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Pharmacology of human experimental anxiety

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Abstract

This review covers the effect of drugs affecting anxiety using four psychological procedures for inducing experimental anxiety applied to healthy volunteers and patients with anxiety disorders. The first is aversive conditioning of the skin conductance responses to tones. The second is simulated public speaking, which consists of speaking in front of a video camera, with anxiety being measured with psychometric scales. The third is the Stroop Color-Word test, in which words naming colors are painted in the same or in a different shade, the incongruence generating a cognitive conflict. The last test is a human version of a thoroughly studied animal model of anxiety, fear-potentiated startle, in which the eye-blink reflex to a loud noise is recorded. The evidence reviewed led to the conclusion that the aversive conditioning and potentiated startle tests are based on classical conditioning of anticipatory anxiety. Their sensitivity to benzodiazepine anxiolytics suggests that these models generate an emotional state related to generalized anxiety disorder. On the other hand, the increase in anxiety determined by simulated public speaking is resistant to benzodiazepines and sensitive to drugs affecting serotonergic neurotransmission. This pharmacological profile, together with epidemiological evidence indicating its widespread prevalence, suggests that the emotional state generated by public speaking represents a species-specific response that may be related to social phobia and panic disorder. Because of scant pharmacological data, the status of the Stroop Color-Word test remains uncertain. In spite of ethical and economic constraints, human experimental anxiety constitutes a valuable tool for the study of the pathophysiology of anxiety disorders.

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Introduction

Animal models of psychopathology are widely used to develop new therapeutic agents as well as to investigate the mechanism of action of psychotherapeutic drugs and the pathophysiology of psychiatric disorders (1). Because of ethical and economical constraints, experiments that induce anxiety states in human beings are less frequently used. Nevertheless, they may constitute a helpful bridge between animal models and clinical disorders.

Experimental anxiety in humans may be induced by either chemical (e.g., caffeine, pentylenetetrazol, yohimbine, CO_2 inhalation) or psychological means. Only the latter will be reviewed here. In this class of tests,

environmental stimuli or contexts are used to induce anxiety states in healthy volunteers. Yet, there are some difficulties in producing anxiety that is sensitive to anxiolytic drugs due to the low level of the anxiety produced that is allowed by ethical constraints. Also, the sedative and anxiolytic effects of drugs are often hard to distinguish (2). In spite of these shortcomings, some procedures have yielded valuable results, which are discussed below.

Aversive conditioning to tones

Classical conditioning plays a pivotal role in theories of anxiety (3). Through this process, originally neutral stimuli or contexts acquire the ability to elicit anxiety after pairing with painful or otherwise unpleasant stimuli.

Emotional states are accompanied by neurovegetative changes, among them an increase in activity of sweat glands of the hands, which are innervated by cholinergic fibers of the sympathetic nervous system. The secretion of such ionic solution increases electrical conductance of the skin, which can be easily measured by means of a galvanometer. As a consequence, recording of skin conductance responses (SCR) to stimuli is often used to measure conditioned anxiety in humans (4). Recently discussed evidence indicates that SCR is an index of activation of the "brain inhibition system", a theoretical construct that Gray and McNaughton (3) equate with anxiety (5).

Vila and Beech (6) developed a procedure of aversive conditioning measuring SCR elicited by a blue light before and after its pairing with a loud white noise. They demonstrated that the association increased the intensity of SCR, an indication of classical conditioning. Later on, Wang (7) used tones, instead of light, as the to-be-conditioned stimulus to study the effect of ethanol abstinence syndrome on aversive conditioning. A modified version of the last procedure has been used in several drug studies performed on healthy volunteers as well as on patients with anxiety disorders (8). This method is briefly described below.

The experimental session is conducted inside a temperature-controlled (22 ± 2ºC) and sound-attenuated room. Skin conductance is measured using a constant voltage (0.6 V) device controlled by a personal computer. Two Ag-Ag Beckman electrodes are connected to the skin of the mid-phalanx of the second and third fingers of the left hand by means of two adhesive patches 1 cm in diameter. The contact is made through an electrically conductive gel.

A sound generator connected to an amplifier produces a white noise of 100-dB loudness and 1-s duration, which elicits a startle response in the volunteer, but is neither unbearable nor harmful. A tone generator produces sounds of specified frequency that are clearly audible, but not unpleasant (80 dB, 1 s). A personal computer controls the presentation of the sound stimuli to the experimental subject through bilateral headphones. The computer also records and later analyzes the SCR elicited by either the tone or the noise, or responses that occur spontaneously.

The following parameters are recorded: 1) magnitude of SCR, defined as a skin conductance fluctuation higher than 0.02 (or 0.05) μS occurring within a 5-s time window following sound presentation; 2) number of spontaneous fluctuations of similar characteristics, but that occur beyond the specified time window; 3) average skin conductance level, measured in the time intervals between stimuli.

After 10 min of adaptation to the laboratory, the experimental session starts with 10 presentations of the tone at pseudo-random intervals (average interval of 58 s, ranging from 40 to 80 s). In this habituation phase, the first tone elicits an SCR of high magnitude, but the intensity of the response steadily declines after each tone presentation. In the

next, conditioning phase, the 11th tone is immediately followed by the white noise - the unconditioned stimulus, which elicits a major SCR. After a 1-min interval, the same tone - now a conditioned stimulus - is again presented 10 times, as before. Due to conditioning, the first conditioned stimulus elicits an SCR of magnitude comparable to that in response to the first tone. As in the first phase, the magnitude of the SCR decreases with recurrence of the tones (extinction), but at a rate far slower than in the habituation phase. Each subject participates in only one experimental session.

In a validation study, healthy volunteers were divided into two groups. The first one followed the above experimental protocol, whereas in the second group the 11th tone was omitted and the white noise was presented alone, so that no tone-noise pairing occurred. As illustrated in Figure 1, only a short-lasting increase in the magnitude of the SCR was observed in the third phase of the experiment, probably due to sensitization, which is a nonassociative process. In contrast, the sustained enhancement that occurs in the standard procedure is likely to be a consequence of the tone-noise association, that is, classical conditioning (8).

Table 1 summarizes reported pharmacological results (9-13).

It may be seen that the test is sensitive to anxiolytic agents such as diazepam, buspirone and ritanserin. Diazepam and buspirone facilitated both the habituation to the neutral tone and the extinction of the conditioned responses, while ritanserin affected extinction, but did not change habituation (Figure 2). Therefore, only ritanserin had a selective effect on aversive conditioning. The gender difference found with diazepam indicates that women are more sensitive to the anxiolytic effect of this drug. Probably, the difference was detected because the dose of diazepam (2 mg) was unusually small, 10 mg being the standard dose. In the opposite direction, the anxiogenic agent methylchlorophenylpiperazine (mCPP) tended to facilitate conditioning. These results suggest that the aversive conditioning test can detect both anxiolytic and anxiogenic effects of drugs.

Most of the studies with the present model were aimed at testing the hypothesis that

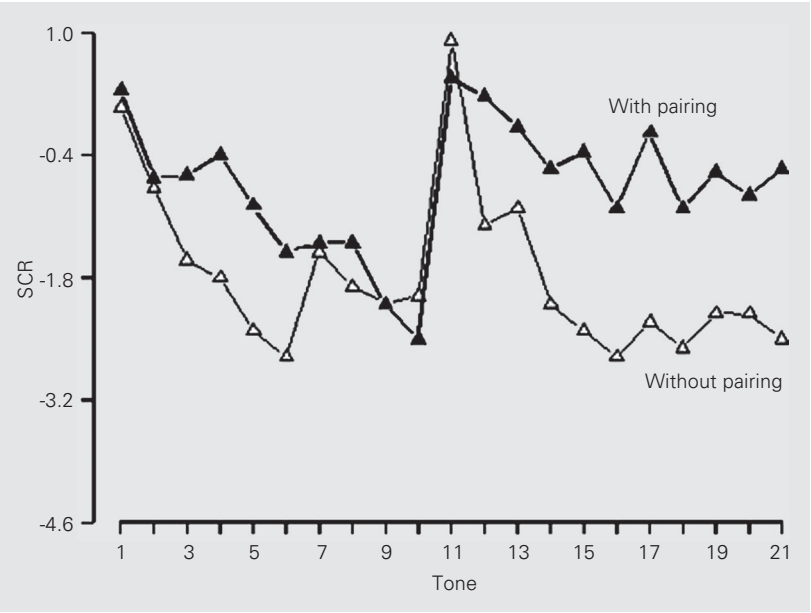

Figure 1. Skin conductance responses (SCR) of healthy volunteers undergoing aversive conditioning to tones. Points indicate the mean value of the natural log of SCR to each tone. In the group "With pairing" (filled triangles) the 11th tone was followed by noise (aversive unconditioned stimulus) presentation. Noise was absent in the group "Without pairing" (open triangles). N = 10. Modified from Ref. 8.

Table 1. Effects of drugs on conditioned skin conductance responses.

Drug	Dose (mg, *po*)	Test phase		Reference
		Habituation	Extinction	
Diazepam	2	+[a]	+[a]	Hellewell et al. (9)
Buspirone	5	+	+	Hellewell et al. (9)
Fluvoxamine	25	+[a]	+[a]	Hellewell et al. (9)
Ritanserin	10	0	+	Hensman et al. (10)
Nefazodone	100	0	0	Silva and Leite (11)
	200	0	0	
d-Fenfluramine	15	0	–[b]	Hetem et al. (12)
	30	0	0	
mCPP	15	0	–[b]	Connel et al. (13)

mCPP = methyl-chlorophenylpiperazine. +, facilitation (anxiolytic); –, impairment (anxiogenic); 0, no change. [a]in women only; [b]nearly significant.

serotonin (5-HT) affects conditioned and unconditioned anxiety in opposite directions (14). In this context, aversive conditioning to tones is viewed as a paradigm of conditioned anxiety, whereas simulated public speaking (see below) is supposed to represent unconditioned anxiety. According to the mentioned hypothesis, 5-HT is supposed to facilitate conditioned anxiety. Therefore, drugs that increase the action of 5-HT are expected to facilitate aversive conditioning. In turn, drugs that decrease the action of 5-HT should inhibit conditioning. From the results summarized in Table 1, it may be concluded that the anxiolytic effect of the 5-$HT_{2A/2C}$-receptor antagonist ritanserin fulfills the predictions of the hypothesis being tested. The tendency to an anxiogenic effect of the 5-HT_{2C}-receptor agonist mCPP and of the 5-HT-releasing agent d-fenfluramine is also consistent with these predictions.

However, the anxiolytic effects of both the 5-HT_{1A}-receptor agonist buspirone and the selective 5-HT reuptake inhibitor fluvoxamine seem to be discordant results. Nevertheless, the authors of this study (9) have argued that reported neurochemical and electrophysiological results indicate that pre-synaptic 5-HT_{1A} receptors would be preferentially stimulated after acute administration of these drugs. These receptors are placed on the neuron bodies of serotonergic neurons of the brain stem raphe nuclei. Their stimulation reduces the firing rate of these neurons, thus decreasing 5-HT release from 5-HT terminals (15,16). As a result, the ultimate functional consequence would be a reduction of 5-HT action on the postsynaptic neurons. Thus, buspirone and fluvoxamine would be expected to decrease anxiety, as observed.

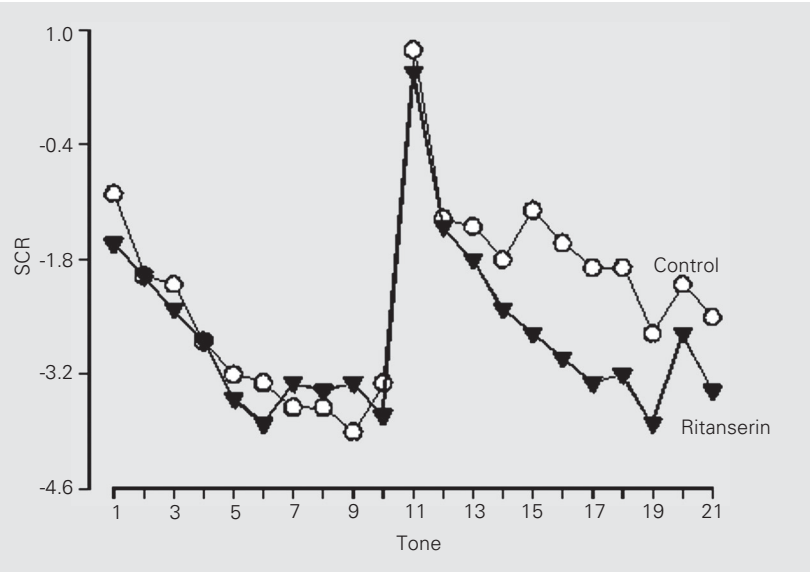

Figure 2. Anxiolytic effect of ritanserin (10 mg, *po*, triangles) measured in healthy volunteers undergoing the aversive conditioning test. Other specifications are given in the legend to Figure 1. SCR = skin conductance responses. Modified from Ref. 10.

The case of nefazodone is more complex, since the drug both blocks 5-$HT_{2A/2C}$ receptors and weakly inhibits 5-HT and noradrenaline reuptake (17). From the effects of ritanserin and fluvoxamine shown in Table 1, an anxiolytic effect of nefazodone would be expected. Yet, the drug did not significantly reduce aversive conditioning measured by the amplitude of the SCR to the tone. Nevertheless, the number of spontaneous fluctuations of skin conductance (not shown in Table 1) was significantly decreased following the highest dose (200 mg) of the drug during both the habituation and the extinction phases of the test (18). Since the number of spontaneous fluctuations is regarded as an index of neurovegetative arousal (4) the effect of this drug may be interpreted to be anxiolytic.

Two studies have applied the aversive conditioning test to patients with anxiety disorders. The first one compared anxious patients to normal controls. Skin conductance level, variability (spontaneous fluctuations) and response amplitudes to tones were significantly greater in patients than controls. Habituation of skin conductance responses to the tone did not differ between groups. All subjects showed enhanced (conditioned) responses to the tones after the conditioning trial, but patients did not show greater conditioning than controls. The results indicate that anxious neurotic outpatients have greater sweat gland activity and

reactivity than controls, but they fail to demonstrate differences in central mechanisms of habituation or conditioning (19). The second study used paired groups of normal subjects and panic patients. As in the preceding study, panic patients showed more spontaneous fluctuations of skin conductance than controls, but conditioning of skin conductance responses to the tone was similar in both groups (20). The last result indicates that panic patients process conditioned anxiety normally, in contrast to unconditioned anxiety (Figure 3).

Simulated public speaking

In 1965, Geer (21) demonstrated that fear of speaking in public is highly prevalent among students. On this basis, McNair and co-workers (22) developed and validated a model of clinical anxiety, named simulated public speaking (SPS), which consists of speaking in front of a video camera. Further studies have shown that fear of speaking in public is fairly constant across genders, races and ages (23). It is the most frequent social fear found in epidemiological studies, being intensified in social phobia (24-26). In addition, the SPS test has been shown to provoke anxiety in healthy volunteers irrespective of trait anxiety level, while another experimental model of anxiety, the Stroop Color-Word test (SCWT, see below), was anxiogenic only in persons with high trait anxiety (27). For these reasons, SPS is believed be a species-specific response.

Later, Guimarães and co-workers (28) modified the original procedure, and this version of the method has been used in several pharmacological studies. In the SPS test, the subject is requested to prepare a speech and then speak in front of a video camera, the performance being recorded on videotape. As in the aversive conditioning model, each subject participates in the SPS only once, but in addition to physiological measures, such as arterial blood pressure and heart rate, psychometric measures of subjective states are taken. These are Spielberger's (29) State-Trait Anxiety Inventory (STAI) and the Visual Analog Mood Scale (VAMS) developed by Norris (30). In this scale, the subject is told to mark a point that identifies his/her present subjective state on a 10-cm strait line placed between two words that describe opposite mood states (e.g., calm - agitated). Factor analysis has grouped the items of the scale into four factors, namely anxiety, mental sedation, physical sedation and other feelings and attitudes (30,31). Reported results have shown that VAMS is more sensitive than STAI-S in the detection of drug effects on anxiety (28,31), provided initial instructions and supervision are given to limit the tendency to extreme choices by the subjects (32). In addition to these anxiety scales, a somatic symptom scale is also used to measure somatic symptoms that interfere with anxiety. These psychometric measures have provided more relevant results than the physiological measures in the drug studies carried out so far. As a result, this review will focus only on the former.

The sequence of the experimental session is summarized in Table 2. After 15 min in the laboratory, initial measures are taken (B). Soon after, the subject swallows the

Table 2. Flowchart of the experimental session in the simulated public speaking (SPS) test as in Zuardi et al. (31).

Time (h:min)	Phases	Measures
-0:25	Adaptation	
-0:10	Initial (B)	VAMS, STAI-T, STAI-S, SSS, BP, HR
0:00	Drug intake	
1:00	Pre-stress (P)	VAMS, STAI-S, SSS, BP, HR
1:10	Instructions	
1:13	Speech preparation	
1:15	Anticipatory anxiety (A)	VAMS, STAI-S, SSS, BP, HR
1:20	Onset of speech	
1:22	Performance anxiety (S)	VAMS, STAI-S, SSS, BP, HR
1:28	Resumes speech	
1:30	End of speech	
2:00	Final (F)	VAMS, STAI-T, STAI-S, SSS, BP, HR

VAMS, Visual Analog Mood Scale; STAI, State (S)-Trait (T) Anxiety Inventory; SSS, Somatic Symptoms Scale; BP, arterial blood pressure; HR, heart rate.

capsule containing drug or placebo. Following a time interval needed to reach maximum plasma drug concentration, pre-stress (P) measures are taken and pre-recorded instructions are played on a video screen. It is explained that the subject has 2 min to prepare a 4-min speech, and that this speech will be recorded on videotape for later analysis by a psychologist. In some of the reported studies the topic was emotionally neutral (e.g., physiological topics for medical students), while in others the subject was requested to talk about particularly anxiety-provoking life events. Anticipatory anxiety measures (A) are taken before speech onset. The address is interrupted after 2 min for taking measures of performance anxiety (S). Speech is resumed soon after and continued for another 2 min. Post-stress measures (F) are taken 15 min later.

Table 3. Effects of drugs on simulated public speaking.

Drug	Dose (mg, *po*)	Test phase			Reference
		Before	During	After	
Diazepam	5	0	0	0	McNair et al. (22)
	10	–	–	–	
	10	0	0	0	Graeff et al. (33)[a]
	10	–	–	–	Guimarães et al. (34)[a]
	10	–	0	–	Zuardi et al. (31)
Lorazepam	2	–	–	0	Guimarães et al. (28)
Flumazenil	1[b]	0	–	0	Kapczinski et al. (35)
Metergoline	12	+[c]	0	0	Graeff et al. (33)[a]
Buspirone	5	0	0	0	Guimarães et al. (34)[a]
Ipsapirone	5	0	–	0	Zuardi et al. (31)
Clomipramine	25	0	+	0	Guimarães et al. (28)
Ritanserin	2.5	0	0	0	Guimarães et al. (36)
	10	0	0	+	
Nefazodone	100	0	0	0	Silva et al. (18)
	200	0	+	0	
d-Fenfluramine	15	0	0	–	Hetem et al. (12)
	30	0	–	0	
- Tryptophan	–	0	+[d]	0	Monteiro-dos-Santos et al. (37)
	–	0	0	0[e]	Shansis et al. (38)
mCPP	15	0	0	0	Connel et al. (13)
Maprotiline	50	–	–	–	Guimarães et al. (28)
Cannabidiol	300	0	0	–	Zuardi et al. (31)

mCPP = methyl-chlorophenylpiperazine. +, increase; –, decrease; 0, no change. [a]audiocassette recorder; [b]*iv*; [c]Spielberger's anxiety scale; [d]in female, but not in male subjects; [e]only male subjects in the study.

Table 3 summarizes the effect of drugs on anxiety measures before (P) during (A and/or S) and after (F) stress (12,13,18,22,28, 31,33-38). It can be seen that the benzodiazepine anxiolytics diazepam and lorazepam consistently decreased anxiety at pre-stress (P). The only exception is the study by Graeff and co-workers (33), which used an audiocassette instead of video recording, and the STAI-S scale, which is less sensitive than VAMS in detecting drug effects (38,39). The level of anxiety during stress may be equal to or lower than in placebo control (Table 3), but in every case the increase in anxiety induced by SPS (phase A or S minus phase P) was not significantly modified by the benzodiazepines (data not shown in Table 3). The noradrenaline reuptake inhibitor maprotiline had a similar effect. In contrast, drugs that primarily affect serotonergic neurotransmission, except for metergoline, do not change pre-stress anxiety. When effective, they either increase or decrease stress-induced anxiety. The difference in drug response between basal anxiety and SPS-induced anxiety indicates that two types of emotional states are generated by each condition. The former seems to be akin to the conditioned anxiety generated by aversive conditioning (see above), whereas the SPS-induced anxiety is a distinct type of emotion, resistant to anxiolytics.

Several drug assays have been carried out to test the dual 5-HT-anxiety hypothesis (14). This hypothesis predicts that drugs that enhance the action of 5-HT may decrease SPS-induced anxiety, while drugs that reduce the action of 5-HT may have the opposite effect. In this way, the pro-anxiety effect of the 5-$HT_{2A/2C}$-receptor blocker ritanserin is consistent with this prediction. Also, the anxiogenic effect observed with either clomipramine or nefazodone (Figure 4) would be an expected result if 5-HT reuptake inhibitors acted mainly by enhancing the action of 5-HT on autosomic 5-HT_{1A} receptors, as discussed above.

However, the absence of a similar effect of buspirone and, even more disturbing, the anxiolytic effect of the similar 5-HT_{1A} agonist ipsapirone do not support the hypothesis being tested. Also, tryptophan depletion, obtained by dietary restriction plus intake of a concentrated amino acid drink devoid of tryptophan, had no effect on SPS-induced anxiety (37,38). Nevertheless, in the former study there was an increase in performance anxiety measured by STAI in female, though not in male subjects (37). The last result is consistent with the dual 5-HT-anxiety hypothesis (14).

In the opposite direction, the 5-HT-releasing agent d-fenfluramine, supposedly increasing 5-HT at postsynaptic receptors, decreased SPS-induced anxiety (Figure 3). The last result is that predicted by the dual 5-HT-anxiety hypothesis.

The SPS test was used in the above comparative study between panic patients and controls, in addition to aversive conditioning (20). The rationale that guided this research was the hypothesis that brain structures responsible for defensive reactions to proximal threat represent the main neural substrate of panic disorder (5,14,39-41). Admitting that SPS measures a species-specific response, panic patients should be different from controls in this model. Indeed, panic patients showed higher baseline levels of VAMS-measured anxiety than controls and, unlike controls, their anxiety failed to increase before and during speech. These results indicate that panic patients process the type of anxiety generated by the SPS test in an abnormal manner (20). Another recent study made use of the SPS test to investigate the response to stress of autistic-like children. The results obtained showed that these children reacted far less than controls to the SPS, as indicated by heart rate and salivary cortisol measures (42). The authors suggested that the impaired responses to psychosocial stress could be the result of limited ability to react adequately to the social environment. The same impairment in stress processing has been found in schizophrenia, and might be a factor in the vulnerability of autistic-like children to developing schizophrenia.

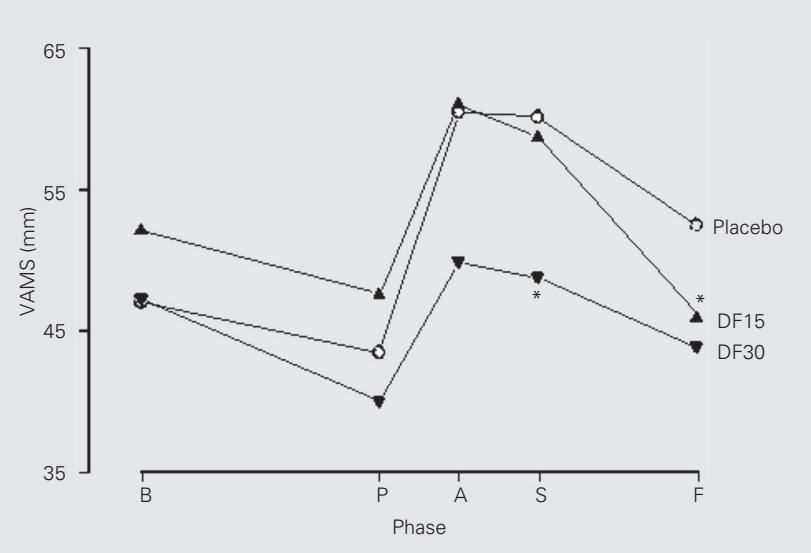

Figure 3. d-Fenfluramine reduces the anxiety induced by simulated public speaking in healthy volunteers. Points indicate mean of 15 subjects in mm of the Visual Analog Mood Scale (VAMS). *B*: initial measurement, *P*: pre-stress, *A*: anticipatory anxiety, *S*: speaking performance anxiety, *F*: final, post-stress measure. DF15: 15 mg, DF30: 30 mg d-fenfluramine. *P<0.05 compared to placebo (Duncan test). Modified from Ref. 12.

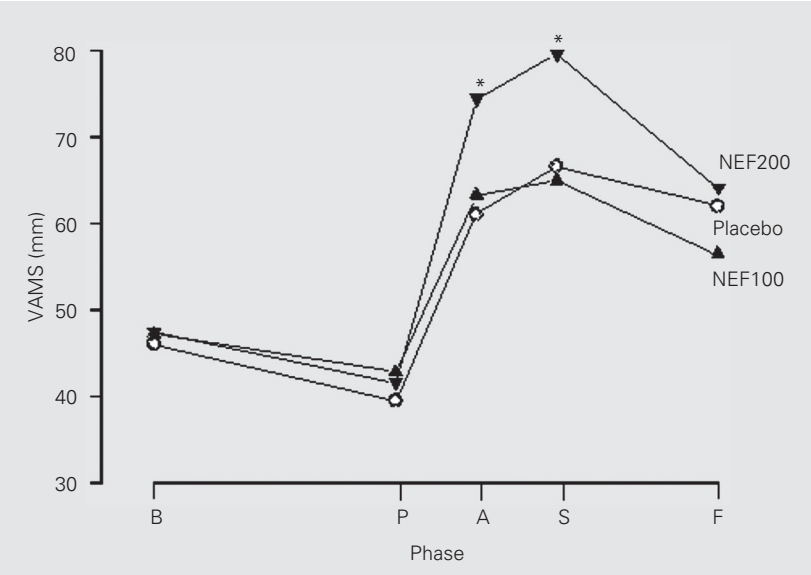

Figure 4. Nefazodone enhances the anxiety induced by simulated public speaking. N = 15. NEF100: 100 mg, NEF200: 200 mg nefazodone. Other specifications are given in the legend to Figure 3. Modified from Ref. 18.

Stroop Color-Word test

The SCWT was originally developed to investigate basic cognitive functions. In 1935, Stroop observed that naming the color of words appearing in another color takes more time than naming the color of a word appearing in the same color. These phenomena became known as Stroop's interference and congruence effects, respectively. Therefore, the SCWT produces a cognitive conflict that may induce anxiety (43,44).

Nakano and co-workers (45) were the first to standardize the instrument, and their method is described next. There are 3 black cards with elements arranged in a 10 x 10 matrix. The first card, named the word card, has words written in white naming five colors: red, blue, green, yellow and purple. The second, the color card, contains only colored circles 8 mm in diameter. In the third, the color-word card, colors and words are discordant; for instance, the word red is printed in green. For the test, the subject is required to read aloud the word card, to name the colors on the color card and to name the colors on the color-word card as fast as possible. The experimenter points out each error - reading the word instead of naming the color - to the subject and records the frequency of errors as well as the time taken to perform the test. It is assumed that psychological stress induces errors and slows the test performance. In the study by Nakano and co-workers (45) volunteers were selected on the basis of trait anxiety. In subjects with high trait anxiety, state anxiety induced by the SCWT was alleviated by diazepam and, to a lesser extent, by nabilone. The latter drug is a synthetic cannabinoid receptor ligand and potential anxiolytic agent (Table 4; 45-47). Tulen and co-workers (47) modified the former procedure using a videotape-recorded presentation in which the words red, green, blue and yellow appear on a TV screen colored in one of these colors, either congruently or incongruently, in a random sequence. The subject is required to write the colors of the words on a sheet of paper as they appear on the TV screen. The results obtained showed that this procedure induces feelings of anxiety and also increases plasma and urinary adrenaline, heart rate, respiration rate, electrodermal activity, and electromyography, and decreases finger pulse amplitude. In a further study the subjects repeatedly performed a 10-min version of the SCWT, with 10 min of rest between tests (46). Lorazepam was administered before each rest period in increasing doses of 0.0, 0.6, 0.13, 0.25 and 0.5 mg (total cumulative dose: 0.94 mg). Heart rate showed a dose-dependent decrease during rest with an ED50 of 0.13 mg lorazepam, while the drug had no effect on the cardiovascular and plasma catecholamine responses to the SCWT. Subjective fatigue and reaction time increased significantly after 0.94 mg lorazepam, while vigor decreased at the same dose. However, the drug did not affect state anxiety measured soon after the SCWT (Table 4). These data show differential effects of lorazepam on cardiovascular, biochemical and psychological function. While heart rate was suppressed at low doses during rest and reaction time and subjective fatigue increased at doses that induced sedation, state anxiety and physiological response patterns to the SCWT were not influenced by lorazepam.

Leite and co-workers (46) recorded the performance of the SCWT on videotape, with the subject being able to see him/herself performing the test on a TV screen. This

Table 4. Effects of drugs on state anxiety in the Stroop Color-Word test.

Drug	Dose (mg, *po*)	Effect	Reference
Diazepam	5	–	Nakano et al. (45)
	5	–	Leite et al. (46)
Lorazepam	0.94[a]	0	Tulen et al. (47)
Nabilone	2	–	Nakano et al. (45)

–, decrease (anxiolytic); 0, no change.
[a]cumulative dose after repeated drug administration and testing.

procedure increased state anxiety in volunteers with normal levels of trait anxiety (between 30 and 50 on STAI-T), and this increase was attenuated by diazepam (Table 4). In contrast, the standard, non-recorded procedure increased state anxiety only in volunteers with high trait anxiety (above 50 on STAI-T). The need to select volunteers with high trait anxiety to obtain measurable increases in state anxiety has also been stressed by Palma and co-workers (27). The results of a further study by Silva and Leite (11) have shown that the video-recorded SCWT procedure induces physiological, in addition to psychological, changes, which may be useful to measure drug effects more objectively.

Overall, the pharmacological results obtained so far with the SCWT are few, and one study with lorazepam yielded negative results (Table 4). The video recording of the SCWT developed by Leite and co-workers (46) combines the SCWT with an element of the SPS test. Further drug studies are needed to determine the type of anxiety generated by such mixed procedure.

Fear-potentiated startle

The jump response of rats to a loud noise increases in the presence of an aversive conditioned stimulus, that is, a previously neutral stimulus that has been paired with an electric foot shock or another primarily aversive stimulus (48). Davis (49) extensively studied this phenomenon, which has been named fear-potentiated startle (FPS). Neurophysiological results obtained in his laboratory indicate that the conditioned stimulus activates the central nucleus of the amygdala through a pathway involving the lateral geniculate nucleus, perirhinal cortex, and lateral and basolateral amygdaloid nuclei. The central nucleus of the amygdala then projects directly to the acoustic startle pathway so as to modulate the startle response. Furthermore, reported pharmacological results qualify this test as an animal model predictive of generalized anxiety disorder (1).

Grillon and co-workers (50) developed a similar model for human subjects. They recorded the eye blink reflex elicited by a loud white noise (106 dB, 40 ms). The protocol described in a later study (51) is as follows. The subject sits on a comfortable chair looking at a front panel provided with a digital timer and two lights, one red and the other green. Electric shocks are delivered through electrodes attached to on one of the wrists. Shocks may occur when the red light is on (threat condition), but never when the green light is on (safe condition). The experimental session is divided into three phases: habituation, threat and recovery. The eye blink reflex is recorded in three trials, separated by 4-min inter-trial intervals. Each trial starts with 6 presentations of the noise alone. Then, the same noise is presented alternatively 6 times under the threat condition and 6 times under the safe condition. The subject is informed that he/she may receive 1 to 3 shocks during the session, of increasing intensity. Actually, only one shock (1.5 mA, 50 s) is delivered during the final 5 s of the last threat condition in trial 2. The subject monitors the duration of each condition by watching the timer, and is informed that the shocks may occur only in the last 10 s of the threat condition. The threat condition lasts 50 s, while the safe condition lasts 60 s.

In contrast to the animal model, few drug studies have been performed so far with the human version of the FPS. Their results are summarized in Table 5 (52-55).

Table 5. Effects of drugs on fear-potentiated startle.

Drug	Dose (mg, *po*)	Effect	Reference
Diazepam	10	–	Patrick et al. (52)
	15	–	
	10	–	Bitsios et al. (53)
Alprazolam	0.25-1	–	Riba et al. (54)
Ethanol	Drink	0	Curtin et al. (55)

–, decrease (anxiolytic); 0, no change.

It may be seen that the two benzodiazepine anxiolytics diazepam and alprazolam consistently attenuated the enhancement of startle caused by the threat condition. Nevertheless, there is a negative result with ethanol. Although more pharmacological investigations are necessary to validate the test, these results are consistent with the animal data (1,49), supporting FPS as a fair candidate to an experimental anxiety test. The face validity of the procedure is also clear and, together with the observed sensitivity to anxiolytics, strongly suggests that the test provokes conditioned, anticipatory anxiety, seemingly related to generalized anxiety disorder (14). Accordingly, Grillon and coworkers (51) have shown that FPS differed in the low and high anxiety subjects. Startle potentiation was larger in the high anxiety group as compared to the low anxiety group, as assessed with the STAI-S (see above). The time course of startle modulation suggested a longer duration of anticipatory anxiety in the high anxiety group. Trait anxiety, assessed with the STAI-T, was not related to individual differences in either baseline or FPS.

As observed for psychopathology, an increase in FPS compared to normal controls has been reported in panic disorder, though only in patients less than 40 years old (56). Increased FPS was also found in post-traumatic stress disorder (57) and in the adolescent offspring of parents with anxiety disorders (58). The last result suggests that FPS may detect vulnerability to anxiety disorders.

Conclusion

From the reviewed evidence, it may be concluded that aversive conditioning to tones and FPS are based on classical conditioning of anticipatory anxiety. Accordingly, their sensitivity to benzodiazepines suggests that they are related to generalized anxiety disorder. On the other hand, the increase in anxiety determined by simulated public speaking is resistant to these drugs and sensitive to drugs affecting serotonergic neurotransmission. This pharmacological profile, together with reported epidemiological evidence, suggests that the emotional state generated by public speaking represents a species-specific response, which may be related to social phobia and panic disorder. The status of the SCWT remains uncertain.

A comprehensive review of animal models of anxiety led to the conclusion that tests with approach-avoidance conflict are the best predictors of drug effects on generalized anxiety disorder (1). Although it may be said that the SCWT generates a cognitive conflict between the color and meaning of the incongruent word, so far no human model with approach-avoidance conflict has been developed.

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