Fear Conditioning in Virtual Reality Contexts: A New Tool for the Study of Anxiety

Johanna M. Baas, Monique Nugent, Shmuel Lissek, Daniel S. Pine, and Christian Grillon

Background: *Context conditioning has been suggested to model clinical anxiety, but context, as manipulated in animal models, has not been translated to human studies. A virtual environment might prove to be the ideal tool for innovative experimental paradigms to study explicitly cued fear and contextual anxiety in humans.*

Methods: *Subjects were guided through a virtual environment that consisted of two rooms connected by a street scene. In each of the rooms, a blue and a yellow panel on a wall served as explicit conditioned stimuli (CS). The panels were displayed several times. One of the panels (CS+) was associated with a shock in one of the rooms (shock room). No shock was administered in the other room (safe room). Acoustic startle stimuli were administered in the presence and in the absence of the panels to assess explicit cued conditioning to the CS and context conditioning to the rooms, respectively.*

Results: *Startle was potentiated by the CS+ in both rooms, which suggests generalization of fear across contexts. After acquisition, startle was potentiated in the shock room, compared with the safe room, in the absence of the CS+.*

Conclusions: *These results support the future use of virtual reality to design new conditioning experiments to study both fear and anxiety.*

Key Words: Virtual reality, classical conditioning, fear conditioning, context conditioning, startle, psychophysiology, anxiety

Abundant research has been devoted to understanding anxiety and anxiety disorders. Animal research has generated detailed information regarding the neurobiology of fear and anxiety; however, more work is needed to translate animal experimentation into human research. The cross-species applicable methodologies of fear conditioning and the startle reflex might provide important avenues toward this end (for a review, see Grillon and Baas 2003). In humans and animals, the startle reflex is potentiated in the presence of a stimulus that has been conditioned with an electric shock (Davis et al 1993; Grillon et al 1991).

Although the basic conditioning procedure is easily translated to human subjects, major questions regarding fear conditioning in humans remain relatively unexplored. Most studies in humans examine processes during acquisition and extinction of phasic fear to an explicit cue, such as a light or a tone. New procedures are needed to study other processes relevant to patient populations, such as context conditioning, generalization of fear (across contexts), and inhibitory fear processes.

During conditioning, associations develop to both explicit (specific) and contextual cues present during acquisition. Contextual cues form the background milieu in which specific cues are encountered and play an important role in the acquisition and inhibition of fear (Bouton 2002). Context conditioning is defined as the display of anxiety in a context in which shocks have been previously administered. Animal research suggests that context conditioning, as opposed to explicitly cued fear conditioning, captures features of a more sustained anxiety response (Davis 1998; Walker et al 2003). Major methodologic hurdles impede efforts to generate clinical measures derived from rodent context conditioning studies, in which differential spatial contexts (different cages) are used. A computer-generated virtual reality environment can function as a tool to create different spatial contexts while keeping the subject stationary in the laboratory or magnetic resonance imaging scanner (Pine et al 2001, 2002). Compared to standard conditioning experiments with isolated stimuli, virtual reality provides a stimulating environment that is engaging to subjects. Although virtual reality has been successfully applied to demonstrate explicitly cued conditioning (Pine et al 2001) and to treat individuals with anxiety disorders (Rothbaum et al 1995), it has not been previously used to demonstrate conditioning to a context.

The aim of this study was to elicit context conditioning with a virtual reality environment comprising two distinct contexts. To dissociate the effects of discrete cues that might become predictive of the shock from more general context effects, shocks were paired with a particular bright-colored light panel in one of the two contexts. These panels were two different colors and were displayed in both contexts. The panels were turned on and off so that they were visible during phasic conditioned stimuli (CS) exposures and invisible during all other time spent in the context. Additionally, during CS presentations, no specific parts of the context were visible because the lighted panel was enlarged to cover most of the screen. Delivering shocks during explicit cues, while none of the contextual cues were visible, protected against the possibility that context conditioning would result from a direct association between the unconditioned stimulus and the specific features of the context. In addition, this design provided an opportunity to assess generalization of fear evoked by the cues across contexts.

Methods and Materials

Subjects

Included in the analysis were 11 women and 11 men (mean age 26.4). One additional subject did not learn to predict the shock and therefore was not included in the analysis. Participants gave written informed consent, which had been approved by the National Institute of Mental Health Human Investigation Review Board.

Stimuli and Apparatus

The virtual environment was adapted from the programmable Duke Nukem game (Pine et al 2002) and consisted of two rooms separated by a street scene. Regular game features were removed, and the rooms were separated from the full environment and decorated as a "magazine store" and an "office." See Figure

From the Mood and Anxiety Disorders Program, National Institute of Mental Health, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland.

Address reprint requests to Christian Grillon, Ph.D., National Institute of Mental Health, Mood and Anxiety Disorders Program, 15K North Drive, Building 15K, Room 113, MSC 2670, Bethesda, MD 20892-2670.

Received August 13, 2003; revised December 30, 2003; revised February 18, 2004; accepted February 20, 2004.

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2004.02.024

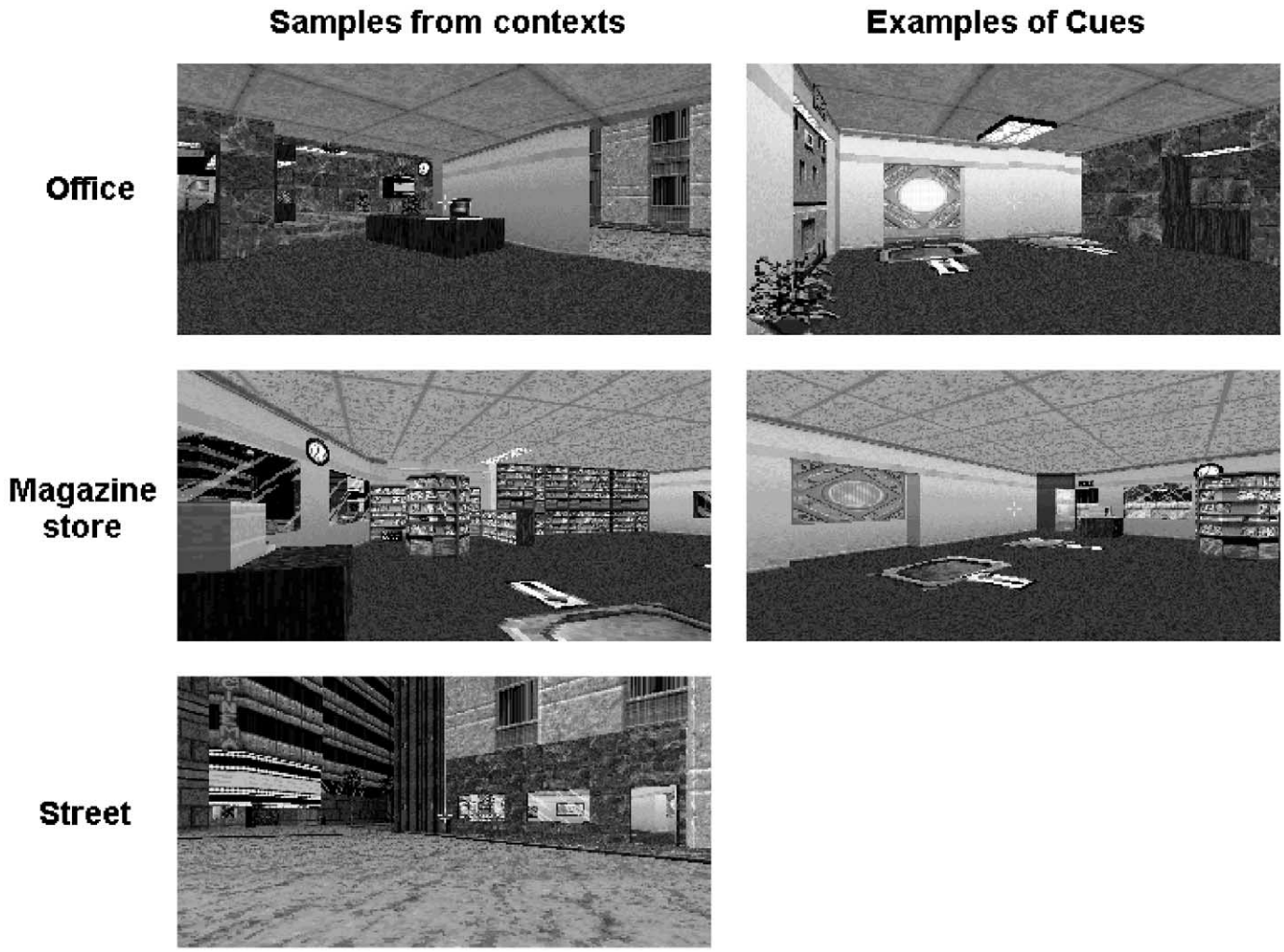

Figure 1. Black-and-white pictures of the virtual environment, illustrating the different contexts. Left column: The different virtual spaces ("office," "magazine store," and "street"). The two indoor spaces differed in furniture (desk and plants vs. checkout counter and magazine shelves), color of the carpet (blue vs. red), shape, and other details. Right column: Locations and general features of the cues. In both pictures, the left panel is activated and the right panel is deactivated. During the experiment, the cues were never viewed from this distance when activated but rather from close up, filling most of the field of view.

1 for black-and-white examples of the rooms. The rooms differed in features, such as shape, color of the carpet (blue vs. red) and furniture (desk, chair, and plants vs. bright-colored magazine shelves). Each room contained two panels (invisible when deactivated) to display either a bright yellow or a blue light. A CS trial consisted of an 8-sec presentation of a yellow or blue panel, which covered most of the screen throughout the 8-sec trial. Startle probes were white noises [50 msec, 100 dB(A)]. Eyeblink electromyogram was recorded with two 6-mm tin electrodes placed under the right eye (band-pass filter 30–200 Hz; Contact Precision Instruments, Cambridge, Massachusetts). Shocks (up to 5 mA) were delivered through two electrodes placed on the left wrist.

Procedure

Shock intensity was set individually at a level that was rated as moderately painful. The experiment consisted of three phases: preacquisition, acquisition, and postacquisition. Subjects were instructed that electric shocks would be delivered and that the shocks would be predictable if they attended to the experiment. Preacquisition consisted of preexposure to all parts of the virtual environment (street, both rooms, and colored panels in both rooms), with nine randomly delivered startle probes to reduce initial startle reactivity (intertrial interval, 18.4 sec). The experiment proper consisted of two runs of acquisition and one run of postacquisition. During these three runs, a prerecorded sequence of movements and events in the environment was played back on a large flat screen in front of the subject in an otherwise dark room. In each run, subjects were navigated into each of the two rooms three times. Each entry in a room lasted two minutes, during which the blue and the yellow panels (CS) were both displayed twice for 8 sec. The CS panels stayed stationary on the screen for the full 8 sec. The order of cue presentation in each room was counterbalanced within subjects. The order of room entries (alternating) and cue presentation (semi-random) was identical for all subjects; however, the CS order was counterbalanced between subjects, by opposite assignment of shock reinforcement. For half of the subjects, the panel associated with shock (CS+) was the yellow panel in the "office" (the first room visited), and for the other half the CS+ was the blue panel in the "magazine store" (the second room visited). By mirroring the assignment of the room and cue associated with the shock

between subjects, counterbalancing of orders occurred between subjects. In the acquisition phase, every occurrence of one of the two panels (i.e., yellow or blue) in only one of the two rooms ("office" or "magazine store") was associated with a shock at offset. No shocks were administered during postacquisition. One to two startle probes were presented in the intervals between CSs in each visit to assess contextual fear. In each visit, one startle probe was presented during one presentation of each of the two CSs. Conditioned stimuli (panels) were probed for only 50% of the presentations. The number of probes during the context was varied to avoid predictability, and to maintain a minimum interprobe interval of 13 sec while assessing startle during both CS and intertrial intervals. Interprobe intervals ranged from 13 sec to 26 sec and were balanced across conditions, with an overall average of 18.9 sec.

After the first acquisition run, subjects answered questions to assess their awareness of the contingency between shocks and the stimuli in the virtual environment. The questions were 1) "Were the shocks predictable (circle one answer)," with answer options "yes," "no," and "I don't know"; and 2) "When did the shocks occur?" If the subject did not indicate the correct and specific contingency, the questionnaire was repeated after the second run. At the end of the experiment, subjects rated their levels of anxiety for each condition on a scale of 1 to 10 (anchors "no anxiety," "extreme anxiety") and the perceived likelihood of receiving a shock on a scale of 1 to 5 (anchors "not at all likely," "very likely"). Questions regarding contingency and anxiety were posed for all conditions, for the context in general, and for the specific cues. Two examples of their format are these: "How anxious were you in the office?" (anxiety, general) and "While in the office, how likely were you to receive a shock following the yellow light?" (contingency, specific). Eyeblink electromyogram was smoothed (20-msec moving window average). Peak amplitude was scored during the 100 msec after noise onset.

Results

Awareness and Anxiety Ratings

All subjects included in the analysis (22 of 23) reported both the correct color of the panel and the correct room that was associated with the shocks. The majority (16) reported both the correct color and the correct room after the first run. Six subjects reported only the correct color of the display associated with the shocks. All of these six subjects also reported the correct room after the second run. The one subject who could not verbalize the contingency explicitly after the second run of acquisition was excluded from further analysis. Table 1 summarizes the ratings taken after the experiment of the likelihood of receiving a shock and of subjective anxiety in the different conditions. Subjects retrospectively reported greater likelihood of receiving a shock in combination with greater anxiety in the room that was associated with the shocks, specifically during the cue that had predicted the shocks. These effects were all significant, with all p values below the .001 level [corresponding F values were as follows: contingency-ratings $F(1,20)$ main effect Room = 104.5, main effect Cue = 158.5, and interaction = 73.4; anxiety-ratings $F(1,21)$ main effect Room = 48.2, main effect Cue = 39.1, and interaction = 26.9].

Eyeblink Startle: Cue

Figure 2 shows startle during cues. A repeated-measures analysis of variance (ANOVA) with the factors Run (acquisition 1, 2), Room (shock, safe), and Cue (CS+, CS−) revealed main effects for Cue [$F(1,21) = 6.8$, $p = .017$] and Run [$F(1,21) = 19.9$, $p = .001$] during acquisition, but not for Room [$F(1,21) < 1.0$, ns). The three-way interaction (Run × Room × Cue) was not significant [$F(1,21) = 2.0$, ns]. The pattern of results was the same in the postacquisition run [main effect Cue $F(1,21) = 5.5$, $p = .029$, no interaction].

Table 1. Contingency and Anxiety Ratings

	Likelihood of Shock ($n = 21$)[a]	Anxiety ($n = 22$)
Street	1.0 (0)	1.6 (1.0)
Shock Room		
Overall	4.3 (1.1)	4.3 (2.5)
CS+	4.5 (1.0)	6.2 (2.9)
CS−	1.4 (.8)	2.4 (1.8)
Safe Room		
Overall	1.3 (.7)	2.3 (1.3)
CS+	1.5 (.7)	2.6 (1.5)
CS−	1.1 (.3)	1.7 (.9)

Summary of subjective measures acquired retrospectively at the end of the experiment. Data are presented as mean (SD). Contingency of receiving a shock with the different parts of the environment was rated on a scale from 1 to 5. Subjective anxiety was rated on a scale from 1 to 10. The "Overall" measure was the subjects' response to the more general question, such as, "How anxious were you in the office?" See Methods section for more details. CS, conditioned stimuli.
[a]Data for one subject missing.

Eyeblink Startle: Context

The mean startle amplitude in the absence of the CS panels is displayed in Figure 3. The test of context conditioning was conducted during postacquisition, when the effect was not confounded by shock administration. Startle amplitude was greater in the shock room compared with the safe room [repeated-measures ANOVA with factor Room (shock, safe) $F(1,21) = 6.3$, $p = .020$]. An additional analysis including the acquisition phase showed that the linear effect of the interaction Room ×

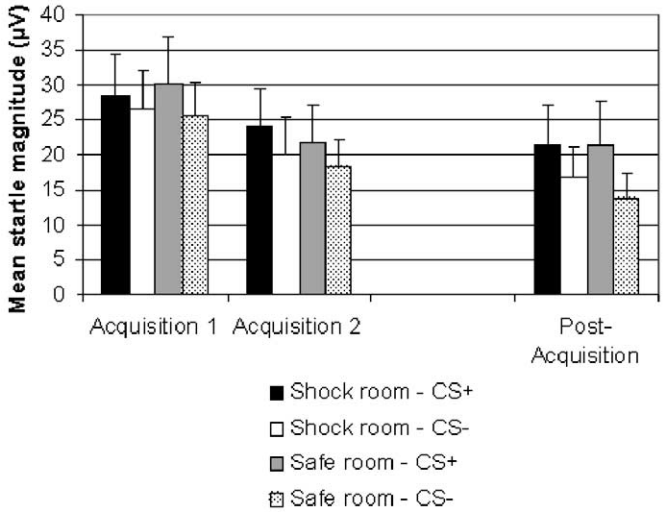

Figure 2. Magnitudes and SEMs of the startle reflex evoked during the conditioned stimuli (CS) in the shock and safe contexts. The CS+ in the shock room was reinforced with a shock at offset during the two acquisition runs. Note the strong generalization of the potentiation effect (from CS+ to CS−) across rooms.

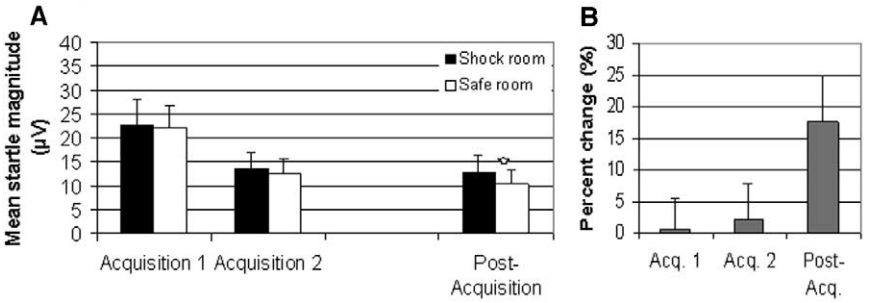

Figure 3. (A) Magnitudes and SEMs of the startle reflex evoked in the shock and safe contexts, when the CS (panels) were not activated. Shock reinforcements were given in the two acquisition runs but not in the postacquisition run. **(B)** Means and SEMs of the percent change in startle magnitude from the safe to the shock room. Acq., acquisition; CS, conditioned stimulus.

Run only reached trend level [$F(1,21) = 2.9$; $p = .10$], with a main effect of Run [$F(2,42) = 8.0$, $p < .001$] and a trend-level effect of Room [$F(1,21) = 3.4$; $p = .08$].

Discussion

This is the first report of the use of virtual reality to document context conditioning in humans. Startle magnitude was increased in individuals returning to a virtual room where they had previously received shocks. The procedure included two different rooms in which two colored panels functioned as explicit CS (see Figure 1 for an impression of the rooms and panels). In one room but not the other, the presentation of one colored panel (CS+) was consistently followed by a shock during the acquisition phase. The main result was that subjects exhibited conditioned fear to both the shock-context and the CS+. This was reflected by greater startle in the shock room compared with the safe room during postacquisition and greater startle during the CS+, compared with the cue that was not associated with the shock (CS−). The latter effect was irrespective of the room, which suggests successful fear conditioning to the cue, in combination with strong stimulus generalization across contexts.

The elevation of startle in the shock room cannot be attributed to a sensitization process because the test of context conditioning took place during the postacquisition phase, when no shocks were administered. It also cannot be attributed to a direct association between discrete features of the context and the shocks. Throughout the 8-sec CS presentation, the majority of the field of view was filled by the CS panel. This view did not contain objects that could serve as contextual cues indicative of the room. In addition, the startle probes used to assess context conditioning were delivered at a time when the CS panels were not activated and thus were invisible.

Although the magnitude of startle potentiation to the context was smaller than startle potentiation to the CS+, it was of the magnitude commonly found in published studies of emotional valence modulation or the effects of darkness (see Grillon and Baas 2003 for a review). The context effect had an average magnitude increase of 17.5% from safe to shock contexts and was highly reliable ($p = .02$). Furthermore, the relatively small size of the effect of contextual conditioning was not unexpected. In general, context conditioning is small in humans (Grillon and Davis 1997). In addition, signaled shocks produce less contextual fear than unsignaled (unpredictable) shocks in animals (Marlin 1981; Rescorla and Wagner 1972) and humans (Grillon 2002; Grillon and Davis 1997). In the present design, the CSs that signaled the shock were very salient and easily overshadowed the context. As a result, subjects were more likely to first learn the association of the shocks with the CS+ (six of 22 reported only the color of the cue after the first run, whereas none reported only the context). In future studies focusing on context conditioning, a design with less salient cues predicting the shock might be preferred to allow for stronger contextual fear.

The lack of differentiation between the two rooms in terms of startle potentiation to the CS+ confirms the notion that excitatory associations generalize across contexts (Bouton 2002). Yet, all subjects became aware that the shock was only presented during the CS+ in one context. Retrospective subjective reports of anxiety correspond with awareness ratings, in that there was greater anxiety during CS+ compared with CS− in the shock room but not in the safe room. Nevertheless, the pattern of startle results indicates that the excitatory tendency of the cue was stronger than the inhibitory properties of the safe context. This is consistent with the view that inhibitory learning is weaker than excitatory learning and takes more time to develop (Bouton 2002; Mineka and Tomarken 1989).

The finding that context conditioning occurs to a virtual room suggests that virtual reality can be used in conjunction with conditioning procedures to model various aspects of anxiety. Context conditioning induces sustained levels of anxiety in contrast to explicitly cued fear (Grillon 2002). We have argued that cue conditioning might model aspects of specific phobias, whereas context conditioning might model more sustained features of anxiety, such as those found in generalized anxiety disorder (Grillon 2002). At the neurobiological level, the amygdala is crucial to explicitly cued fear conditioning. The amygdala is also involved in context conditioning, but other structures, such as the bed nucleus of the stria terminalis (Davis 1998; Walker and Davis 1997) and hippocampus (Holland and Bouton 1999; Phillips and LeDoux 1992) have also been found to play a role. In addition, experimental models for fear and anxiety can help elucidate differences between these responses in aspects such as time course and susceptibility to pharmacologic intervention (Baas et al 2002; De Jongh et al 2003). Virtual reality is a new tool that might enable us to extend these investigations to humans to further our understanding of anxiety and conditioning processes. Exploring generalization across contexts and inhibitory learning in a safe context will be an important next step, because patient populations have been hypothesized to be deficient in the formation of inhibitory associations or in the proper use of safety signals. In addition, virtual reality also offers a means for studying avoidance learning by having subjects navigate in the virtual environment.

Virtual reality is a promising tool for the study of context conditioning, fear generalization, and inhibitory learning. The development of new innovative designs for human research might aid future efforts to bridge the gap between animal and human research.

We thank Harvey Iwamoto for his indispensable help with programming the environment; Meghan Donlevy and Megan Lawley for their practical assistance in setting up and running the study; and Dr. Jonathan Sporn for medical supervision of the study.

Baas JM, Grillon C, Bocker KB, Brack AA, Morgan CA, III, Kenemans JL, Verbaten MN (2002): Benzodiazepines have no effect on fear-potentiated startle in humans. *Psychopharmacol* 161:233–247.

Bouton ME (2002): Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biol Psychiatry* 52:976–986.

Davis M (1998): Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry* 44:1239–1247.

Davis M, Falls WA, Campeau S, Kim M (1993): Fear-potentiated startle: A neural and pharmacological analysis. *Behav Brain Res* 58:175–198.

De Jongh R, Groenink L, Van der Gugten J, Olivier B (2003): Light-enhanced and fear-potentiated startle: Temporal characteristics and effects of α-helical corticotropin-releasing hormone. *Biol Psychiatry* 54:1041–1048.

Grillon C (2002): Associative learning deficits increase symptoms of anxiety in humans. *Biol Psychiatry* 51:851–8.

Grillon C, Ameli R, Woods SW, Merikangas K, Davis M (1991): Fear-potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiol* 28:588–595.

Grillon C, Baas J (2003): A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clin Neurophysiol* 114:1557–1579.

Grillon C, Davis M (1997): Fear-potentiated startle conditioning in humans: Explicit and contextual cue conditioning following paired vs. unpaired training. *Psychophysiol* 34:451–458.

Holland PC, Bouton ME (1999): Hippocampus and context in classical conditioning. *Curr Opin Neurobiol* 9:195–202.

Marlin NA (1981): Contextual associations in trace conditioning. *Anim Learn Behav* 9:519–523.

Mineka S, Tomarken AJ (1989): The role of cognitive biases in the origin and maintenance of fear and anxiety disorders. In: Archer T, Nilsson LF, editors. *Aversion, Avoidance, and Anxiety*. Hillsdale, NJ: Lawrence Erlbaum, 195–221.

Phillips RG, LeDoux JE (1992): Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 106:274–285.

Pine DS, Fyer A, Grun J, Phelps EA, Szeszko PR, Koda V, et al (2001): Methods for developmental studies of fear conditioning circuitry. *Biol Psychiatry* 50:225–228.

Pine DS, Grun J, Maguire EA, Nurgess N, Zarahn E, Koda V, et al (2002): Neurodevelopmental aspects of spatial navigation: A virtual reality fMRI study. *Neuroimage* 15:396–406.

Rescorla RA, Wagner AR (1972): A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement on nonreinforcement. In: Black AH, Prokasy WF, editors. *Classical Conditioning II: Current Theory and Research*. New York: Appleton-Century-Crofts, 64–99.

Rothbaum BO, Hodges LF, Kooper R, Opdyke D, Williford JS, North M (1995): Effectiveness of computer-generated (virtual reality) graded exposure in the treatment of acrophobia. *Am J Psychiatry* 152:626–628.

Walker DL, Davis M (1997): Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J Neurosci* 17:9375–9383.

Walker DL, Toufexis DJ, Davis M (2003): Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur J Pharmacol* 463:199–216.