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The fear-inhibited light reflex: importance of the anticipation of an aversive event

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Abstract

Rationale: It has been shown previously that the amplitude of the pupillary light reflex response decreases when subjects anticipate an aversive stimulus (i.e. electric shock), compared to periods when subjects are resting ('fear-inhibited light reflex'). *Objective:* To compare the effects of the anticipation of an electric shock (putative aversive event) and of an acoustic stimulus (putative neutral event) on the light reflex. *Methods:* Twelve healthy volunteers participated in a training session and an experimental session. Pupil diameter was monitored with infra-red binocular television pupillometry. The experimental session consisted of 14 blocks of 3 light simuli. 'Relaxation' (no anticipation) and 'anticipation' (electrical or acoustic stimulus) blocks alternated. Mood and feelings were self-rated on visual analogue scales. *Results:* The anticipation of the electrical stimulus was associated with increases in initial pupil diameter and subjectively rated 'anxiety' and 'alertness', and a decrease in the amplitude of the pupillary light reflex response, whereas anticipation of the acoustic stimulus was associated with increases in initial pupil diameter and subjective 'alertness' only. *Conclusions:* The increase in initial pupil diameter is related to the anticipation of any stimulus, whereas the decrease in the amplitude of the light reflex response is associated with the aversiveness of the anticipated stimulus.

Keywords: Fear-inhibited light reflex; Television pupillometry; Acoustic stimulus

1. Introduction

We have shown previously that the threat posed by the anticipation of an electric shock increases the initial diameter of the pupil and decreases the amplitude of the light reflex response compared to periods when subjects are resting (Bitsios et al., 1996, 1998a,b, 1999). These changes in pupillary activity are accompanied by increases in subjective alertness and anxiety. Furthermore, the decrease in the amplitude of the light reflex response, but not the increase in the initial diameter of the pupil, correlates negatively with the increase in subjective anxiety (Bitsios et al., 1996). We termed this phenomenon 'fear-inhibited light reflex' and proposed that the threat-induced decrease in light reflex response amplitude could be a potential laboratory model for human anxiety. The paradigm of the fear-inhibited light reflex is methodologically and conceptually similar to the paradigm of the fear-potentiated startle reflex, a well-known paradigm of animal (Davis et al., 1993) and human

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(Grillon et al., 1991) anxiety, with which it may share a common mechanism.

The acoustic startle response is the contraction of a large number of voluntary muscles in response to a sudden loud acoustic stimulus. In humans the startle response includes an eye blink and is recorded by EMG electrodes placed over the orbicularis oculi muscle. It has been shown that the amplitude of this response is augmented when the acoustic stimulus is presented in the presence of a cue (conditioned stimulus) that has been associated with an electric shock (Grillon et al., 1991). Similarly, we have found that the amplitude of the light reflex response is reduced when the light stimulus is presented in the presence of a cue (e.g. a tone) that has been previously associated with an electric shock (Bitsios et al., 1996). In both tests the conditioned response is considered to be a state of fear. The conditioned fear in humans can thus be operationally defined as the augmentation of the startle reflex, or the inhibition of the light reflex, in the presence of a cue associated with a shock. Indeed, we have shown that, while simultaneously recording the startle and the light reflexes, the cue signalling the possibility of the delivery of a shock modifies both reflexes in the predicted direction (Bitsios et al., 1999). Moreover, we have shown that the fear-inhibited light reflex, in common with the fear-potentiated startle reflex, is dose-dependently sensitive to the anxiolytic drug diazepam (Bitsios et al., 1998b, 1999), suggesting that a common mechanism may mediate the effect of fear in the case of both reflex paradigms (Bitsios et al., 1999).

Following the administration of the threat-signalling cue, apart from a reduction in light reflex amplitude, there is also an increase in initial pupil diameter (Bitsios et al., 1996). Despite the close temporal proximity of the two pupillary changes, there is mounting evidence that the two effects of the cue on the pupil may reflect the operation of separate neural mechanisms (Bitsios et al., 1996, 1998a,b, 1999, 2002). Firstly, the two measures do not covary, and only the light reflex amplitude, but not the initial pupil diameter, correlates with subjectively rated anxiety (Bitsios et al., 1996, 2002). Perhaps the most compelling evidence for a separation between the two pupillary variables emerges from pharmacological studies. It has been reported that diazepam fails to affect the threat-induced increase in initial pupil diameter, while it is effective in reducing subjectively rated anxiety and attenuating the threat-induced reduction in light reflex amplitude (Bitsios et al., 1998b). On the other hand, clonidine has been shown to reduce initial pupil diameter and subjective alertness, but not subjective anxiety. Furthermore, although clonidine can enhance light reflex amplitude, this effect does not seem to be threat-specific (Bitsios et al., 1998a).

Previous studies of the psychophysiology of the pupil have centered largely on pupillary dilatation time-locked to the performance of a task. A number of tasks, from arithmetic problems of varying difficulty to memory and language-based tasks have been used (Beatty, 1982; Steinhauer and Hakerem, 1992; Loewenfeld, 1993). The magnitude of the peak pupillary dilatation during a cognitive task appears to be a function of processing load or 'mental effort' required to perform the cognitive task even when the composition of processing resources differs between tasks (Kahneman, 1973).

The role of emotional and motivational factors on the peak pupillary dilatation during a cognitive task has been less extensively studied. Normal subjects had increased peak pupillary dilatations during a cognitive task when they were threatened with a shock (Polt, 1970); however, this was interpreted in terms of increased recruitment of 'mental effort', rather than as an indication of the involvement of CNS structures mediating anxiety. Bernick and Overlander (1968) have considered the possibility that subjects may always experience some anxiety associated with apprehension about evaluation on the part of the experimenter, which, at least in some subjects, may contribute to the magnitude of the peak pupillary dilatation during a cognitive task. In this context, it is interesting that patients with 'audience anxiety' (today diagnosed as 'social phobia') were found to have increased magnitudes of the peak pupillary dilatation during a cognitive task (Simpson and Molloy, 1971). Therefore, the peak pupillary dilatation during a cognitive task may reflect both cognitive and affective processes.

The aim of the present study was to examine whether the changes in the pupillary measures evoked by the threat of the delivery of an electric shock are specific to the anxiety with which the threat of a shock is presumably associated, or whether they could be caused by the anticipation of any external, not necessarily harmful, event. To address this question, we examined the effect of the anticipation of an electric shock (putative aversive event) and the effect of anticipation of an acoustic stimulus (putative neutral event) on the light reflex.

2. Materials and methods

2.1. Subjects

Twelve healthy volunteers (6 male, 6 female) aged 18–35 years (mean ± S.D.; 24.4 ± 5.6) participated in the study. Subjects were all medication-free non-smokers, 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 only occasional social alcohol consumers. The instructions given to the subjects prior to the experiment are described in detail in Section 2.3. They were all tested in the morning hours (09.00–13.00 h). 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.

2.2. Tests and apparatus

2.2.1. Pupillometry

An infra-red binocular television pupillometer (TVP 1015B Applied Science Laboratories, Waltham, MA) was used to record the light reflex response in darkness, in previously dark-adapted eyes. The sampling rate of the pupillometer was 60 Hz and the detection accuracy was better than 0.05 mm (or 0.5%). The stimuli were light flashes (green, 565-nm peak wavelength) of 200-ms duration, delivered via a light emitting diode positioned 1 cm from the cornea of the subject's right eye, providing 'full face' light stimulation. The incident light intensity (illuminance) measured 1 cm from the source was 0.43 mW cm^{-2}. The recordings took place in a dark, sound-attenuated room and the subjects fixed their gaze on a dim red spot of light positioned approximately 2.5 m in front of them. Stimulus presentation was controlled by a microcomputer, and pupillary measures were digitized and stored on a floppy disk for off-line analysis. The parameters studied were initial diameter (i.e. diameter of the pupil before the application of the light stimulus) and amplitude of light reflex response (Fig. 1). The baseline ('initial pupil diameter') was defined as the mean pupil diameter recorded over 500 ms prior to the onset of the light stimulus. The light stimulus was applied 3000 ms following the onset of the warning tone.

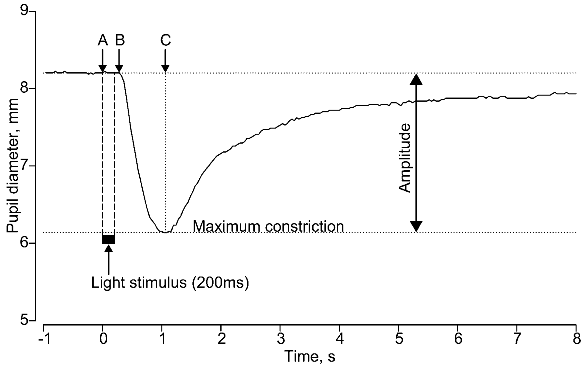

Fig. 1. An example of a light reflex response recorded by infra-red television pupillometry. Ordinate: pupil diameter millimetre (mm), abscissa: running time (s), measured from the onset of the light stimulus. The recording was done in the dark on a dark-adapted pupil. After the attainment of a stable pupil diameter, a brief light stimulus was applied. Following a latency, the pupil constricted, and then gradually re-dilated to its pre-stimulation diameter. (A) Onset of light stimulus, (B) onset of constrictor response, (C) attainment of maximum pupil constriction.

2.2.2. Subjective ratings

The subjects' mood and feelings were self-rated on visual analogue scales (VAS) (Aitken, 1969; Norris, 1971) on several occasions throughout the session (for details see Section 2.3). For each subject, the raw values (mm) for each item were weighted by multiplication with their respective factor loading, and the weighted values for each item were then allocated to 'alertness', 'discontentment' and 'anxiety' factors, based upon a principal component analysis (Bond and Lader, 1974). The average of the weighted values for each factor was entered in the statistical analysis.

2.3. Procedures

The experiment consisted of a training session and an experimental session.

2.3.1. Training session

Upon their arrival in the laboratory, the subjects received a detailed description of all procedures and a demonstration of all apparatuses. Then the subjects underwent a brief training session (application of a few light flashes in the dark to evoke the pupillary light reflex), in order to familiarise them with pupillometry. They were then exposed to a mild electric stimulus (1.5 mA, 50 ms) delivered to the skin of their left wrists, which is known to cause only minimal discomfort (Bitsios et al., 1996). A constant current square pulse (1.5 mA, 50 ms) was delivered twice to the skin overlying the median nerve of the left wrist, through disposable silver surface electrodes by a Grass stimulator (S.D. 9). The subjects were also exposed to a loud acoustic stimulus (2 kHz, 100 dB, 200 ms) delivered via headphones over a 70-dB background noise. They were informed at this point that the shock in the experimental session would be 50 times stronger and, therefore, more painful than the one they had just experienced, and the acoustic stimulus 50 times weaker and, therefore, more difficult to detect than the one they had just heard. Finally, the subjects were given instructions about the experimental session; however, no further demonstration of electric and acoustic stimuli occurred in the training session.

2.3.2. Experimental session

This took place 1 or 2 days after the training session. First, the subjects adapted to dim red illumination using red goggles (20 min). After a brief adaptation phase (3 blocks of 4 light flashes; 12 light flashes in total) the headphones were placed on the subjects' ears, the background noise was turned on and the electrodes were applied after preparation of the skin on the subjects' left wrists. The headphones and the electrodes remained fixed throughout the rest of the session.

The experimental recording consisted of two parts. Each part comprised seven identical blocks of three light stimuli of the same intensity and duration (21 light flashes per part, 42 flashes in total). Each block was associated either with a Relaxation or Anticipation condition. The first block was always associated with the Relaxation condition, and responses recorded in this block were excluded from the analysis. The Relaxation and Anticipation conditions alternated regularly in the remaining six blocks. Half of the subjects started with Part 1 and half with Part 2. Anticipation blocks were associated with the anticipation of an electric stimulus ('shock') in Part 1 and with the anticipation of an acoustic stimulus in Part 2. One single mild electric stimulus (1.5 mA, 50 ms) was delivered at the end of the last Anticipation block (i.e. block 6) in Part 1, and one single low intensity acoustic stimulus (2 kHz, 72 dB, 200 ms over a 70-dB background noise) was delivered at the end of the last Anticipation block (i.e. block 7) in Part 2. An acoustic cue, lasting for 500 ms, signalled the onset of anticipation 3 s prior to the light flashes in the Anticipation blocks only. Subjects who started with a Relaxation block in Part 1 also started with a Relaxation block in Part 2, and similarly, subjects who started with an Anticipation block in Part 1 also started with an Anticipation block in Part 2. Thus, although Parts 1 and 2 and the Anticipation and Relaxation blocks were all counter-balanced, subjects were examined under the same conditions for both the electric and the acoustic stimuli. The subjects were informed 30 s prior to the onset of each block about the nature of the condition with which the block was associated. The inter-stimulus interval within a block was kept constant at 25 s. Each block ended 10 s after delivery of the third light flash; thus, the duration of each block was 60 s. In order to investigate changes in mood and feelings from Relaxation to the next Anticipation condition, the subjects were asked to rate themselves retrospectively, immediately after each

Relaxation and Anticipation block, with a mood/feelings battery of VAS. The interblock interval was 90–120 s, to allow sufficient time for the completion of the VAS. Thus, the experimental recording lasted 35–40 min.

2.3.3. Instructions to subjects

Following application of headphones and electrodes, an electrical or an acoustic stimulus was delivered as in the training session (see above), depending on the Part (1 or 2) of the recording session to which the subject was allocated. It was then emphasized again, as in the training session, that the shock would be 50 times stronger and the sound 50 times weaker. In order to convince the subjects, a pseudo-switch on the shock box was turned to a 50-fold higher shock intensity and a real switch on the sound generator was turned to a 50-fold lesser sound intensity. In the Relaxation condition the subjects were instructed to relax and were told that no electric stimuli (in Part 1) or acoustic stimuli (in Part 2) would be administered. In the Anticipation blocks of Part 1, the subjects were instructed to anticipate a total of one to three electric stimuli of increasing intensity, delivered to their left wrists during the 3 s elapsing between the 500-ms warning tone and the light flash. In the Anticipation blocks of part 2, the subjects were similarly instructed to anticipate a total of one to three acoustic stimuli of decreasing intensity, delivered by the headphones, during the 3 s elapsing between the 500-ms warning tone and the light flash. The subjects did not know the exact number of electric or acoustic stimuli or in which Anticipation block(s) in Part 1 and 2, respectively, it/they would occur. The shocks were described by the experimenter as painful stimuli inducing a short-lived localised unpleasant sensation on the wrist. The acoustic stimuli were described as low in intensity, difficult to detect short-lived sounds, the detection of which would demand all of the subjects' attention. To make sure that subjects would make the effort to attend to the acoustic stimuli, they were instructed to report at the end of each Anticipation block of Part 2 whether they heard any sound(s).

2.4. Data reduction and analysis

The pupillary measures (initial pupil diameter and light reflex response amplitude) for each block were obtained by averaging the light reflex responses in the block by computer, and taking the measures from the averaged response. The VAS measures were obtained as described above (Section 2.2.2), and the average of the weighted values for factors 'alertness' and 'anxiety' was entered in the statistical analysis.

Data for each pupillary and VAS measure were collapsed across blocks for the two conditions (Anticipation, Relaxation) and the two stimulus types (electric, acoustic). Two-way analyses of variance with stimulus type and condition as within-subject factors were used to analyse the pupillary and VAS data. In the case of a significant interaction, the two stimulus types were compared under each condition with the least significant difference test (criterion, $P<0.05$).

Pearson's Product Moment Correlation was used to study the relationship between changes in anxiety/alertness ratings and changes in light reflex amplitude, following anticipation of an electric shock or sound stimulus.

3. Results

3.1. Subjective ratings

The group means of subjective anxiety and alertness (collapsed data, averaged across the blocks for the two stimuli and the two conditions) obtained with the VAS are displayed in Fig. 2. It is apparent that 'anxiety' was greater under the Anticipation condition than that under the Relaxation condition, and that shock anticipation caused a greater increase of 'anxiety' compared to anticipation of the acoustic stimulus. ANOVA of the 'anxiety' data revealed significant main effects of stimulus type (F: 11.01, d.f.: 1.11; $P<0.007$) and condition (F: 17.97, d.f.: 1.11; $P<0.001$), as well as a significant interaction (F: 10.16, d.f.: 1.11; $P<0.01$). Post hoc comparisons with the least significant difference test showed that 'anxiety' did not differ significantly between the two Relaxation conditions and that a significant increase in

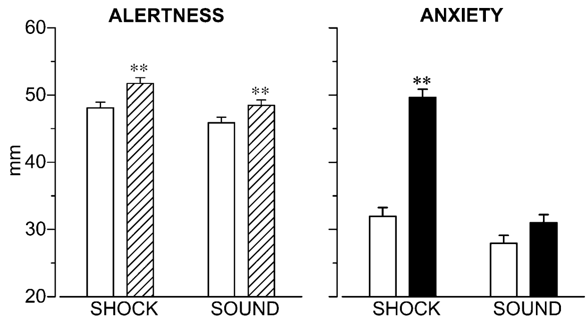

Fig. 2. Results of subjective ratings (mm) obtained on a battery of VAS. The height of each column corresponds to the mean obtained in the group ($n=12$), vertical bars are S.E.M. Open bars: relaxation, closed bars: anticipation (electric shock or sound stimulus, as indicated). Asterisks indicate statistically significant differences between anticipation and relaxation (**$P<0.001$).

'anxiety' under the Anticipation condition was associated with anticipation of the electrical stimulus only.

Both shock and sound anticipation were associated with an increase in 'alertness' compared to the relaxation periods. ANOVA of the 'alertness' data revealed significant main effects of stimulus type (F: 18.13, d.f.: 1.11; $P<0.001$) and condition (F: 17.9, d.f.: 1.11; $P<0.001$), but no significant interaction ($F<1$).

3.2. Pupillary measures

The pupillary measures used were initial pupil diameter and light reflex response amplitude (Fig. 1). Fig. 3 shows the group means of these measures, averaged across the blocks for the two stimulus types and the two conditions.

Initial pupil diameter was greater under the Anticipation condition than that under the Relaxation condition, for both the electric and acoustic stimuli. Moreover, initial pupil diameter was greater for the anticipation of the electric than the acoustic stimulus. ANOVA revealed significant main effects of stimulus (F: 12.05, d.f.: 1.11; $P<0.005$) and condition (F: 21.8, d.f.: 1.11; $P<0.001$) and a significant interaction (F: 12.1, d.f.: 1.11; $P<0.005$). Post hoc comparisons showed that initial diameter did not differ significantly in the two Relaxation conditions and that anticipation of either the electric or the acoustic stimulus was associated with a significant increase in the initial diameters. In order to compare the magnitude of the effects of electric vs. acoustic stimulus anticipation on initial pupil diameter, the differences were calculated between each subject's initial pupil diameter following electric or acoustic stimulus anticipation and their initial pupil diameter at the respective relaxation periods. These differences were compared with Student's t-test (paired comparisons). This analysis showed that the effect of electric stimulus anticipation on initial diameter was significantly greater than the effect of acoustic stimulus anticipation for this measure ($t=3.5$, d.f. $=11$; $P<0.005$).

The amplitude of the light reflex response was smaller under the Anticipation condition than that under the Relaxation condition for both stimuli. Moreover, the amplitude was smaller for the anticipation of the electric than the acoustic stimulus. ANOVA revealed significant main effects of stimulus (F: 34.2, d.f.: 1.11; $P<0.001$) and condition (F: 68.2, d.f.: 1.11; $P<0.001$) as well as a significant interaction (F: 25.9, d.f.: 1.11; $P<0.001$). Post hoc comparisons showed that amplitude did not differ significantly between the two Relaxation conditions and that a significant reduction in the

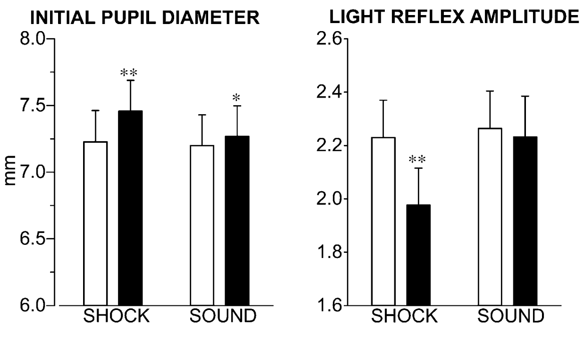

Fig. 3. Initial pupil diameter (mm) and light reflex amplitude (mm) obtained in the dark-adapted pupils of the subjects. The height of each column corresponds to the mean obtained in the group ($n=12$), vertical bars are S.E.M. Open bars: relaxation, closed bars: anticipation (electric shock or sound stimulus, as indicated). Asterisks indicate statistically significant differences between anticipation and relaxation (**$P<0.001$, *$P<0.05$).

Table 1
Correlation (r) between changes in ratings of anxiety/alertness and changes in light reflex amplitude, following anticipation of electric shock or sound stimulus

	Anxiety vs. amplitude	Alertness vs. amplitude
Shock anticipation	-0.68^{*}	-0.32
Sound anticipation	-0.02	-0.11

* $P<0.05$.

response amplitude under the Anticipation condition was associated with anticipation of the electric stimulus only.

3.3. Relationship between subjective ratings and pupillary measures

Table 1 shows the correlation between changes in ratings of anxiety/alertness and changes in light reflex amplitude, following anticipation of electric shock or sound stimulus. There was a significant correlation between the reduction in light reflex response amplitude and increase in anxiety when the administration of an electric shock was anticipated by the subjects. However, the reduction in light reflex response amplitude did not correlate with any change in alertness, and there was no significant correlation between the subjective ratings of anxiety/alertness and the reduction in light reflex response amplitude when the delivery of a sound stimulus was anticipated.

4. Discussion

Anticipation of either the electric or the acoustic stimulus was associated with a significant increase in the initial pupil diameter, suggesting that this measure is sensitive to anticipation of any stimulus. The experimental design ensured that anticipation of a stimulus, rather than its actual delivery, was the relevant independent variable, since, although the stimulus (sound or electric) was delivered after the third Anticipation block, the subjects expected it to occur at any time during the duration of either part of the experimental session (Section 2.3.3).

As could be expected, the anticipation of the more aversive stimulus resulted in a greater increase in both initial pupil diameter and subjectively rated alertness. Furthermore, anticipation of the electrical stimulus also resulted in a significant reduction in light reflex response amplitude, together with an increase in subjective anxiety, suggesting that the anticipation of the shock was anxiogenic. It is noteworthy that light reflex response amplitude was affected only by the anticipation of the shock and not by the anticipation of a sound, suggesting that this measure was specifically sensitive to the threat of shock. Indeed, there was a significant correlation between the reduction in light reflex response amplitude and subjectively rated anxiety only when the delivery of an electric shock was anticipated, but not when the delivery of a sound stimulus was anticipated.

The anticipation of the acoustic stimulus did not result in any increase in subjective anxiety, consistent with our hypothesis that sound alone is a neutral stimulus and anticipation of an acoustic stimulus would not be an anxiety-provoking condition. The anticipation of the acoustic stimulus resulted in a significant increase in subjective alertness and, interestingly, it also increased the initial pupil diameter, albeit to a lesser extent than the anticipation of a shock. The small increase (0.1–0.2 mm) in initial pupil diameter in anticipation of the sound is of the same order as that seen in psychophysiological experiments in response to increased attention or mental effort (for reviews see Kahneman, 1973; Beatty, 1982; Steinhauer and Hakerem, 1992; Loewenfeld, 1993; Steinhauer et al., 2000).

In this study ‘full face’ light stimulation of the pupil was used. A possible drawback of this strategy is that changes in pupil diameter may influence the amount of light reaching the retina. Thus, light reflex response amplitude would be expected to increase with larger initial pupil diameters. However, it is unlikely that ‘full face’ light stimulation contaminated the results of the present study, since in the Anticipation conditions, when there was a small increase in initial pupil diameter, the light reflex response either remained unchanged (anticipation of sound) or was attenuated (anticipation of electric shock).

In the present experiment, a warning tone was used in the anticipation blocks, to signal the possibility of the delivery of the stimulus, either electric or sound, prior to the application of the light flash. Therefore, the question arises whether the warning tone itself may have had an effect on the size of the light reflex response and thus may have contaminated the results. The role of the warning tone was to act as the conditioned stimulus in the paradigm used to generate 'conditioned fear' (Davis et al., 1993). This paradigm of 'fear conditioning' is based upon animal experiments (Davis et al., 1993) and has been successfully adopted for human studies (Grillon et al., 1991; Bitsios et al., 1996, 1998a,b, 1999). In the present experiment, the same protocol was used in order to maintain consistency with previous experiments. It is generally assumed that the role of the warning tone is to signal the aversiveness of an impending stimulus presentation, and thus it should not have any effect on the light reflex response, if the signal is not associated with the threat of an electric shock. Indeed, this was the case in the present experiment: light reflex response amplitude was affected only in Part 1 (when the tone signalled the possibility of receiving an electric shock), but not in Part 2 (when the tone signalled the possibility of receiving a sound stimulus). Therefore, we can conclude that the warning tone itself was without any effect on the amplitude of the light reflex response.

It is of interest that it has been reported that mental effort, apart from leading to an increase in initial pupil diameter, can also reduce the amplitude of the light reflex response (Steinhauer et al., 2000). The lack of effect of the anticipation of the acoustic stimulus on the amplitude of the light reflex response in the present experiment may be due to the fact that the mental effort involved was not of sufficient magnitude to affect this measure. Furthermore, it may be argued that a more substantial mental effort is likely to be accompanied by an increase in the level of anxiety, and this anxiety may be reflected in the modification of the light reflex response.

In summary, the present results are consistent with the widely accepted notion that electric shock is an unconditional aversive stimulus and anticipation of a shock, which has been widely and effectively used over decades in animal models of anxiety and human psychophysiology, has much face validity as an anxiety-provoking condition (Deane, 1969; Reiman et al., 1989). Moreover, our results suggest that it is the aversiveness of an anticipation that decreases the amplitude of the light reflex response, rather than the anticipation of any event (i.e. anticipation per se). Finally, the present results are in agreement with previous reports showing a dissociation between the two pupillary measures: while initial pupil diameter is affected by level of arousal and attention, only light reflex amplitude is sensitive to the level of anxiety (Bitsios et al., 1996). Interestingly, the anxiolytic drug diazepam selectively antagonises the threat-induced reduction in light reflex amplitude without affecting the threat-induced increase in initial pupil diameter (Bitsios et al., 1998b, 1999), whereas clonidine, a sedative drug with anxiolytic property, is effective in antagonising the effect of threat on both pupillary measures (Bitsios et al., 1998a).

Finally, the present experiment has bearing on the general issue of the relationship between arousal and anxiety, indicating that, while the two functions are closely related, they can be separated experimentally. The increase in initial pupil diameter is likely to reflect an increase in sympathetic activity, whereas the reduction in light reflex response amplitude can be related to a decrease in parasympathetic outflow to the iris. The locus coeruleus, the noradrenergic nucleus of the brain stem, is likely to be involved in both effects: apart from directly contributing to sympathetic outflow, it exerts a tonic inhibitory influence on the Edinger-Westphal nucleus (Szabadi and Bradshaw, 1996). However, sympathetic activation can also arise directly from the hypothalamus without involving the locus coeruleus (Loewy, 1990): activation of this system could cause an increase in initial pupil diameter without any change in the light reflex. These relationships may provide the neurobiological basis for our findings: anticipation of a neutral event may lead to sympathetic activation without involving the locus coeruleus, whereas conditioned fear may activate the locus coeruleus, resulting both in sympathetic activation and parasympathetic inhibition. Indeed, there is evidence that the amygdala, the brain structure crucial to fear conditioning (Davis, 1992), sends a rich output to the locus coeruleus (Cederbaum and Aghajanian, 1978).

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