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Characterization of a psychophysiological model of classical fear conditioning in healthy volunteers: influence of gender, instruction, personality and placebo

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Abstract. Two experiments are described which evaluate the role of associative mechanisms and placebo effects on aversively conditioned skin conductance responses in groups of healthy volunteers. In both experiments, skin conductance level (SCL), variability (spontaneous fluctuations, SF) and amplitude (SCR) were recorded during a sequence of tone stimuli (80 dB, 1 s, 360 Hz). All the variables habituated during the first ten presentations of the tones. Tone 11 was immediately followed by a loud (100 dB) aversive brief (1 s) white noise UCS. The conditioning trial significantly enhanced SCRs to a further ten presentations of the tones and increased SCL and variability (SF). No enhancement of SCRs occurred when tone 11 was omitted and the UCS occurred in temporal isolation (experiment 1). Thus enhanced SCRs to tones following paired tone-noise presentation involves an associative mechanism. Increased "spontaneous" variability was shown to involve both conditioning and sensitization following the UCS. In both experiments females showed greater conditioned SCRs than males. In experiment 2 no effect of "anxiolytic" placebo could be discerned and there were no general relationships between questionnaires scores of extraversion or neuroticism with skin conductance measures in a group of 40 volunteers. The results question the role of conditionability and autonomic lability as major determinants of extraversion and neuroticism.These studies validate the use of the psychophysiological model of aversive conditioning in pharmacological studies of the mechanisms of habituation, conditioning and sensitization.

Key words: Skin conductance – Aversive conditioning – Habituation – Extinction – Placebo – Extraversion – Neuroticism – Gender effects

Studies in animals suggest that brain 5TH systems may be involved in mediating components of the behavioural response to aversive stimuli such as freezing and inhibition of other on-going behaviours (Deakin 1983). 5HT punishment theories are largely based on findings that drugs or lesions that interfere with 5HT neurotransmission release behaviour inhibited by fear-inducing stimuli. No such tests of 5HT punishment theories have been carried out on humans. This paper characterizes a putative model of classical fear conditioning which has been used to investigate 5HT mechanisms in human anxiety.

Classical conditioning plays a central role in many theories of the acquisition of fear responses (Gray 1982, 1987). Innocuous stimuli which predict an aversive event acquire the ability to elicit anticipatory fear responses when subsequently encountered. According to Gray, aversively conditioned stimuli share with fear-inducing unconditioned stimuli, novel stimuli, and the paradigm of frustative non-reward, the ability to activate a brain system which mediates behavioural inhibition, autonomic activation, vigilance and orienting responses.

One autonomic component of orienting and fear responses is increased sweat-gland activity, usually measured as an increase in skin conductance. The skin conductance response (SCR) is a convenient, reliable and objective measure of an individual's response to novel or significant stimuli. The SCR has been studied extensively as an index of classical conditioning in humans (Campbell et al. 1964; Maltzman et al. 1979; Pitman and Orr 1986).

Vila and Beech (1977) developed an aversive conditioning paradigm in which SCRs to a blue light stimulus were enhanced by light-loud noise pairings. Wang (1986) used a similar method but employing neutral tone CS instead of lights, to investigate the effects of alcohol withdrawal on fear conditioning. We have employed this methodology to investigate 5HT mechanisms in human anxiety (Wang et al. 1988).

Few studies of SCR conditioning have a complete set of control experiments which exclude non-Pavlovian mechanisms of response enhancement. This study investigated the untested assumption that contiguous tone (CS)-aversive noise (UCS) pairings are necessary for

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enhancement of SCRs to subsequent tone CSs, in Wang's version of the Vila and Beech paradigm.

The prevalence of anxiety disorders is considerably greater in females than males, and this raises the possibility of important gender differences in the process of acquisition, incubation or extinction of conditioned responses or in the magnitude of fear responses themselves. Vila and Beech (1977) demonstrated premenstrual enhancement of SCRs to a blue light CS which have previously been paired with an aversive loud noise stimulus (UCS), suggesting an influence of gender on conditioning.

In addition to gender, individual differences in conditioning mechanisms may be related to personality traits and vulnerability to anxiety states. According to Eysenck (1979), the personality dimensions of introversion and neuroticism are related, respectively, to conditionability and autonomic lability. These dimensions are likely to relate to brain neurotransmitter systems, but little is known about the neurochemical basis of classical conditioning or of personality. These questions may be important in the pathogenesis of anxiety states and in understanding the mechanism of action of anxiolytics drugs. We have therefore investigated possible influences of gender, personality and placebo in evaluating the psychophysiological model of fear conditioning we have used in pharmacological studies.

Material and methods

Experiment 1

Subjects. Twenty healthy medical students, ten males and ten females, aged between 21 and 36 years, participated as unpaid volunteers. All subjects were drug free at the time of the experiment with the exception of oral contraceptives.

Apparatus. The experiments were conducted in a sound attenuated, temperature controlled ($22 \pm 2°$ C) room. Skin conductance was measured using a computer-controlled, constant voltage (0.6 V), skin conductance module with automatic back off (Contact Precision Instruments). Beckman silver-silver chloride electrode were used with an adhesive ring (1 cm diameter) which exposed the skin to the conducting medium (KY jelly).

The white noise aversive stimulus was generated by a DAWE white noise generator (model 419C) and amplified by a stereo integrated amplifier (JVC A10X). The neutral tones were generated by a computer controlled tone generator (Contact Precision Instruments). Both tones and white noise were delivered via Koss stereo Headphones (model C101).

Calibration using an "artificial ear" showed that the tones had an intensity of 80 dB, frequency of 360 Hz and duration of 1 s. The white noise had an intensity of 100 dB, frequency band width of 0.1–10 KHz and duration of 1 s.

Presentation of the stimuli was controlled by an IBM–AT computer, which also recorded and automatically processed the skin conductance data. The minimum criterion for response detection was 0.02 μmhos. The computer was programmed to score the following parameters: amplitude of the skin conductance responses (SCR) (responses that began up to 5 s after the stimulus); number of spontaneous fluctuations (SF) between each stimulus (responses that began in a period outside the 5 s after each stimulus); and the mean skin conductance level (SCL) between each stimulus.

The volunteers were monitored in a control room by means of a videoscan monitor with an Ikegama CTC–4300 camera. Eagle wireless intercom (T123GW) gave direct communication between the psychophysiology laboratory and the control room. All subjects tested and all their results were included in the analysis.

Procedure. Subjects washed their hands before being taken to the psychophysiology laboratory (Venables and Christie 1980). Subjects sat in a comfortable armchair and electrodes were attached to the medial phalanges of the second and third fingers of the left hand (Venables and Christie 1980). Calibration and gain adjustments were then carried out using a keyboard and monitor located in the laboratory.

Subjects listened to the following instructions: "First you will have 10 min where you will hear nothing at all. This is so that you can relax and get used to being in the room by yourself. Then you will hear some noises through the phones with different intervals between then. During the session you will also hear a very loud noise through the phones. Try to ignore the sounds and just relax. Keep your eyes open so that you do not fall sleep. The whole session will last for about 30 min. Please wait where you are until I come back to unwire you".

Then the experimenter went to the control room and after 10 min began the experimental session. This lasted a further 22 min and the stimulus sequence consisted of ten habituation trials (neutral tones) presented between pseudorandom intertrial intervals (mean = 58 s); one acquisition trial (neutral tone 11 immediately followed by a 1 s burst of white noise); and ten subsequent extinction trials (the same neutral tones) presented again between pseudorandom intervals (mean = 62 s). In one of the experimental groups tone 11 was omitted (UCS only group). The volunteers were randomly distributed between the UCS only and the paired (CS–UCS) group, counterbalanced by sex.

Statistical analysis. Values for the SCR and SCL were not normally distributed and were converted to their natural log (logn) equivalents (with the addition of the constant value of 0.01) resulting in a normal distribution.

Data were analyzed by multivariate analysis of variance (MANOVA) using the Statistical Package for Social Science (SPSS/PC+) version 3.0. The degrees of freedom of the univariate analysis were corrected by the Huynh-Feldt epsilon. The corrected degrees of freedom are indicated by the symbol*.

The main factors analyzed in the different measurements were: group, sex, conditioning period (skin conductance parameters during tones 1–10 versus tones 12–21, i.e. habituation versus extinction) and trials.

Experiment 2

Subjects. Forty healthy subjects, 20 males and 20 females, aged between 18 and 30 years, participated in this study as paid volunteers. All subjects were drug free by the time of the experiment with the exception of oral contraceptives.

Procedure. The procedure was similar to that described for experiment 1. All the volunteers received paired CS–UCS presentation. Fifty minutes before being taken to the laboratory the subjects, counterbalanced by sex, received either a capsule containing placebo or no pill at all. In the former case they were told that the medication had anti-anxiety effects and might make then feel quite relaxed. The investigator was unaware of the treatment received by the subject. The verbal instructions before the experiment differed slightly from experiment 1: "First there will be a 10 min rest period during which time you should try to relax. Stay seated and try to keep still. You will hear sounds over the headphones. Keep your eyes open and do not go to sleep. The experiment will be over in about 25 min. See you then".

Questionnaires. All volunteers completed the Eysenck Personality Inventory (EPI) (1964).

Statistical analysis. As for experiment 1.

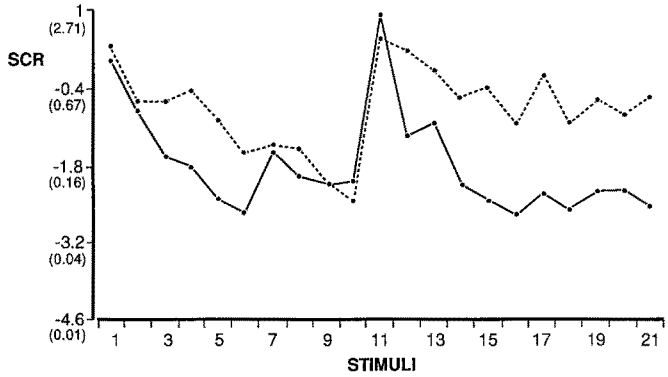

Fig. 1. Mean amplitude (logn) and detransformed mean (μmho) of the skin conductance response (SRC) to each stimulus in the paired (CS–UCS-*dotted line*) and unpaired (UCS only-*solid line*) groups

Results

Experiment 1

Skin conductance responses (SCR). The mean skin conductance response amplitude can be seen in Fig. 1. There was no significant effect of conditioning ($F_{1,16}=0.14$), but there was a significant interaction of group × conditioning ($F_{1,16}=6.21$, $P=0.024$), indicating that SCRs to tones were greater in the CS–UCS group than in the UCS only group, but only in the post-UCS period. A significant trials effect ($F_{9,144}=9.77$, $P<0.001$) was also observed, indicating a decrease of SCR with trials in both the habituation and extinction periods, although with a faster rate in habituation than extinction (trials × conditioning interaction, $F_{9,144}=2.03$, $P=0.039$).

Females, particularly in the CS–UCS group, had greater SCRs than males (sex factor, $F_{1,16}=4.72$, $P=0.045$; sex × trials interaction, $F_{9,144}=4.35$, $P<0.001$; group × sex interaction, $F_{1,16}=7.37$, $P=0.015$). The mean SCRs for females and males can be seen in Table 1.

No difference between groups ($F_{1,16}=0.27$, NS) or sex ($F_{1,16}=0.03$, NS) was found in the SCR to the UCS (white noise).

Spontaneous fluctuations (SF). The mean number of SFs before and after the UCS can be seen in Table 2. Males had more SFs than females, especially in the UCS only group (sex factor, $F_{1,16}=9.75$, $P=0.007$; group × sex interaction, $F_{1,16}=6.31$, $P=0.023$). SF increased in both groups after the UCS but the increase was greater in the CS–UCS group (conditioning, $F_{1,16}=9.43$, $P=0.007$; group × conditioning interaction, $F_{1,16}=5.37$, $P=0.034$). This suggests that the increase in SFs after the UCS may relate to associative conditioning to the tones. Spontaneous fluctuations showed a tendency to occur immediately after SCRs to the tones in the CS–UCS group after the CS–UCS pairing than in the group where the UCS was presented alone (Fig. 2).

Table 2. Mean (+/−SD) number of spontaneous fluctuations before and after unconditioned stimulus

CS–UCS group			UCS only group		
Group	Male	Female	Group	Male	Female
BEFORE UCS					
33.0(21)	39.2(29)	26.8(11)	40.5(37)	67.4(32)	13.6(15)
AFTER UCS					
46.6(21)	46.0(30)	47.2(09)	42.4(34)	69.6(70)	15.2(12)

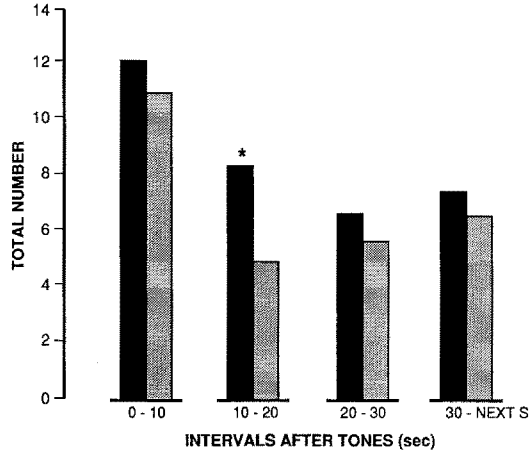

Fig. 2. Temporal distribution of skin conductance responses and spontaneous fluctuations averaged over the ten post-conditioning tone presentations and adjusted by covariance for the equivalent pre-conditioning responses. In the paired (CS–UCS-*black column*) group there are a greater number of spontaneous fluctuations after the tone than in the UCS-only *(dotted column)* group. *$P<0.05$ ANCOVA

Table 1. Mean (+/−SD) skin conductance response amplitude in logn μmho before and after unconditioned stimulus (UCS): effect of paired UCS presentation. [ ]=detransformed means

CS–UCS group			UCS only group		
Group	Male	Female	Group	Male	Female
BEFORE UCS					
−1.1(1.6) [0.33]	−1.1(2.1) [0.33]	−1.1(1.0) [0.33]	−1.7(1.5) [0.18]	−0.5(1.0) [0.61]	−2.8(1.1) [0.06]
AFTER UCS					
−0.5(1.5) [0.61]	−0.8(1.9) [0.45]	−0.2(1.1) [0.81]	−2.1(1.8) [0.12]	−0.6(1.1) [0.54]	−3.6(1.9) [0.03]

The minimal detectable response was −3.9 logn μmho [0.02 μmho]. Note that smaller negative numbers indicate greater responses

Table 3. Experiment 2. Mean (+/−SD) skin conductance response amplitude in logn μmhos before and after unconditioned stimulus: effect of placebo. [ ]=detransformed means

No-pill			Placebo		
Group	Male	Female	Group	Male	Female
BEFORE UCS					
−2.7(1.3) [0.07]	−2.9(1.3) [0.05]	−2.4(1.3) [0.09]	−2.8(1.5) [0.06]	−2.9(1.6) [0.05]	−2.7(1.6) [0.07]
AFTER UCS					
−1.6(1.3) [0.20]	−1.9(1.1) [0.15]	−1.3(1.4) [0.27]	−2.3(1.3) [0.10]	−2.8(1.5) [0.06]	−1.9(1.1) [0.15]

Skin conductance level (SCL). There was no difference between the groups in the skin conductance level (group, $F_{1,16}=1.64$, NS; group × conditioning interaction, $F_{1,16}=1.13$, NS). The conditioning factor, however, was significant ($F_{1,16}=7.14$, $P=0.017$), reflecting an increase in SCL after the UCS in both groups. Significant time ($*F_{2,32}=19.56$, $P<0.001$) and trials × conditioning factors ($*F_{7,116}=7.70$, $P<0.001$) shows that the SCL tended to decrease during the whole session, although this effect is greater during the habituation period.

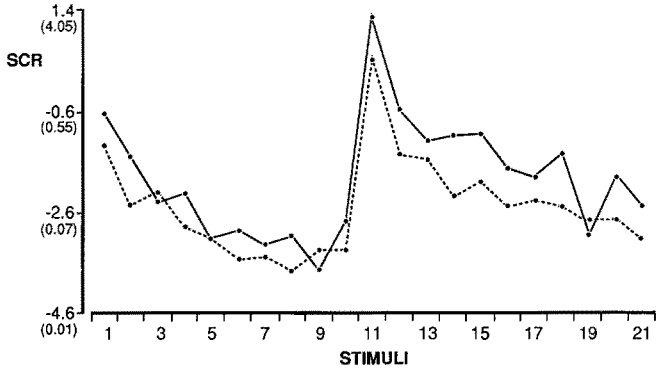

Fig. 3. Experiment 2. Mean amplitude (logn) and detransformed mean (μmho) of the skin conductance response (SCR) to each stimulus in males *(dotted line)* and females *(solid line)*

Table 4. Mean (+/−SD) number of spontaneous fluctuations before and after unconditioned stimulus

No-pill			Placebo		
Group	Male	Female	Group	Male	Female
BEFORE UCS					
16.0(20)	13.6(18)	18.5(22)	14.8(16)	15.2(08)	14.4(16)
AFTER UCS					
27.1(20)	23.0(11)	31.2(27)	29.2(22)	24.3(17)	34.2(25)

Experiment 2

Skin conductance responses (SCR). Mean SCRs can be seen in Table 3. No difference was found between placebo and no-pill groups ($F_{1,36}=1.31$, $P=0.26$). The conditioning ($F_{1,36}=17.24$, $P<0.001$), trials ($*F_{8,289}=26.22$, $P<0.001$) and trials × conditioning ($*F_{7,257}=2.79$, $P<0.05$) effects were significant, confirming the findings of experiment 1. As in experiment 1 there was evidence that females had significantly greater SCRs than males after the UCS (Fig. 3). Multivariate tests of significance showed a significant sex × conditioning × trials interaction ($F_{9,28}=2.58$, $P=0.026$).

No significant sex difference was seen in the responses to the UCS, although the females tended to have greater responses ($F_{1,36}=3.89$, $P=0.059$).

Spontaneous fluctuations (SF). The mean number of SFs can be seen in Table 4. The conditioning effect was again highly significant ($F_{1,36}=24.81$, $P<0.001$). No significant group or sex effects appeared.

Skin conductance levels (SCL). Confirming experiment 1, both conditioning ($F_{1,36}=33.5$, $P<0.001$) and trials ($*F_{2,72}=8.06$, $P<0.001$) effects were significant. No differences between groups or sexes were found.

Comparison between experiments. The analysis shows that both SCR ($F_{1,56}=6.95$, $P<0.011$), SF ($F_{1,56}=11.92$, $P<0.001$) and SCL ($F_{1,56}=4.45$, $P=0.038$) were increased in experiment 1 as compared to experiment 2.

Eysenck personality inventory (EPI). The results of the EPI can be seen int Table 5. The no-pill group scored higher on the neuroticism factor than the placebo group ($F_{1,36}=5.12$, $P=0.030$). No differences were found in extraversion or lie scores.

Correlations. A non-parametric (Spearman) correlation matrix was computed for the EPI factors and number of

Table 5. Mean scores (+/−SD) for extraversion (EXT), neuroticism (NEU) and lie (LIE) EPI scales

	No-pill			Placebo		
	Group	Male	Female	Group	Male	Female
EXT	14.7(4.3)	15.3(3.3)	14.2(5.3)	14.3(4.1)	15.2(3.0)	13.5(5.0)
NEU	9.5(3.5)	10.1(3.5)	9.0(3.5)	6.9(4.0)	5.3(2.7)	8.5(4.6)
LIE	2.9(1.8)	2.8(1.4)	3.1(2.1)	3.0(1.2)	2.7(1.3)	3.3(1.1)

Table 6. Spearman correlation coefficients between extraversion (EXT), neuroticism (NEU) and the mean skin conductance responses (SCR) for the first (A) and last (B) three tones before the UCS, the unconditioned response (UCR), the mean SCR for the first (C) and last (D) three tones after the unconditioned stimulus (UCS), and the number of spontaneous fluctuations before (SF1) and after (SF2) the UCS in no pill and placebo groups (* = $P < 0.05$)

	No-pill		Placebo	
	EXT	NEUR	EXT	NEUR
A	0.04	0.06	0.22	−0.04
B	−0.17	−0.18	−0.05	−0.06
UCR	0.01	0.01	−0.07	0.09
C	−0.03	0.30	0.27	−0.23
D	0.23	0.55*	0.33	−0.09
SF1	−0.04	0.17	−0.01	−0.05
SF2	0.11	0.47*	0.04	0.06

spontaneous fluctuations before (SF1) and after the UCS (SF2), SCR to the UCS (UCR), and mean responses for the first (A) and last (B) three tones before the UCS and the first (C) and last (D) three tones after the UCS. No significant correlations were seen between the EPI factors and the psychophysiological parameters. However, separate analysis of the no-pill and placebo groups showed that in the former neuroticism is positively correlated with the number of SFs after the UCS and the mean SCR amplitudes of the last three tones (Table 6).

Discussion

Experiment 1 (Fig. 1) showed that paired CS–UCS (tone-noise) presentation results in considerably greater SCRs to subsequent tones than presentation of the UCS alone (80 s after tone 10). This suggests that an associative process maintains SCRs to tones after paired CS–UCS presentations. In contrast to SCR, SCL increased after both CS–UCS and UCS-only presentations, suggesting a non-associative sensitization process. The increase in SFs after the UCS is probably related to both process, since the increase occurred following paired and unpaired UCS presentation (sensitization) but to a greater extent in the CS–UCS group (conditioning). A conditioning effect in the SF is also suggested by the temporal distribution of responses (Fig. 2).

Significant gender differences were seen in both experiments. Females had greater SCRs, particularly after UCS presentation. However, there was no evidence of faster habituation in females as reported in earlier studies (Korn and Mayer 1968; Castleman et al. 1978; Maltzman et al. 1979).

The finding that presentation of the aversive UCS enhances sex differences is compatible with studies which reported greater skin conductance responsivity in females than males when a shock was threatened (Kopacz and Smith 1971) or during public speaking (Puigcerver et al. 1989). In experiment 1 females had greater SCRs than males before UCS presentation as well as an enhanced difference after the UCS. The instructions in experiment 1 may have been more threatening than in experiment 2, since they referred to a "loud noise". This may explain why females had greater SCRs before the UCS in experiment 1 as well as the enhanced increase after the UCS seen in both experiments. Both studies suggest that females acquire greater conditioned SCRs than males. Gender differences in associative mechanisms may thus be one mechanism for the greater incidence of anxiety disorders in women.

It is well known that placebos may give rise to strong psychological and physiological effects in accordance with suggestion. For example, Frankenhauser et al. (1963), using normal female subjects, were able to detect both "depressant" and "stimulant" effects of placebo on heart rate, blood pressure, reaction time as well as on subjective mood.

In the present studys, no differences in any skin conductance parameter were found between "anxiolytic" placebo and no-pill groups. However, the pattern of correlations between EPI scores and skin conductance variables differed in the no-pill and placebo groups. In the no-pill but not in the placebo group, neuroticism correlated with SCR amplitude at the end of the session and SFs after UCS presentation (Table 6). This finding is compatible with Eysenck's suggestion that neuroticism involves greater incubation of conditioned fear responses. The placebo might have abolished this relationship because of the expectation of an anxiolytic effect. However, no evidence of incubation was seen in that there was no progressive increase in conditioned SCRs, although it could be argued that incubation balanced extinction. The evidence for effects of placebo, neuroticism and incubation in these data is clearly weak, and further experiments are needed.

The skin conductance parameters in this study (SCR, SF and SCL) were greater in experiment 1 than 2. Different instructions used could be partially responsible for this. It has been previously been shown that knowledge of forthcoming stimulus events can affect physiological activity (Farha and Sher 1989). Other factors, however, such as differences in the experimental subjects (medical students versus other volunteers) cannot be ruled out.

In conclusion, the results show that:

1. SCR and SF reliably detect associative effects in a one-trial aversive classical conditioning paradigm in healthy volunteers. SF and SCL are also influenced by non-associative effects of noxious stimulation (sensitization).

2. Females have greater aversive conditioned responses than males in the paradigm used.

3. No significant placebo effect occurred in the skin conductance parameters.

4. The instructions given to the subjects probably have strong influences on skin conductance parameters.

5. Positive correlations between neuroticism, SF and SCR after the acquisition trial were found in the no-pill group, but not in the "anxiolytic" placebo. This is compatible with Eysenck's theory that neuroticism relates to incubation of conditioned fear responses.

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