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Relationship of the 'fear-inhibited light reflex' to the level of state/trait anxiety in healthy subjects

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Abstract

Rationale: It has been shown 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 examine whether the sensitivity of the pupillary light reflex to the threat of an electric shock is related to the pre-existing levels of state and trait anxiety. *Methods:* Thirty-two healthy volunteers participated in one experimental session. The possibility of an electric shock to the wrist was signalled by a tone. There were six blocks of three light stimuli: three SAFE blocks (no tone applied) and three THREAT blocks (tone applied). The State–Trait Anxiety Inventory was completed at the beginning and at the end of each session. *Results:* There was a positive correlation between the state anxiety scores and the within-subject (SAFE-THREAT) difference in light reflex amplitude ($P < 0.05$). There was no significant correlation between the trait anxiety scores and the within-subject differences in light reflex amplitude. *Conclusions:* Individual differences in state anxiety associated with the threat of an electric shock are reflected in the amplitude of the pupillary light reflex response. This observation strengthens the validity of the fear-inhibited light reflex as a model of human anxiety.

Keywords: Pupil; Light reflex; Fear-inhibited light reflex; State/trait anxiety; Human volunteers

1. Introduction

It has been shown previously that pupil diameter increases and light reflex amplitude decreases when subjects are in anticipation of an aversive event (i.e. electric shock), compared to periods when they are resting (Bitsios et al., 1996a). Furthermore, the decrease in light reflex amplitude, but not the increase in initial pupil diameter, during anticipation of shock ('threat condition') correlates negatively with the increase in anxiety, suggesting dissociation between the two pupillary

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measures. We called this phenomenon the 'fear-inhibited light reflex', and suggested that it may be a potential laboratory model of human anxiety. Diazepam, which is thought to reduce fear in behavioural tests in experimental animals (Gray, 1977), attenuated subjective anxiety, together with the threat-induced reduction in light reflex amplitude in a dose-dependent manner (Bitsios et al., 1998a). The effect of diazepam was 'threat-specific', since it only attenuated the threat-induced reduction in the light reflex amplitude, but not the threat-induced increase in pupil diameter; while it did not have any effect on pupil diameter and the light reflex in the absence of the threat ('safe condition') at the doses used. These results strengthen the validity of this paradigm as a model of human anxiety.

The aim of the present study was to explore the effects of individual differences in anxiety on the light reflex and the fear-inhibited light reflex. The trait and state forms of anxiety were explored. The former represents an enduring predisposition and the latter is a more labile phenomenon (Cattell and Scheier, 1958), which in this study was induced by the threat of electric shock. A common approach in previous studies was to select subjects who presented with extreme scores on questionnaires, thus excluding many subjects who were falling between these extremes (Klorman and Ryan, 1980; Cook et al., 1991). As in this study the aim was to study anxiety in a normal sample, a correlational approach was adopted, which included responses from the entire sample.

2. Materials and methods

2.1. Subjects

Thirty-two healthy volunteers (24 males, 8 females) aged 18–22 years (mean ± S.D.; 20.4 ± 1.5) 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 32 subjects, 20 were non-smokers and 12 were occasional smokers. All of them were occasional caffeine and only occasional social alcohol consumers. They were all tested in the morning (09.00–13.00 h). The University of Nottingham Medical School Ethics Committee approved the study protocol. 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 infrared binocular television pupillometer (TVP 1015B Applied Science Laboratories, Waltham MA, USA) was used for the recording of the light reflex in darkness, in previously dark-adapted eyes. 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 subjects' right eye; the incident 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.

2.2.2. Electrical stimulation

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

2.2.3. Subjective ratings

The State and Trait forms of the State–Trait Anxiety Inventory (Spielberger, 1983) were used to assess subjects' state and trait anxiety (for details see Section 2.3).

2.3. Procedures

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 apparatus. 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 familiarize them with pupillometry.

2.3.2. Experimental session

This took place 1 or 2 days after the training session. It was divided into two phases: adaptation phase; and main phase, separated by a 10-min interval. In the adaptation phase, the subjects first adapted to dim red illumination using red goggles (10 min). During the following 10 min, the light reflex was elicited by the application of a few light flashes, in order to enable the subjects to adapt to pupillometry. At the end of the adaptation phase (approx. 20 min after arrival in the laboratory) the skin on the subjects' left wrist was prepared and the electrodes were applied. Following this, the State form of the State–Trait Anxiety Inventory (STAI-state) (Spielberger, 1983) was administered to assess subjects' anxiety in relation to the main phase of the experimental session. The main phase was started immediately after the completion of the STAI-state questionnaires.

The main phase comprised seven identical, consecutive blocks of three light flashes of the same intensity and duration (21 light flashes in total). 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 65 s. The interblock interval was 40 s. The main phase, therefore, lasted less than 15 min. Responses in each block were recorded either under anticipation of an electric shock (THREAT condition) or without anticipation (SAFE condition). 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 six blocks. 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. 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 s elapsing between a 500-ms 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 at the end of the experiment, since it was anticipated that it is the psychological threat produced by the instructions rather than the delivery of the shock that affects the light reflex (Bitsios et al., 1996a). In the SAFE condition the subjects were told that no electric shocks would be administered. At the end of the experiment the Trait form of the STAI (STAI-trait) was used to assess subjects' trait anxiety.

2.3.3. Data reduction and data analysis

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. For each of the 32 individuals the mean initial pupil diameter and the mean light reflex amplitude in the threat condition were calculated by averaging the responses from the three threat blocks. Identical procedures were followed for the three safe blocks. The relationships between mean pupillary measures in the threat as well as in the safe condition and STAI scores were examined by separate linear regression analyses (least squares, product moment correlation). Furthermore, for each pupillary measure, the within-subject (THREAT–SAFE) differences obtained for each pair of threat/safe blocks (three pairs) were calculated and their mean was then taken for each of the 32 individuals. This mean within-subject (THREAT−SAFE) difference for each individual was defined as this individual's response to threat. The relationship between these individual differences (for both pupillary measures) and individual scores of subjective ratings were examined by

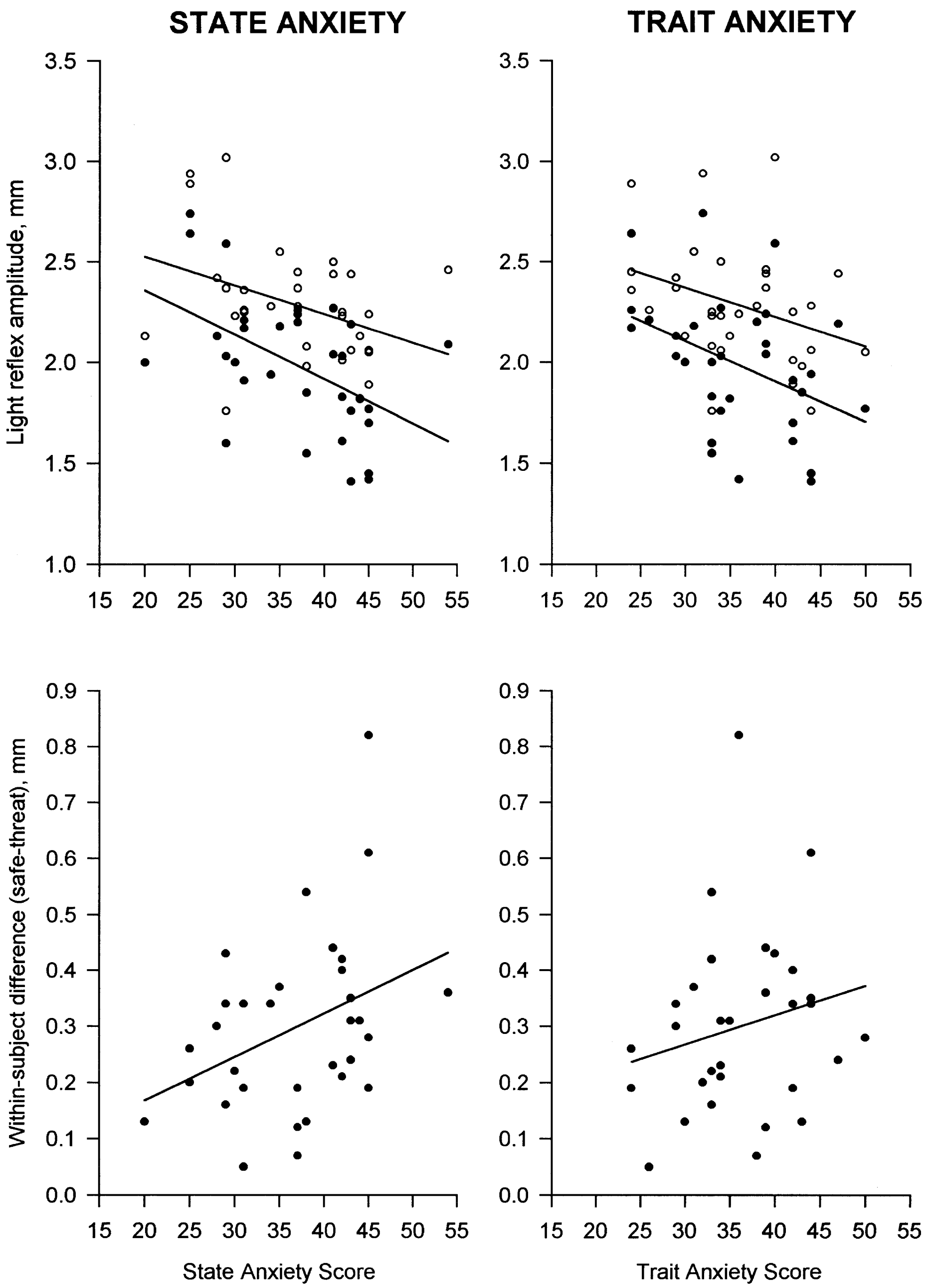

Fig. 1. Relationship between levels of state anxiety (left-hand graphs) and trait anxiety (right-hand graphs), and light reflex amplitude (top) recorded in the SAFE (open circles) and THREAT (closed circles) conditions, and the threat-evoked decrease in light reflex amplitude (bottom). The lines are best-fit linear functions. See text for details.

separate linear regression analyses (least squares, product moment correlation).

3. Results

Fig. 1 (top left panel) shows the relationship between state anxiety scores and light reflex amplitude in the SAFE and THREAT conditions. It can be seen that light reflex amplitude in the SAFE condition was attenuated with increasing state anxiety and that this relationship was even more prominent for light reflex amplitude in the THREAT condition. The linear regression analysis of these data showed that there was a significant negative correlation between state anxiety scores and light reflex amplitude in the SAFE condition ($r = -0.38$; d.f. $= 30$; $P < 0.05$), as well as between state anxiety scores and light reflex amplitude in the THREAT condition ($r = -0.50$; d.f. $= 30$; $P < 0.05$). When the effect of trait anxiety was partialled out, only the correlation between state anxiety scores and light reflex amplitude in the THREAT condition remained significant ($r = -0.37$; d.f. $= 29$; $P < 0.05$). There was no significant relationship between state anxiety scores and initial pupil diameter in the SAFE ($r = +0.16$; d.f. $= 30$, $P > 0.1$) or in the THREAT condition ($r = +0.11$; d.f. $= 30$; $P > 0.1$) (data not shown in figure).

Fig. 1 (bottom left panel) also shows the relationship between state anxiety scores and the within-subject difference (THREAT−SAFE) in light reflex amplitude, which was defined as the individual's response to threat. It can be seen that the individual's response to threat increased with increasing levels of state anxiety. The linear regression analysis of these data showed a significant positive correlation ($r = +0.375$; d.f. $= 30$; $P < 0.05$) between state anxiety and the threat-evoked decrease in light reflex amplitude. When the individual's response to threat was defined as the within-subject difference (THREAT−SAFE) in initial pupil diameter no such significant correlation was observed ($r = -0.17$; d.f. $= 30$, $P > 0.1$).

Fig. 1 (top right panel) shows the relationship between trait anxiety scores and light reflex amplitude in the SAFE and THREAT conditions. It can be seen that light reflex amplitude in the SAFE condition was attenuated with increasing levels of trait anxiety and that this relationship was even more prominent in the THREAT condition. The linear regression analysis of these data showed a non-significant negative correlation between trait anxiety scores and light reflex amplitude in the SAFE condition ($r = -0.34$; d.f. $=$ 30; $0.05 < P < 0.06$) and a significant negative correlation between trait anxiety scores and light reflex amplitude in the THREAT condition ($r = -0.41$; d.f. $= 30$; $P < 0.03$). However, when state anxiety was partialled out, the above relationship was no longer significant ($r = -0.18$; d.f. $= 29$; $P > 0.1$). There was no significant relationship between trait anxiety scores and initial pupil diameter in the SAFE ($r = +0.09$; d.f. $= 30$; $P > 0.1$), or in the THREAT ($r = +0.03$; d.f. $= 30$; $P > 0.1$) conditions (data not shown in figure).

Fig. 1 (bottom right panel) shows that there was no significant relationship between trait anxiety scores and subjects' responses to threat defined either as within-subject differences in light reflex amplitude ($r = +0.23$; d.f. $= 30$; $P > 0.1$), or as within-subject differences in initial pupil diameter ($r = -0.19$; d.f. $= 30$; $P > 0.1$; data not shown in figure).

4. Discussion

The experiment described in this paper was conducted in the dark using dark-adapted pupils. There are several advantages associated with carrying out this type of experiment in the dark. Firstly, it is possible to ensure that the baseline is relatively stable and is not disturbed by oscillations (hippus) (Loewenfeld, 1993a,b). Secondly, using a relatively large pupil diameter in the dark is the best safeguard against the curtailment of the light reflex response by a 'floor effect' (Szabadi, 1977; Longmore et al., 1987). Therefore, studies of the light reflex are routinely carried out in the dark using infrared pupillometer. It should be borne in mind, however, that working with dark-adapted pupils carries the risk of curtailing possible mydriasis evoked by experimental manipulations. Indeed, in the present experiment the experimental variable (i.e. threat) evoked not only decreases in the amplitude of the pupillary light reflex response, but also increases in initial pupil diameter. Therefore, the possibility cannot be excluded that the enhancement of the pupil diameter might have been curtailed by a 'ceiling effect' (Szabadi, 1977; Longmore et al., 1987). However, it should be noted that psychophysiological experiments investigating the effects of cognitive load on pupil diameter are often conducted in the dark, presumably in order to ensure a stable baseline (Steinhauer and Hakerem, 1992). In these experiments, and also in our present study, measurable increases in pupil diameter could be obtained in the dark.

In agreement with previous reports from our laboratory (Bitsios et al., 1996a,b, 1998a,b), the threat of an electric shock increased initial pupil diameter and decreased the amplitude of the light reflex response. The present study addressed the question whether the degree to which the two pupillary measures are affected by the threat is related to the subject's pre-existing levels of state and trait anxiety.

The amplitude of the light reflex response appeared to be sensitive to the pre-existing level of state anxiety, as evidenced by a negative correlation between state anxiety level and light reflex response amplitude. Furthermore, the sensitivity of light reflex response amplitude to the level of state anxiety was observable both in the THREAT and SAFE conditions. The effect of the level on-going anxiety on the amplitude of the light reflex response is illustrated by a previous report which has shown that patients suffering from generalized anxiety disorder have attenuated light reflex amplitudes compared to healthy control subjects (Bakes et al., 1990).

The relationship between the level of trait anxiety and the amplitude of the light reflex response was much weaker than that found for state anxiety. Although qualitatively similar relationships were found, the negative correlation between the level of trait anxiety and light reflex response amplitude achieved statistical significance only in the THREAT condition, and the significance of even this correlation disappeared when the contribution of state anxiety was partialled out. The relatively low sensitivity of the light reflex response amplitude to the level of trait anxiety may, at least partially, reflect the fact that there was relatively small variance in the level of trait anxiety within the sample. It is of interest, however, that in another laboratory model of human anxiety, the fear-potentiated acoustic startle response, only the level of state anxiety showed a significant positive correlation with the size of the threat-evoked response, while the level of trait anxiety failed to do so (Grillon et al., 1993).

In agreement with previous reports (Bitsios et al., 1996a,b, 1998a,b), the threat of an electric shock not only reduced the amplitude of the pupillary light reflex response, but also caused an increase in initial pupil diameter. However, neither initial pupil diameter nor its threat-evoked enhancement were related to either state or trait anxiety. This observation seems to support our earlier contention about the dissociation between the two pupillary measures (Bitsios et al., 1996a). The results of the present and earlier studies suggest that while light reflex amplitude is a physiological correlate of anxiety, initial pupil diameter is more related to the general level of arousal. Firstly, we found that while light reflex amplitude in the SAFE and THREAT conditions correlated well with anxiety scores, there was no such correlation between initial pupil diameter and anxiety scores (Bitsios et al., 1996a). Secondly, when the two pupillary measures were compared between a group of patients suffering from generalized anxiety disorder and a group of age- sex-matched healthy subjects, the patients displayed attenuated light reflex responses, but there was no difference in initial pupil diameter between the two groups (Bakes et al., 1990). Thirdly, preliminary results from our laboratory showed that anticipation of an emotionally neutral stimulus (acoustic tone) increased initial pupil diameter and subjectively rated alertness, without affecting the amplitude of the light reflex response and subjectively rated anxiety, in contrast to the anticipation of an electric shock which not only increased initial pupil diameter, but also reduced the amplitude of the light reflex response and increased subjectively rated anxiety (Bitsios et al., 1996b). Finally, the most compelling evidence for dissociation between the threat-induced increase in initial pupil diameter and the threat-induced reduction in light reflex amplitude comes from the effects of drugs. The anxiolytic drug diazepam antagonized the threat-induced decrease in light reflex amplitude, and the threat-evoked increase in subjectively rated anxiety, without affecting the threat-induced increase in initial pupil diameter (Bitsios et al., 1998a). However, the sympatholytic drug clonidine antagonized the threat-induced increase in initial pupil diameter, without affecting subjectively rated anxiety (Bitsios et al., 1998b).

In conclusion, the present results show that the fear-inhibited light reflex, like another laboratory model of human anxiety, the fear-potentiated startle reflex (Grillon et al., 1993), is modulated by the level of state anxiety. The neural mechanisms underlying this modulation remain to be elucidated. However, it is likely that two central nuclei, the amygdala and the locus coeruleus are involved, since both the amygdala (Davis 1992; Davis et al., 1993) and the locus coeruleus (Charney et al., 1995) have been implicated in mediating fear-related responses. Furthermore, both nuclei send inhibitory efferents to the Edinger–Westphal nucleus, the parasympathetic pupillomor nucleus of the midbrain (for discussion, see Bitsios et al., 1996a; Szabadi and Bradshaw 1996). A heightened level of state anxiety would be expected to be associated with increased neuronal activity in both the amygdala and the locus coeruleus, which in turn would lead to an increase of the tonic inhibition of the pupillary light reflex by these structures. The phasic effect of anticipatory anxiety associated with the threat of an electric shock would be superimposed on the tonically increased supranuclear inhibition of the Edinger–Westphal nucleus, leading to enhancement of the threat-evoked attenuation of the pupillary light reflex response.

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