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It has been shown that the eye-blink response evoked by an abrupt loud white noise ('acoustic startle') is potentiated when the subjects anticipate an aversive stimulus, e.g. an electric shock ('fear-potentiated startle'). It has been proposed that this paradigm may be a useful laboratory model of human anxiety. We examined whether the threat of an electric shock, as used in the fear-potentiated startle paradigm, would affect the pupillary light reflex, in 12 healthy volunteers. Light stimuli (0.32 mW/cm^2, 200 msec) were generated by a light-emitting diode, and pupil diameter was monitored by computerized binocular infrared television pupillometry in the dark. The light reflex was recorded during either the anticipation of a shock ('threat' blocks) or periods in which no shocks were anticipated ('safe' blocks). The shock consisted of a single square wave current pulse (1.5 mA, 50 msec) applied to the median nerve. At the end of each 'threat' or 'safe' block, subjects rated their anxiety using visual analogue scales. Two-factor analysis of variance (condition × block) showed that in the 'threat' condition there was a consistent increase in initial pupil diameter, a decrease in light reflex amplitude and an increase in alertness and anxiety ratings. These effects were observable before the subjects received any shock (a single stimulation of the median nerve). These results show that the anticipation of an electric shock can modify not only the startle reflex response but also the pupillary light reflex, suggesting that the inhibition of the light reflex by threat may be another suitable laboratory model of human anxiety.

Key words: pupil; light reflex; startle reflex; fear; anxiety; humans

Introduction

It has been shown in experimental rats that the amplitude of the acoustic startle response (i.e. a sudden jump in response to an abrupt loud white noise) is increased by presenting the eliciting acoustic stimulus together with a light signal (conditioned stimulus) that has been paired previously with an electric shock (unconditioned stimulus) (for reviews see Davis, 1992; Davis *et al.*, 1993). Recently a human equivalent of this 'fear-potentiated startle response' paradigm has been described. It has been shown that a light signal associated with the possibility ('threat') of an electric shock increases the amplitude and shortens the latency of the eye-blink response evoked by an abrupt loud white noise (Grillon *et al.*, 1991). As the effect of threat on the acoustic startle response is susceptible to both anxiogenic (e.g. yohimbine, Morgan *et al.*, 1993) and anxiolytic (e.g. alcohol, Grillon, Sinha and O'Maley, 1994) drugs, it has been proposed that the fear-potentiated acoustic startle response could be used as a laboratory model of human anxiety (Grillon *et al.*, 1991; Davis *et al.*, 1993).

Anxiety disorder is characterized by profound changes in autonomic functions that are usually related to an increase in the activity of the sympathetic nervous system (see Szabadi and Bradshaw, 1988). However, more recent evidence indicates that apart from sympathetic overactivity an inhibition of parasympathetic activity can also be detected in anxious patients. Thus, Yeragani *et al.* (1990) have reported that patients suffering from panic disorder show an attenuation of physiological heart rate variability, which is likely to reflect a reduction in vagal activity (Ewing *et al.*, 1980).

The human pupil is eminently suitable to study the relationship between sympathetic and parasympathetic activity in human subjects. The diameter of the pupil at any one time reflects the relationship between the opposing sympathetic and parasympathetic inputs, the sympathetic dilating and the parasympathetic constricting the pupil. The physiological modulation of pupil diameter is mainly due to light via the pupillary light reflex. This reflex pathway starts at the photoreceptor cells of the retina; important synapses include the pretectal nucleus of the midbrain, Edinger–Westphal nucleus, ciliary ganglion and the sphincter muscle of the iris. The diameter of the pupil can be monitored on-line using infrared television pupillometry and the light reflex can be evoked by suitably applied light stimuli. By measuring the kinetic parameters of the light reflex response (Fig. 1), it is possible to infer changes in the parasympathetic and sympathetic inputs to the iris on the basis of evidence that the latency

and the amplitude of the response reflect parasympathetic activation, whereas the time required for the pupil to attain its original size after a light stimulus ('recovery time') is greatly influenced by sympathetic activation (Smith, 1992). Thus the light reflex can be used to detect the effects of drugs on the autonomic nervous system, anticholinergic drugs prolonging the latency and reducing the amplitude (Theophilopoulos *et al.*, 1995), sympatholytic drugs prolonging (Morley, Bradshaw and Szabadi, 1991; Mortlock *et al.*, 1996) and sympathomimetic drugs shortening (Theophilopoulos *et al.*, 1995) the recovery time of the response. Apart from its usefulness in clinical neurology (e.g. diagnosis of Horner's syndrome indicating unilateral sympathetic lesion, light refractory pupils indicative of neurosyphilis; see pp. 225–264 in Adams and Victor, 1993) the light reflex response has been used successfully in clinical research. In particular attenuation of the reflex response is a useful indicator of subtle lesions to autonomic nerves as in multiple systems atrophy and diabetes (Smith, 1992).

In the present study we examined whether the amplitude of the pupillary light reflex can be modified by the threat of an electric shock, using the same protocol (Grillon *et al.*, 1991) as described for the potentiation of the acoustic startle response by threat. The prediction was that threat may reduce the amplitude of the response since it has been shown that the amplitude of the pupillary light reflex response is reduced in generalized anxiety disorder (Bakes, Bradshaw and Szabadi, 1990).

Materials and Methods

Subjects

Twelve healthy volunteers (eight males, four females) aged 18–22 years (mean ± SD 20.5 ± 2.6 years) participated in the study. Subjects were all medication-free and were requested to stop smoking and to avoid drinking alcohol, coffee and other caffeine-containing beverages for at least 12 h before the experimental session. Of the 12 subjects 11 were non-smokers and one was an occasional smoker. 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 under Procedures. They were all tested in the morning (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.

Tests and apparatus

Pupillometry

An infrared binocular television pupillometer (TVP 1015B Applied Science Laboratories Waltham, MA, USA) was used for recording the light reflex in darkness in previously dark-adapted eyes. The stimuli were light flashes (green, 565 nm peak wavelength) of 200 msec duration, delivered via a light emitting diode positioned 1 cm from the cornea of the subject's right eye. The incident light intensity measured 1 cm from the source was 0.43 mW/cm^2. The recordings took place in a dark, sound-attentuated 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); minimal diameter (i.e. pupil diameter measured at the time of maximum pupil constriction); and amplitude of light reflex response (i.e. the difference between the initial and minimal pupil diameters) (see Fig. 1).

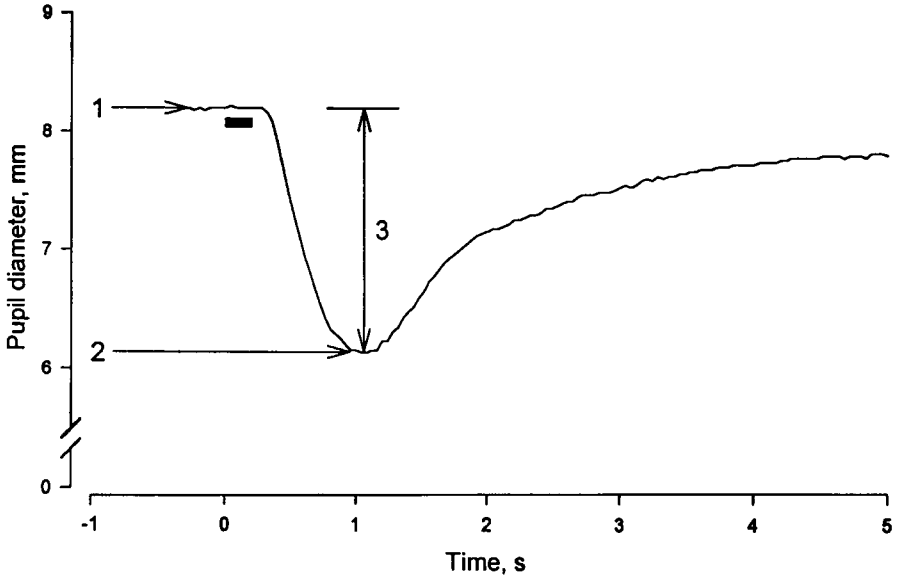

Figure 1 An example of a computer-generated pupillary light reflex response to illustrate the parameters measured. The light stimulus is represented by the horizontal bar. 1: Initial diameter ('baseline'); 2: minimal diameter; 3: amplitude of the light reflex response

Electrical stimulation
A constant current square pulse (1.5 mA, 50 msec) was delivered only once during the session to the skin overlying the median nerve of the left wrist through disposable silver surface electrodes using a Grass stimulator (SD 9).

Subjective ratings
The subjects' moods and feelings were self-rated on a 16-item visual analogue scales (VAS) (Aitken, 1969; Norris, 1971). The raw values (mm) for each item and each subject were weighted by multiplication with their respective factor loading and the weighted values for each item and subject were then allocated to 'alertness', 'discontentment' and 'anxiety' factors, based on a principal component analysis (Bond and Lader, 1974). The average of the weighted group values for each factor was entered in the statistical analysis.

Subjects rated their level of anxiety on the State–Trait Anxiety Inventory (STAI–S) (Spielberger, 1983) and the level of 'expected' pain and 'perceived' pain on a one-item VAS (no pain–extreme pain).

Procedures

Training session
On arrival in the laboratory the subjects received a detailed description of all procedures and a demonstration of all apparatus. The subjects then underwent a brief training session (application of a few light flashes in the dark to evoke the pupillary light reflex) in order to familiarize them with pupillometry. Finally, the subjects were given instructions about the experimental session, and completed a STAI–S form and the one-item VAS for 'expected' pain.

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). The session was divided into two phases, phase 1 (adaptation phase) and phase 2 (main session), separated by a 15 min interval. Each phase comprised 12 identical blocks of three light flashes of the same intensity and duration (36 light flashes per phase, 72 flashes in total). The average amplitude of the three light reflexes within a block was taken as the measure of the response in that block. The inter-stimulus interval within a block was kept constant at 25 msec. Each block ended 10 sec after delivery of the third light flash; thus, the duration of each block was 65 sec. The inter-block interval was 40 sec in phase 1; thus this phase lasted 20 min. After completion of phase 1 the skin on the subjects' left wrists was prepared and the electrodes applied.

In phase 2 (main session) responses in each block were recorded either under anticipation of an electric shock (THREAT condition) or without anticipation (SAFE condition). There were a total of 13 blocks. The first block was always associated with the SAFE condition, responses recorded in this block were not entered in the analysis. After recording responses from the first block, half of the subjects started with a SAFE block, and the remaining half with a THREAT block. The SAFE and THREAT conditions alternated regularly in the remaining 12 blocks. The subjects were informed 30 sec prior to the onset of each block about the nature of the condition with which the block was associated. In the THREAT blocks the subjects were instructed to anticipate a total of one to three electric shocks, delivered to their left wrists during the 3 sec elapsing between a 500 msec warning tone and a light flash. The 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 one single electric shock was delivered, always 2 sec before the first light stimulus in the block associated with the fourth THREAT condition (block 7 or 8). In an attempt to maintain the level of anticipation, it was stressed that the second and third shocks, if delivered, would be more intense and prolonged than the preceding shock(s). In the SAFE condition the subjects were told that no electric shocks would be administered and to support this statement the wires were removed from the wrist. The interblock interval was 90–120 sec to allow sufficient time for the completion of the VAS, thus phase 2 lasted 35–40 min.

Administration of rating scales
The 16-item mood and feelings VAS were administered after each block in phase 2. The Spielberger STAI–S scale and the one-item VAS ('expected' pain) were administered in the training session, prior to the onset of the experimental session and prior to the onset of phase 2 of the experimental session, and on completion of the experimental session. On this last occasion, however, the one-item VAS referred to 'perceived' pain rather than to 'expected' pain. Two different scales were used to rate anxiety (VAS and STAI–S), firstly to cross-validate the two scales against each other and secondly to provide measures both for shorter term (VAS) and longer term (STAI–S) changes in anxiety.

Statistical analysis

The scores of the STAI–S and pain scales across the three occasions (i.e. training session, beginning of experimental session and beginning of phase 2 of experimental session) were analysed by one-way analysis of variance (ANOVA). The pupillary measures (initial diameter and light reflex 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. Two-way ANOVA (condition, block) with repeated measures on both factors was used to analyse the pupillary measures and the mood/feeling VAS.

The relationship between changes in light reflex amplitude and initial pupil diameter was analysed by analysis of covariance (ANCOVA) (multi condition × block, with initial diameter as covariate). The relationship between pupillary measures and scores of anxiety ratings on the VAS were examined by linear regression analysis (least squares, product moment correlation). Individual values obtained in the 12 subjects on each of the six SAFE and THREAT occasions were entered in the analysis.

Results

Pupillary measures

Initial pupil diameter and minimal pupil diameter (group means) for each of the six SAFE and six THREAT occasions are displayed in Fig. 2, and the individual subject values averaged across the blocks for the two conditions are shown in Table 1. It can be seen that both initial and minimal pupil diameters were greater under the THREAT condition than under the SAFE condition. ANOVA of the initial diameter data revealed significant main effects of condition ($F=68.2$; df = 1, 11; $p<0.001$) and block ($F=6.6$; df = 5,55; $p<0.001$), but no significant interaction ($F=1.1$; df = 5, 55; $p>0.1$). Similarly, ANOVA of the minimal diameter data revealed significant main effects of condition ($F=140.1$; df = 1, 11; $p<0.001$), and block ($F=12.85$; df = 5, 55; $p<0.001$), but no significant interaction ($F<1$; df = 5, 55).

Light reflex amplitude data (group means) for each of the six SAFE and six THREAT occasions are given in Fig. 3 and the individual subject values averaged across the blocks for the two conditions are shown in Table 1. There was a progressive increase in amplitude during the experimental session, but in each block amplitude was smaller under the THREAT than under the SAFE condition (see Fig. 3). ANOVA of these data revealed significant main effects of condition ($F=58.0$, df = 1, 11; $p<0.001$) and block ($F=8.3$; df = 5, 55; $p<0.001$), but no significant interaction ($F=1.15$; df = 5, 55; $p>0.1$).

In order to address the possibility that the changes in amplitude were secondary to changes in initial diameter, an ANCOVA of the amplitude data (condition × block with initial diameter as the covariate) was carried out. This analysis did not reveal a significant effect of the regression in the case of condition ($F<1$), block ($F=1.43$; df = 1, 54; $p>0.1$) or the interaction ($F=3.6$; df = 1, 54; $p>0.05$).

To exclude the possibility that the differences observed between the THREAT and SAFE conditions were due to the delivery of the electric shock, the data obtained prior to the shock were also analysed separately. This analysis revealed significant differences between the two conditions in the cases of initial and minimum diameters and amplitude of light reflex response as was found for the whole session (see earlier).

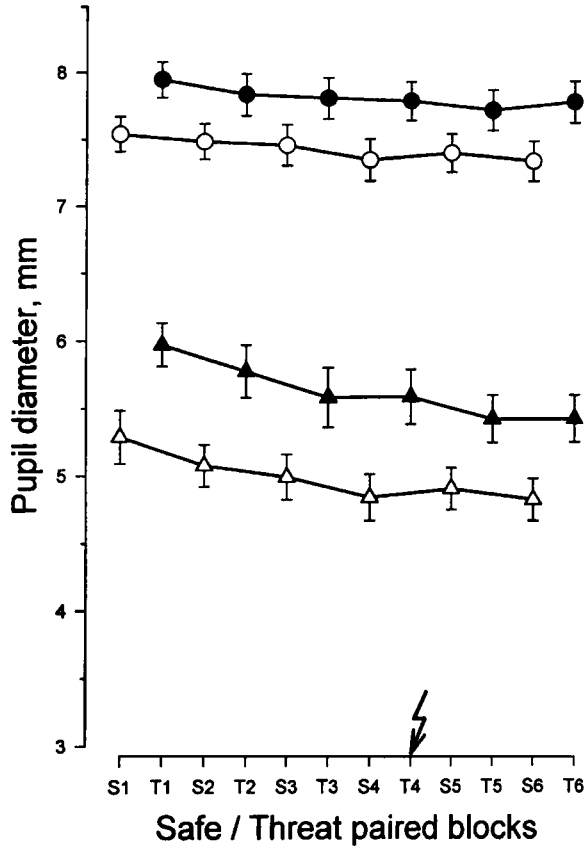

Figure 2 Initial (circles) and minimal (triangles) pupil diameters recorded in Phase 2 of the experimental session. T: THREAT; S: SAFE. Jagged arrow indicates the delivery of a mild electric shock to the median nerve. The data points are mean values obtained in the group of 12 subjects; open symbolis: SAFE condition; closed symbols: THREAT condition. Both initial and minimal diameters were increased in the THREAT blocks, and all pupil diameters slightly declined in the course of the session

Subjective ratings

The results obtained with the mood/feelings VAS for 'anxiety' and 'alertness' are shown in Fig. 4. It is apparent that both 'anxiety' and 'alertness' were greater under the THREAT condition than under the SAFE condition. ANOVA of the 'anxiety' data revealed significant main effects of condition ($F=20.4$; df = 1, 11; $p<0.001$) and block ($F=8.99$; df = 5, 55; $p<0.001$) but no significant interaction ($F=1.43$; df = 5, 55; $p>0.1$) ANOVA for the 'alertness' data revealed a significant main effect of condition ($F=12.34$; df = 1, 11; $p<0.005$) but not of block ($F=1.14$; df = 5, 55; $p>0.1$); there was no significant interaction ($F=1.26$; df = 5, 55; $p>0.1$). There was no significant difference between the SAFE and THREAT conditions for the 'discontentment' data.

As for the pupillary measures (see earlier), the data obtained prior to the shock were also analysed separately. Again, the pre-shock data revealed significant differences between the SAFE and THREAT conditions for 'alertness' and 'anxiety' as found for the whole session (see earlier).

The results obtained with the STAI–S and VAS for 'expected–perceived' pain are shown in Fig. 5. There was an increase in state anxiety as the shock became more imminent ($F=19.6$; df = 2, 22; $p<0.001$). The increase in state anxiety was accompanied by an increase in the VAS score for 'expected' pain ($F=8.43$; df = 2, 22; $p<0.002$). As can be seen in Fig. 5, on completion of the experiment, state anxiety dropped to baseline levels, and subjects reported that the 'perceived' pain from the shock was far less intense than expected.

Relationship between pupillary measures and subjective ratings

The results of the linear regression analysis are shown in Fig. 6. There was a significant negative correlation between anxiety scores on the VAS and light reflex amplitude. However, there was no significant relationship between anxiety scores and initial pupil diameter.

Table 1 Individual initial and minimal pupil diameter and light reflex amplitude measures averaged across blocks

	Initial diameter (mm)					Minimal diameter (mm)					Amplitude (mm)				
	Safe		Threat			Safe		Threat			Safe		Threat		
Subject	Mean	SD	Mean	SD	% change	Mean	SD	Mean	SD	% change	Mean	SD	Mean	SD	% change
1	7.42	0.13	7.78	0.09	4.76	5.06	0.08	5.53	0.08	9.13	2.37	0.07	2.26	0.13	−4.60
2	7.77	0.19	7.90	0.15	1.64	5.17	0.24	5.59	0.18	8.04	2.60	0.46	2.31	0.38	−11.10
3	7.18	0.35	7.63	0.23	6.23	4.88	0.29	5.61	0.15	14.96	2.30	0.43	2.02	0.25	−12.34
4	7.02	0.14	7.32	0.17	4.26	4.86	0.07	5.43	0.18	11.78	2.16	0.06	1.89	0.28	−12.64
5	6.84	0.18	7.37	0.21	7.74	4.35	0.08	5.04	0.14	15.79	2.49	0.07	2.34	0.20	−6.31
6	7.75	0.09	8.48	0.16	9.37	4.74	0.04	5.69	0.15	19.83	3.01	0.07	2.79	0.23	−7.12
7	6.51	0.10	6.93	0.13	6.54	4.05	0.06	4.60	0.11	13.65	2.46	0.06	2.34	0.15	−5.14
8	7.16	0.10	7.47	0.11	4.30	4.79	0.06	5.25	0.07	9.60	2.37	0.12	2.22	0.13	−6.36
9	7.63	0.14	8.00	0.12	4.83	4.95	0.10	5.65	0.09	14.05	2.68	0.16	2.36	0.13	−12.18
10	7.89	0.07	8.31	0.14	5.30	5.80	0.04	6.66	0.15	15.00	2.10	0.04	1.65	0.31	−21.48
11	8.20	0.09	8.64	0.20	5.39	5.99	0.08	6.86	0.10	14.37	2.20	0.15	1.78	0.20	−19.12
12	7.62	0.07	7.76	0.07	1.91	5.14	0.04	5.55	0.06	8.04	2.48	0.08	2.21	0.12	−10.79

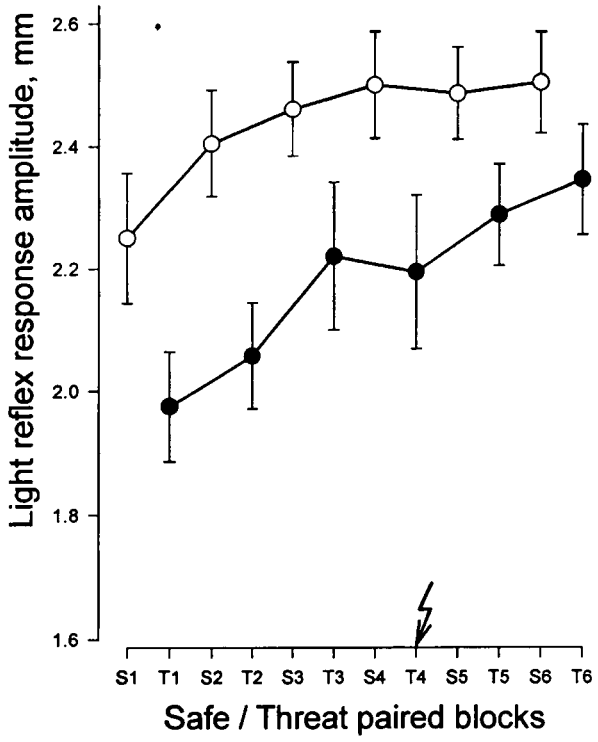

Figure 3 Light reflex amplitude recorded in phase 2 of the experimental session. T: THREAT; S: SAFE. Jagged arrow indicates the delivery of a mild electric shock to the median nerve. The data points are mean values obtained in the group of 12 subjects; open symbols: SAFE condition; closed symbols: THREAT condition. Light reflex response amplitude was decreased in the THREAT blocks and slightly increased in the course of the session both in the SAFE and THREAT blocks

Discussion

The results show that the threat of an electric shock had significant effects on all three pupillary measures, resulting in increases in initial and minimal diameters, and a decrease in light reflex amplitude. These effects of the threat were paralleled by increases in subjectively rated 'alertness' and 'anxiety'. The threat also had consistent effects on the ratings of state anxiety (STAI–S) and expected/perceived pain in relation to the electric shock, the ratings on both measures increasing as the possibility of receiving the shock became more imminent and declining on the final removal of the threat. The differences observed in the pupillary measures and subjective ratings between the SAFE and THREAT conditions were present throughout phase 2 of the experiment, and were likely to be related to the threat rather than to the shock itself since all the differences between the two conditions were present prior to the application of the shock.

The diameter of the pupil, at any one time, reflects the relationship between sympathetically mediated pupil dilatation and parasympathetically mediated pupil constriction. It is of interest that the initial pupil diameter, reflecting a steady-state tonic relationship between the two influences and the amplitude of the light reflex, reflecting a fast phasic response, changed in opposite directions in response to threat, initial diameter increasing and light reflex amplitude decreasing in the THREAT condition. Furthermore, in the course of the session initial diameter declined and light reflex amplitude increased. This raises the possibility that the changes in light reflex amplitude may be secondary to changes in initial diameter. However, this is unlikely for the following reasons. Firstly, the ANCOVA failed to detect any significant association between the two variables. Secondly, there is independent evidence from the literature showing that initial (or 'baseline') pupil diameter and light reflex amplitude can change independently. Thus Loewenfeld (1958), has shown in the monkey that electrical stimulation of the cervical sympathetic chain results in an increase in initial diameter without any significant change in the amplitude of the light reflex response, whereas thalamic stimulation results in little change in initial diameter but completely abolishes the light reflex response. Thirdly, different drugs can have differential effects on initial diameter and light reflex amplitude. For example, while both morphine and clonidine dilate the pupil in the cat, morphine reduces and clonidine enhances the amplitude of the light reflex response (Sharpe, 1990); amphetamine has only a negligible effect on initial diameter in darkness in humans, but it attenuates the

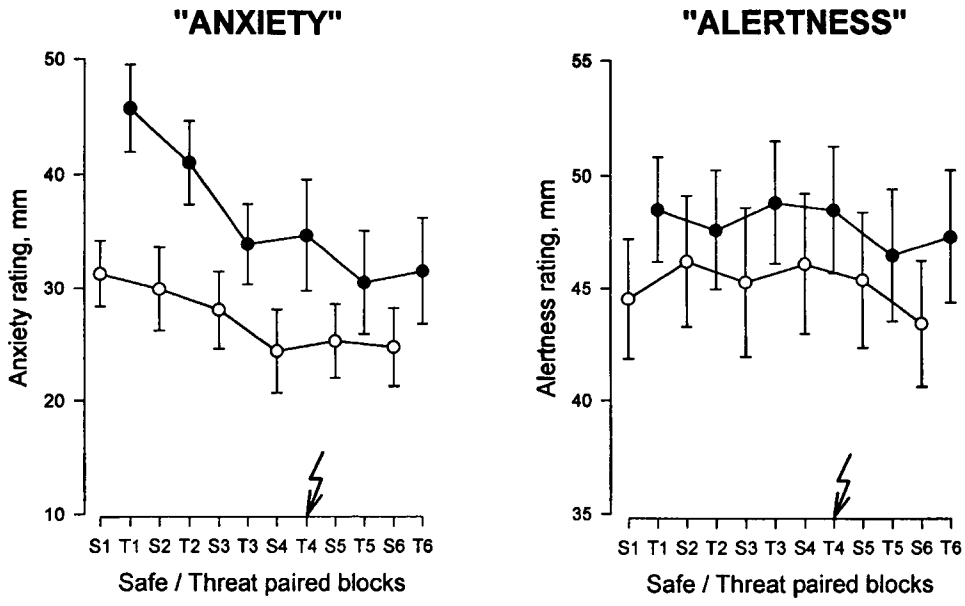

Figure 4 Subjective ratings on a battery of visual analogue scales of 'anxiety' and 'alertness' in phase 2 of the experimental session. T: THREAT; S: SAFE. The data points are mean values obtained in the group of 12 subjects immediately after the recording of the light reflex response; open symbols: SAFE condition; closed symbols: THREAT condition. Both 'anxiety' and 'alertness' ratings were increased in the THREAT condition, and 'anxiety' ratings showed a slight gradual decline in the course of the session

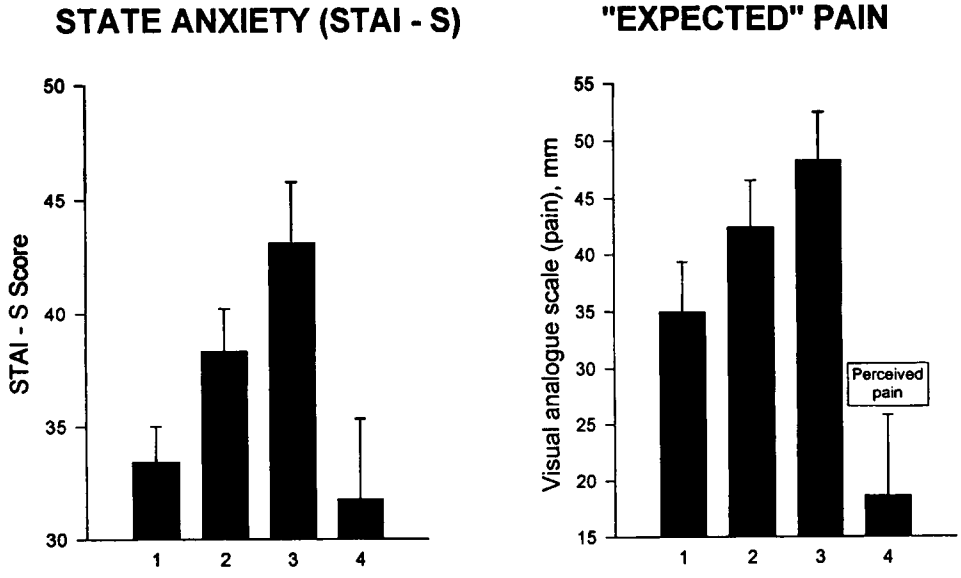

Figure 5 Subjective ratings of state anxiety (STAI–S) (left) and expected/perceived pain (one single VAS) (right) at four time points in the course of the experiment. 1: Training session; 2: beginning of experimental session; 3: beginning of phase 2 of experimental session; 4: on completion of experiment. Each bar represents the mean scores (±SEM) obtained in the group of 12 subjects. Measures on both scales increased as the threat of the electric shock became more imminent and decreased on removal of the threat

light reflex response significantly (see pp. 774–777 of Loewenfeld, 1993). Finally, it has been reported that patients suffering from generalized anxiety disorder have reduced light reflex amplitudes although their initial pupil diameters do not differ from those of healthy controls (Bakes, Bradshaw and Szabadi, 1990).

All three pupillary measures changed in the course of the session, initial and minimal diameters showing a gradual

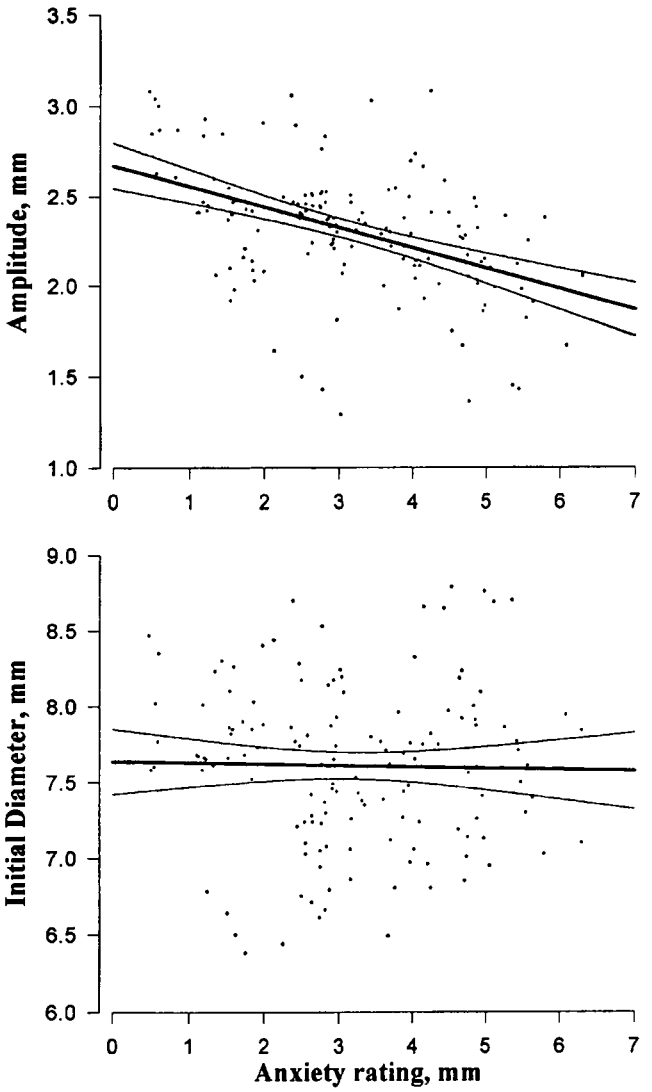

Figure 6 Relationship between the anxiety scores obtained on the VAS and the amplitude of the pupillary light reflex response (top) and pupil diameter immediately prior to the onset of the light stimulus ('initial diameter', bottom). The data points correspond to individual data obtained from each subject on each of the six SAFE and six THREAT occasions. The lines were fitted with linear regression (see Table 1 for details); faint lines correspond to 95% CI. Amplitude versus anxiety: slope ± SE estimate: -0.115 ± 0.018; intercept ± SE estimate: 2.673 ± 0.064; $r = -0.463$; $p < 0.001$. Initial diameter versus anxiety: slope ± SE estimate: -0.009 ± 0.031; intercept ± SE estimate: 7.638 ± 0.109; $r = -0.025$; $p > 0.5$

decline and light reflex amplitude a gradual increase, both in the SAFE and THREAT conditions, as evidenced by a significant effect of 'block' in the ANOVA. The gradual time-dependent slight decline in baseline pupil diameter is well documented (Lowenstein, Feinberg and Loewenfeld, 1963; Simpson, 1969; Yoss, Moyer and Hollenhorst, 1970; Peavler, 1974; Steinhauer, 1982), and is generally attributed to a gradual reduction in the level of arousal (Lowenstein, Feinberg and Loewenfeld, 1963; Yoss, Moyer and Hollenhorst, 1970; for review see pp. 480–497 of Loewenfeld, 1993). Within-session changes in pupillary responses to repeated light stimuli have been noted previously (see pp. 482–495 of Loewenfeld, 1993), however, it is not clear from the literature whether the responses change systematically over time, or whether any time-dependent change can be related to a change in initial pupil diameter. In the present study, light reflex amplitude increased gradually in the course of the session: through the first three blocks in the SAFE condition and through all six blocks in the THREAT condition. This change is unlikely to be secondary to the gradual decline in initial pupil diameter, firstly because the decline in initial diameter occurred at an even rate in the course of the session and was not restricted to the first three blocks in the SAFE condition, and secondly, because a decline in initial (or 'baseline') pupil diameter is expected to be associated with a decrease rather than an increase in the amplitude of the pupillary light reflex response. This latter prediction is based on the relationship between the baseline and the response size in a physiological/pharmacological test system ('law of initial values', see Szabadi, 1977; Longmore *et al.*, 1987). Finally as discussed above, baseline pupil diameter and light reflex amplitude are controlled by separate physiological mechanisms. It is of interest that both 'threat' and the initial blocks in the SAFE condition were associated with lower light reflex amplitudes compared to those seen in later SAFE blocks, suggesting that a common mechanism might operate in the two situations. It is an intriguing possibility that this common mechanism underlies subjectively experienced anxiety as well, since there was a significant negative correlation between anxiety ratings and pupillary light reflex amplitude (Fig. 6; for discussion see later). Thus, the 'habituation' of the anxiety response was paralleled by the 'habituation' of the reduction in the light reflex amplitude in the course of the session. The relatively high anxiety scores on the VAS in the SAFE condition at the beginning of the session (Fig. 4), paralleled by a reduction in light reflex amplitude (Figs 3 and 6), probably reflect the design of the experiment: half the subjects started before having experienced any of the THREAT blocks and thus presumably experienced high levels of anticipatory anxiety. It should be noted that no habituation of the acoustic startle response was observed by Grillon *et al.* (1991). The discrepancy regarding habituation of the response between our results and those of Grillon *et al.* (1991) may be due to the fact that we studied an autonomic reflex involving smooth muscle contraction, whereas Grillon *et al.* (1991) recorded a fast somatic response involving the contraction of voluntary striated muscles. Further work is needed, however, to investigate the relationship between the effects of 'threat' on the two different reflex responses.

The relationship between the anticipation of an electric shock ('threat') and the modification of the pupillary measures remains to be elucidated. In the case of the potentiation of the acoustic startle response by 'threat', it has been proposed that the same physiological mechanisms may operate in humans as have been demonstrated for the fear-potentiated startle response paradigm in experimental animals (Grillon *et al.*, 1991; Davis *et al.*, 1993). It has been shown that the amygdala plays a crucial role in mediating the modification of the acoustic startle response by threat (for reviews see Davis, 1992; Davis *et al.*, 1993). With respect to our observations it is relevant that there is an anatomical/physiological linkage

between the amygdala and the midbrain pupillomotor centre (Edinger–Westphal nucleus). Thus the hypothalamus receives an afferent input from the central nucleus of amygdala (LeDoux *et al.*, 1988) and there is a well-documented inhibitory projection from the posterior hypothalamus to the Edinger–Westphal nucleus (Loewenfeld, 1958; Koss, 1986). Indeed, electrical stimulation of the amygdala evokes a mydriatic response (Koikegami and Yoshida, 1953; Fernandez de Molina and Hunsberger, 1962).

Although it is likely that there is a neural substrate for the inhibition of the light reflex response by 'threat' (see earlier), the possibility should be considered that the pupillary changes were brought about by the release of catecholamines associated with anxiety induced by the threat of the electric shock. Indeed, in our experiment, there was an increase in the level of subjectively rated anxiety in the THREAT condition, and it is known that anxiogenic stimuli can cause an acute increase in the concentrations of circulating adrenaline and noradrenaline (for review see Szabadi and Bradshaw, 1988). Furthermore, it is well documented that adrenaline, and to a lesser extent noradrenaline are potent mydriatics when injected into the blood stream (for review see Arnold, 1980). Thus, theoretically, it is possible that the increase in initial diameter in the THREAT condition was due to a direct effect of circulating catecholamines on the dilator muscle of the iris. This is unlikely, however, since the effect disappeared in the SAFE conditions which were interspersed between the THREAT conditions at 2-min intervals, and it is improbable that the hormonal response would follow such a fast time course. Furthermore, as argued above, the inhibition of the light reflex response cannot be explained on the basis of an increase in initial ('baseline') pupil diameter.

Finally, the relationship between the pupillary measures and subjectively rated anxiety is of great interest since this relationship could have bearings on the applicability of the pupillary measures as a potential laboratory model of human anxiety. It is noteworthy that a significant negative correlation was found between light reflex amplitude and anxiety scores, whereas initial pupil diameter did not correlate with anxiety scores. The dissociation between light reflex amplitude and initial pupil diameter in the correlation analysis may reflect the separate physiological regulation of these two pupillary measures: the increase in initial pupil diameter is probably mediated mainly by the activation of the sympathetic innervation to the iris, whereas the reduction in light reflex amplitude is likely to reflect the inhibition of the parasympathetic control of the light reflex (for discussion of evidence, see earlier). It is of interest that in a separate study, a reduction in light reflex amplitude was also observed in a group of patients suffering from generalized anxiety disorder, compared to a group of non-anxious subjects, without any difference in baseline ('initial') pupil diameter between the two groups (Bakes, Bradshaw and Szabadi, 1990). This observation in clinical anxiety disorder suggests that patients show an inhibition of the parasympathetic innervation of the iris without any tonic increase in the sympathetic influence. The frequently quoted observation that anxious patients have larger pupils than healthy subjects (Bond, James and Lader, 1974) may only be applicable in an illuminated room where the large pupil could reflect a tonically inhibited light reflex rather than an increase in sympathetic tone. Since in the present experiment there was an increase in the initial diameter of the pupil in the THREAT condition, it is possible that this sympathetic response habituated with time and it was no longer detectable in patients presenting with an ongoing anxiety disorder. It should be pointed out, however, that there were also some methodological differences between the present study and that of Bakes, Bradshaw and Szabadi (1990), the most important being that in the present study all comparisons were made in the same subjects, whereas Bakes, Bradshaw and Szabadi (1990) examined two different groups of individuals.

In conclusion, the results presented here show that the threat of an electric shock, which is known to modify a somatic reflex (i.e. acoustic startle), can also influence an autonomic reflex (i.e. pupillary light response). These findings may have implications for human anxiety. In our experiment, a reduction in the amplitude of the pupillary light reflex response in the THREAT condition was paralleled by increases in subjectively rated anxiety both on the STAI–S and on the VAS for anxiety, suggesting that the inhibition of the light reflex may constitute a valid laboratory model of anxiety. It is of interest that the acute phasic changes in light reflex amplitude in response to 'threat' could also be detected in patients suffering from chronic ongoing anxiety disorder without exposure to acute 'threat' (Bakes, Bradshaw and Szabadi, 1990). This strengthens the validity of the laboratory model. Further research is needed to explore the susceptibility of the 'threat-inhibited pupillary light reflex' model to anxiogenic and anxiolytic drugs, as has been demonstrated in the case of the 'fear-potentiated startle reflex' model.

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