by a benzodiazepine on the basic and the fear-potentiated startle responses are of equal intensity, and whether or not the drug shows a predominant, i.e., selective, effect on either. *Methods:* The effects of three increasing doses of the benzodiazepine alprazolam (0.25, 0.5, and 1.0 mg) were assessed on the human baseline and fear-potentiated startle responses. Twelve healthy volunteers attended the laboratory on four experimental days and received either alprazolam or placebo according to a double-blind crossover balanced design. Startle recordings were undertaken 2 h after drug intake. Fear potentiation was implemented by means of an electric-shock-anticipation experimental procedure. Additionally, subjective self-reports of sedation and anxiety and psychomotor performance were obtained at 2 and 3 h, respectively, after drug administration. *Results:* Alprazolam dose-dependently impaired psychomotor performance and produced increases in subjective anxiolytic activity and sedation, although the latter did not reach statistical significance. Additionally, the drug reduced the magnitude of the startle response both in the absence and in the presence of a threat-related cue, although a differentially greater inhibitory effect was seen on the fear-potentiated response as the dose increased. *Conclusions:* Alprazolam showed a greater inhibitory effect on the fear-potentiated startle than on the baseline reflex, suggesting a more selective action of the drug on those structures mediating potentiation of the behavioral response by anxiety.

Keywords Fear-potentiated startle · Subjective effect · Psychomotor performance · Alprazolam · Human

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Introduction

Benzodiazepines are currently the most commonly used pharmacological treatment for anxiety disorders. Despite their safety profile and established utility in this field, they are not devoid of undesired effects such as rebound phenomena after treatment cessation, addictive potential, and sedation, a consequence of the non-specific depression of the central nervous system they elicit (Shader and Greenblatt 1993). This latter effect, which is not unique to the benzodiazepines, is characterized by a reduction in overall activation, which can lead to both subjective feelings of drowsiness and objective impairments in performance (Hindmarch 1994a; Rihoux and Donnelly 1999). Research efforts to develop newer anxiolytics with fewer sedative effects could benefit from experimental models able to objectively distinguish between a drug's potential anxiety-relieving properties and its sedative activity, and provide a quantitative measure of the former. Human psychopharmacological research has classically relied on the degree of impairment in behavioral tasks (psychomotor performance) and the subjective perception of drug effects (self- and examiner-reported questionnaires and scales) to study the actions of drugs on the noopsyche (cognitive functions) and the tymopsyche (emotional

functions), respectively, usually in experimental contexts free of anxiety-provoking cues. These approaches have consequently focused on the assessment of relevant, though undesired, side effects, and are probably not adequate to specifically study the therapeutic potential of a drug, i.e., its anxiety-relieving properties. Additionally, although decreases in psychomotor performance and consequently the behavioral toxicity of a drug can be reliably measured (Hindmarch 1994b), these parameters do not always correlate with the subject's own perception of impairment (Rihoux and Donnelly 1999). This inevitably leads to difficulties in establishing clear differences between the true anxiolytic potential of a drug under investigation, and its sedative activity in early phases of clinical research, when it is administered to healthy subjects. However, valuable information could be obtained if the anxiolytic profile of a drug could be established before it is administered to patient populations. Pharmacological models of anxiety have been developed to induce anxiety- or even panic-like states in humans that are subsequently to be reverted by the potential anxiolytic (Gorman et al. 1987; Lines et al. 1995). Nevertheless, this approach has also presented several limitations. Firstly, the non-physiological nature of the anxiety state elicited, and secondly drugs either directly antagonizing the anxiogenic effects at the binding site of the eliciting drug, or acting on the same neurotransmitter system, will prove the most effective in the model.

In recent years, the classic conditioning of the startle reflex, a simple neurophysiological response relatively easy to elicit and record, has gained acceptance as an animal model for psychopharmacological research with anxiety-relieving drugs. The startle reflex originates in structures located in the brainstem following the presentation of sudden intense stimuli, and pertains to the mammal's protective behavior repertoire (Davis 1984). The basic startle response can be effectively modulated by descending neural pathways, thus being modified by the subject's psychological state, and has been considered to provide information on various cognitive and affective processes (Dawson et al. 1999). Additionally, the similarities between species regarding the nature of the response and its eliciting cues, together with the existence of a large corpus of preclinical data on its pharmacological modulation, have made it an attractive dependent variable for human clinical research. Thus, animal and more recently human investigations have shown that the basic response can be potentiated by behavioral manipulations causing fear and anxiety (Davis et al. 1999; Bradley et al. 1999). This "fear-potentiated startle" paradigm has been used as an animal model to evaluate the effects of both anxiogenic and anti-anxiety drugs. Anxiety-provoking drugs such as yohimbine and piperoxane, increase "fear-potentiated startle" in rodents, whereas it is reduced by drugs that relieve anxiety, such as benzodiazepines, clonidine, buspirone, and morphine (for a review see Davis 1986). Research in humans has found anxiolytics to reduce the basic (Kumari et al. 1996; Abduljawad et al. 1997; Rodriguez-Fornells et al. 1999) and the fear-potentiated startle response (Patrick et al. 1996; Bitsios et al. 1999), suggesting that the drugs tested show activity in two different emotional states, the former reflecting a resting condition and the latter more akin to pathological anxiety. Nevertheless, data are scarce as to whether the magnitude of response inhibition in a given subject is equal in both the baseline and the fear-potentiated conditions or whether anxiolytics show a more selective activity on one of the two conditions. As a working hypothesis we postulated that for drugs claiming an anxiety-relieving activity, rather than a general depressant one, the inhibition of the fear-potentiated startle should predominate over inhibition of the basic response. For those drugs showing a mixed anxiolytic and depressant activity, their inhibitory profile would vary, showing more or less selectivity for either response, depending on the dose administered.

Using a within-subject design, the present study intended to examine to which extent the reductions induced by a benzodiazepine on the basic and the fear-potentiated startle responses are of equal intensity, and whether or not the drug showed a predominant effect on either. Thus, dose-response data regarding the effects of alprazolam on the baseline and fear-potentiated responses were gathered. Drug effects on the magnitude and onset latency of the responses were assessed by means of a previously developed experimental paradigm, known to simulate anticipatory anxiety in healthy humans (Grillon et al. 1993). Additionally, data concerning alprazolam's effects on self-reported drowsiness and anxiety, as well as psychomotor performance were assessed by means of visual analog scales (VAS) and a choice reaction time (CRT) task, respectively.

Materials and methods

Subjects

The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the Hospital Ethics Committee and the Spanish Ministry of Health. Twelve healthy volunteers, six males and six females, (mean age 23.75 years, range 20–32 years) were recruited, and their written informed consent was obtained. None of them had a history of psychiatric or neurological disorders. They did not take any medication during the 2 weeks preceding the study and they abstained from alcohol, tobacco, and caffeinated drinks 48 h before each experimental day. Prior to their inclusion in the study, volunteers answered two personality questionnaires: the Eysenck Personality Inventory (EPI; Eysenk and Eysenk 1963), and the trait-anxiety scale from the State-Trait Anxiety Inventory (STAI-T; Spielberger et al. 1970). The purpose of personality screening was to exclude extreme scorers on anxiety and neuroticism (Cook et al. 1991). Only subjects with a STAI-T score within mean ±1 SD were selected. Mean values obtained were 38.67, range 32–49, for the STAI-T, 6.4, range 3–11, for the EPI neuroticism subscale, and 12.9, range 10–20, for the EPI extraversion subscale.

Study design and experimental procedure

The experiment was carried out according to a double-blind randomized crossover placebo-controlled design. Oral doses of 0.25,

0.50, and 1.0 mg alprazolam or placebo were administered in a balanced order. Treatment administration was carried out according to a randomization table comprising a 4×4 Latin square repeated three times. Experimental days were separated by a 1-week washout period.

The volunteers participated on four separate experimental days, on which they were given a capsule containing one of the three alprazolam doses or lactose (placebo). Upon arrival in the laboratory under fasting conditions a urine sample was obtained to test for illicit drug intake, a cannula was placed in the cubital vein of the left arm for drawing blood samples, and medication was given. During each recording session volunteers remained in a quiet room, sitting in a comfortable reclining chair, and were asked to stay alert throughout the experiment. Blood samples were drawn at baseline and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 24 h after administration in order to establish the drug's pharmacokinetic profile. The determination of alprazolam plasma levels was carried out by a high-performance liquid chromatography method previously described in the literature (Rieck and Platt 1992).

Measurements

Psychophysiological measures

Stimulus. The acoustic startle stimulus administered throughout the experiment was a 1-KHz pure tone of 116 dB [A], with a 50-ms duration and an instantaneous rise/fall time, presented binaurally through air headphones. No background noise was presented to the subjects during the experiment.

Recording. The startle reflex was recorded by means of two 0.5-cm-diameter silver surface disc electrodes. The first electrode was placed 1 cm inferior to the external canthus of the left eye, and the second 1 cm medial to the first, following the guidelines set by Fridlund and Cacioppo (1986). Spontaneous and voluntary blinking was also controlled by means of two electrodes placed above and below the right eye and a ground electrode was placed on the forehead. Impedance level was maintained below 5 KΩ. Special care was taken to ensure the accurate positioning of the electrodes in order to avoid placement errors between the four treatment conditions. The electromyographic signal (EMG) was AC amplified with a Grass 8 plus amplifier, using a 10–500 Hz bandpass analog filter. The EMG signal was digitized at a 1000 Hz rate.

Procedure. Fear-potentiation was implemented following a methodology similar to that described by Grillon et al. (1993). Each recording session began with a pretask habituation procedure in which 15 acoustic startle stimuli were delivered at variable time intervals (mean 20 s, range 18–22 s) without threat of shock. Immediately after the pretask habituation phase, shock electrodes were placed on the subject's wrist. This was followed by a series of 6 additional habituation startle stimuli, and subsequently by 16 alternating conditions comprising 8 threat and 8 no-threat conditions. The threat and no-threat conditions had durations of 35 and 30 s, respectively. A blue and a green square presented on the computer screen signaled the threat and no-threat conditions. Color assignment to each condition was counterbalanced across subjects. In two of the four experimental sessions the recordings began with a threat condition and in the other two sessions they began with a no-threat condition. In the threat condition a digital timer appeared on the upper right corner of the color square and counted seconds down from 35 to 0. The acoustic startling stimuli were administered at different time points: 5, 10, 15, 20, and 30 s (±2 s) into the threat condition and 5, 10, 15, 20, and 25 s (±2 s) into the no-threat condition, totaling 10 different trial types. Startle stimuli were administered a total of four times at each time point. Thus, 20 startle stimuli were delivered during each condition (threat/no-threat), and a total of 40+21 startle stimuli in the course of an experimental session.

Subjects were told that they could randomly receive from zero to three electric shocks in the course of an experimental session, but only during the last 10 s of the threat condition, as the timer counted from 10 to 0. In order to maintain expectancy throughout the experiment, volunteers were also informed that the intensity of the shocks would increase as they appeared within a session and also from session to session. All subjects did in actual fact receive five brief electrical shocks in the course of the study: two in the first session (7 and 10 mA), two in the second session (13 and 16 mA), and one in the third session (19 mA). All shocks were 0.5 ms in duration. No shocks were delivered in the fourth experimental session. The psychophysiological recording session was undertaken 2 h after treatment administration.

Subjective ratings

At baseline (prior to drug administration) and immediately before each psychophysiological recording session (+2 h), participants were asked to rate their level of subjective drowsiness and anxiety by means of VAS. These were three 100-mm horizontal lines with the following labels: "Active-Passive", "Awake-Drowsy", and "Anxious-Calm".

Psychomotor performance

A computerized CRT task was implemented. Subjects were instructed to respond by pressing a button with either their right or left hand depending on whether an "H" or an "S" was presented on a computer screen 800 ms following a warning signal (an asterisk presented for 600 ms). Response-hand/letter assignment was counterbalanced across subjects and was the same on all experimental days for a given volunteer. A total of 600 trials was presented, with an intertrial interval of 2250 ms (range 2000–2500 ms). The CRT task was undertaken 3 h after treatment administration.

Data analysis

Psychophysiological measures. The recorded EMG signal was full-wave rectified off-line and smoothed using a five-point moving average filter. Startle latency onset was defined as the first increment of EMG level 2 SD above the average baseline, not followed by a return to baseline within the next 10 ms. Peak eyeblink amplitude was defined as the highest point in the EMG response within a time window of 120 ms after stimulus administration. Baseline EMG was computed as the mean EMG in the 30 ms preceding stimulus onset. Reactivity was defined as the value of blink amplitude for each subject in the first startle trial. Trials in which the apparent response had an onset latency of less than 20 ms after stimulus administration and/or a rise time greater than 95 ms were rejected. In those trials in which no response was detected, amplitude was scored as 0 μV, whereas latency was scored as a missing value and was excluded from further calculations. Two of the 12 subjects were excluded from the startle amplitude analysis as they showed a large number of artifacted epochs. Amplitude values were obtained for the 10 remaining subjects and latency values only in a subgroup of 9 volunteers, as an additional subject showed numerous epochs without detectable responses.

Blink magnitude (0 μV amplitude epochs included in the analysis) and onset latency values were averaged for each time point (i.e., four trials for each of the five trial types in each condition) and these data were subjected to a 4×2×5 (treatment, experimental condition, and trial type) analysis of variance (ANOVA) with repeated measures. Additionally, reactivity and global blink probability (measured inversely considering the number of zero response trials) were evaluated by means of a one-way repeated measures ANOVA with treatment as factor. Finally, habituation (magnitude decreases across blocks of trials in the pretask habituation procedure) was also analyzed by means of a two-way repeated measures ANOVA with treatment and block as factors.

Subjective ratings. VAS data at 2 h after administration were transformed into differences from baseline. Transformed values were analyzed by means of a one-way repeated measures ANOVA with treatment as factor.

Psychomotor performance. The variable derived from the CRT task was mean reaction time in all correctly responded trials. This variable was analyzed by means of a one-way ANOVA with treatment as factor.

Alprazolam plasma levels. Experimental C_{max} and T_{max} values, and mean plasma levels at 2 and 3 h after dosing are given for alprazolam at the three doses administered.

In all ANOVAs performed, Greenhouse-Geisser epsilon was used to correct possible violations of the sphericity assumption and to reduce type I errors (Jennings 1987). *P* values after correction are shown. When ANOVA showed significant differences between treatments, pairwise comparisons were carried out by means of *t*-tests, followed by Bonferroni correction.

Results

As no gender effects were found in the statistical analyses performed, this factor is omitted in the results reported below.

Psychophysiological measures

Blink probability, reactivity, and habituation

Blink response probability was affected by treatment, and decreased as the alprazolam dose increased. The number of non-response trials increased linearly with dose [$F(3,27)=6.03$, $P<0.01$, $\varepsilon=0.811$; linear tendency $F(1,9)=10.19$, $P<0.05$; number of non-responsive epochs as mean ± SEM: placebo 2.2±1.3, low dose 4.4±2.3, medium dose 5.5±3.0, and high dose 11.2±3.7].

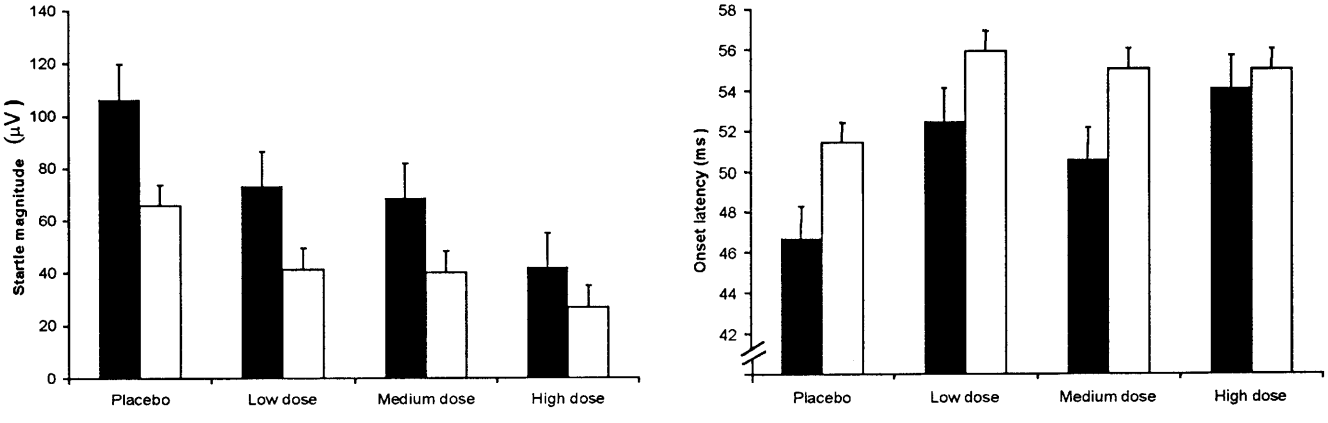
A significant effect of treatment was also observed for reactivity in the ANOVA, [$F(3,27)=14.49$, $P<0.001$, $\varepsilon=0.717$]. Mean amplitude (μV) ± SEM for the first eyeblink was 199.2 μV±35.2 for placebo, 114.7 μV±29.9 for the low dose, 121.2 μV±30.7 for the medium dose, and 90.4 μV±36.1 for the high dose. The linear tendency contrast was significant [$F(1,9)=27.57$, $P<0.01$].

The 15 trials in the pretask habituation procedure were grouped in three blocks of five trials and their mean values were subjected to a two-way ANOVA with treatment and block as factors. Results showed main effects of treatment [$F(3,27)=7.75$, $P<0.01$, $\varepsilon=0.480$], block [$F(2,18)=21.78$, $P<0.001$, $\varepsilon=0.645$], and their interaction [$F(6,54)=4.52$, $P<0.05$, $\varepsilon=0.495$] in the ANOVA. The mean amplitude of the eyeblink magnitude showed a significant linear [$F(1,9)=25.46$, $P<0.01$] reduction tendency across treatments [placebo: 108.8 μV±23.8, low dose: 68.8 μV±12.6, medium dose: 73.7 μV±21.5, and high dose: 47.9 μV±14.6]. Regarding block, polynomial contrasts showed both linear [$F(1,9)=23.46$, $P<0.01$] and quadratic [$F(1,9)=10.5$, $P<0.01$] reduction tendencies (mean amplitude for the first block was 99.3 μV±20.7, second block 68.4 μV± 16.9, and third block 56.6 μV±13.9). The interaction between treatment and block revealed a reduction in the habituation slope associated with the administration of alprazolam.

Magnitude of the eyeblink reflex

Figure 1 (left panel) presents mean eyeblink magnitude values obtained in the four treatment conditions. Startle magnitude was affected by alprazolam [$F(3,27)=9.05$, $P<0.01$, $\varepsilon=0.467$; linear reduction contrast $F(1,9)=21.47$, $P<0.01$; mean magnitude (μV) ± SEM for the four different treatment conditions were placebo 86.4 μV±20.5, low dose 57.3 μV±11.0, medium dose 54.3 μV±14.7, and high dose 34.4 μV±10.1]. The magnitude of the startle eyeblink response was found to be increased in the threat vs the no-threat condition. This was seen as a significant experimental condition (threat/no-threat) effect [$F(1,9)=12.10$, $P<0.01$; mean magnitude in the threat condition 72.6 μV±15.9; no-threat condition 43.5 μV±11.8]. The interaction between treatment by experimental condition was also found to be significant [$F(3,27)=5.02$, $P<0.05$].

Fig. 1 The *left* and *right panels*, respectively, show mean startle magnitude values in microvolts (*μV*) and mean onset latency values in milliseconds (*ms*), in the threat (■) and no-threat (□) conditions for each of the four administered treatments. *n*=10 for startle magnitude and *n*=9 for onset latency. *Error bars* denote 1 SEM



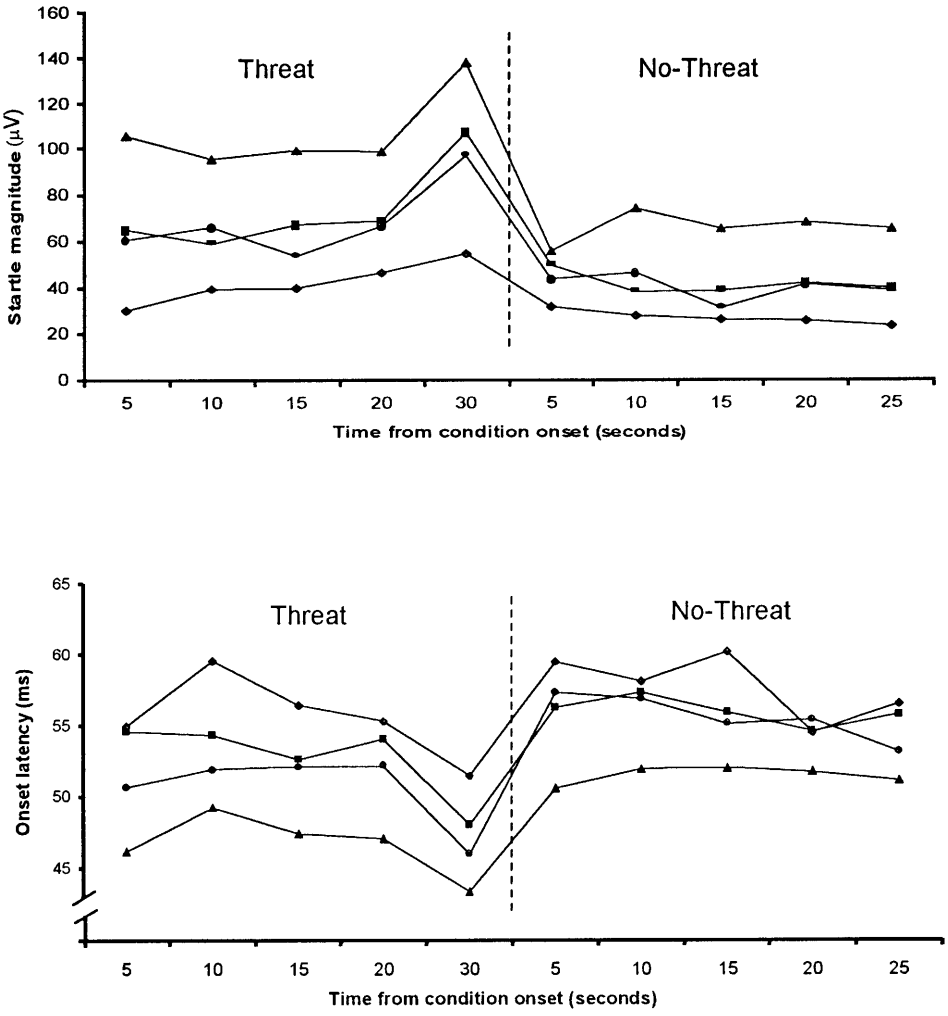


Fig. 2 Time course of effects. The *upper panel* shows mean startle magnitude values in microvolts (μV) at each of the set time points at which startle stimuli were delivered in the alternating threat and no threat conditions (n=10). The *lower panel* shows mean onset latency values in milliseconds (*ms*) at the same time points (n=9). Symbols: ▲ placebo, ■ 0.25 mg alprazolam, ● 0.5 mg alprazolam, and ◆ 1.0 mg alprazolam

This interaction points out that magnitude differences between the threat and the no-threat condition decreased as the alprazolam dose increased, and was corroborated by a significant linear contrast for this interaction [$F(1,9)=14.34$, $P<0.01$].

Given the differences observed in eyeblink response probability associated to treatment, a reanalysis was performed considering non-response trials as missing values. This criterion left out an additional subject, but similar effects were found in the ANOVA. Statistically significant effects were found for treatment [$F(3,24)=8.59$, $P<0.01$, $\varepsilon=0.461$; linear reduction tendency $F(1,8)=19.90$, $P<0.01$], experimental condition [$F(1,8)=12.76$, $P<0.01$], and their interaction [$F(3,24)=5.44$, $P<0.05$, $\varepsilon=0.638$; linear reduction tendency $F(1,8)=17.41$, $P<0.01$].

The time course of the above effects is presented in Fig. 2 (upper panel) and described as follows. The magnitude of the reflex increased as the end of the threat condition approached, as evidenced by a significant 'trial' effect [$F(4,36)=6.33$, $P<0.05$, $\varepsilon=0.460$; a significant quadratic tendency was found $F(1,9)=29.28$, $P<0.001$ and a less intense linear contrast $F(1,9)=6.10$, $P<0.05$; mean trial values (from the beginning to the end of the threat condition): 65.3 μV±16.0, 64.7 μV±15.7, 64.7 μV±15.4, 70.1 μV±17.4, and 99.0 μV±18.0] and experimental condition by trial interaction [$F(4,36)=7.37$, $P<0.01$, $\varepsilon=0.528$]. This last interaction reflects an abrupt increase in the eyeblink magnitude in the last 10 s of the threat condition [linear $F(1,9)=10.06$, $P<0.05$ and quadratic tendencies $F(1,9)=14.43$, $P<0.01$]. Finally, the interaction treatment by trial was not found to be significant [$F(12,108)=1.01$], nor was the treatment by experimental condition by trial [$F(12,108)=0.77$].

Onset latency of the eyeblink reflex

Figure 1 (right panel) presents the mean eyeblink onset latency values for nine subjects in the four treatment conditions. Onset latency was affected by alprazolam, as shown by a significant treatment effect [$F(3,24)=5.0$, $P<0.05$, $\varepsilon=0.869$; linear tendency $F(1,8)=13.59$, $P<0.01$; mean onset latency (ms) ± SEM for the four different treatments was placebo 49.0 ms ±1.3, low dose 54.2 ms ±3.0, medium dose 52.8 ms ±2.1, and high dose 54.6 ms ±3.2]. Onset latency was reduced in the threat vs the no-threat condition. This was seen as a significant

Table 1 Pairwise comparisons performed on the startle magnitude and latency values, subjective ratings, and psychomotor performance. *n*=10, except for startle onset latency, where *n*=9. Student's *t*-tests were followed by Bonferroni correction. *ns* Not significant

Variable	ANOVA *P* value	Student's *t*-test					
		Placebo			0.25 mg		0.50 mg 1.0
		0.25	0.50	1.0	0.50	1.0	
Startle magnitude							
Global	**	ns	***	***	ns	****	ns
No-threat	**	ns	***	***	ns	***	ns
Threat	**	ns	***	***	ns	****	ns
Onset latency							
Global	ns	–	–	–	–	–	–
No-threat	ns	–	–	–	–	–	–
Threat	*	ns	ns	ns	ns	ns	ns
Subjective ratings (VAS)							
Active-Passive	ns	–	–	–	–	–	–
Awake-Drowsy	ns	–	–	–	–	–	–
Anxious-Calm	*	ns	***	***	ns	ns	ns
Psychomotor performance							
CRT	**	ns	***	****	ns	***	ns

* $P<0.05$
** $P<0.01$
*** $P<0.05$ after Bonferroni correction
**** $P<0.01$ after Bonferroni correction

experimental condition (threat/no-threat) effect [$F(1,8)=15.92$, $P<0.01$; mean latency in the threat condition 51.0 ms ±2.2; no-threat condition 54.4 ms ±2.1]. The interaction treatment by experimental condition was not found to be significant [$F(3,24)=1.50$].

The time course of the above-described effects is presented in Fig. 2 (lower panel) and described as follows. Onset latency of the eyeblink response decreased as the end of the threat condition approached, as shown by a significant 'trial' effect [$F(4,32)=4.44$, $P<0.05$, $\varepsilon=0.711$]. The interaction experimental condition by trial showed a trend toward statistical significance [$F(4,32)=2.90$, $P=0.06$, $\varepsilon=0.685$], reflecting the abrupt decrease in latency onset in the last 10 s of the threat condition. Finally, neither the interaction treatment by trial [$F(12,96)=0.75$] nor the treatment by experimental condition by trial [$F(12,96)=0.39$] were found to be significant.

Pairwise comparisons among the different treatments for global eyeblink magnitude and onset latency, and for values in the two conditions (threat and no threat) are shown in Table 1.

Subjective ratings

Figure 3 (upper panels and lower left panel) shows mean VAS scores at +2 h (differences from baseline) in the four treatment conditions. A significant treatment effect was observed in the statistical analyses performed (ANOVAs) only for the Anxious-Calm item [$F(3,27)=5.52$, $P<0.05$, $\varepsilon=0.756$; placebo: –0.20 mm ±4.0, low dose: 9.2 mm ±4.24, medium dose: 17.6 mm ±4.04, and high dose: 11.00 mm ±3.56]. No statistically significant results were found for the Awake-Drowsy [$F(3,27)=1.96$] or the Active-Passive [$F(3,27)=2.39$] items. In all three items, a decrease in the mean score was seen between the medium and high doses. Pairwise comparisons among treatments for the Anxious-Calm item are shown in Table 1.

Psychomotor performance

Figure 3 (lower right panel) presents mean reaction time values obtained in the four treatment conditions. Reaction time was affected by alprazolam [$F(3,27)=15.79$, $P<0.001$, $\varepsilon=0.791$; linear contrast $F(1,9)=49.0$, $P<0.001$; mean reaction time (ms) ± SEM for the four different treatment conditions were placebo: 330.5 ms ±13.3, low dose: 345.4 ms ±14.0, medium dose: 359.2 ms ±13.4, and high dose: 384.5 ms ±16.6]. Pairwise comparisons among treatments for reaction time are shown in Table 1.

Alprazolam plasma levels

At the three doses administered, maximum alprazolam plasma concentrations (T_{max}) were reached at 60 min after drug administration. C_{max} values were (mean ± SD) 3.02±0.44 ng/ml for the low dose, 5.58±1.24 ng/ml for the medium dose, and 10.73±3.18 ng/ml for the high dose. Plasma concentrations immediately before the psychophysiological recording session (+2 h) were (mean ± SD) 2.72±0.34 ng/ml for the low dose, 4.78± 0.61 ng/ml for the medium dose, and 9.82±2.17 ng/ml for the high dose. Plasma concentrations immediately before the CRT task (+3 h) were (mean ± SD) 2.49± 0.31 ng/ml for the low dose, 4.37±0.75 ng/ml for the medium dose, and 9.57±1.94 ng/ml for the high dose.

Discussion

In the present study, the effects of three different doses of alprazolam on a previously developed human psychophysiological model of anticipatory anxiety (Grillon et al. 1993) were evaluated. Additionally, drug effects on subjective ratings of drowsiness and anxiety, and on a CRT task were assessed. As reported by other researchers (Grillon et al. 1991, 1993; Curtin et al. 1998; Grillon and Ameli 1998; Bitsios et al. 1999), the anticipation of an electric shock in the threat condition induced elevations in the magnitude of the electromyographic response, elicited by an acoustic startling stimulus, and recorded at the level of the orbicularis oculi muscle. Additionally, in all treatment conditions the magnitude of the response showed a time-dependent pattern with a final

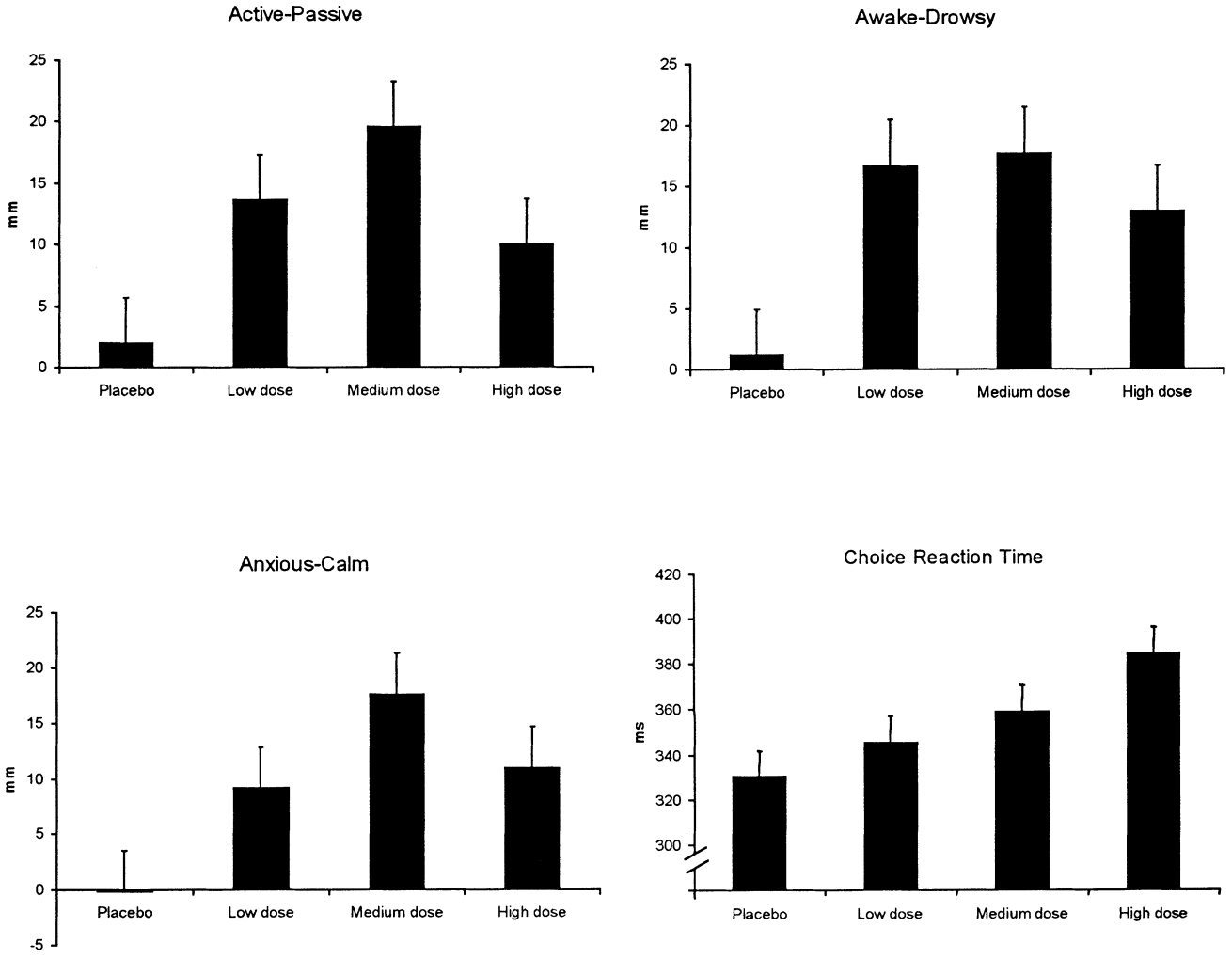


Fig. 3 The *upper panels* and *lower left panel* show mean scores (differences from baseline) obtained for the three self-report visual analog scales (VAS) in millimeters (*mm*) at 2 h after administration in the four treatment conditions. Increases in subjective sedation (*upper panels*) and anxiolytic activity (*lower left panel*) are reflected as greater VAS scores. The *lower right panel* shows mean choice reaction time (CRT) values in milliseconds (*ms*) at 3 h after administration in the four treatment conditions. Increases in psychomotor impairment are reflected as greater CRT values. *n*=10; *error bars* denote 1 SEM

maximal increase, coinciding with the time window where the volunteers expected the shock, as previously described by Grillon and coworkers (1993) in the absence of a pharmacological challenge. An inverse pattern was found for onset latency. The time to onset of the EMG response was decreased in the threat vs the no-threat condition, also showing a time-dependent pattern with a downward-pointing peak corresponding to the response recorded within the last 10 s of the threat-condition. This effect of the threat-of-shock on onset latency also replicates the results obtained by other research groups (Grillon et al. 1991, 1993; Curtin et al. 1998; Grillon and Ameli 1998; Bitsios et al. 1999).

The principal finding that can be drawn from the results obtained in the present study is the differential modulation of the threat-potentiated and baseline startle reflex magnitudes at the three different doses used. Interestingly, the characteristic curve obtained under placebo, analogous to that described by Grillon and coworkers (1993), was modified in a dose-dependent manner by alprazolam, which produced reductions both of the baseline and potentiated responses, with magnitude differences between the two conditions gradually decreasing, at the medium and high doses. As the dose increased, the effects of the drug were more evident on the potentiated response (threat condition). Thus, the potentiated reflex appeared to be more sensitive to the effects of alprazolam than was the baseline response. Alprazolam also produced dose-dependent increases in latency onset, but the interaction between treatment and experimental condition in the ANOVA was not significant.

Drug administration increased scorings on the three self-report VAS used. These increases, however, only reached statistical significance for the Anxious-Calm item, and all three VAS showed a decrease between the medium and high doses, reflecting a saturation effect. On the contrary, the CRT task showed a significant dose-dependent effect, with the highest psychomotor impairment obtained after the high 1.0 mg alprazolam dose. Thus, analogous dose-response variations were obtained

between the psychomotor performance and the psychophysiological approaches, whereas subjective self-report measures failed to show a linear increase with dose. Regarding pharmacokinetic data, a linear increase with dose was seen for alprazolam's C_{max} values and plasma levels, in line with data previously reported in the literature (Dawson et al. 1984). Additionally, experimental T_{max} was independent of the administered alprazolam dose.

The attenuation of startle magnitude induced by alprazolam both in the threat and in the no-threat conditions is in agreement with results from previous studies with humans that have demonstrated inhibitory effects of benzodiazepines on both the baseline (Abduljawad et al. 1997; Bitsios et al. 1999; Rodríguez-Fornells et al. 1999) and the fear-potentiated startle (Patrick et al. 1996; Bitsios et al. 1999). In the present study, this inhibitory effect was already evident at the low 0.25 mg dose, although at this dose it did not reach statistical significance. However, at the medium and high doses, alprazolam produced statistically significant reductions in the reflex's magnitude both in the threat and no-threat conditions.

Our results for the potentiated startle are in line with data from research with animals, which demonstrate inhibitory effects of anxiety-relieving drugs on the potentiated response (Davis 1986). Thus, barbiturates (Chi 1965), benzodiazepines (Davis 1979a; Berg and Davis 1984), morphine (Davis 1979b), alcohol (Williams 1960 in Davis 1986), serotonergic anxiolytics (Davis et al. 1985), and clonidine (Davis et al. 1979) have shown predominantly selective actions on the potentiated response without a significant inhibition of the baseline reflex. This lack of a significant effect on baseline startle is in contrast with results obtained in the present experiment, and in several other human studies in which anxiolytics/depressants other than benzodiazepines, such as alcohol (Stritzke et al. 1995; Curtin et al. 1998) and clonidine (Kumari et al. 1996; Abduljawad et al. 1997), have demonstrated an inhibitory effect on the basic response.

Regarding onset latency, drug-induced increases have been found in some previous experiments with benzodiazepines (Rodríguez-Fornells et al. 1999) but not in others (Patrick et al. 1996; Abduljawad et al. 1997; Bitsios 1999). Significant latency increases have also been described after the administration of alcohol (Stritzke et al. 1995) to humans.

From a neurophysiological perspective, the drug's differential effect on the baseline and potentiated startle is suggestive of a predominant action of alprazolam at the higher doses on secondary structures responsible for the reflex's affective potentiation. Rosen and coworkers (1991) have described a direct neural pathway originating in the central nucleus of the amygdala which projects onto the nucleus reticularis pontis caudalis, the site where the reflex response is thought to originate (Davis 1996). Electrical stimulation of the amygdala in the rat has been found to elicit a behavioral state analogous to fear (Davis 1996). Conversely, lesions selectively affecting the pathway connecting the central nucleus of the amygdala with the nucleus reticularis pontis caudalis have been shown to block fear-potentiated startle (Hitchcock and Davis 1991). The differential effects of alprazolam could be reflecting a lower effect of the drug on baseline responsiveness, than on the combination of baseline responsiveness plus increased responsiveness caused by anxiety. Previous studies have shown a good agreement between startle potentiation by fear in humans and greater increases in subjective anxiety ratings measured simultaneously (Bitsios et al. 1999), and the independence of startle increases induced by fear from other phenomena such as attention (Grillon et al. 1993). Additionally, the predominant effects of alprazolam on the potentiated response are in agreement with previous results obtained for diazepam in a similar experimental paradigm (Bitsios et al. 1999), and taken together suggest a greater action of benzodiazepines upon those structures mediating potentiation of the response. These findings consequently support the approach of using the difference between potentiated and baseline startle as an index of drug effects on anxiety alone. However, as has been suggested by other authors (Bitsios et al. 1999), baseline startle could already be potentiated by the unspecific anxiety caused by the experimental setting, in such a way that drug-induced reductions seen in the no-threat condition could also be reflecting decreases in fear-potentiated startle. This possibility of baseline startle being increased due to contextual factors suggests that assimilating drug-induced reductions in baseline startle with a general CNS depressant activity should be done with caution. On the other hand, baseline startle has been shown to be reduced after a 2 mg alprazolam dose in an experimental setting free of fear-conditioned cues (Rodríguez-Fornells et al. 1999).

According to our model, startle measures indicated that alprazolam predominantly attenuated, at the higher doses, the response increases caused by anticipatory anxiety. Consistently, subjective self-report measures showed a significant effect of alprazolam on the item indicating decreases in anxiety (Anxious-Calm), with nonsignificant increases for those items reflecting unspecific depressant effects (Active-Passive and Awake-Drowsy). However, all three items, including the first, showed a saturation between the medium and high doses. Additionally, a linear increase pattern was obtained for the CRT, which contrary to subjective self-assessment did not show a saturation effect. Interestingly enough, CRT increases have been classically considered to reflect sedation (Hindmarch 1994b). CRT results obtained suggest that the general depressant effects of the drug actually increased from the medium to the high alprazolam dose. Thus, the integration of results obtained through the three approaches used appears to be far from simple, even though discrepancies between drowsiness self-perception and objective performance measures have already been pointed out by other authors (Rihoux and Donnelly 1999). In the specific case of the benzodiazepines this effect of the drug on what has been termed

metacognition (Lister et al. 1988) has been previously described at least for triazolam (Roache and Griffiths 1985), lorazepam (Roache and Griffiths 1987) and diazepam (Barbanoj 1991).

To conclude, we would like to note that in the present study it was not feasible to obtain self-ratings of anxiety and drowsiness specifically for the time windows when the threat and no-threat conditions were present, or measures of autonomic responses simultaneously with startle recordings. Both technical and study design constraints precluded such an approach. Although the results obtained are promising and seemingly indicate that the benzodiazepines display predominant effects on the potentiated vs the baseline startle response, additional data are needed in order to validate this methodology as a human model capable of selectively measuring anxiolytic activity. Future studies could benefit from the simultaneous recording of autonomic and subjective variables, and the comparative evaluation of other drug classes with different profiles of anxiolytic vs depressant activity, in order to further assess the strengths and limitations of this model.

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