

# Journal of Psychopharmacology

<http://jop.sagepub.com>

---

## **Diazepam suppresses the acquisition but not the expression of 'fearpotentiation' of the acoustic startle response in man**

J. C. Scaife, R. W. Langley, C. M. Bradshaw and E. Szabadi  
*J Psychopharmacol* 2005; 19; 347  
DOI: 10.1177/0269881105053285

The online version of this article can be found at:  
<http://jop.sagepub.com/cgi/content/abstract/19/4/347>

---

Published by:



<http://www.sagepublications.com>

On behalf of:



[British Association for Psychopharmacology](#)

**Additional services and information for *Journal of Psychopharmacology* can be found at:**

**Email Alerts:** <http://jop.sagepub.com/cgi/alerts>

**Subscriptions:** <http://jop.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.co.uk/journalsPermissions.nav>

**Citations** <http://jop.sagepub.com/cgi/content/refs/19/4/347>

# Diazepam suppresses the acquisition but not the expression of 'fear-potential' of the acoustic startle response in man

*Journal of Psychopharmacology*  
19(4) (2005) 347–356  
© 2005 British Association  
for Psychopharmacology  
ISSN 0269-8811  
SAGE Publications Ltd,  
London, Thousand Oaks,  
CA and New Delhi  
10.1177/0269881105053285

J. C. Scaife *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

R. W. Langley *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

C. M. Bradshaw *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

E. Szabadi *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

## Abstract

Sudden auditory stimuli elicit a short-latency muscular response (acoustic startle response) which is enhanced during presentation of a Pavlovian conditioned stimulus (CS) that has previously been paired with an aversive unconditioned stimulus (US) ('fear-potential'). In rodents, acute treatment with benzodiazepines blocks both the acquisition of fear-potential and the expression of fear-potential induced by prior exposure to CS/US pairing. We examined the effect of diazepam on the acquisition and expression of fear-potential of the acoustic startle response in man. Forty-six male volunteers (18–30 years) participated in two sessions separated by 7 days. In session 1, they were exposed to 20 2-s presentations of a light (CS), 50% of which terminated with an electric shock to the wrist (1.8 mA, 50 ms; US). Somatosensory potentials evoked by the US were recorded from the scalp at Cz, and skin conductance responses from electrodes taped to the second and fourth fingers. In session 2, the CS was presented 20 times without the US; a random 50% of CS presentations terminated with a sound pulse (40-ms 115-dB 1-kHz); an equal number of sound pulses was presented without the CS. Electromyographic responses of the orbicularis oculi muscle to the acoustic stimuli were recorded from electrodes placed on the lower eyelid, late-latency auditory evoked potentials were recorded at Cz, and skin conductance responses from electrodes taped to the second and

fourth fingers. In each session, alertness was measured using visual analogue self-rating scales and critical flicker fusion frequency. Subjects received placebo or diazepam 10 mg in the two sessions in a double-blind protocol: group 1 ( $n = 12$ ) placebo/placebo; group 2 ( $n = 11$ ) placebo/diazepam; group 3 ( $n = 12$ ) diazepam/placebo; group 4 ( $n = 11$ ) diazepam/diazepam. Diazepam reduced alertness as measured by visual-analogue self-rating scales and critical flicker fusion frequency. In session 1, diazepam reduced the amplitude of the somatosensory potentials and skin conductance responses evoked by the CS. In session 2, the acoustic startle response, the N1/P2 auditory evoked response and the skin conductance response evoked by the sound stimuli were enhanced in the presence of the CS. This fear-potential was attenuated in subjects who received diazepam in session 1, but was not affected by the treatment given in session 2. The results indicate that diazepam blocks the acquisition of fear-potential of startle responses in man, as in animals, but does not prevent the expression of a previously learned response.

## Keywords

startle reflex, diazepam, fear-potentiated startle, Pavlovian conditioning

## Introduction

Sudden intense auditory stimuli elicit a sequence of involuntary responses (startle responses). The initial short-latency response, which consists of contraction of the skeletal and facial musculature, is believed to be mediated by two or three synaptic relays between the sensory receptors and the motoneurons, the principal

relay being the caudal pontine reticular nucleus (for review, see Koch, 1999). This response may be recorded in man as the electromyographic (EMG) response of the orbicularis oculi muscle. The fast component is followed by slow autonomic responses, including a rise in skin conductance, which is maximal approximately 6 s after the acoustic stimulus (Turpin *et al.*, 1999; Graham *et al.*, 2005).

*Correspondence author:* C. M. Bradshaw, Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Floor B, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK. Email: c.m.bradshaw@nottingham.ac.uk

In both animals and man, the EMG startle response is enhanced during anticipation of an aversive event, such as a mild electric shock, a phenomenon known as fear-potentiated startle (Davis *et al.*, 1993). Two procedures have been used to elicit the fear-potentiated startle: (i) exposure to an experimental situation ('context') in which the aversive event has previously been experienced; (ii) presentation of a discrete stimulus (conditioned stimulus, CS) that has previously been paired with the aversive event (unconditioned stimulus, US) in a Pavlovian conditioning protocol. Both procedures are effective in potentiating the short-latency startle response in man (e.g. Grillon *et al.*, 1991; Lipp *et al.*, 1994; Bitsios *et al.*, 1999; Baas *et al.*, 2002).

Anxiolytic benzodiazepines have been found to attenuate fear potentiation of the acoustic startle response in some human volunteer studies (Patrick *et al.*, 1996; Bitsios *et al.*, 1999; Graham *et al.*, 2005), but not in others (Baas *et al.*, 2002). Baas *et al.* (2002) have proposed that the apparent inconsistency between different studies may arise from the differential sensitivities of contextual and 'cue-specific' fear conditioning to benzodiazepines, the former, but not the latter, being suppressed by these drugs.

The present experiment further explored the effect of a benzodiazepine, diazepam, on the fear-potentiated startle response in man. The particular question addressed in this experiment was whether diazepam would block the *acquisition* of fear-potentiation, and/or the *expression* of fear-potentiation evoked by a previously conditioned CS. There is evidence that benzodiazepines can disrupt some forms of associative learning both in animals (Harris and Westbrook, 1996) and in man (Gorissen and Eling, 1998; Curran, 1999); however there do not appear to have been any previous studies of the effects of benzodiazepines on the acquisition of fear-potentiation of startle responses in man. It was predicted that acute treatment with a benzodiazepine would attenuate the acquisition of fear-potentiation; however, on the basis of Baas *et al.*'s (2002) proposal that cue-specific fear may be relatively insensitive to benzodiazepines, it was anticipated that the benzodiazepine would be ineffective in suppressing the expression of previously established fear-potentiation.

In addition to the EMG and skin conductance startle responses, we also recorded a late-latency component of the auditory evoked potential, the N1/P2 complex. This potential can be evoked by low-intensity auditory stimuli that are too weak to elicit the EMG startle response (Lewine *et al.*, 2002); therefore it is not considered to be part of the startle cascade. There is evidence that the N1/P2 response, like the EMG startle response, is subject to modulation by prior stimulus presentation (prepulse inhibition: Abduljawad *et al.*, 1999; Graham *et al.*, 2001; 2002; 2004; 2005). We were interested in whether the N1/P2 auditory evoked potential might also be susceptible to modulation by Pavlovian fear conditioning. It has already been established that visual evoked potentials can be classically conditioned (Skrandies and Jedynak, 2000); however, to our knowledge, there have been no previous investigations of the susceptibility of auditory evoked potentials to fear conditioning.

## 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

Forty-six healthy male volunteers aged 18–30 years (mean  $\pm$  s.d.:  $20.8 \pm 3.0$  years) and weighing 47–100 kg ( $73.6 \pm 11.5$  kg) participated in the study. (Forty-eight subjects were initially recruited; however electrophysiological data were lost for two subjects in one session, and these were subsequently excluded from all analyses.) The subjects were recruited by advertisement, and gave their written informed consent before inclusion in the study; they received remuneration for their participation in the experiment at a level approved by the Ethics Committee. Before entering the study, each subject underwent a medical interview, a physical examination and a hearing test. Subjects were excluded if they had a history of any psychiatric or neurological disease, or a hearing threshold above 20 dB[A]. None of the subjects had a hearing threshold above 10 dB[A] at 1 kHz. Three subjects declared themselves to be 'light smokers' (<5 cigarettes a day); the others were non-smokers. All subjects undertook to abstain from alcohol for 48 h before and 24 h after each session. Smoking and the consumption of caffeine-containing beverages or solid food were prohibited during the experimental sessions, and consumption of caffeine-containing beverages was also prohibited during the 24 h preceding each experimental session.

## Treatments and design

Before the start of the experiment, each subject participated in a training session in which his hearing threshold was assessed, and the procedures explained and demonstrated. The experiment consisted of two 4-h sessions, one week apart. The subjects were randomly allocated to four groups that received different treatments in the two sessions. Group 1 ( $n = 12$ ) received placebo in both sessions; Group 2 ( $n = 11$ ) received placebo in session 1 and diazepam 10 mg in session 2; Group 3 ( $n = 12$ ) received diazepam 10 mg in session 1 and placebo in session 2; and Group 4 ( $n = 11$ ) received diazepam 10 mg in both sessions. There were no significant differences between the ages or body weights of the subjects in the four groups. The treatments were administered orally in matching capsules according to a double-blind protocol.

## Tests and apparatus

Recordings took place in the same room in both sessions. The subject was seated in a comfortable arm-chair.

**'Fear conditioning' stimuli (session 1)** The CS was a 2-s presentation of red light (2.5 W) delivered by a light-emitting diode placed 2 m directly in front of the subject. The US was an electric shock (constant current square pulse, 50 ms, 1.8 mA) delivered via silver/silver chloride electrodes to the skin overlying the median nerve of the left wrist.

**Acoustic stimuli (session 2)** Acoustic stimuli were generated by a Kamplex AC30 clinical audiometer (PC Werth Ltd, London,

UK) and were presented to the subject binaurally. A background 70-dB[A] 1-kHz tone was presented throughout the recording period. The sound pulses consisted of 40-ms, 1-kHz tones of intensities 115 dB[A].

**Electrophysiological recording** *EMG recording.* 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+ 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). *Skin conductance recording.* Recordings were made via two 8-mm diameter silver-silver chloride surface electrodes taped to the terminal phalanges of the second and fourth digits of the right hand. Skin conductance was monitored continuously via an CED 2502-SA interface connected to the same CED 1401+ computer that was used to control the acoustic stimuli. *Recording of evoked potentials.* Both the somatosensory evoked potentials (session 1) and auditory evoked potentials (session 2) were obtained using single channel recordings from the scalp at the Cz (vertex) position. 0.5-cm disc electrodes were placed on the scalp at Cz and the left mastoid, with the ground electrode placed on the forehead. A CED 1902 interface was used to record the potentials. The vertex potentials were displayed in a 'positive up' configuration.

**Critical flicker fusion frequency** A Flicker Fusion Monitor, model 1199 (System 696 Ltd, London, UK) was used. Subjects viewed the stimulus through a 2-mm 'artificial pupil'. Four measurements of the threshold were made, two with increasing and two with decreasing frequencies (see Graham *et al.*, 2004; 2005).

**Subjective ratings** Before treatment, and following the recording sessions, the subjects completed a battery of 16 100-mm visual analogue rating scales (Norris, 1971) presented on a computer monitor; the subjects moved the cursor to a position on the line that indicated their subjective state on each scale. The polarity of the scales was randomized between treatments to eliminate potential learning effects.

## Procedure

After arrival in the laboratory, subjects rested for 15 min before undergoing pre-treatment recordings of critical flicker fusion frequency and completing the visual analogue self-ratings; these tests took 15 min to complete. After completing the tests, the capsule was ingested, followed 60 min later by a recording session (see below). Following the recording session, subjects repeated the same tests as used in the pre-treatment assessment.

**'Fear conditioning' (session 1)** The 8.5-min recording session consisted of 20 trials. In ten trials, the CS and US were both presented, the US being delivered at the offset of the CS. In the

remaining ten trials, the CS was presented without the US. The CS/US and CS-alone trials occurred in a pseudo-random order with the constraint that there was one sequence of three CS-alone trials in succession during the session. (The reason for using a random 50% reinforcement schedule was to render it less likely that the subjects would deduce that no shocks were delivered in the testing session (session 2): see below.) Somatosensory evoked potentials and skin conductance responses to the US were recorded.

**Startle response recording (session 2)** The 10-min recording session started with a 180-s adaptation period in which the background sound was presented. A single 115-dB pulse was presented (the response to this initial pulse was discarded). The remainder of the recording period consisted of 24 trials, separated by inter-trial intervals varying between 15 and 35 s (mean 25 s). The stimuli presented in the trials were: (i) a single 40 ms, 115-dB sound pulse (startle alone); (ii) a 2-s presentation of the CS (CS alone); and (iii) a 2-s presentation of the CS immediately followed by a 40–115-dB sound pulse (CS/startle). There were eight trials of each type, presented in a pseudo-random sequence. EMG, skin conductance and auditory evoked responses to the sound pulse were recorded.

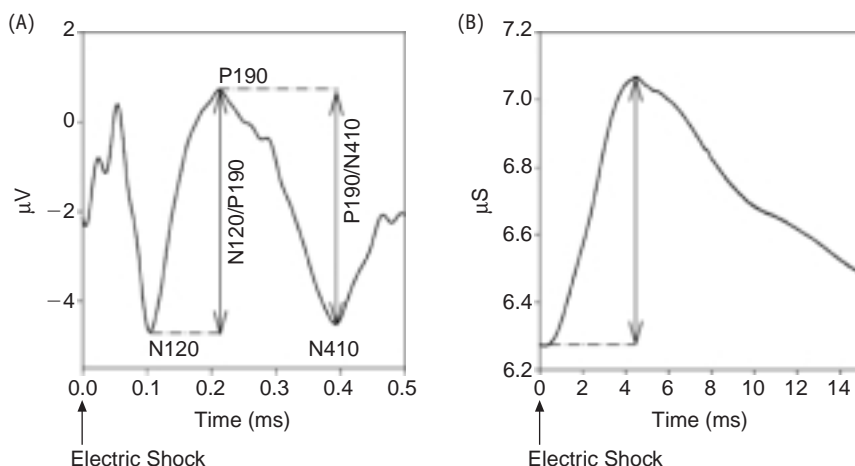
## Data analysis

**Session 1** The subjects were divided according to the treatment received in session 1 (Groups 1 and 2 received placebo, and Groups 3 and 4 diazepam 10 mg). Comparisons between placebo- and diazepam-treated subjects were made using Student's *t*-test (criterion,  $p < 0.05$ ).

The somatosensory evoked potentials recorded following the shock stimulus were averaged across the ten trials in which the shock was delivered using Spike-2 software (Cambridge Electronic Design Ltd, Cambridge, UK). The following components of the evoked responses were identified from the averaged recordings (values in parentheses indicate the range of latencies to the peak of the wave following stimulus onset): N120 (60–180 ms), P190 (160–240) (Allison *et al.*, 1992), N410 (370–480). The amplitude of the N120/P190 complex was measured as the amplitude difference from the peak of the N120 wave to the peak of the P190 wave (Thrauf *et al.*, 1994). In the same manner, the amplitude of the P190/N410 complex was measured as the amplitude difference from the peak of the P190 wave to the peak of the N410 wave (see Fig. 1a).

The changes in skin conductance evoked by the electric shock were measured from the onset of the response to the maximum conductance achieved within 10 s of the stimulus delivery. Changes in skin conductance following the CS in CS-alone trials were measured in the same way.

Pre-/post-treatment changes in 'alertness' were obtained from the weighted change scores on the individual visual-analogue scales using the factor weightings derived by Bond and Lader (1974) (Phillips *et al.*, 2000). Pre-/post-treatment changes were also calculated for the critical flicker fusion frequency.



**Figure 1** Measurement of somatosensory potential and skin conductance response evoked by the US. The traces show responses of one placebo-treated subject, averaged across ten US presentations. (A) Somatosensory evoked potential recorded at Cz; arrows indicate amplitudes of N120/P190 and P190/N410 potentials. (B) Skin conductance response recorded from second and fourth digits of the right hand; arrow indicates response amplitude

**Session 2** Pre-/post-treatment changes in 'alertness' and critical flicker fusion frequency were analysed in the same way as in session 1. The other measures (see below) were analysed as follows. An initial analysis of the EMG response data in the individual CS and no-CS trials of session 2 indicated that there was a diminution of the CS-induced increase in response amplitude during the session (CS/no-CS  $\times$  time:  $F(7,308) = 2.6$ ;  $p < 0.02$ ). It was considered likely that this represented extinction of conditioned fear-potentiation due to the lack of US presentation in session 2. The stimulus presentation schedule in session 1 included three successive CS-alone trials, with the aim of delaying the subjects' awareness that the CS no longer signalled imminent shock delivery. Therefore, averaged recordings derived from the first half of the recording session were used in all subsequent analyses. Each measure was first subjected to a three-factor analysis of variance (stimulus condition [presence/absence of CS]  $\times$  session 1 treatment [placebo/diazepam]  $\times$  session 2 treatment [placebo/diazepam]) with repeated measures on the first factor (criterion,  $P < 0.05$ ). In the case of a significant effect of stimulus condition, separate two-factor analyses of variance (session 1 treatment  $\times$  session 2 treatment) were performed on the baseline response amplitude data, and on the CS/no-CS difference data. In the case of a significant interaction between the session 1 and session 2 treatment factors, multiple comparisons were made against Group 1 (placebo/placebo), using Dunnett's test (Winer, 1991).

The amplitudes of the EMG responses to the acoustic stimuli were measured using Spike-2 software (see Graham *et al.*, 2005). The mean amplitude was derived for the no-CS (sound pulse alone) trials and the CS (light + sound pulse) trials. The enhancement of the EMG response amplitude following the CS was calculated as CS/no-CS difference.

The auditory evoked potentials were measured using Spike-2 software. The following components of the evoked responses were identified from the averaged recordings (values in parentheses indicate the range of latencies to the peak of the wave following stimulus onset) N1 (70–150 ms) and P2 (140–220 ms). The amplitude of the N1/P2 complex was measured as the amplitude dif-

ference from the peak of the N1 wave to the peak of the P2 wave (Senkowski *et al.*, 2003) (see Fig. 1b).

The skin conductance responses to the acoustic stimuli were measured in the same way as the skin conductance responses to the US in session 1 (see above).

## Results

### Measures of arousal (sessions 1 and 2)

**Visual analogue scales** Table 1 shows the mean ( $\pm$ SEM) pre-treatment baseline values and pre-/post-treatment changes in 'alertness' for placebo- and diazepam-treated subjects in each session. There were no significant differences between the pre-treatment baseline values for either session [session 1:  $t(44) = 1.4$ ;  $p > 0.1$ ; session 2:  $t < 1$ ]. Diazepam reduced alertness, compared to placebo, both in session 1 [ $t(44) = 2.6$ ;  $p < 0.05$ ] and in session 2 [ $t(44) = 2.8$ ;  $p < 0.01$ ].

**Critical flicker fusion frequency** Table 1 shows the mean ( $\pm$ SEM) pre-treatment baseline values and pre-/post-treatment changes in flicker fusion threshold for the placebo- and diazepam-treated subjects in each session. There were no significant differences between the pre-treatment baseline values for either session [ $t < 1$  in each case]. Diazepam significantly reduced the threshold, compared to placebo, both in session 1 [ $t(44) = 3.9$ ;  $p < 0.001$ ] and in session 2 [ $t(44) = 4.0$ ;  $p < 0.001$ ].

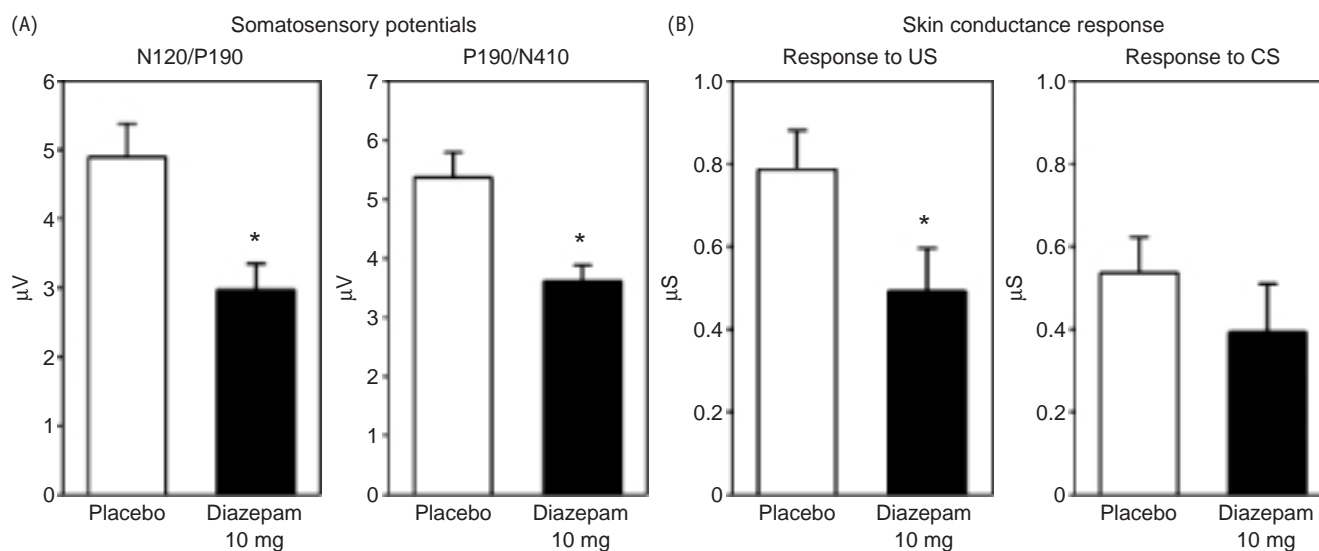
### Responses to the shock US (session 1)

**Somatosensory evoked potentials** Data from three subjects were excluded, as their recordings were contaminated by movement artefacts. Fig. 2A shows the mean ( $\pm$ SEM) amplitudes of the potentials evoked by the US under the two treatment conditions. Diazepam reduced the amplitude of both the N120/P190 [ $t(41) = 3.1$ ;  $p < 0.01$ ] and the P190/N410 responses [ $t(41) = 3.6$ ;  $p < 0.001$ ].

**Table 1** Mean ( $\pm$ SEM) pre-/post-treatment changes in 'alertness and critical flicker fusion frequency' (CFFF)

| Measure                | Session 1 (conditioning) |                |                              |                  | Session 2 (fear-potentiation testing) |                |                              |                  |
|------------------------|--------------------------|----------------|------------------------------|------------------|---------------------------------------|----------------|------------------------------|------------------|
|                        | Placebo (Groups 1, 2)    |                | Diazepam 10 mg (Groups 3, 4) |                  | Placebo (Groups 1, 3)                 |                | Diazepam 10 mg (Groups 2, 4) |                  |
|                        | Baseline                 | Change         | Baseline                     | Change           | Baseline                              | Change         | Baseline                     | Change           |
| 'Alertness' factor, cm | 48.6 $\pm$ 1.7           | -4.9 $\pm$ 2.2 | 44.7 $\pm$ 2.0               | -12.6 $\pm$ 2.0* | 45.4 $\pm$ 2.5                        | -5.0 $\pm$ 2.4 | 43.0 $\pm$ 2.2               | -17.4 $\pm$ 3.0* |
| CFFF, Hz               | 21.5 $\pm$ 0.4           | 0.0 $\pm$ 0.1  | 22.2 $\pm$ 0.3               | -1.6 $\pm$ 0.3*  | 21.5 $\pm$ 0.4                        | -0.3 $\pm$ 0.2 | 21.2 $\pm$ 0.3               | -1.6 $\pm$ 0.3*  |

Significantly different from corresponding change score for placebo-treated groups \*  $p < 0.05$  (see text for details).



**Figure 2** Somatosensory evoked potentials and skin conductance responses in session 1. (A) Amplitudes of N120/P190 and P190/N410 somatosensory evoked potentials evoked by the US ( $\mu V$ ); (B) Amplitudes of skin conductance responses evoked by the US and the CS ( $\mu S$ ). Columns show mean data; vertical bars indicate SEM. *Open columns*, placebo; *filled columns*, diazepam 10 mg. Significance of difference from placebo condition: \* $p < 0.05$  (see text for statistical analysis)

**Skin conductance response** Fig. 2B shows the mean ( $\pm$ SEM) amplitudes of the skin conductance response to the US under the two treatment conditions. Diazepam significantly reduced the response evoked by the US [ $t(44) = 2.1$ ;  $p < 0.05$ ].

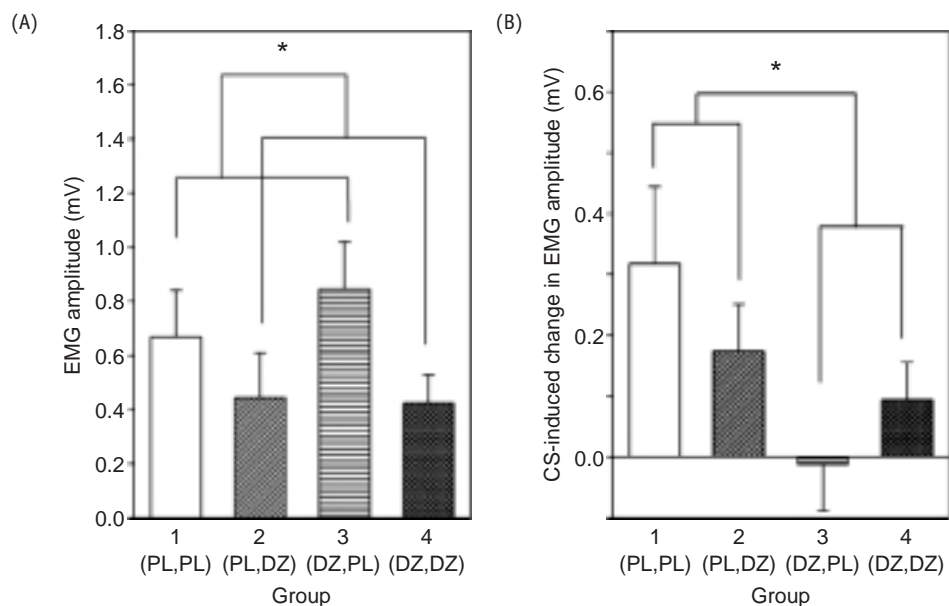
### Responses to the light CS (session 1)

The mean ( $\pm$ SEM) amplitudes of the skin conductance changes following CS presentation in the CS-alone trials are shown in the right-hand panel of Fig. 2B. Diazepam had no significant effect on response amplitude [ $t(44) = 1.0$ ;  $p > 0.2$ ].

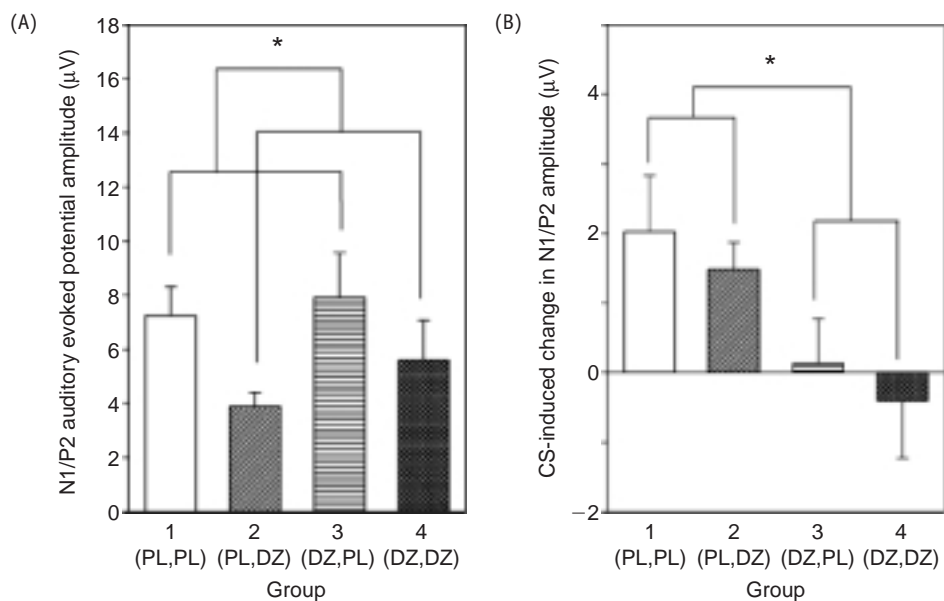
### Responses to the sound stimuli (session 2)

**EMG responses** The mean ( $\pm$ SEM) EMG responses in the absence of the CS are shown in the left-hand panel, and the

CS/no-CS amplitude differences in the right-hand panel of Fig. 3. The initial three-factor analysis of variance (CS condition  $\times$  session 1 treatment  $\times$  session 2 treatment, with repeated measures on the first factor) revealed a significant main effect of the CS condition [ $F(1,42) = 9.4$ ;  $p < 0.01$ ], reflecting an increase in response amplitude induced by the CS; there was no significant main effect of session 1 treatment [ $F < 1$ ], but the main effect of session 2 treatment was significant [ $F(1,42) = 4.1$ ;  $p < 0.05$ ]; there was a significant interaction between CS condition and session 1 treatment [ $F(1,42) = 4.7$ ;  $p < 0.05$ ], but not between CS condition and session 2 treatment [ $F < 1$ ]. The three-way interaction term was not statistically significant [ $F(1,42) = 2.1$ ;  $p > 0.1$ ]. Analysis of the simple main effects showed that the amplitude of the 'baseline' response measured in the absence of the CS was significantly reduced by diazepam administered in session 2 [ $F(1,42) = 4.1$ ;  $p < 0.05$ ], but was not affected by the



**Figure 3** EMG responses of orbicularis oculi muscle to sound pulses in session 2. (A) Baseline EMG response amplitudes (mV) in the absence of the CS. (B) CS-induced increases in response amplitude (mV). Columns show mean data for the four groups; vertical bars indicate SEM. The treatments given in the two sessions are indicated for each group (PL, placebo; DZ, diazepam 10 mg). Diazepam 10 mg administered in session 2 (Groups 2 and 4) significantly reduced baseline response amplitude; diazepam 10 mg administered in session 1 (Groups 3 and 4) significantly reduced the CS-induced increase in response amplitude:  $*p < 0.05$  (see text for statistical analysis)



**Figure 4** N1/P2 auditory evoked responses (Cz) evoked by sound pulses in session 2. (A) Baseline response amplitudes ( $\mu V$ ) in the absence of the CS. (B) CS-induced increase in response amplitude ( $\mu V$ ). Columns show mean data for the four groups; vertical bars indicate SEM; conventions as in Fig. 3. Diazepam 10 mg administered in session 2 (Groups 2 and 4) significantly reduced baseline response amplitude; diazepam 10 mg administered in session 1 (Groups 3 and 4) significantly reduced the CS-induced increase in response amplitude:  $*p < 0.05$  (see text for statistical analysis)

treatment administered in session 1 [ $F < 1$ ]. In contrast, the increase in response amplitude induced by the CS (CS/no-CS difference score) was significantly reduced by diazepam administered in session 1 [ $F(1,42) = 4.7$ ;  $p < 0.05$ ], but was not affected by the treatment administered in session 2 [ $F < 1$ ].

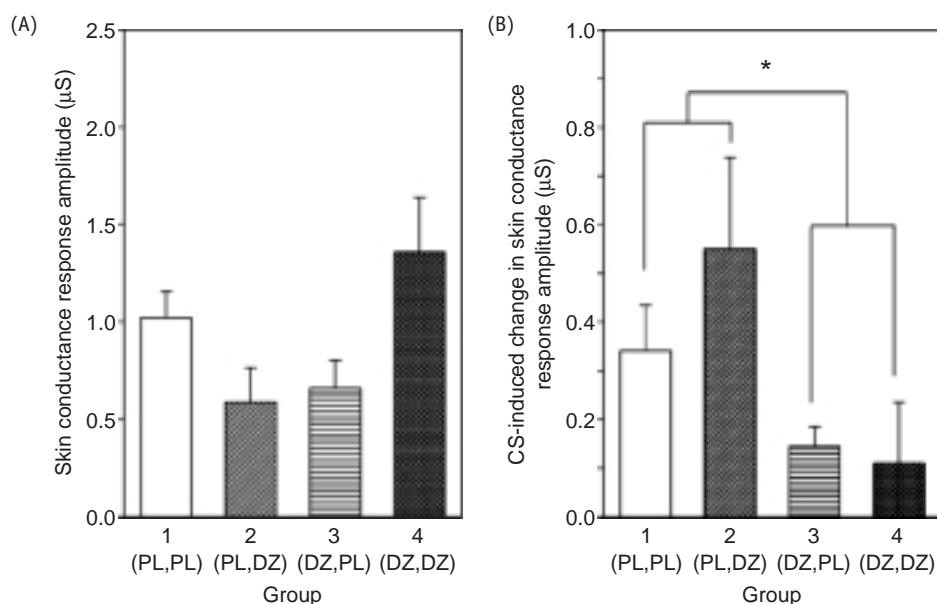
**Auditory evoked potentials** The left-hand panel of Fig. 4 shows the mean ( $\pm$ SEM) amplitude of the baseline (no-CS) N1/P2 responses, and the right-hand panel the mean ( $\pm$ SEM) CS/no-CS amplitude differences. The initial three-factor analysis of variance revealed a significant main effect of CS [ $F(1,42) = 5.4$ ;  $p < 0.05$ ], reflecting enhancement of the response by the CS; the main effect of session 1 treatment was not significant [ $F < 1$ ], but there was a significant main effect of session 2 treatment [ $F(1,42) = 6.8$ ;  $p < 0.05$ ]; there was a significant interaction between CS condition and session 1 treatment [ $F(1,42) = 7.4$ ;  $p < 0.01$ ], but not between CS condition and session 2 treatment [ $F < 1$ ]. The three-way interaction term was not statistically significant [ $F < 1$ ]. Further analyses showed that the baseline (no-CS) response amplitude was significantly reduced by diazepam administered in session 2 [ $F(1,42) = 5.0$ ;  $p < 0.05$ ], but was not affected by the treatment administered in session 1 [ $F < 1$ ]. In contrast, the CS-induced increase in N1/P2 response amplitude was unaffected by session 2 treatment [ $F < 1$ ], but was significantly attenuated by diazepam administered in session 1 [ $F(1,42) = 6.3$ ;  $p < 0.05$ ].

**Skin conductance responses** The left-hand panel of Fig. 5 shows the mean ( $\pm$ SEM) amplitude of the baseline (no-CS) skin conductance responses evoked by the acoustic stimuli, and the right-hand

panel the mean ( $\pm$ SEM) CS/no-CS amplitude differences. The three-factor analysis of variance revealed a significant main effect of the CS condition [ $F(1,44) = 22.6$ ;  $p < 0.001$ ], reflecting enhancement of the response in the presence of the CS; the main effects of session 1 treatment [ $F(1,42) = 1.2$ ;  $p > 0.1$ ] and session 2 treatment [ $F < 1$ ] were not statistically significant. There was a significant interaction between CS condition and session 1 treatment [ $F(1,42) = 7.0$ ;  $p < 0.05$ ], but not between CS condition and session 2 treatment [ $F < 1$ ]. The session 1 treatment  $\times$  session 2 treatment interaction was significant [ $F(1,42) = 7.1$ ;  $p < 0.05$ ]. The three-way interaction was not significant [ $F(1,42) = 1.0$ ;  $p > 0.1$ ]. Analysis of the baseline (no-CS) data showed no significant main effect of either session 1 treatment [ $F(1,42) = 1.2$ ;  $p > 0.1$ ], or session 2 treatment [ $F < 1$ ]. There was a significant interaction between the sessions [ $F(1,42) = 9.0$ ;  $p < 0.01$ ]; however, multiple comparisons (Dunnett's test) showed that none of the groups that received active treatments differed significantly from Group 1 (placebo/placebo). Analysis of the CS/no-CS differences showed that diazepam administered in session 1 attenuated the CS-induced enhancement of the skin conductance response [ $F(1,42) = 7.0$ ;  $p < 0.05$ ], whereas the treatment administered in session 2 had no significant effect [ $F < 1$ ].

## Discussion

Acute treatment with diazepam 10 mg produced significant reductions of subjectively rated alertness and critical flicker fusion frequency, an objective measure of arousal (Hindmarch, 1980).



**Figure 5** Skin conductance responses evoked by the sound pulses in session 2. (A) Baseline response amplitudes ( $\mu$ S) in the absence of the CS. (B) CS-induced increase in response amplitude ( $\mu$ S). Columns show mean data for the four groups; vertical bars indicate SEM; conventions as in Fig. 3. Diazepam 10 mg administered in session 2 (Groups 2 and 4) did not significantly alter the baseline response amplitude; diazepam 10 mg administered in session 1 (Groups 3 and 4) significantly reduced the CS-induced increase in response amplitude:  $*p < 0.05$  (see text for statistical analysis)

In subjects who experienced repeated pairings of the CS and US under the placebo treatment condition in session 1 (Groups 1 and 2), presentation of the CS before a startle probe in session 2 significantly potentiated the EMG and skin conductance startle responses, and enhanced the N1/P2 auditory evoked potential. Potentiation of the EMG eyeblink startle response in human volunteers by presentation of a discrete aversive Pavlovian CS has been previously reported by Lipp *et al.* (1994). The EMG startle response can also be enhanced by more diffuse aversive contextual stimuli, including verbal threat of electric shock (Grillon *et al.*, 1991; Bitsios *et al.*, 1999; Graham *et al.*, 2005), darkness (Baas *et al.*, 2002) and presentation of unpleasant pictures (Hamm *et al.*, 1991; Patrick *et al.*, 1996; Bradley and Lang, 2000) or film clips (Kaviani *et al.*, 1999, 2004). We have previously found that the skin conductance component of the startle response is enhanced by an aversive context (threat of electric shock: Graham *et al.*, 2005). The present results suggest that this late component of the startle cascade is also susceptible to Pavlovian fear conditioning. The enhancement of the N1/P2 auditory evoked potential in the presence of a Pavlovian CS appears to be a novel finding.

Diazepam administered in session 2 failed to prevent the expression of the fear-potentiated startle responses in those subjects who were trained under the placebo condition in session 1 (Group 2). In other words, diazepam did not significantly reduce fear-potentiation of the EMG and skin conductance startle responses, nor did it attenuate fear-potentiation of the N1/P2 complex. At first sight this appears to be inconsistent with the results of previous studies which found that diazepam (Patrick *et al.*, 1996; Bitsios *et al.*, 1999) and lorazepam (Graham *et al.*, 2004b) suppressed fear-potentiation of the EMG startle response. It is likely that this apparent discrepancy reflects methodological factors. As pointed out by Baas *et al.* (2002), the experimental protocols used in previous studies in which suppression of fear-potentiation has been observed have generally entailed relatively diffuse aversive contexts (e.g. threat of shock occasioned by attachment of electrodes to the wrist), rather than discrete Pavlovian CSs. The present finding that diazepam did not prevent the elicitation of fear-potentiation by a Pavlovian CS is consistent with Baas *et al.*'s (2002) finding that benzodiazepines had no effect on 'cue-specific' fear-potentiation of the EMG startle response. The validity of the distinction between 'contextual' and 'cue-specific' fear-potentiation is supported by a substantial body of evidence indicating that these two paradigms have distinct neural substrates (see Walker *et al.*, 2003). It has recently been proposed that cue-specific and contextual fear may constitute valid models of phobic and generalized anxiety, respectively (Grillon, 2002; Mineka and Ohman, 2002; Grillon and Baas, 2003). In this context, it is of interest to note that the apparently selective effect of benzodiazepines on contextual fear coincides with a greater clinical efficacy of benzodiazepines in suppressing generalized anxiety than phobic anxiety in man (see Grillon and Baas, 2003).

It is of interest to consider whether the treatment conditions themselves might have functioned as contexts for conditioned fear. It is well established that, in animals, conditioned responses may be preferentially expressed in the presence of a drug that was present at the time of conditioning (state-dependent learning: see

Colpaert, 1990; File *et al.*, 1993; Harris and Westbrook, 2001). State-dependent learning might be advanced as an explanation of the apparently greater fear-potentiation shown by Group 4, which experienced diazepam in both sessions, compared to Group 3, which experienced diazepam in session 1 and placebo in session 2. It should be noted, however, that the apparent difference between these two groups was not substantiated statistically. Moreover, an explanation in terms of state-dependent learning fails to account for the greater degree of fear-potentiation exhibited by Group 2, which received placebo in session 1 and diazepam in session 2, than by Group 3. In this context, it is of interest that Pain *et al.* (2002) recently reported that another benzodiazepine, midazolam, administered before the CS/US training, disrupted subsequent expression of fear conditioning in rats, whether or not the drug was also administered before the retention test, indicating that state-dependent learning could not account for the reduced responding to the CS.

Diazepam significantly reduced the amplitude of the baseline EMG startle response and N1/P2 auditory evoked potential. This result is in accord with several previous studies which found that not only benzodiazepines (Abduljawad *et al.*, 1997, 2001; Bitsios *et al.*, 1999; Baas *et al.*, 2002), but also sedative drugs belonging to a variety of other pharmacological classes, including the  $\alpha_2$  adrenoceptor agonist clonidine (Kumari *et al.*, 1996; Abduljawad *et al.*, 1997, 2001), the 5-HT<sub>2</sub> receptor antagonist ketanserin (Graham *et al.*, 2002), the tricyclic antidepressant amitriptyline (Phillips *et al.*, 2000), and the atypical antipsychotics clozapine and quetiapine (Graham *et al.*, 2001, 2004) all reduced the amplitude of the baseline EMG startle response. These findings strongly suggest that suppression of the startle response is a rather non-specific effect of sedative drugs. However, this may not be true of the suppression of the N1/P2 auditory evoked potential. Thus, whilst the benzodiazepines diazepam (Abduljawad *et al.*, 2001) and lorazepam (Pooviboonsuk *et al.*, 1996) reduce baseline N1/P2 amplitude, this effect is not shared by many other sedative drugs, including clonidine (Abduljawad *et al.*, 1997), quetiapine (Graham *et al.*, 2004), ketanserin (Graham *et al.*, 2002), clozapine (Graham *et al.*, 2001), scopolamine (Curran *et al.*, 1998) and diphenhydramine (Curran *et al.*, 1998).

It is possible that suppression of the baseline EMG response by benzodiazepines was partly due to the anxiolytic effect of these drugs, since the startle probe stimuli are themselves aversive for many subjects. However, this does not explain why other sedative drugs without known anxiolytic properties also suppress the EMG startle response (see above). Nor does it account for the fact that the skin conductance response elicited by the acoustic stimuli is insensitive to benzodiazepines (diazepam: present experiment; lorazepam: Graham *et al.*, 2005).

Perhaps the most striking outcome of this experiment was the finding that in subjects who experienced the paired presentation of CS and US in the presence of diazepam (Groups 3 and 4), subsequent presentation of the CS in session 2 produced significantly less enhancement of the EMG and skin conductance startle responses and the N1/P2 auditory evoked potential than was seen in those subjects who experienced CS/US pairing in the presence of placebo (Groups 1 and 2). This suggests that diazepam

impaired the acquisition of conditioned fear. It is known that benzodiazepines can prevent the acquisition of conditioned fear responses in rats (Harris and Westbrook, 1996; Pain *et al.*, 2002) and mice (Cole, 1986), although there do not appear to have been any previous reports of this effect in the case of human subjects.

It is widely believed that acquisition of conditioned fear responses entails an initial learning of the CS/US association, followed by a period of 'consolidation', when the association becomes established in long-term memory (Maren, 2001; McGaugh, 2004). The present results provide some circumstantial evidence that diazepam did not totally prevent the formation of a CS/US association, in that the rise in skin conductance following presentation of the CS alone in session 1 did not differ significantly between placebo- and diazepam-treated subjects. However, further experiments will be needed to establish whether the hypothetical processes of association formation and consolidation are equally sensitive to disruption by diazepam. In the present experiment, the conditioning session was carried out 1 h after ingestion of diazepam, the approximate time of peak plasma concentration of the drug (Greenblatt *et al.*, 1980; Friedman *et al.*, 1992). In view of the relatively slow elimination of diazepam ( $t_{1/2} = 43$  h; Friedman *et al.*, 1992), it is likely that an effective concentration of diazepam was present during the period of consolidation. One way of separating effects on conditioning from effects on consolidation might be to 'switch off' the effect of diazepam with a benzodiazepine antagonist (e.g. flumazenil) immediately after exposure to CS/US pairing, thereby obviating any effect of diazepam on consolidation. There is some evidence from experiments on animals that benzodiazepines may cause retrograde amnesia, suggesting a disruption of memory consolidation. For example, Jensen *et al.* (1979) found that flurazepam administered immediately after a conditioning session blocked subsequent expression of a passive avoidance response. However, although diazepam's effect on episodic memory in man is well-known, it has proved difficult to demonstrate retrograde amnesiac effects of benzodiazepines in man, comparable to that seen in animals (see Curran, 1999).

More than one putative mechanism might be advanced to account for a disruptive effect of diazepam on the formation of CS/US associations. Two plausible candidates are reduced attention to the CS during the conditioning trials, and a reduction of the aversiveness of the US. In the present experiment, circumstantial evidence for the latter mechanism is provided by the somatosensory potential and skin conductance responses evoked by the US, both of which were significantly reduced by diazepam. Suppression of somatosensory potentials by benzodiazepines is well-known (Thrauf *et al.*, 1994); however, it remains controversial whether this is appropriately described as an analgesic effect, or whether it should be regarded as a manifestation of a general sedative action of the benzodiazepines (Thrauf *et al.*, 1994; Nishiyama, 1995; Quevedo *et al.*, 1997; Cox and Collins, 2001). In either case, it seems possible that in the present experiment, diazepam could have reduced the subjective discomfort caused by the electric shock, thereby reducing its effectiveness as a Pavlovian US. Direct evidence for reduction of shock-induced subjective discomfort could be obtained in future experiments by taking pain thresh-

old measurements under each treatment condition. Although the role of benzodiazepines' sedative and/or analgesic effect on fear conditioning does not appear to have been examined previously in humans, there is some evidence that mnemonic rather than analgesic effects are responsible for the suppression of fear conditioning in rats. Thus, Pain *et al.* (2002) demonstrated that midazolam suppressed the effect of pre-exposure to the CS on subsequent conditioning; since the CS was entirely innocuous at the time of pre-exposure, Pain *et al.* (2002) argued that midazolam's effect could not have been mediated by a reduction of the aversiveness of the stimulus.

In summary, the present results indicate that acute treatment with diazepam impairs the acquisition of a conditioned aversive response, the fear-potentiated startle response, in man, but does not prevent the expression of a previously learned response.

## References

- Abduljawad K A J, Langley R W, Bradshaw C M, 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 K A J, Langley R W, Bradshaw C M, Szabadi E (1999) Effects of bromocriptine and haloperidol on prepulse inhibition: comparison of the acoustic startle eyeblink response and the N1/P2 auditory evoked response in man. *J Psychopharmacol* 13: 3–9
- Abduljawad K A J, Langley R W, Bradshaw C M, Szabadi E (2001) Effects of clonidine and diazepam on prepulse inhibition of the acoustic startle response and N1/P2 auditory evoked potential in man. *J Psychopharmacol* 15: 237–242
- Allison T, McCarthy G, Wood C C (1992) The relationship between long-latency somatosensory evoked potentials recorded from the cortical surface and from the scalp. *Electroenceph Clin Neurophysiol* 84: 301–314
- Baas J M P, Grillon C, Bocker K B E, Brack A A, Morgan C A, Kenemans J L, Verbaten M N (2002) Benzodiazepines have no effect of fear-potentiated startle in humans. *Psychopharmacology* 161: 233–224
- Bitsios P, Philpott A, Langley R W, Szabadi E, Bradshaw C M (1999) Comparison of the effects of diazepam on the fear-potentiated startle reflex and the fear-inhibited light reflex in man. *J Psychopharmacol* 13: 226–234
- Bond A J, Lader M H (1974) The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47: 211–218
- Bradley M M, Lang P J (2000) Affective reactions to acoustic stimuli. *Psychophysiol* 27: 513–522
- Cole S (1986) Effects of benzodiazepines on acquisition and performance: a critical assessment. *Neurosci Biobehav Rev* 10: 265–272
- Colpaert F C (1990) Amnesic trace locked into the benzodiazepine state of memory. *Psychopharmacology* 102: 28–36
- Cox R F, Collins M A (2001) The effects of benzodiazepines on human opioid receptor binding and function. *Anesth Analg* 93: 354–358
- Curran H V (1999) Effects of anxiolytics on memory. *Human Psychopharmacol Clin Exp* 14: S72–S79
- Curran H V, Pooviboonsuk P, Dalton J A, Lader M H (1998) Differentiating the effects of centrally acting drugs on arousal and memory: an event related potential study of scopolamine, lorazepam and diphenhydramine. *Psychopharmacology* 135: 27–36
- Davis M, Falls W A, Campeau S, Kim M (1993) Fear-potentiated startle: a neural and pharmacological analysis. *Behav Brain Res* 58: 175–198
- File S E, Goodall E M, Mabbutt P S, Harris A, Skelly A M (1993) State-dependent retrieval and midazolam. *Human Psychopharmacol* 8: 243–251
- Friedman H, Greenblatt D J, Peters G R, Metzler C M, Charlton M D,

- Harmatz J S, Antal E J, Sanborn E C, Francom S F (1992) Pharmacokinetics and pharmacodynamics of diazepam: effect of dose, plasma concentration, and time. *Clin Pharmacol Ther* 52: 139–150
- Gorissen M E E, Eling P A T M (1998) Dual task performance after diazepam intake: can resource depletion explain the benzodiazepine-induced amnesia? *Psychopharmacology* 138: 354–361
- Graham S J, Langley R W, Bradshaw C M, Szabadi E (2001) Effects of haloperidol and clozapine on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. *J Psychopharmacol* 15: 243–250
- Graham S J, Scaife J C, Verdusco A M B, Langley R W, Bradshaw C M, Szabadi E (2004) Effects of quetiapine and haloperidol on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. *J Psychopharmacol* 18: 173–180
- Graham S J, Scaife J C, Langley R W, Bradshaw C M, Szabadi E, Xi L, Crumley T, Calder N, Gottesdiener K, Wagner J A (2005) Effect of lorazepam on fear-potentiated startle responses in man. *J Psychopharmacol*, 19: 249–258
- Graham S J, Verdusco A M B, Langley R W, Bradshaw C M, Szabadi E (2002) Effects of ketanserin and haloperidol on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. *J Psychopharmacol* 16: 15–22
- Greenblatt D J, Allen M D, Harmatz J S, Shader R I (1980) Diazepam disposition determinants. *Clin Pharmacol Ther* 27: 301–312
- Grillon C (2002) Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biol Psychiat* 52: 958–997
- 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, Baas J (2003) A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clin Neurophysiol* 114: 1557–1579
- Hamm A O, Globisch J, Cuthbert B N, Vaitl D (1991) Startle reflex modulation in simple phobics and normals. *Psychophysiology* 28: S28
- Harris J A, Westbrook R F (1996) Midazolam impairs the acquisition of conditioned analgesia if rats are tested with an acute but not a chronic noxious stimulus. *Brain Res Bulletin* 39: 227–233
- Harris J A, Westbrook R F (2001) Contextual control over the expression of fear in rats conditioned under a benzodiazepine. *Psychopharmacology* 156: 92–97
- Hindmarch I (1980) Psychomotor function and psychoactive drugs. *Br J Clin Pharmacol* 10: 189–209
- Jensen R A, Martinez J L, Vasquez B J, McGaugh J L (1979) Benzodiazepine alter acquisition and retention of an inhibitory avoidance response in mice. *Psychopharmacology* 64: 125–126
- Kaviani H, Gray J A, Checkley S A, Kumari V, Wilson G D (1999) Modulation of the acoustic startle by emotionally-toned film-clips. *Int J Psychophysiol* 32: 47–54
- Kaviani H, Gray J A, Checkley S A, Raven P W, Wilson G D, Kumari V (2004) Affective modulation of the startle response in depression: influence of the severity of depression, anhedonia, and anxiety. *J Affect Dis* 83: 21–31
- Koch M (1999) The neurobiology of startle. *Prog Neurobiol* 59: 107–128
- Kumari V, Cotter P, Corr P J, Gray J A, Checkley S A (1996) Effect of clonidine on the human acoustic startle reflex. *Psychopharmacol* 123: 353–360
- Lewine J D, Thoma R J, Provencal S L, Edgar C, Miller G A, Canive J M (2002) Abnormal stimulus response intensity functions in posttraumatic stress disorder: an electrophysiological investigation. *Am J Psychiat* 159: 1689–1695
- Lipp O V, Sheridan J, Siddle D A T (1994) Human blink startle during aversive and nonaversive Pavlovian conditioning. *J Exp Psychol: Anim Behav Proc* 20: 380–389
- McGaugh J L (2004) The amygdale modulates the consolidation of memories of emotionally arousing experiences. *Ann Rev Neurosci* 27: 1–28
- Maren S (2001) Neurobiology of Pavlovian fear conditioning. *Ann Rev Neurosci* 24: 897–931
- Mineka S, Ohman A (2002) Phobias and preparedness: the selective, autonomic and encapsulated nature of fear. *Biol Psychiat* 52: 927–937
- Nishiyama T (1995) The post-operative analgesic action of midazolam following epidural administration. *Eur J Anaesthesiol* 12: 369–374
- Norris H (1971) The action of sedation on brain-stem oculomotor systems in man. *Neuropharmacology* 10: 181–191
- Pain L, Launoy A, Fouquet N, Oberling P (2002) Mechanisms of action of midazolam on expression of contextual fear in rats. *Br J Anaesth* 89: 614–621
- Patrick C J, Bertholt B D, Moore J D (1996) Diazepam blocks fear potentiated startle in humans. *J Abn Psychol* 105: 89–96
- Phillips M A, Langley R W, Bradshaw C M, Szabadi E (2000) The effects of some antidepressant drugs on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. *J Psychopharmacol* 14: 40–45
- Pooivoonsuk P, Dalton J A, Curran H V, Lader M H (1996) The effects of single doses of lorazepam on event-related potentials and cognitive function. *Human Psychopharmacology* 11: 241–252
- Quevedo J, Moretto P, Moretto A, Roesler R, Ferreira M B C (1997) Antinociceptive effects induced by diazepam at different ages. *Med Sci Res* 25: 479–480
- Senkowski D, Linden M, Zubragel D, Bar T, Gallinat E (2003) Evidence for disturbed cortical signal processing and altered serotonergic neurotransmission in generalized anxiety disorder. *Biological Psychiatry* 53: 304–314
- Skrandies W, Jedynak A (2000) Associative learning in humans – conditioning of sensory-evoked brain activity. *Behav Brain Res* 107: 1–8
- Thrauf N, Ditterich W, Kopal G (1994) Different sensitivity of pain-related chemosensory potentials evoked by stimulation with CO<sub>2</sub> tooth pulp event-related potentials, and acoustic event related potentials to the tranquilizer diazepam. *J Clin Pharmacol* 38: 545–555
- Turpin G, Schaefer F, Boucsein W (1999) Effects of stimulus intensity, risetime, and duration on autonomic and behavioral responding: implications for the differentiation of orienting, startle, and defense responses. *Psychophysiol* 36: 453–463
- Walker D L, Toufexis D J, Davis M (2003) Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress and anxiety. *Eur J Pharmacol* 463: 199–216
- Winer B J (1991) *Statistical Principles in Experimental Design*, 2nd edn. Wiley, New York, NY.