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## Comparison of the effects of diazepam on the fear-potentiated startle reflex and the fear-inhibited light reflex in man

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It has been shown previously that the amplitude of the acoustic startle reflex is enhanced, and the amplitude of the light reflex reduced, when subjects anticipate an aversive event, compared to periods when subjects are resting ('fear-potentiated startle reflex' and 'fear-inhibited light reflex'). We examined whether the anxiolytic diazepam would reverse the effects of threat on the startle and pupillary reflexes. Twelve male volunteers participated in three weekly sessions in which they received oral treatment with placebo, diazepam 5 mg and diazepam 10 mg, according to a balanced crossover double-blind design. One hour after ingestion of the treatments, miotic responses to light pulses and electromyographic responses of the orbicularis oculi muscle to sound pulses were elicited during alternating periods in which the threat of an electric shock (electrodes attached to the subject's wrist) was present (THREAT) and absent (SAFE). The THREAT condition was associated with a significant increase in the amplitude of the electromyographic (EMG) response, a significant reduction of the miotic response amplitude, and an increase in self-rated anxiety. Diazepam attenuated all these effects of THREAT. Diazepam did not affect the amplitude of the miotic response under the SAFE condition, but did suppress the EMG response under this condition. These results confirm the validity of the fear-potentiated startle reflex and fear-inhibited light reflex as laboratory models of human anxiety, and reveal some differences between the effects of diazepam on the two reflexes.

**Key words:** anxiety; diazepam; fear; light reflex; startle reflex

### Introduction

The startle reflex consists of contraction of the skeletal and facial musculature in response to a sudden intense stimulus, such as a loud sound (acoustic startle). Elicitation of the basic startle reflex entails structures below the level of diencephalon; in the rat, there is evidence that as few as three or four synapses are involved in the reflex (Davis *et al.*, 1982; Yeomans and Frankland, 1996). It is well established that, in animals, the amplitude of the startle reflex is enhanced in the presence of cues previously paired with noxious events ('fear-potentiated startle reflex'; Davis, 1979). Fear-potential of the startle reflex is believed to reflect the influence of an excitatory pathway projecting from the amygdala to the pontine relay nucleus of the startle reflex arc (nucleus reticularis pontis caudalis; Davis *et al.*, 1993). The fear-potentiated startle reflex has been proposed as a laboratory model of anticipatory anxiety, a proposal that has been supported by numerous reports that, in rats, the potentiation can be reversed by divers anxiolytic drugs, including benzodiazepines, 5-HT<sub>1A</sub> receptor agonists and clonidine (Davis *et al.*, 1993).

The acoustic startle response can also be reliably evoked in adult humans, a convenient measure being the electromyographic (EMG) response of the orbicularis oculi muscle (startle eyeblink response; Braff *et al.*, 1978; Morgan *et al.*, 1995). The EMG response can be enhanced by exposure to

'threatening' stimuli, for example, cues signalling the imminent delivery of an electric shock (Grillon *et al.*, 1991, 1993; Patrick *et al.*, 1996), or slides depicting distressing or frightening scenes (Vrana *et al.*, 1988; Bradley *et al.*, 1990; Hamm *et al.*, 1993). There have been very few attempts to study the pharmacological sensitivity of the 'fear-potentiated' startle reflex in man. However, Patrick *et al.* (1996) recently reported that diazepam (10 mg and 15 mg) attenuated the enhancement of the startle response induced by threat of an electric shock.

Recent work in our laboratory has examined the effect of threat of an electric shock on the pupillary light reflex (Bitsios *et al.*, 1996, 1998). The light reflex is a parasympathetically mediated response which serves to regulate the illumination of the retina. The reflex arc involves the retinal ganglion cells, the pretectal nuclei, the mesencephalic Edinger–Westphal (pupillomotor) nucleus, the ciliary ganglion, and the pupillary sphincter muscle (Loewenfeld, 1958). Light is the exclusive unconditioned physical stimulus that drives this reflex and the amplitude of the response is regarded as a pure measure of parasympathetic activity (Loewenfeld, 1993a,b). Under physiological conditions, the Edinger–Westphal nucleus is believed to be under tonic inhibition from the locus coeruleus, either directly (Koss *et al.*, 1984; Koss, 1986) or indirectly from the coeruleo–hypothalamic and coeruleo–cortico–hypothalamic loops (Szabadi and Bradshaw, 1996).

We have found that the amplitude of the light reflex response decreases when normal subjects anticipate an electric shock compared with periods when no shock is anticipated (Bitsios *et al.*, 1996). We termed this phenomenon the 'fear-inhibited light reflex', and suggested that it may have potential as a laboratory model of human anxiety. Recently, we found that the fear-inhibited light reflex could be attenuated by the anxiolytic diazepam in doses that did not affect the light reflex in a 'safe' condition when no shock was anticipated.

The fear-potentiated startle reflex and the fear-inhibited light reflex constitute two simple laboratory models of anxiety in man, both of which hold promise for psychopharmacological investigation. However, to date there has been no attempt to compare the pharmacological sensitivity of these paradigms in the same group of subjects. The aim of this study was twofold: first, to explore the feasibility of simultaneous recording of the fear-potentiated startle and the fear-inhibited light reflexes in the same group of human subjects and, second, to observe their concurrent modulation by the anxiolytic diazepam.

## Methods

The study protocol was approved by the University of Nottingham Medical School Ethics Committee. All volunteers gave their written consent following a verbal explanation of the study and after reading a detailed information sheet.

### Subjects

Twelve healthy male volunteers aged 18–28 years (mean  $\pm$  SD,  $21.3 \pm 0.6$  years) and weighing 70–99 kg ( $78.0 \pm 2.5$  kg) participated in the study. Three other subjects were recruited, but were subsequently excluded because they failed to produce consistent EMG responses to the 115-dB sound pulses. Before entering the study, their hearing thresholds at 0.5, 1, 2 and 4 kHz were measured; none had thresholds above 20 dB at any of these frequencies. Subjects were all medication-free and were requested to avoid drinking alcohol, coffee and other caffeine-containing beverages for at least 12 h before the experimental session. All of them were occasional caffeine and/or social alcohol consumers. Three of the subjects were regular cigarette smokers; smoking was not permitted during the experimental sessions. Similarly, eating was not permitted during the sessions, but no restriction was placed on food consumption prior to the sessions.

### Drugs and design

Before the start of the experiment, each subject participated in a training session in which their hearing thresholds were assessed and the procedures were explained and demonstrated. The experiment consisted of three experimental sessions, each lasting approximately 2 h, in which the three treatments (placebo, diazepam 5 mg and diazepam 10 mg) were administered orally in matching capsules according to a balanced double-blind protocol.

### Tests and apparatus

#### *Acoustic startle reflex*

The method used was similar to that used by Abduljawad *et al.* (1997, 1998). Acoustic stimuli were generated by an AC30 Clinical Audiometer (Kamplex Ltd, London, UK) and were presented to each subject binaurally. A background 70-dB[A] 1-kHz tone was present throughout the recording period. The sound pulses consisted of 40-ms 1-kHz tones of intensity 115 dB[A]. EMG responses of the orbicularis oculi muscle of the left eye were recorded via two 0.5-cm diameter silver surface disc electrodes placed approximately 0.5 cm below the lower eyelid. The ground electrode was placed over the left mastoid. A CED 1401<sup>+</sup> computer with a 1902 interface (Cambridge Electronic Design Ltd, Cambridge, UK) was used to record the EMG (rectified input, via a 1-Hz high-pass filter, with a notch filter set at 50 Hz to minimize mains electrical interference).

#### *Pupillary light reflex*

The method used was similar to that used by Bitsios *et al.* (1996, 1998). Recordings were made with the head positioned in an ophthalmic head-rest. An infrared binocular television pupillometer (TVP 1015B; Applied Science Laboratories, Waltham, MA, USA) was used to record the miotic responses to brief light stimuli, in previously dark-adapted eyes. The stimuli were light pulses (green, 565 nm peak wavelength) of 200 ms duration, delivered via a light emitting diode positioned 1 cm from the cornea of the right eye; the incident light intensity measured 1 cm from the source was  $0.43 \text{ mW cm}^{-2}$ . Stimulus delivery was controlled by the same CED 1401<sup>+</sup> computer that was used to control the acoustic stimuli. A second 1902 interface unit was used to capture the output signal from the pupillometer.

#### *Electric shock*

A constant current square pulse (1.5 mA, 50 ms) was delivered to the skin overlying the median nerve of the left wrist through disposable silver surface electrodes using a Grass stimulator (SD 9) (Grass Instruments, Quincy, MA, USA).

#### *Subjective ratings*

At various times during the recording period, subjects rated their subjective anxiety with verbal reports according to an imaginary 0–100 numerical scale (0 = not at all anxious; 100 = extremely anxious). At the end of each session, subjects completed a battery of 16 100-mm visual analogue rating scales (Norris, 1971; Bond and Lader, 1974); nine of these were used for the assessment of subjective 'alertness' (see Abduljawad *et al.*, 1997).

### Procedure

After arrival in the laboratory, each subject rested for 10 min before ingesting the capsule. Some 40 min later, subjects entered the darkened test cubicle for a 20-min accommodation period. During the last 5 min of this accommodation period, the EMG recording electrodes and the shock delivery electrodes were attached, headphones were placed over the ears of the subject, and the head was positioned in the ophthalmic head rest, and the light stimulus source was

positioned in front of the right eye. A single acoustic pulse and four light pulses separated by 10-s intervals were delivered; responses to these initial stimuli were discarded. The recording period started 3 min later, 63 min after ingestion of the capsule. The time-course of the session was based on the pharmacokinetic profile of diazepam. Diazepam is absorbed rapidly from the gut, and peak plasma concentration is attained approximately 1 h after ingestion (see Baldessarini, 1990; Cooper, 1995).

The recording period comprised six consecutive 90-s blocks, each including four acoustic pulses and three light flashes. Acoustic and light stimuli alternated within each block (always starting with an acoustic stimulus). The interval between successive acoustic stimuli varied between 19 and 31 s (mean 25 s); the interval between successive light stimuli was kept constant at 25 s. Responses in each block were recorded either during anticipation of an electric shock (THREAT condition) or without such anticipation (SAFE condition), the two conditions being associated with attachment and removal of the leads connecting the shock source to the wrist electrodes. For half the subjects, the first block was a SAFE block, whereas for the remaining subjects it was a THREAT block. The SAFE and THREAT conditions alternated regularly in the remaining five blocks. At the end of each SAFE and THREAT block, subjects were asked to rate their anxiety verbally, on a 0–100 numerical scale. These reports did not require removal of their head from the ophthalmic head-rest, thus minimizing the need for camera and light-source readjustments. The inter-block interval was 30 s, to allow time for removal or re-attachment of the wrist electrodes. The recording period lasted approximately 12 min. After the completion of the recording period, the electrodes were removed and the subject completed the visual analogue self-rating scales.

### Instructions to subjects

Before the start of each block, subjects were informed about the nature of the condition with which the block was associated (i.e. SAFE or THREAT). In the SAFE condition, the leads connecting the shock source to the wrist electrodes were removed. In the THREAT condition, the electrodes were re-connected and each subject was instructed to anticipate a total of one to three electric shocks, delivered to their wrist at any time during that block. Subjects did not know the exact number of shocks, or in which THREAT block(s) it/they would occur. The shocks were described by the experimenter as mildly painful stimuli inducing a short-lived localized unpleasant sensation on the wrist. In fact, only two 1.5-mA electric shocks were delivered in the entire experiment. Previously, we found that the threat of the shock, rather than the delivery of the shock, was responsible for the changes in light reflex amplitude (Bitsios *et al.*, 1996). Therefore, no shock was delivered in the first session. To restore threat in the second session, it was emphasized to each subject that 'although shock delivery had been judged unnecessary the first time, one to three electric shocks' as previously instructed, 'would now definitely occur'. One 1.5-mA shock was delivered at the end of the second session. Previously, we found that subjects did not judge a 1.5-mA shock to be painful (Bitsios *et*

*al.*, 1996). Therefore, in order to restore an effective threat in the third session, subjects were informed that on this occasion the shock(s) would be at least 50 times stronger than the shock they had received in the second session; in fact, no further shock was administered.

### Data reduction and analysis

#### *Acoustic startle reflex*

The latency and amplitude of the EMG response to each acoustic stimulus were measured using Spike-2 software (Cambridge Electronic Design Ltd, UK). Trials in which the apparent response had an onset latency of less than 10 ms from the stimulus onset and/or a rise time greater than 95 ms were discarded (Grillon *et al.*, 1991). The latency and amplitude of the EMG responses were averaged across all the trials under the SAFE condition and under the THREAT condition.

#### *Pupillary light reflex*

The baseline pupil diameter before each light stimulus ('initial pupil diameter'), and the amplitude of each miotic response were measured using Spike-2 software (Cambridge Electronic Design Ltd, UK). Trials in which a spontaneous blink occurred during stimulus presentation were discarded. Initial pupil diameter and miotic response amplitude were averaged across all the trials under the SAFE condition and under the THREAT condition.

#### *Subjective anxiety ratings*

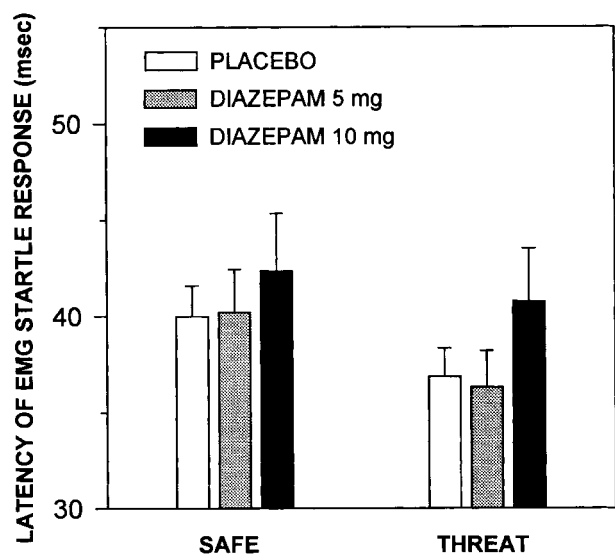
The mean verbal anxiety rating was calculated for the SAFE and THREAT blocks under each treatment condition.

#### *Subjective alertness ratings*

An 'alertness' score was derived for each subject under each treatment condition by multiplying the raw scores on the individual visual analogue scales by their respective loadings on the 'alertness' factor, as described by Bond and Lader (1974), and averaging the weighted scores (see Abduljawad *et al.*, 1997). The resulting 'alertness' score has a range 0–100.

#### *Statistical analyses*

The mean latencies and amplitudes of the EMG response, the mean initial pupil diameters, the mean amplitudes of the miotic responses, and the subjective anxiety ratings in the SAFE and THREAT conditions were analysed by two-factor analyses of variance (treatment  $\times$  condition) with repeated measures on both factors. In the case of a significant main effect of treatment or a significant interaction, comparisons between placebo and each dose of diazepam were made using Dunnett's test (d.f. = 22;  $k = 3$ ; criterion,  $p < 0.05$ ). In the case of the EMG and miotic response amplitudes and the subjective anxiety ratings, the effect of the THREAT was expressed as the difference between the amplitudes in the SAFE and THREAT conditions; these data were subjected to one-factor analyses of variance (treatment) with repeated measures, followed by comparisons between placebo and each dose of diazepam using Dunnett's test (d.f. = 22;  $k = 3$ ; criterion,  $p < 0.05$ ). 'Alertness' ratings were analysed by a one-factor analysis of variance (treatment) with repeated measures, followed by comparisons between placebo and each dose of



**Figure 1** Latencies of the electromyographic (EMG) acoustic startle response in the SAFE and THREAT conditions following each treatment. Columns show mean data; vertical bars indicate SEM ( $n = 12$ ). See text for statistical analysis.

diazepam using Dunnett's test (d.f. = 22;  $k = 3$ ; criterion,  $p < 0.05$ ).

**Results**

**Acoustic startle reflex**

The latencies of the EMG responses are shown in Fig. 1. Latency was shorter under the THREAT condition than under

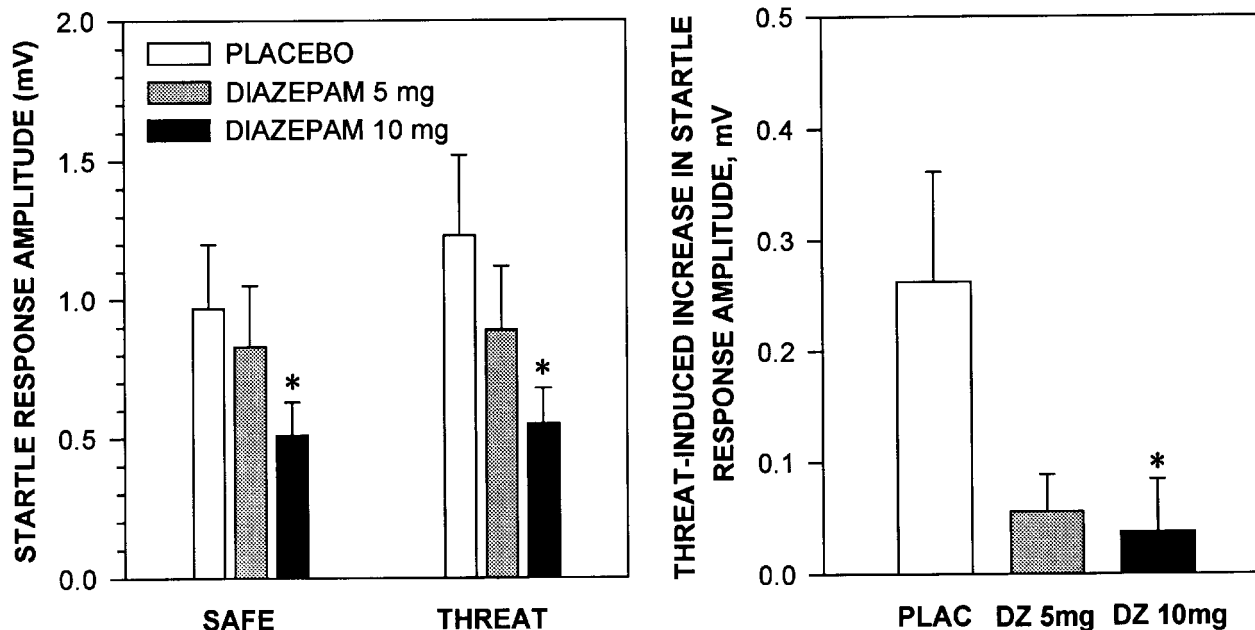
the SAFE condition, under all three treatments. Analysis of variance of these data revealed a significant main effect of condition [ $F(1,11) = 14.6$ ;  $p < 0.01$ ], but no significant main effect of treatment [ $F(2,22) = 1.5$ ;  $p > 0.1$ ] and no significant interaction [ $F(2,22) = 1.4$ ;  $p > 0.1$ ].

The amplitudes of the EMG responses are shown in Fig. 2 (left-hand panel). The responses were greater under the THREAT condition than under the SAFE condition. Under each condition, the response amplitude was smaller following treatment with diazepam than following treatment with placebo. Analysis of variance of these data revealed a significant main effect of condition [ $F(1,11) = 8.6$ ;  $p < 0.02$ ]; the main effect of treatment was 'borderline' [ $F(2,22) = 3.4$ ;  $p = 0.05$ ], and there was a significant interaction [ $F(2,22) = 3.8$ ;  $p < 0.05$ ]. *Post hoc* comparisons using Dunnett's test revealed significant differences between the EMG response amplitudes following treatment with diazepam 10 mg and placebo ( $p < 0.05$ ), under both the SAFE and THREAT conditions.

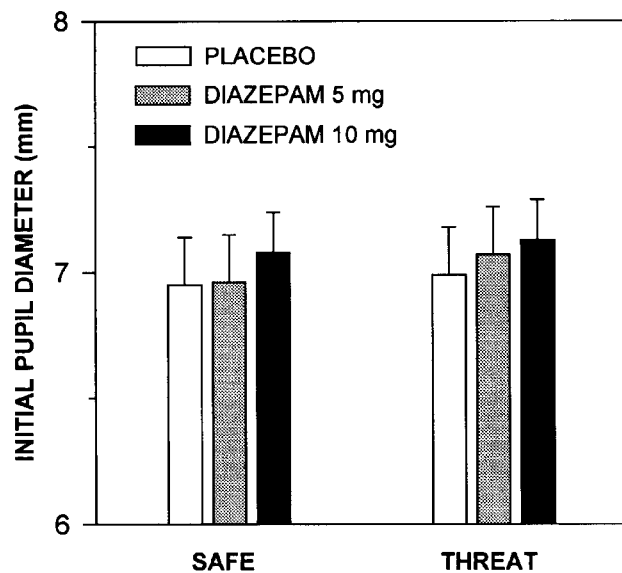
Figure 2 (right-hand panel) shows the SAFE-THREAT differences in startle response amplitude following each of the three treatments. The effect of THREAT was reduced following treatment with diazepam. Analysis of variance revealed a significant effect of treatment [ $F(2,22) = 3.8$ ;  $p < 0.05$ ]; *post hoc* comparisons revealed a significant difference between the diazepam 10 mg and placebo treatments ( $p < 0.05$ ).

**Pupillary measures**

The initial pupil diameters under the SAFE and THREAT conditions following the three treatments are shown in Fig. 3. Initial pupil diameter appeared to be somewhat larger under the THREAT than under the SAFE condition, under all three treatments. However, analysis of variance failed to reveal a significant main effect of treatment [ $F(2,22) = 1.1$ ;  $p > 0.1$ ] or



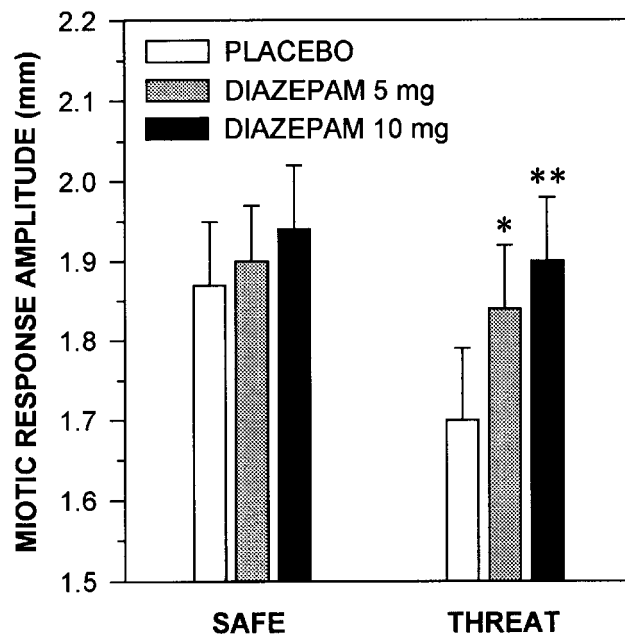
**Figure 2** Left-hand panel: Amplitudes of the electromyographic (EMG) acoustic startle response in the SAFE and THREAT conditions following each treatment. Right-hand panel: Threat-induced increase in EMG response amplitude (difference between amplitude of response in the SAFE and THREAT conditions) following each treatment. Columns show mean data; vertical bars indicate SEM ( $n = 12$ ). Significance of differences from corresponding placebo condition: \* $p < 0.05$  (see text for statistical analysis).



**Figure 3** Initial pupil diameters in the SAFE and THREAT conditions following each treatment. Columns show mean data; vertical bars indicate SEM ( $n=12$ ). See text for statistical analysis.

condition [ $F(1,11)=2.1$ ;  $p>0.1$ ], or a significant interaction [ $F(2,22)=1.2$ ;  $p>0.1$ ].

Figure 4 (left-hand panel) shows the light reflex amplitude data. The amplitude of the miotic response was smaller under the THREAT condition than under the SAFE condition, and diazepam reduced the size of the response in the THREAT condition in a dose-dependent fashion. Analysis of variance of these data revealed significant main effects of treatment [ $F(2,22)=7.8$ ;  $p<0.01$ ] and condition [ $F(1,11)=10.1$ ;



**Figure 4** Left-hand panel: Amplitudes of the miotic responses to light stimulation in the SAFE and THREAT conditions following each treatment. Right-hand panel: Threat-induced decrease in miotic response amplitude (difference between amplitude of response in the SAFE and THREAT conditions) following each treatment. Columns show mean data; vertical bars indicate SEM ( $n=12$ ). Significance of differences from corresponding placebo condition: \* $p<0.05$ , \*\* $p<0.02$ . See text for statistical analysis.

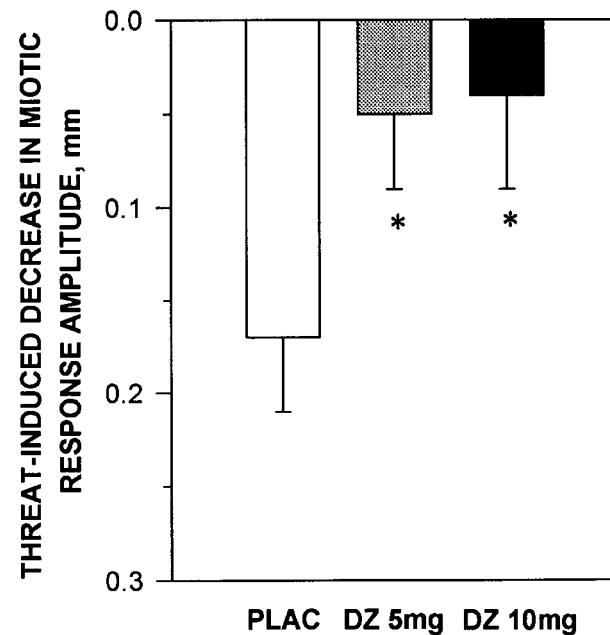
$p<0.01$ ]; the interaction term was of 'borderline' significance [ $F(2,22)=2.9$ ;  $p=0.07$ ]. *Post hoc* comparisons showed that both doses of diazepam were associated with a significant increase in the response amplitude under the THREAT condition ( $p<0.05$ ) but not under the SAFE condition.

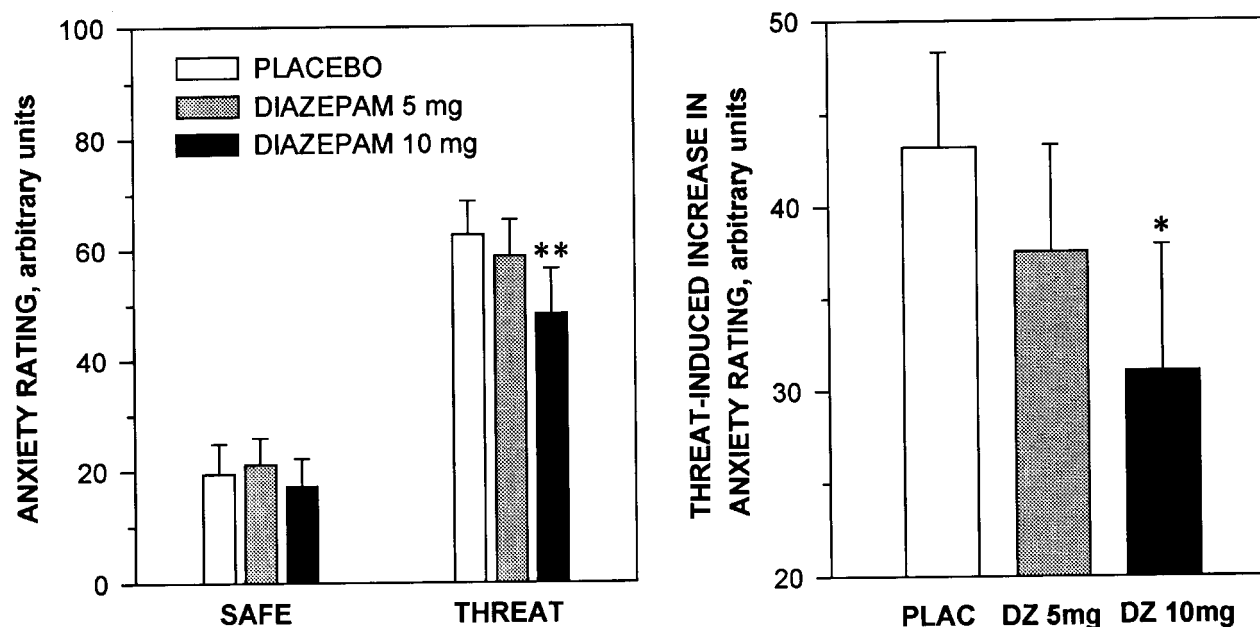
Figure 4 (right-hand panel) shows the SAFE-THREAT differences in light reflex amplitude following each of the three treatments. The effect of THREAT was reduced following treatment with diazepam. Analysis of variance revealed that the effect of treatment was of 'borderline' significance [ $F(2,22)=2.9$ ;  $p=0.07$ ]; *post hoc* comparisons revealed a significant difference between the placebo treatment and both the diazepam 5 mg and diazepam 10 mg treatments ( $p<0.05$ ).

#### Anxiety ratings

Figure 5 (left-hand panel) shows the anxiety self-ratings. 'Anxiety' scores were greater under the THREAT condition than under the SAFE condition, and diazepam treatment decreased 'anxiety' in the THREAT condition in a dose-dependent fashion. Analysis of variance of these data revealed significant main effects of treatment [ $F(2,22)=6.1$ ;  $p<0.01$ ] and condition [ $F(1,11)=44.9$ ;  $p<0.001$ ], and a significant interaction [ $F(2,22)=5.3$ ;  $p<0.02$ ]. *Post hoc* comparisons showed that only treatment with diazepam 10 mg was associated with a significant decrease in 'anxiety' under the THREAT condition, compared to treatment with placebo ( $p<0.05$ ).

Figure 5 (right-hand panel) shows the SAFE-THREAT differences in self-rated anxiety following each of the three treatments. The effect of THREAT was reduced following treatment with diazepam. Analysis of variance revealed that





**Figure 5** Left-hand panel: Self-rated 'Anxiety' scores in the SAFE and THREAT conditions following each treatment. Right-hand panel: Threat-induced increase in 'Anxiety' rating (difference between amplitude of response in the SAFE and THREAT conditions) following each treatment. Columns show mean data; vertical bars indicate SEM ( $n=12$ ). Significance of difference from corresponding placebo condition: \* $p<0.05$ , \*\* $p<0.02$ . See text for statistical analysis.

the effect of treatment was statistically significant [ $F(2,22)=5.3$ ;  $p=0.02$ ]; *post hoc* comparisons revealed a significant difference between the diazepam 10 mg treatment and placebo treatment ( $p<0.05$ ).

#### Alertness ratings

The group mean ( $\pm$ SEM) values of the 'alertness' factor were  $43.6 \pm 1.9$  (placebo),  $32.8 \pm 2.9$  (diazepam 5 mg) and  $33.7 \pm 2.7$  (diazepam 10 mg). Analysis of variance revealed a significant effect of treatment [ $F(2,22)=5.8$ ;  $p=0.01$ ]; *post hoc* comparisons revealed significant differences between the diazepam 5 mg and diazepam 10 mg treatments and the placebo treatment ( $p<0.05$ ).

#### Discussion

In this experiment, we adapted the protocol used in our previous investigations of the 'fear-inhibited light reflex' (Bitsios *et al.*, 1996, 1998) to enable this reflex to be recorded simultaneously with the acoustic startle reflex in healthy volunteers. Although the simultaneous recording of the two reflexes did present some minor technical difficulties, none of these was insurmountable. For example, adequate separation of light and sound stimulation was obviously required in order to ensure that the application of the sound stimulus and the occurrence of the startle eyeblink response did not conflict with the following pupillary light reflex trial. Furthermore, in our experience, variable intertrial intervals are advantageous in the case of the acoustic startle reflex, which is highly susceptible to habituation (Grillon *et al.*, 1991; Cadenhead *et al.*, 1993; Kumari *et al.*, 1996; Abduljawad *et al.*, 1997), whereas a constant intertrial interval maintains better consistency of the

pupillary light reflex (e.g. Bitsios *et al.*, 1996); these considerations could be accommodated in the sequence of trials used in each block. Some subjects found the combination of EMG electrodes, headphones and sustained immobility in the ophthalmic head-rest somewhat uncomfortable, and it is doubtful whether a recording period much longer than 12 min would have been tolerable to most of our subjects.

The THREAT condition was evidently successful in inducing subjective anxiety, as indicated by the significant increase in numerical 'anxiety' scores generated by the subjects. Similar findings have been reported by other workers using comparable 'threatening' conditions (Grillon *et al.*, 1991; Bitsios *et al.*, 1996, 1998; Patrick *et al.*, 1996). Bitsios *et al.* (1996) used the 'State' component of the State-Trait Anxiety Inventory (STAI-S; Spielberger, 1983) and a battery of visual analogue scales (Bond and Lader, 1974) to assess changes in subjective anxiety in response to the THREAT condition. In the present experiment, a single numerical verbal self-report scale was used for practical reasons (the need to avoid removing the subject's head from the ophthalmic head-rest after each block of trials). The results indicate that this very simple approach was adequate to reveal the anxiogenic effect of the THREAT condition.

In agreement with numerous previous studies with human subjects (Grillon *et al.*, 1991, 1993; Morgan *et al.*, 1995; Patrick *et al.*, 1996), the amplitude of the EMG startle response was enhanced during anticipation of electric shock. This was accompanied by a small but significant reduction in the latency of the response. The same THREAT condition was also associated with a significant suppression of the amplitude of the miotic response to light, confirming our previous findings with this method (Bitsios *et al.*, 1996, 1998).

In previous experiments, we have observed time-dependent reductions of the amplitude of the startle response (Abduljawad *et al.*, 1997) and time-dependent increases in the amplitude of the light reflex (Bitsios *et al.*, 1998); neither of these time-dependent effects was influenced by diazepam (Abduljawad *et al.*, 1997; Bitsios *et al.*, 1998). The time-dependent change in startle response amplitude is usually ascribed to habituation (e.g. Davis *et al.*, 1977); however, the origin of the time-dependent change in light reflex amplitude is uncertain (for a discussion, see Bitsios *et al.*, 1996). In the present experiment, we used relatively short recording periods (12 min) and relatively few trials in each session (24 acoustic stimuli and 18 light stimuli), in order to minimize the influence of such time-dependent changes.

In agreement with our previous findings using the present protocol (Bitsios *et al.*, 1998), diazepam (5 and 10 mg) attenuated the anxiogenic effect of the THREAT condition. This is consistent with results obtained with other 'anxiety models', confirming that the anxiolytic effect of single acute doses of benzodiazepines can be detected in normal subjects subjected to anxiety-provoking situations under laboratory conditions (e.g. McNair *et al.*, 1982; Guimarães *et al.*, 1987).

Diazepam significantly reduced the amplitude of the EMG response, and in addition attenuated the potentiation of the response induced by the THREAT condition. The suppression of the baseline startle response seen in this experiment is consistent with our previous experience with diazepam (Abduljawad *et al.*, 1997), but differs from the findings of Patrick *et al.* (1996), who found that diazepam (10 mg and 15 mg) blocked fear-potentiation without affecting the baseline startle response. There are a number of methodological differences between our experiments and the study of Patrick *et al.* (1996). For example, Patrick *et al.* (1996) used a between-groups experimental design, in contrast to our use of within-subjects designs. However, it is not immediately clear why such differences should have contributed to the different findings. Another, admittedly speculative, explanation is that the different findings may reflect differences between the subject samples used in the two studies. It is noteworthy that the EMG responses recorded in the present experiment were considerably larger than those recorded by Patrick *et al.* (1996). It is possible that in our subjects, the startle reflex was to some extent 'potentiated' even in the SAFE condition (perhaps due to anxiety occasioned by the whole experimental context), and that the effect of diazepam on the baseline startle response reflected suppression of this potentiation. In this context, it may be noted that exaggerated baseline startle responses as well as enhanced fear-potentiation have been found in patients suffering from some clinical anxiety disorders (post-traumatic stress disorder: Butler *et al.*, 1990; Orr *et al.*, 1995; Morgan *et al.*, 1996; Shalev *et al.*, 1997).

In agreement with our previous finding (Bitsios *et al.*, 1996), diazepam attenuated the 'fear-inhibited' pupillary light reflex. In contrast to its effects on the acoustic startle reflex, diazepam in the doses used in this experiment blocked the threat-induced change in the miotic response without affecting the baseline response.

It is an intriguing possibility that similar mechanisms may underlie the fear-inhibited light reflex and the fear-potentiated

startle reflex. There is now a large body of evidence showing that the potentiation of the startle reflex is mediated by the amygdala, a structure implicated in fear and anxiety (see Davis, 1992). The central nucleus of the amygdala, which has direct neural connections with the nucleus reticularis pontis caudalis (an obligatory point of the startle reflex pathway), has been especially implicated in the potentiation of the startle reflex (see Davis, 1992). Thus, lesions of the central nucleus of the amygdala block the fear-potentiated startle reflex, without affecting the baseline startle reflex (Hitchcock and Davis, 1986, 1991).

It is known that the pupillary light reflex is under tonic inhibitory control from the hypothalamus (Loewenfeld, 1958, 1993a) via connections between the hypothalamus and the Edinger–Westphal nucleus (Saper *et al.*, 1976). It is known that there are excitatory amygdalo–hypothalamic connections (Le Doux *et al.*, 1988; Davis, 1992; Falls and Davis, 1995), activation of which may result in enhanced inhibition of the Edinger–Westphal nucleus. This mechanism may account for the finding that stimulation of the amygdala causes pupillary dilatation in the cat (Koikegami and Yoshida, 1953; de Molina and Hunsberger, 1962). It is thus possible that stimulation of the amygdala by conditioned aversive stimuli enhances the inhibitory influence of the hypothalamus on the Edinger–Westphal nucleus, resulting in enhancement of the inhibition of the pupillary light reflex.

While the hypothalamus may play an important role in mediating the effect of fear on the pupillary light reflex, it is unlikely to be involved in the fear-potentiated startle, since destruction of amygdalo–hypothalamic connections does not disrupt this response (Davis *et al.*, 1993). If diazepam's effects on the fear-inhibited light reflex and the fear-potentiated startle reflex are mediated by an action on a single neural structure, this structure is likely to be within the amygdaloid complex, since lesions of the central nucleus of the amygdala do prevent expression of the fear-potentiated startle response (Davis *et al.*, 1993). However, the present results, and other findings based on systemic drug treatment, cannot exclude the possible involvement of multiple sites of action of diazepam in mediating the suppression of 'fear-related' behaviours.

In conclusion, the present results showed that experimentally induced anxiety in normal human subjects was accompanied by potentiation of the acoustic startle response and suppression of the pupillary light reflex. Both these effects were sensitive to the anxiolytic diazepam, supporting the notion that both effects may have utility as laboratory models of human anxiety. Future investigation of the comparative pharmacological sensitivity of these two responses may help to shed further light on the mechanisms underlying the somatic manifestations of anxiety in man.

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## References

- Abduljawad KA J, Langley RW, Bradshaw CM, Szabadi E (1997) Effects of clonidine and diazepam on the acoustic startle response and on its inhibition by 'prepulses' in man. *J Psychopharmacol* 11: 29–34
- Abduljawad KA J, Langley RW, Bradshaw CM, Szabadi E (1998) Effects of bromocriptine and haloperidol on prepulse inhibition of the acoustic startle response in man. *J Psychopharmacol* 12: 239–245
- Baldessarini RJ (1990) Drugs and the treatment of psychiatric disorders. In Goodman Gilman A, Goodman LS, Rall TW, Murad F (eds), *The pharmacological basis of therapeutics*, 7th edn. Macmillan, New York, pp. 387–445
- Bitsios P, Szabadi E, Bradshaw CM (1996) The inhibition of the pupillary light reflex by the threat of an electric shock: a potential laboratory model of human anxiety. *J Psychopharmacol* 10: 279–287
- Bitsios P, Szabadi E, Bradshaw CM (1998) The sensitivity of the fear-inhibited light reflex to diazepam. *Psychopharmacology* 135: 93–98
- Bond A J, Lader MH (1974) The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47: 211–218
- Bradley M M, Cuthbert B N, Lang P J (1990) Startle reflex modification: emotion or attention? *Psychophysiology* 27: 513–522
- Braff D L, Stone C, Callaway E, Geyer M A, Glick I, Bali L (1978) Pre-stimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 15: 339–343
- Butler R W, Braff D L, Rausch J L, Jenkins M A, Sprock J, Geyer M A (1990) Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. *Am J Psychiatry* 147: 1308–1312
- Cadenhead K S, Geyer M A, Braff D L (1993) Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. *Am J Psychiatry* 150: 1862–1867
- Cooper S J (1995) Anxiolytics, sedatives and hypnotics. In King D J (ed.), *Seminars in clinical psychopharmacology*. Gaskell, London, pp. 103–137
- Davis M (1979) Diazepam and flurazepam: effects on conditioned fear as measured with the potentiated startle paradigm. *Psychopharmacol* 62: 1–7
- Davis M (1992) The role of the amygdala in conditioned fear. In Aggleton J (ed.), *The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction*. Wiley-Liss, New York, pp. 255–305
- Davis M, Cederbaum J M, Aghajanian G K, Gendelman D S (1977) Effects of clonidine on habituation and sensitization of the acoustic startle in normal, decerebrate and locus coeruleus lesioned rats. *Psychopharmacol* 51: 243–253
- Davis M, Gendelman D S, Tischler M, Gendelman P M (1982) A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci* 6: 791–808
- Davis M, Falls W A, Campeau S, Kim M (1993) Fear-potentiated startle: a neural and pharmacological analysis. *Behav Brain Res* 58: 175–198
- de Molina A F, Hunsberger R W (1962) Organisation of the subcortical system governing defence and flight reactions in the cat. *J Physiol* 160: 200–213
- Falls W A, Davis M (1995) Lesions of the central nucleus of the amygdala block conditioned excitation, but not conditioned inhibition of fear as measured with the fear-potentiated effect. *Behav Neurosci* 109: 379–387
- Grillon C, Ameli R, Woods S W, Mericangas K, Davis M (1991) Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology* 28: 588–595
- Grillon C, Ameli R, Foot M, Davis M (1993) Fear-potentiated startle: relationship to the level of state/trait anxiety in healthy subjects. *Biol Psychiatry* 33: 566–574
- Guimarães F S, Zuardi A W, Graeff F G (1987) Effect of chlorimipramine and maprotiline on experimental anxiety in humans. *J Psychopharmacol* 1: 184–192
- Hamm A O, Greenwald M K, Bradley M M, Lang P J (1993) Emotional learning, hedonic change, and the startle probe. *J Abnorm Psychol* 102: 453–465
- Hitchcock J M, Davis M (1986) Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behav Neurosci* 100: 11–22
- Hitchcock J M, Davis M (1991) The efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behav Neurosci* 105: 826–844
- Koikegami H, Yoshida K (1953) Pupillary dilation induced by stimulation of amygdaloid nuclei. *Folia Psychiatr Neurolog Jpn* 7: 109–125
- Koss M C (1986) Pupillary dilation as an index of central nervous system  $\alpha_2$ -adrenoceptor activation. *J Pharmacol Meth* 15: 1–19
- Koss M C, Gherezghiher T, Nomura A (1984) CNS adrenergic inhibition of parasympathetic oculomotor tone. *J Auton Nerv Syst* 10: 55–68
- Kumari V, Cotter P, Corr P J, Gray J A, Checkley S A (1996) Effect of clonidine on the human acoustic startle reflex. *Psychopharmacology* 123: 353–360
- LeDoux J E, Iwata J, Cicchetti P, Reis D J (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci* 8: 2517–2529
- Loewenfeld I E (1958) Mechanisms of reflex dilatation of the pupil. *Documenta Ophthalmol* 12: 185–448
- Loewenfeld I E (1993a) Reflex integration: physiologic mechanisms. In *The pupil*. Iowa State University Press/Amos Wayne State University Press, Detroit, pp. 407–480
- Loewenfeld I E (1993b) Pupillary pharmacology. In *The pupil*. Iowa State University Press/Amos Wayne State University Press, Detroit, pp. 683–827
- McNair D M, Frankenthaler I M, Czerlinsky T, White T W, Sasson S, Fisher S (1982) Simulated public speaking as a model of clinical anxiety. *Psychopharmacology* 77: 7–10
- Morgan C A, Grillon C, Southwick S M, Davis M, Charney D S (1995) Fear-potentiated startle in posttraumatic stress disorder. *Biol Psychiatry* 38: 378–385
- Morgan C A, Grillon C, Southwick S M, Davis M, Charney D S (1996) Exaggerated acoustic startle reflex in Gulf War veterans with posttraumatic stress disorder. *Am J Psychiatry* 153: 64–68
- Norris H (1971) The action of sedation on brain-stem oculomotor systems in man. *Neuropharmacology* 10: 181–191
- Orr S P, Lasko N, Shalev A, Pitman R K (1995) Physiologic responses to loud tones in Vietnam veterans with PTSD. *J Abnorm Psychol* 104: 75–82

- Patrick C J, Bertholt B D, Moore J D (1996) Diazepam blocks fear-potentiated startle in humans. *J Abnorm Psychol* 105: 89-96
- Saper C B, Loewy A D, Swanson L W, Cowan W M (1976) Direct hypothalamo-autonomic connections. *Brain Res* 117: 305-312
- Shalev A Y, Peri T, Orr S P, Bonne O, Pitman R K (1997) Auditory startle responses in help-seeking trauma survivors. *Psychiat Res* 69: 1-7
- Spielberger C D (1983) Manual for the state-trait anxiety inventory. Consulting Psychologists Press, Palo Alto, CA
- Szabadi E, Bradshaw C M (1996) Autonomic pharmacology of  $\alpha_2$ -adrenoceptors. *J Psychopharmacol* 10 (Suppl 3): 6-18
- Vrana S R, Spence E L, Lang P J (1988) The startle probe response: a new measure of emotion? *J Abnorm Psychol* 97: 487-491
- Yeomans J S, Frankland P W (1996) The acoustic startle reflex: neurons and connections. *Brain Res Rev* 21: 301-314