

Fear Thou Not: Activity of Frontal and Temporal Circuits in Moments of Real-Life Courage

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SUMMARY

How does the brain encode courage in a real-life fearful situation that demands an immediate response? In this study, volunteers who fear snakes had to bring a live snake into close proximity with their heads while their brains were scanned using functional magnetic resonance imaging (fMRI). Bringing the snake closer was associated with a dissociation between subjective fear and somatic arousal. Activity in the subgenual anterior cingulate cortex (sgACC) and the right temporal pole was positively correlated with such action. Further, activity in the sgACC was positively correlated with the level of fear upon choosing to overcome fear but not upon succumbing to it. Conversely, activity in a set of inter-related temporal lobe structures, including the amygdala, was attenuated as the level of fear increased when choosing to overcome fear. We propose how the internally reinforced fast representational shift, in which the courageous-response representation gains control over behavior, takes place.

INTRODUCTION

The Roman philosopher Seneca considered all humans slaves to fear (Seneca, 1969). Yet given the right circumstances, ordinary people can set themselves free of this bondage and act courageously. How is this achieved? To act courageously may mean in ordinary discourse anything from rescuing a child from turbulent waters to selecting a risky academic career. Such a broad spectrum of behaviors hence spans different situations and time intervals. However, the wide spectrum of courageous behaviors does have a common denominator, which is the performance of voluntary action opposed to that promoted by ongoing fear (Rachman, 1984, 1990, 2004a). This suggests that rather different manifestations of courage might share some core brain mechanisms. It is noteworthy that courage as here conceived focuses on action (in spite of fearfulness) that is observed in the general population rather than on an exceptional trait (fearlessness). Thus, by gauging properly defined

actions of either overcoming fear or succumbing to it in an acute controllable fearful situation, one can render certain neural substrates of courage amenable to investigation in a brain research laboratory setting.

Because it has to do with action to overcome fear, the study of brain mechanisms of courage is expected to overlap with the prolific research on brain mechanisms of fear (LeDoux, 1996), fear extinction (Quirk et al., 2006), and cognitive control of emotion (Ochsner and Gross, 2005). There are, however, notable differences. Experimental extinction deals with gradual reconditioning to appreciate that a conditioned fear response is not warranted any more because the original source of fear has vanished, whereas courageous action as considered here involves prompt voluntary overcoming of the fear reaction to an acute, on-line source of fear. And although courage clearly involves emotion control, in research protocols of cognitive control of emotion, participants are commonly instructed to use specific cognitive strategies to regulate emotion, while success or failure of this regulation is not instrumental in generating a behavioral outcome, nor does it affect the unfolding of the experimental protocol. In contrast, real-life courage involves uninstructed idiosyncratic regulation strategies leading to behavioral outcomes that in turn influence future decisions and actions. To the best of our knowledge, to date, no functional brain imaging studies have been reported that allow a choice between succumbing to a naturalistic source of fear or overcoming it and acting on the choice; hence, there are none that have probed brain mechanisms of swift decision and action to overcome ongoing fear.

In this study we devised a paradigm that enables induction of a sustained acute ecological fear ambience within a functional magnetic resonance imaging (fMRI) scanner, while allowing participants instant choices between overcoming and succumbing to the ongoing fear and a behavioral expression of these choices. We selected a snake as the fear-eliciting stimulus because fear of snakes, often intense, is common in the general population (Agras et al., 1969). Specifically, a live snake (corn snake, *Elaphe guttata*, ~1.5 m long) or a toy bear (a control stimulus intended to evoke no fear) were secured to the top of a trolley that could travel step-wise on a conveyer belt spanning the distance between the far end of the scan room and close proximity to the participant's head within the scanner. Participants were instructed that their task was to reach maximal proximity to the objects, while overcoming to the best of their ability

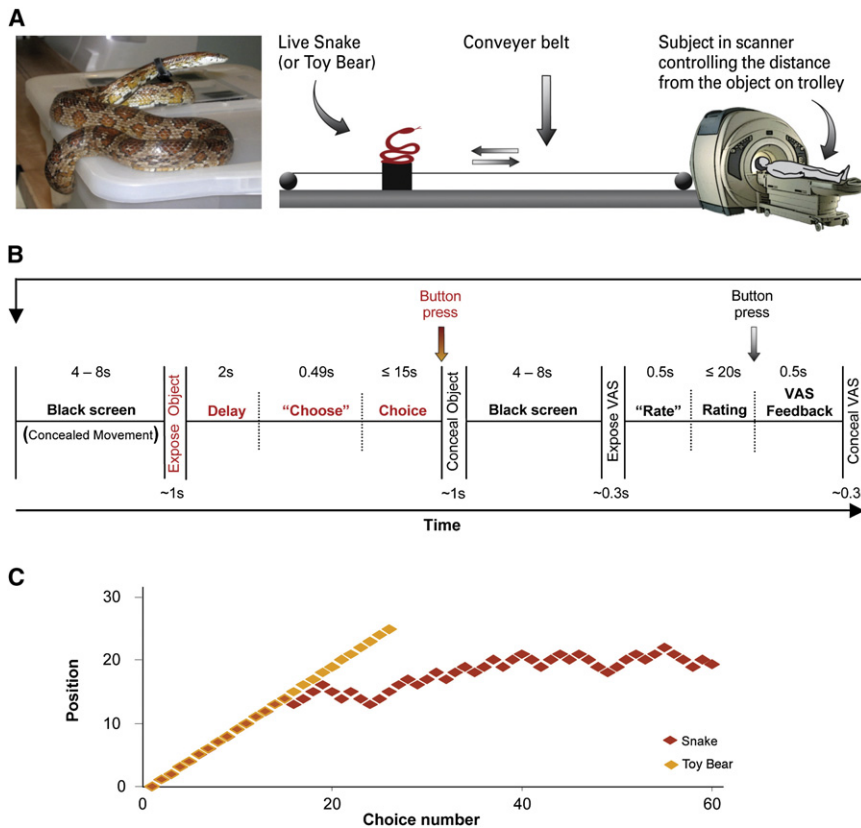


Figure 1. The Experimental Setup and Protocol

(A) The participants' task was to reach maximal proximity to either a live snake (*Snake*) or a toy bear (*Toy Bear*), by repeatedly choosing whether to bring the object closer (*Advance*) or move it away (*Retreat*), while undergoing fMRI brain scanning. (B) Each trial began with a black screen occluding the participant's view (*Black screen*), after which it was lowered (*Expose Object*) to allow full view of the object on the trolley. Following removal of the screen a brief delay was enforced (*Delay*), after which the word "Choose" was sounded via earphones. The participant then expressed the choice (*Advance* or *Retreat*) by pressing one of two response device buttons (*Choice*). The time between beginning of exposure of the object and the choice button press was defined as the choice event of interest (denoted in red). Following the choice the black screen was raised (*Conceal Object*) to again occlude the participant's view (*Black screen*). Next, the black screen was lowered so as to expose only a Visual Analog Scale (VAS) display (*Expose VAS*) and the participant heard the word "Rate." The participant then indicated the fear level felt during the last choice by using the response device (*Rating*), after which the VAS cursor changed color (*VAS feedback*) to indicate that the rating had been recorded. The black screen was then again raised (*Conceal VAS*) to completely block the participant's view, after which occurred the concealed movement of the trolley according to the last choice. If no choice was made within the set time limit, the trolley retreated. The trials were repeated until the trolley

reached maximal proximity to the participant or until a minimum of 20 *Advance* and 20 *Retreat* (or no choice) selections were made. (For further details see [Supplemental Information](#).)

(C) Illustrative behavior of a *Fearful (FF)* participant, depicting the position of the object (*Snake*, *Toy Bear*) relative to the participant (starting from 0, most distant from the participant, to 25 (maximum) or less, nearest to the participant), at all consecutive choices made by the participant. Thus, each descent of the line in the graph signifies a retreat choice and each ascent an approach choice. The first 15 choices in the *Snake* condition made by this participant were *Advance* after which *Retreat* and *Advance* choices were made intermittently.

the fear they might experience. In each trial, the participants were prompted to choose whether to move the object one step closer (*Advance*) or further away (*Retreat*) by pressing a response button while their brains were scanned using fMRI (Figure 1). During scanning, the subjective fear rating and skin conductance response (SCR) for each decision were recorded as well. The participants were also evaluated for trait anxiety in general and for state anxiety in the experiment. Two groups of volunteers took part in the study, selected to permit multiple intra- and intergroup analyses of behavioral performance, physiological reaction, and brain activity: *Fearful*, composed of healthy individuals who fear snakes and who were selected by using a validated snake anxiety questionnaire (SNAQ, Klorman et al., 1974), and *Fearless*, composed of individuals accustomed to handling snakes. In the following, we describe behavioral, physiological, and brain correlates of the task. By combining our findings with current models of fear regulation, we propose a model of brain mechanisms that could account for what we observe when individuals are engaged in moments of real-life courage.

RESULTS

Behavior and Physiology Self-Reported Fear, Anxiety, and Somatic Response

We selected self-reported fear ratings as a measure of subjective fear because no other measure is considered to provide a direct correlate of subjective emotional experience (Wager et al., 2008). Furthermore, self-reported fear ratings and state anxiety scores for the snake condition, acquired independently, were highly correlated ($r = 0.84$, $p < 10^{-12}$), supporting the reliability of these subjective emotion measures in our study. Fear ratings for the snake (*Snake*) were significantly higher than for the toy bear (*Toy Bear*) in the *Fearful* group (*FF*), but not in the *Fearless* group (*FL*) (Figure 2A; *FF*: *Snake* (mean \pm SEM) = 58.14 ± 3.84 ; *Toy Bear* = 0.65 ± 0.25 ; $p < 0.0005$, *FL*: *Snake* = 0.21 ± 0.12 ; *Toy Bear* = 0.21 ± 0.11 ; $p > 0.05$, interaction effect: $F_{1, 45} = 115.04$, $p < 10^{-14}$). State-anxiety scores (STAI-S; Spielberger, 1983) for the two experimental conditions exhibited a similar pattern (Figure 2B; *FF*: *Snake* = 50.81 ± 2.23 ; *Toy Bear* = 30.29 ± 1.25 ; $p < 0.0005$, *FL*: *Snake* = 24.75 ± 1.13 ; *Toy Bear* = 23.94 ± 0.92 ;

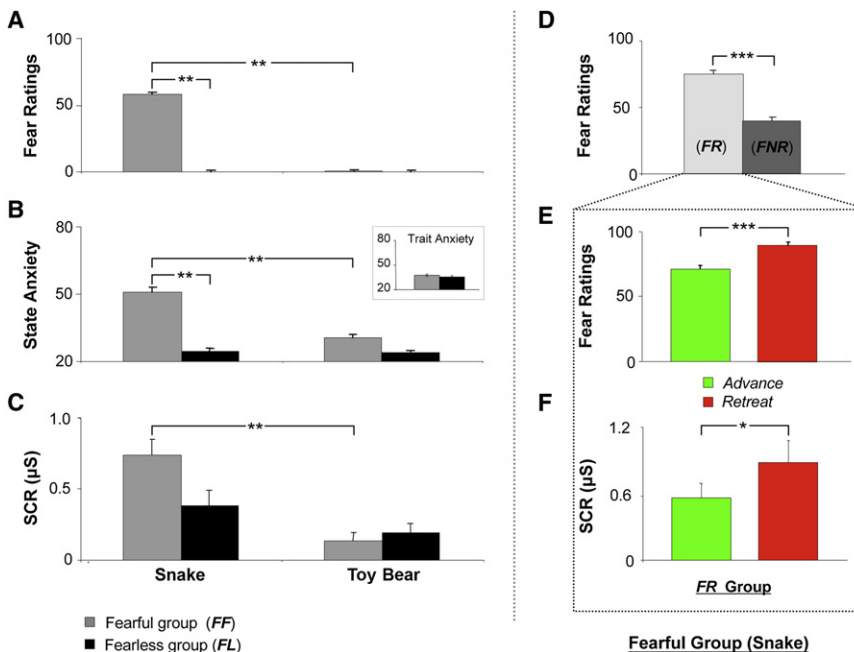


Figure 2. Fear and Anxiety Evoked by the Experimental Stimuli

(A–C) Mean of self-reported fear ratings (A), state anxiety (B), and choice-related skin conductance responses (SCRs) (C) of the *Fearful* (FF) and *Fearless* (FL) groups for *Snake* and *Toy Bear*.

(D) Comparison of mean fear ratings for *Snake* in the two FF subgroups (*Fearful Retreaters*, FR, and *Fearful Nonretreaters*, FNR).

(E and F) Mean fear ratings (E) and choice-related SCRs (F) of the FR group for *Advance* and *Retreat* choices in the *Snake* condition. Error bars here and below represent SEM. * $p < 0.05$, ** $p < 0.0005$, *** $p < 0.000005$.

$p > 0.05$, interaction effect: $F_{1, 45} = 33.97$, $p < 0.000005$). The two groups, however, did not significantly differ in trait anxiety (STAI-T; Spielberger, 1983) (Figure 2B, inset; FF = 37.58 ± 1.41 ; FL = 35.38 ± 1.63 ; $p > 0.05$). Choice-related SCR levels were consistent with the subjective fear ratings and were significantly higher for *Snake* than for *Toy Bear* in the FF, but not the FL (Figure 2C; FF: *Snake* = 0.74 ± 0.12 micro siemens [μ S]; *Toy Bear* = 0.14 ± 0.05 μ S; $p < 0.0005$, FL: *Snake* = 0.38 ± 0.11 μ S; *Toy Bear* = 0.19 ± 0.06 μ S; $p > 0.05$, interaction effect: $F_{1, 44} = 6.73$, $p < 0.05$). Additionally, both fear ratings and state-anxiety scores for *Snake* were significantly higher in the FF as compared with those in the FL (Figures 2A and 2B; $p < 0.0005$ for both comparisons). SCRs displayed a similar trend (Figure 2C; $p = 0.059$).

Fearful Retreaters and Nonretreaters

Examination of choice (*Advance/Retreat*) patterns revealed that all the FL participants made only *Advance* choices in both the *Snake* and *Toy Bear* conditions. Similar behavior was observed in the FF in the *Toy Bear* condition. However, in the *Snake* condition, the FF group appeared to consist of two subgroups: participants who made no or a few (up to three) *Retreat* choices; and those that made a substantial number (seven or above) of *Retreat* choices. We dub these two subgroups “*Fearful Nonretreaters*” (FNR) and “*Fearful Retreaters*” (FR), respectively. To examine whether the different behaviors of these two FF subgroups were a result of the FNR displaying more courage (i.e., overcoming similar levels of fear more successfully) or of experiencing less fear as compared with the FR, we compared the fear ratings of the two groups (Figure 2D). Significantly lower fear ratings in the FNR as compared with those of the FR revealed that the second was the case (FR = 75.68 ± 2.68 ; FNR = 39.42 ± 3.44 ; $p < 0.000005$). We then compared the fear ratings and SCRs for *Advance* and *Retreat* choices within

the FR (Figures 2E and 2F). Both fear ratings and SCRs for *Retreat* choices were significantly higher than those for *Advance* choices, suggesting that within this group exceeding a certain threshold of fear was correlated with a decision to choose *Retreat* (fear ratings: *Advance* = 70.13 ± 3.13 ; *Retreat* = 88.51 ± 2.32 , $t_{15} = 7.4$, $p < 0.000005$; SCRs: *Advance* = 0.56 ± 0.13 μ S; *Retreat* = 0.88 ± 0.2 μ S, $t_{15} = 3.26$, $p < 0.006$).

Relationship of Self-Reported Fear to Proximity of the Snake

A significant correlation between proximity of the object and self-reported fear ratings was found only for the *Snake* condition in the FF (mean $r = 0.86 \pm 0.03$). In a postexperiment debriefing questionnaire (see Supplemental Information, available online), the score for the question “Did you need to make a larger effort to overcome your fear as your proximity to the snake increased?” in the FF was 3.96 ± 0.15 on a scale of 1–5, indicating that higher fear levels necessitated a larger degree of “mental effort” in order to overcome fear and execute *Advance* choices. The average score for the question “To what extent did you try to overcome your fear?” was 3.81 ± 0.16 , indicating that such an effort was indeed made. No parallel measures were obtained for the FL because they reported feeling no fear in the experiment. Collectively, the above results indicate that the *Snake* condition in the FF alone involved ongoing fear that increased with proximity to the snake, necessitated increased effort in order to overcome fear and execute *Advance* choices, and, within the FR, led to *Retreat* choices when this effort was unsuccessful.

Brain Correlates

Advance versus Retreat Contrasts

We proceeded to identify brain areas whose activity correlated with overcoming fear by contrasting the blood-oxygen-level-dependent (BOLD) response of *Advance* and *Retreat*. Since there was no *Retreat* in the FL and FNR in both the *Snake* and *Toy Bear* conditions, or in the FF in the *Toy Bear* condition, this contrast was only possible in the *Snake* data of the FR. The *Retreat* > *Advance* contrast yielded a network of regions,

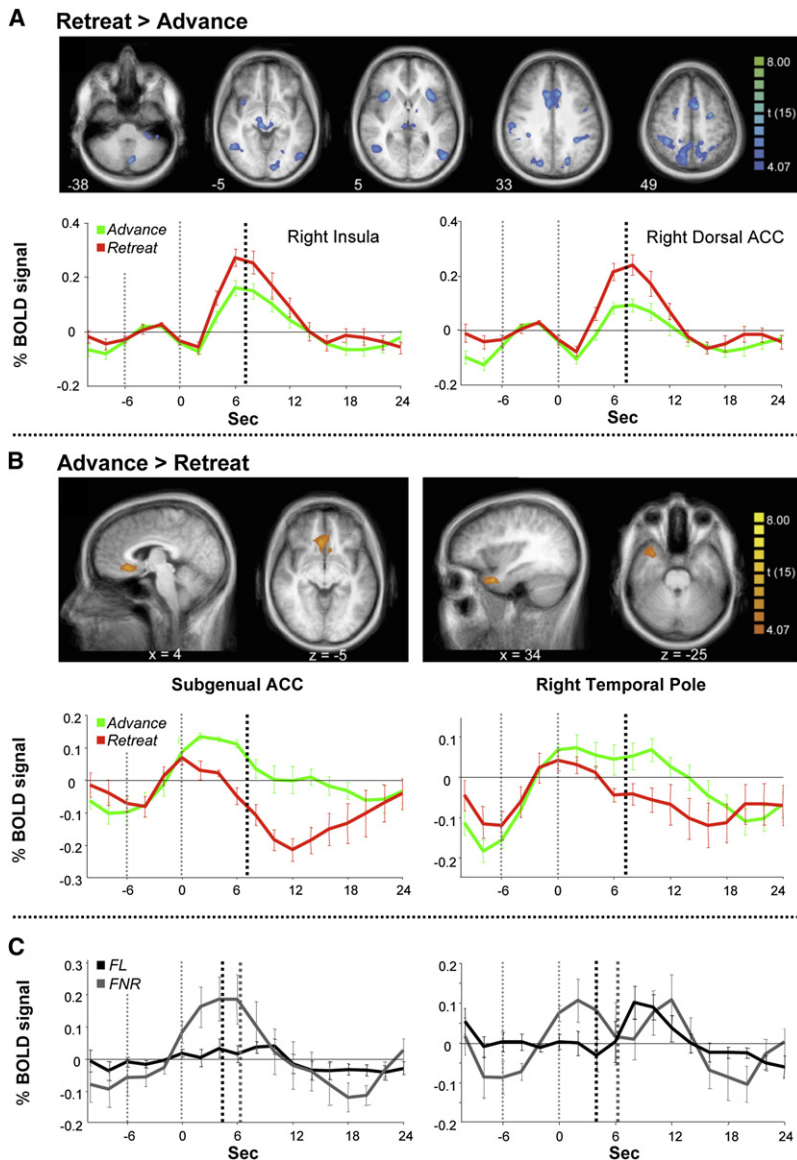


Figure 3. Brain Activity Differentiating Advance from Retreat Choices

(A and B) Statistical maps (radiological orientation, top panels) and average percent BOLD signal change (PSC, bottom panels) for the *Retreat > Advance* (A) and *Advance > Retreat* (B) contrasts for *Snake* choices of the *FR* group. Maps (obtained with a threshold of $p < 0.001$, corrected at $\alpha = 0.05$ for cluster-level false-positive rate, minimal cluster size $>270 \text{ mm}^3$) are overlaid on axial (A and B) and sagittal (B) slices of the group-averaged anatomical map of all *FR* subjects (z/x coordinates are indicated on each image). For the *Retreat > Advance* contrast (A), the PSC of two representative areas (right dorsal ACC and right insula) is shown. All other ROIs derived from this contrast exhibited a similar PSC pattern. In all PSC graphs the heavy dotted line represents the average time of the subjects' choice button press (for the *FR* the same line signifies both *Advance* and *Retreat* choices because average duration of these choices was very similar; see text). Time 0 (right light dotted line) indicates the beginning of the choice event of interest as defined by us a priori. Time -6 (left light dotted line) indicates the average beginning of the prechoice black screen period (for the protocol, see Figure 1B). (C) PSC in the sgACC (left panel) and rTP (right panel) ROIs of the *FNR* and *FL* groups in *Advance* choices of the *Snake* condition. Black and gray heavy dotted lines represent the choice button presses of the *FL* and *FNR* groups, respectively.

See also Figure S1.

an additional analysis in which for each participant only a number of *Advance* choices equal to the number of *Retreat* choices of the participant was used. We further utilized this analysis to examine whether subjective fear could per se differentiate between *Advance* and *Retreat*. For that purpose, for each participant, the *Advance* choices used when equating the numbers of *Advance* and *Retreat* choices were those with the highest fear ratings. Comparison of the average fear of the *Advance* and *Retreat* choices used in this control analysis revealed no significant difference (*Advance* = 87.49 ± 2.42 ; *Retreat* = 88.25 ± 2.33 ; $t_{15} = 0.84$, $p > 0.05$), indicating that high levels of subjective fear alone were not sufficient to induce *Retreat* choices. An *Advance* versus *Retreat* contrast using the equated number of *Advance* and *Retreat* choices yielded statistical maps similar to those obtained when using all *Advance* choices for both the *Retreat > Advance* and *Advance > Retreat* contrasts (Figures S1A and S1B available online). Thus, the different activity in *Advance* and *Retreat* observed in the original contrast did not stem from the larger number of *Advance* choices.

Event-Locked Kinetics of the Differential BOLD Signal in sgACC and rTP

In both *Advance* and *Retreat*, the BOLD signal in sgACC and rTP started to rise approximately 6 s before exposure to the snake (exposure which was originally defined as the onset of the choice event; see Figure 1B) (Figure 3B, middle panel). This time

including visual processing areas, motor-related areas, left inferior frontal gyrus, bilateral dorsal anterior cingulate cortex (ACC), bilateral anterior insula, bilateral red nucleus, and cerebellum (Figure 3A, top panel; Table 1). In contrast, the *Advance > Retreat* contrast yielded two regions only: the subgenual ACC (sgACC) and the right temporal pole (rTP) (Figure 3B, top panel and Table 1).

The differential activity between *Advance* and *Retreat* was not a consequence of the differences in the duration of choices of each type (*Advance* = $7712 \pm 446 \text{ ms}$; *Retreat* = $7534 \pm 464 \text{ ms}$; $t_{15} = 0.57$, $p > 0.05$). Due to the nature of the experimental paradigm, however, the average number of *Advance* choices per participant was significantly larger than that of *Retreat* choices (*Advance* = 35.94 ± 1.02 , *Retreat* = 16.63 ± 1.24 ; $t_{15} = 13.87$, $p < 10^{-9}$). To control for this difference, we conducted

Table 1. Regions Showing Differences between Advance and Retreat in the FR

	Region	x	y	z	mm ³	t value	p value
<i>Retreat > Advance</i>	L fusiform gyrus (BA 19) ^a	-30	-76	-14	2377	7.67	0.000001
	L lingual gyrus (BA 19)	-24	-64	-2	256	5.04	0.0001
	R precuneus (BA 7)	15	-76	40	9145	6.90	0.00005
	L precuneus (BA 7) ^b	-24	-79	25	8637	9.93	<10 ⁻⁶
	R inferior parietal lobule (BA 40)	36	-43	43	6080	7.96	0.000001
	L inferior parietal lobule (BA 40)	-27	-46	43	2461	6.58	0.000009
	R post central gyrus (BA 3)	57	-25	28	2222	6.00	0.00002
	R inferior temporal gyrus (BA 37) ^c	45	-55	-8	3816	6.13	0.00002
	L inferior temporal gyrus (BA 37)	-51	-70	1	2805	7.33	0.000002
	L inferior temporal gyrus (BA 37)	-48	-61	-17	337	5.56	0.00005
	L superior temporal gyrus (BA 22)	57	-40	19	298	5.38	0.00008
	L inferior frontal gyrus (BA 9)	-45	2	19	392	6.37	0.00001
	L pre central gyrus (BA 6)	-33	2	28	608	8.82	<10 ⁻⁶
	R middle frontal gyrus (BA 6)	24	-1	55	789	6.22	0.00002
	L middle frontal gyrus (BA 6)	-27	-7	49	867	7.12	0.000004
	R dorsal ACC (BA 32)	6	5	37	4621	6.22	0.00002
	L dorsal ACC (BA 32)	-9	5	49	5571	7.13	0.000004
	R insula (BA 13)	30	20	10	3163	8.51	<10 ⁻⁶
	L insula (BA 13)	-33	14	7	2721	6.94	0.000005
	R red nucleus ^d	6	-22	-8	1937	7.55	0.000002
	L red nucleus ^e	-9	-13	-8	1244	6.03	0.00002
	L cerebellar inf semi lunar lobule	-9	-64	-42	944	7.87	0.000001
L cerebellar tonsil	-27	-52	-32	434	6.08	0.00002	
L cerebellar tonsil	-30	-34	-38	397	5.53	0.00006	
<i>Advance > Retreat</i>	subgenual ACC (BA 32, 24, 25)	3	26	-5	2351	5.94	0.00003
	R temporal pole (BA 38)	33	11	-26	1541	6.68	0.000007

Coordinates in Talairach space (Talairach and Tournoux, 1988) and associated t scores for the peak voxel in ROIs that display differential BOLD activity between *Advance* and *Retreat*. Size of the whole ROI is indicated. The ROIs were obtained with a threshold of $t < -4.07$, $p < 0.001$, corrected at $\alpha = 0.05$ for cluster-level false-positive rate (minimal cluster size >270 mm³).

^a Extending into left lingual gyrus (BA18).

^b Extending into BA18.

^c Extending into right middle temporal gyrus (BA 37).

^d Extending into right pulvinar nucleus.

^e Extending into left pulvinar nucleus.

coincides with the average beginning of the prechoice black screen rest period (see Figure 1B). In contrast, activity in the regions of interest (ROIs) derived from the *Retreat > Advance* contrast followed the exposure to the snake in both *Advance* and *Retreat* (Figure 3A, bottom panel). This suggests that the sgACC and rTP were engaged in some form of choice-related activity similarly for both *Advance* and *Retreat* as early as the rest period prior to the choice. Nevertheless, a divergence of the *Advance*- and *Retreat*-related activations began around the exposure to the snake. At that point in time, in *Advance* the activity continued to rise and maintained a high level until the choice button press, while in *Retreat* it rapidly declined. We hypothesized that if the aforementioned *Advance* associated pattern of activity in the sgACC and rTP of the *FR* was indeed related to overcoming fear, it should also be observed in these ROIs in *Snake Advance* choices of the *FNR*. This is because these choices necessitated overcoming fear as well. Concomi-

tantly, it should be observed neither in *Snake Advance* choices of the *FL* nor in *Toy Bear Advance* choices of all groups, because these did not involve overcoming fear.

In line with this hypothesis, *Snake Advance* choices of the *FNR*, but not the *FL*, were correlated with the aforementioned pattern of activity in the sgACC and rTP ROIs (Figure 3C). No such activity was observed in these ROIs in *Toy Bear Advance* choices in any of the groups (Figure S1C). Notably, a rise in activity in the rTP following the choices button press was observed in all groups in both conditions (Figures 3B, 3C, and S1C), suggesting that this region may also be involved in postchoice processing for all types of choices. The finding that activity in the sgACC and rTP started to rise prior to the a priori defined choice event of interest prompted us to evaluate a second general linear model (GLM) in which the choice event of interest was modeled as the time between beginning of the prechoice rest and the choice button press (see Figure 1B). This was done in an attempt to

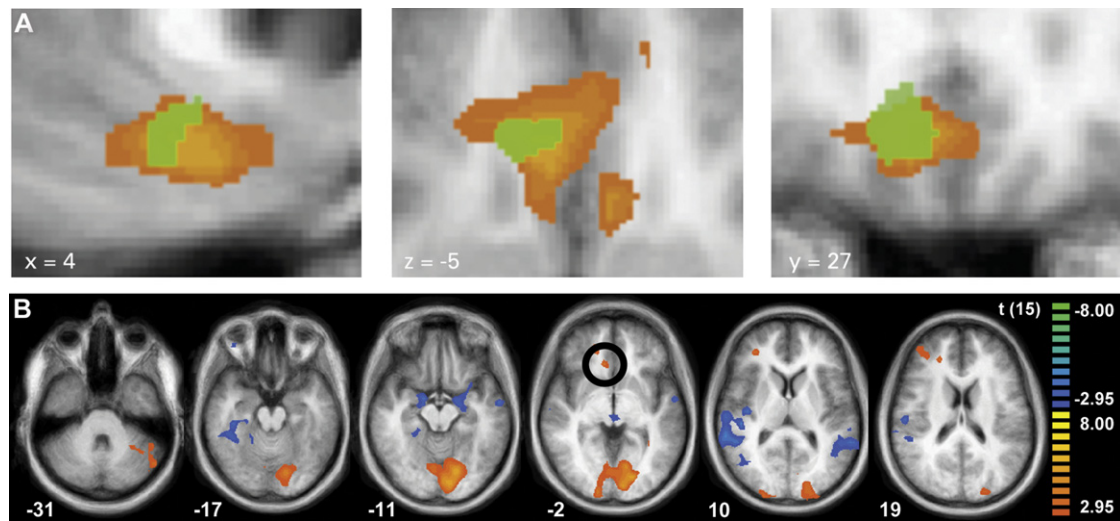


Figure 4. Brain Activity Correlated with Subjective Advance-Related Fear

(A) Statistical maps of the conjunction between [*Advance* > *Retreat*] and [*PFA* > *Baseline*] (light green) overlaid on the sgACC ROI (*x*, *z*, and *y* coordinates are indicated) derived from the original *Advance* > *Retreat* contrast shown in Figure 3B. A cluster of BOLD activity was found only within this ROI. Conjunction maps were obtained with a threshold of $t > 2.4$, $p < 0.03$.

(B) Statistical maps (radiological orientation) for the [*PFA* > *Baseline*] contrast in *Snake* choices of the *FR* group. Maps (obtained with a threshold of $t < -2.95$, $t > 2.95$, $p < 0.01$, corrected at $\alpha = 0.05$ for cluster-level false-positive rate, minimal cluster size >270 mm³) are overlaid on axial slices of the group-averaged anatomical map of all *FR* subjects (*z* coordinates are indicated on each image). Encircled is activity in the sgACC ROI.

search for additional choice-related regions displaying prechoice activity that might have been missed using the original GLM. No such regions were identified, and the original *Advance* > *Retreat* ROIs were replicated (Figure S1D).

Parametric Analysis of Fear-in-Advance

Higher activity in the sgACC and rTP in *Advance* > *Retreat* did not simply reflect higher levels of subjective fear, as evident from the finding that average fear ratings accompanying *Retreat* were higher than those accompanying *Advance* (see above). We reasoned that if, however, activity in these areas reflected the mental effort necessary to overcome fear, the level of this activity may positively correlate with the level of fear within *Advance*. This is because *Advance* choices involving greater fear also necessitated a larger effort to overcome the fear (see above). To examine this hypothesis we added an additional predictor (“*Parametric Fear-in-Advance*,” *PFA*) to the GLM, enabling detection of voxels in which activity was correlated with the level of reported fear experienced in *Advance* (for further details see [Experimental Procedures](#)). A conjunction analysis of the [*Advance* > *Retreat*] and [*PFA* > *Baseline*] contrasts (*Baseline* defined as activity in the black screen periods, Figure 1B), yielded a cluster within the sgACC ROI (Figure 4A, Table 2). This is in line with the hypothesis that activity in the sgACC was correlated with the mental effort exerted by participants in successful attempts to overcome fear. Fear levels in *Retreat* were consistently high and within a narrow range, thus excluding the possibility of a parallel analysis for *Retreat* choices.

Apart from the sgACC, which was the only region displaying activity positively correlated with both *Advance* > *Retreat* and *PFA*, a number of additional regions exhibited *PFA*-correlated activity (Figure 4B, Table 2). Positively correlated activity (i.e., greater activity with rising fear levels in *Advance*) was observed

in occipital areas, right supplementary motor area, right precuneus, right inferior parietal lobule, right middle frontal gyrus, and left cerebellum. Negatively correlated activity (i.e., lesser activity with rising fear levels in *Advance*) was observed in bilateral superior temporal sulcus (STS, extending on both sides to adjacent medial temporal gyrus (MTG) and on the right also to adjacent insula), right parahippocampal gyrus (rPHG), right inferior temporal gyrus (rITG) and, notably, bilateral amygdala.

Unique Correlation of sgACC Activity with Fear and Action

To directly compare the activity in low- and high-fear-associated *Advance* choices and in *Retreat* choices in the abovementioned regions, we utilized the GLM used when controlling for the number of *Advance* and *Retreat* choices (see above and [Experimental Procedures](#)). This enabled extraction of a separate activity measure for *Low-Fear Advance* (*LFA*) choices (mean fear rating = 54.94 ± 4.46 , $n = 20.4 \pm 0.9$), *High-Fear Advance* choices (*HFA*) (mean fear rating = 87.49 ± 2.42 , $n = 16.63 \pm 1.24$), and *Retreat* choices (mean fear rating = 88.25 ± 2.42 , $n = 16.63 \pm 1.24$) in the different ROIs. The analysis revealed that, with the exclusion of the sgACC, in all regions in which we observed activity that positively correlated with *PFA*, the correlation was with subjective fear per se (or alternatively with proximity to the object or chronology in the experiment, with which changes in subjective fear within the *FR* were concomitant; see [Figures S2A–S2C](#)), regardless of the behavioral outcome (*Advance* or *Retreat*). I.e., high activations were observed for both *HFA* and *Retreat*, in which fear levels were highest (Figure 5A). Thus, the only region displaying activity positively correlated with fear levels exclusively in *Advance* choices was the sgACC. Within this region activity was greater in *HFA* than in *LFA* and dropped considerably in *Retreat* (Figure 5A).

Table 2. Regions Displaying Parametric Fear-in-Advance-Correlated Activity in the FR

	Region	x	y	z	mm ³	t value	p value
Positively correlated activity	L lingual gyrus (BA 18) ^a	-15	-73	-8	17025	6.19	0.00002
	R middle occipital gyrus (BA 18)	24	-97	7	609	4.34	0.0006
	R precuneus (BA 7)	12	-70	49	567	3.79	0.002
	R inferior parietal lobule (BA 40)	54	-40	43	280	3.58	0.003
	R superior frontal gyrus (BA 6)	24	-4	64	321	3.90	0.001
	subgenual ACC (BA 24)	9	26	-2	570	4.49	0.0004
	R middle frontal gyrus (BA 9) ^b	21	38	19	1246	4.48	0.0004
	L cerebellar tonsil	-36	-49	-38	1482	4.32	0.0006
	Negatively correlated activity	R superior temporal sulcus (BA 22)	60	-40	10	5363	-4.90
L superior temporal sulcus (BA 22)		-60	-46	10	3020	-4.34	0.0006
R middle temporal gyrus (BA 19)		39	-64	13	316	-3.43	0.004
L middle temporal gyrus (BA 21)		-57	-7	-11	485	-4.22	0.0007
R insula (BA 13)		39	-16	7	572	-5.13	0.0001
R inferior temporal gyrus (BA 20)		36	-34	-20	1723	-4.47	0.0004
R parahippocampal gyrus (BA 36)		21	-37	-14	327	-3.38	0.004
R amygdala		15	-4	-11	839	-4.38	0.0005
L amygdala		-15	-7	-8	1190	-4.32	0.0006

Coordinates in Talairach space (Talairach and Tournoux, 1988) and associated t scores for the peak voxel in ROIs that displayed *Parametric-Advance*-correlated activity. Size of the whole ROI is indicated. The ROIs were obtained with a threshold of $t < -2.95$, $t > 2.95$, $p < 0.01$, corrected at $\alpha = 0.05$ for cluster-level false-positive rate (minimal cluster size >270 mm³).

^a Extending into right lingual gyrus (BA18).

^b Extending into BA10.

In contrast, in all regions in which we observed activity negatively correlated with *PFA*, the negative correlation was exclusive to *Advance* choices; i.e., in these regions activity in *Retreat* was greater than that in *HFA* (Figure 5B). Thus, these regions exhibited diminishing activity with rising subjective fear levels (and concomitantly, with a growing effort to overcome fear) when the effort to overcome fear was successful, and a relative rise in activity upon its failure, an opposite pattern to that observed in the sgACC. For a parallel analysis of the sgACC ROI activity in *Snake Advance* choices of the *FNR*, we used two predictors to model *LFA* choices (mean fear rating = 19.67 ± 2.70 , $n = 14.27 \pm 1.13$) and *HFA* choices (mean fear rating = 62.33 ± 3.59 , $n = 12.00 \pm 1.16$) in this group. The analysis revealed a similar trend of rise in activity in the sgACC of the *FNR* in *HFA* as compared to *LFA* choices (inset in Figure 5A, top panel; $t_{13} = 1.66$, $p = 0.058$). No such rise in sgACC activity was observed when either comparing late versus early *Snake Advance* choices of the *FL* group or late versus early *Toy Bear Advance* choices of all groups (Figures S2D–S2G). Together, these results demonstrate that a rise in sgACC activity was observed only in correlation with rising fear levels in *Advance* choices, in line with a role for the sgACC in successful efforts to overcome fear.

Dissociation of Self-Reported Fear and Somatic Arousal and Its Brain Correlates

Our finding that the transition between *Advance* and *Retreat* choices could not be explained by a rise in subjective fear levels alone (see above) prompted us to examine the SCR level in the different choice bins of the *FR*. This analysis revealed that oppo-

site to the pattern of sgACC activity, and mirroring the pattern of activity in the ROIs displaying activity negatively correlated with subjective fear levels within *Advance* choices, SCR levels were lower in *HFA* than in *LFA* choices and exhibited an increase in *Retreat* over *HFA* choices (Figure 6A). To further characterize the relation between the changes in sgACC activity and in SCRs in the transition between the different states, we plotted the changes in these measures against each other for all participants ($[HFA \beta - LFA \beta]$ versus $[HFA SCR - LFA SCR]$ and $[Retreat \beta - HFA \beta]$ versus $[Retreat SCR - HFA SCR]$; Figure 6B). This revealed that the transition between *LFA* and *HFA* was consistently accompanied by a rise in sgACC activity and a drop in SCR. Conversely, the transition between *HFA* and *Retreat* was consistently accompanied by a decrease in sgACC activity and an increase in SCR. Thus, for both transitions, inverted patterns of change in sgACC activity and in SCR level were observed at the individual participant level (Sign test: $Z = 3.33$, $p < 0.005$).

Due to the well established role of the amygdala in mediating fear-related physiological arousal (LeDoux, 1996), we conducted a similar analysis for the amygdala ROIs. This revealed that opposite to the pattern observed in the sgACC, the transition between *LFA* and *HFA* was consistently accompanied by a drop in both amygdala activity and SCR, whereas the transition between *HFA* and *Retreat* was consistently accompanied by a rise in both amygdala activity and SCR (Figures 6C and 6D). Thus, for both transitions, similar change patterns in amygdala activity and in SCR level were observed at the individual participant level (left amygdala: Sign test: $Z = 0.7$, $p > 0.05$; right amygdala: Sign test: $Z = 1.15$, $p > 0.05$). As implied by the above,

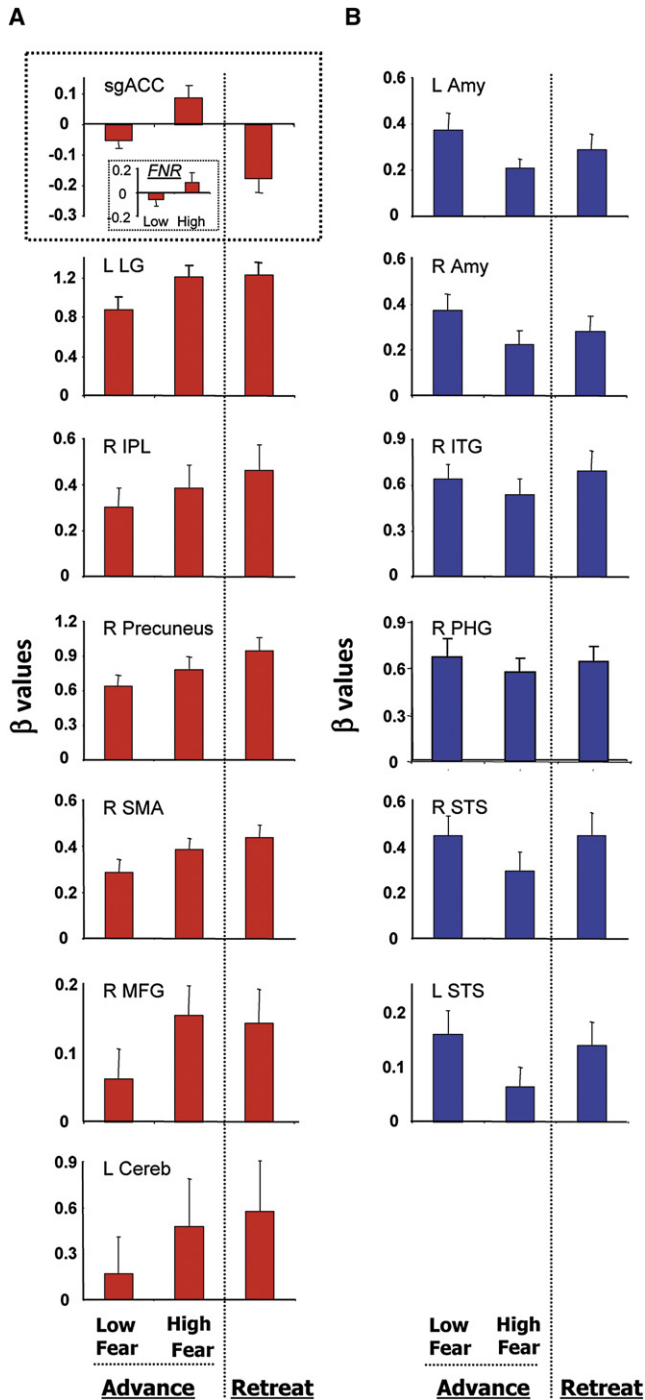


Figure 5. The sgACC Is the Only Region Displaying Activity Positively Correlated with Fear Levels Exclusively in Advance Choices (A and B) Beta values representing BOLD activity levels in Low- and High-Fear Advance and Retreat choices of the FR, in ROIs displaying significant positively (A) or negatively (B) PFA-correlated activity. ROIs from top to bottom are as follows: (A) sgACC, left lingual gyrus (LLG), right inferior parietal lobule (R IPL), right (R) precuneus, right supplementary motor area (R SMA), right middle frontal gyrus (R MFG), and left cerebellar tonsil (L cereb); (B) left amygdala (L Amy), right amygdala (R Amy), right inferior temporal gyrus (R ITG), right parahippocampal gyrus (R PHG), right superior temporal sulcus (R STS), and left superior temporal sulcus (L STS). The inset in the sgACC panel (A, top panel) depicts the beta values for Low- and High-Fear Advance choices in the sgACC of the FNR; see text. See also Figure S2.

plotting the changes in sgACC versus those in the amygdala demonstrated a pattern of inverse concomitant changes in activity within these regions in the transition between choices pertaining to the different behavioral bins (left amygdala: Sign test: $Z = 3.02$, $p < 0.005$; right amygdala: Sign test: $Z = 2.85$, $p < 0.005$, Figure S3).

DISCUSSION

In this study we examined the behavioral, physiological, and brain correlates of overcoming fear or succumbing to it in an acute fearful ambience. Specifically, we confronted snake-fearing individuals with decisions whether to move a live snake away or bring it closer to their heads. Each instance of overriding the fear and engaging in an action that is opposed to that promoted by this fear is defined by us, following others (Rachman, 2004a), as an instance of courage. Some might add that courage could also be considered in terms of perseverance (Rachman, 1984). In this respect, our experimental protocol taps into multiple manifestations of perseverance, ranging from that required to overcome the fear on each decision point to that required to keep making fear-overriding decisions over time. At the behavioral and physiological level, our results demonstrate that overcoming fear was associated with a dissociation between subjective fear (assessed by fear self-ratings) and somatic arousal (assessed by SCR). Hence either high somatic arousal accompanied by low subjective fear or high subjective fear accompanied by low somatic arousal were accompanied by decisions to bring the snake closer. In contrast, congruence of the two measures, i.e., high subjective fear accompanied by high somatic arousal, was associated with moving the snake farther away.

At the whole-brain level, our data demonstrate that activity in only a small set of areas, the sgACC and the rTP, was positively correlated with choosing to overcome fear. Furthermore, activity in the sgACC correlated positively with the level of subjective fear when choosing to overcome fear but not when choosing to succumb to it. In contrast, activity in a set of temporal lobe structures, including bilateral amygdala, bilateral STS, rPHG, and rITG, was attenuated as the level of subjective fear increased when choosing to overcome fear. This pattern was inverted when participants failed to overcome fear (*Retreat* choices). In these instances, the aforementioned regions exhibited a relative increase in activity as compared with that observed after *HFA* choices, whereas the sgACC displayed a sharp decrease in activity. Hence, our results point to the sgACC as the only brain region detected in which the pattern of activity matched that which may be expected of a region involved in a successful mental effort to overcome fear. Specifically, this region exhibited greater activity in instances where the overcoming of fear was displayed, as compared with activity in instances of succumbing to fear. Furthermore, within such successful instances of overcoming fear, those involving greater subjective fear and concomitantly necessitating a larger mental effort to overcome that fear also involved greater activity in this region.

left superior temporal sulcus (L STS). The inset in the sgACC panel (A, top panel) depicts the beta values for Low- and High-Fear Advance choices in the sgACC of the FNR; see text. See also Figure S2.

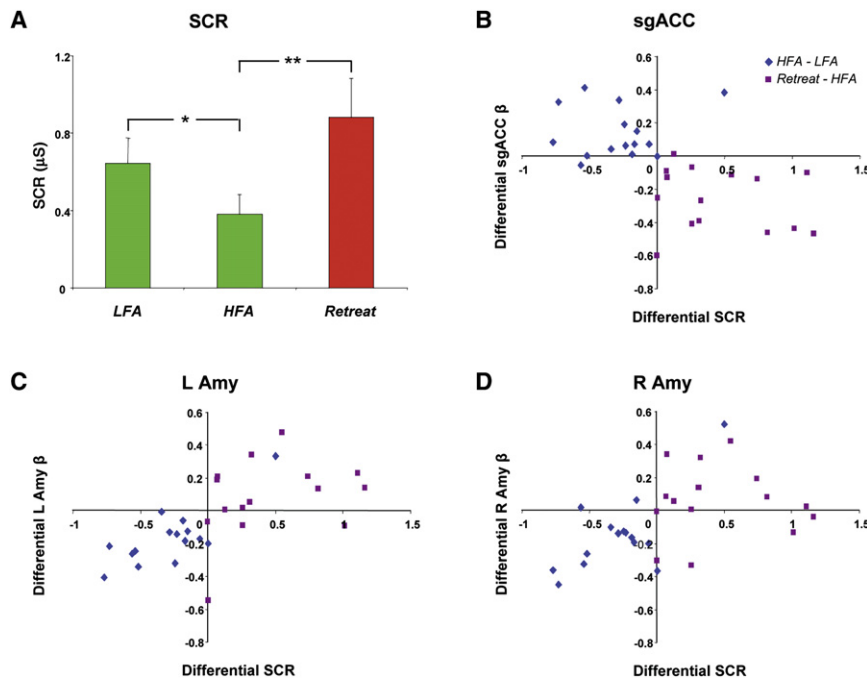


Figure 6. Somatic Arousal Associated with Choices Taken by the Fearful-Retreaters and Its Relation to Activity of the sgACC and Amygdala

(A) SCR levels in Low-Fear-Advance (LFA), High-Fear Advance (HFA), and Retreat choices of the FR group in the Snake condition.

(B–D) Changes in BOLD activity (beta values) and in SCR in the transition between choices pertaining to the different choice bins, plotted against each other ($[HFA \beta - LFA \beta]$ versus $[HFA SCR - LFA SCR]$ and $[Retreat \beta - HFA \beta]$ versus $[Retreat SCR - HFA SCR]$) for the sgACC (B) and amygdala (C and D), for each participant. * $p < 0.05$, ** $p < 0.005$.

See also Figure S3.

The ACC has been implicated in regulation of both cognitive and emotional processing (Bush et al., 2000). Specifically, the ventral region of the ACC encompassing the sgACC is considered to be activated by tasks that relate to affective or emotional content (Bush et al., 2000; Allman et al., 2001). Highly relevant to the study of courage, studies of cognitive control of emotion (Ochsner and Gross, 2005) have demonstrated a role of the sgACC in regulation of affect associated with negative autobiographical memories (Kross et al., 2009), in regulation of conditioned-fear-related SCR (Delgado et al., 2008), and in a deficit in effortful downregulation of emotion in response to phobic stimuli in spider phobics (Hermann et al., 2009). Additionally, the ventromedial prefrontal cortex (vmPFC), encompassing the sgACC, has been reported to be hypoactive in posttraumatic stress disorder (Etkin and Wager, 2007).

A role for the sgACC in overcoming fear is also in line with studies of experimental extinction of fear conditioning, which report a role for vmPFC in retrieval of the inhibitory association (Morgan et al., 1993; Milad and Quirk, 2002; Phelps et al., 2004; Milad et al., 2005, 2007a; Kalisch et al., 2006). However, in our protocol, fear increases over time, whereas in extinction, the fear—which stems from a past conditioned association—decreases. Furthermore, whereas in most extinction studies, the vmPFC was implicated only after a consolidation period and not during conditioned fear acquisition or extinction training (Milad and Quirk, 2002; Phelps et al., 2004; Santini et al., 2004; but see Milad et al., 2007a), in our study the sgACC displayed on-line fluctuations in activity, correlated with choosing to overcome or succumb to ongoing fear. Nevertheless, our findings suggest that instances of courageous action in a naturalistic fearful situation that demands immediate response may require swift recruitment of some brain substrates that also subserve

incremental, training-dependent inhibition of the expression of fear in experimental extinction.

How then might activity in the sgACC contribute to overcoming the fear of the snake in our paradigm? Our data demonstrate that changes in somatic arousal were opposite to those in sgACC activity.

That is, somatic arousal was attenuated as the level of subjective (i.e., self-reported) fear increased when choosing to overcome fear, but intensified in the transition between high subjective-fear-related instances of overcoming fear (HFA choices) and high subjective-fear-related instances of succumbing to fear (Retreat choices). This suggests a mechanism by which activity in the sgACC may serve to inhibit fear-related somatic arousal, which contributes to the promotion of Advance choices.

In line with this proposal, the sgACC was implicated in regulation of the autonomic nervous system by increasing parasympathetic tone (Matthews et al., 2004; Tang et al., 2009). Furthermore, the sgACC is densely interconnected with subcortical structures that control autonomic and visceral responses, such as the hypothalamus, orbitofrontal cortex, and notably, the amygdala (Johansen-Berg et al., 2008; Rudebeck et al., 2008). The sgACC may thus reduce such responses by exerting an inhibitory influence on these structures. Indeed, it has been proposed that the role of mPFC in expression of experimental extinction might involve inhibition of the amygdala (Quirk et al., 2003; Rosenkranz et al., 2003). This inhibition has later been specifically attributed to the infralimbic region, within the ventral part of mPFC (i.e., vmPFC) (Vidal-Gonzalez et al., 2006). A similar mechanism was suggested to underlie a reduction in conditioned-fear-related SCR following the use of a reappraisal cognitive control strategy (Delgado et al., 2008). Likewise, functional decoupling between cognitive-control-related prefrontal regions and amygdala has been suggested to underlie symptom provocation in a specific phobia (Ahs et al., 2009). Activity in the amygdala in our paradigm displayed a pattern opposite to that observed in the sgACC, and similar to that observed for SCR, i.e., attenuation with increasing levels of subjective fear in Advance. This finding may seem counterintuitive at first, given the well established role of amygdala in the expression of fear

(LeDoux, 1996; Killcross et al., 1997; Herry et al., 2008; Likhtik et al., 2008). The possibility that this attenuation merely reflected time-dependent habituation seems unlikely, because amygdala activity was higher in *Retreat* than in *HFA* (Figure 5), whereas the chronology of these two types of choices within the experimental timeline did not differ (Figure S2). Hence, in line with the above, a plausible account might also involve increased inhibition of the amygdala as a consequence of greater mental effort invested in overcoming fear (*Advance* choices), reflected in greater sgACC activity, when fear levels were higher. All in all we propose that dampening of fear-related somatic arousal by sgACC, through either or both of the aforementioned direct and indirect pathways, may in turn promote overcoming ongoing fear. In this regard it is noteworthy that fear extinction studies in humans have commonly used a change in SCR as a measure of extinction success, whereas our data support the notion that reported fear feeling, fear-related somatic arousal, and fear driven action may under certain circumstances exhibit dissociation (Leitenberg et al., 1971; Lang et al., 1983; Rachman, 2004a).

The only other region apart from the sgACC that displayed *Advance* > *Retreat* activity was the rTP. The TP is interconnected with all sensory systems represented in the temporal lobe (visual, auditory, and olfactory) and has accordingly been implicated in high-level perception (Olson et al., 2007). It is also reciprocally connected to the amygdala and the basal forebrain (including orbitofrontal and medial networks) and projects to the hypothalamus and periaqueductal gray (PAG), implicating it in both emotional processing and visceral modulation (Kondo et al., 2003). Concurrent with a role in emotion processing, activations in the TP concomitant with emotions evoked by visual stimuli have been reported (Olson et al., 2007). In accord with a role in visceral modulation, stimulation of the TP has been shown to produce changes in respiratory rate and blood pressure (Kaada et al., 1949). In line with the above, it has been suggested that together with medial prefrontal networks, TP is part of a system that modulates visceral emotional functions in response to emotionally evocative perceptual stimuli (Kondo et al., 2003; Olson et al., 2007). This may account for the analogous patterns of activity observed in the sgACC and rTP in instances of *Advance* and *Retreat* in our paradigm. It has further been suggested that the TP may be reactivated when emotions are perceived or imagined (Olson et al., 2007). Such a function, as well as potential postaction evaluation and monitoring, may be related to the rise in activity observed in our paradigm in this region following the choice button press in all groups in both experimental conditions (*Snake* and *Toy Bear*). That is, following each choice subjects had to report the level of fear they experienced in the period leading up to the choice. Hence, it is possible that the rise in activity in this area following the choice reflected this process of self-evaluation of fear.

A small number of other temporal lobe regions, namely, rPHG, STS, and rITG, exhibited activity patterns similar to those observed in the amygdala. All these regions interconnect with the amygdala (Aggleton et al., 1980). Parahippocampal cortex was reported to provide a source of highly processed cortical information directly into the amygdala (Gallagher, 2000). STS activity has been implicated in the processing of biological motion. Additionally, the STS has been implicated in viewing

eye movements, and specifically in judgments of gaze direction and opening and closing movements of the mouth. ITG has also been implicated as part of an occipito-temporal network involved in gaze perception (Wicker et al., 1998). It is noteworthy that the aforesaid functions attributed to these temporal lobe regions were observed mainly in the context of human interactions with conspecifics. Nevertheless, such functions may also play a role in assessing the threat imposed by a nearby snake. Indeed, the involvement of STS in the processing of biological motion has been suggested to play a role in deciphering intention in predators (Puce and Perrett, 2003). Taken together, it is plausible that activity in these regions was related to subjects' assessments of the snake's behavior in terms of movement and assumed intention when deciding whether to choose *Advance* or *Retreat*.

Both dorsal ACC and anterior insula, two regions implicated in processing fear, exhibited greater activity in *Retreat* than in *Advance*. In humans, the dorsal ACC has been shown to play a role in conditioned fear acquisition and expression as assessed by SCR (Milad et al., 2007b), possibly through generation of autonomic responses (Critchley, 2005). Furthermore, electrical stimulation of this region led to reports of intense feelings of fear, whereas its ablation reduces anxiety symptoms (Meyer et al., 1973). Concomitant with the above, a role for the proposed rat homolog of the dorsal ACC, the prelimbic cortex (PL), in expression of conditioned fear and failure of extinction has been reported (Quirk et al., 2006; Burgos-Robles et al., 2009). The anterior insula has been suggested to encode autonomic and visceral responses and support their interaction with perceived threat signals, leading to conscious subjective emotional experiences such as feelings of fear (Critchley, 2005). The anterior insula has also been implicated in the perception of disgust (Jabbi et al., 2008), an emotion that could be evoked in the proximity of snakes and augment fear, though ranked by the *FR* as much weaker than fear per se (data not shown). In line with the present findings, both the dorsal ACC and insula have been reported to display reduction of hyperactivity in spider phobics after cognitive behavioral therapy (Straube et al., 2006; Goossens et al., 2007).

No activity differentiating *Advance* and *Retreat* choices was observed by us in prefrontal areas that are traditionally implicated in cognitive control of emotion, such as ventrolateral, dorsolateral, or dorsomedial PFC (Ochsner and Gross, 2005). One account for this null finding may be that, as opposed to studies of cognitive control of emotion that instruct participants regarding specific cognitive strategies to be used, in our study individuals used idiosyncratic strategies when deciding whether to overcome or succumb to fear. To the extent that different strategies may recruit different prefrontal regions (Ochsner and Gross, 2005), no particular region might be expected to come up in our group analysis. If correct, such an account points to the sgACC as a common mediator of different cognitive control strategies in instances of overcoming fear. A second alternative may be that decisions to both overcome and succumb to fear involve similar activity levels within one of the abovementioned PFC regions, and only differentiate in the details of the processing carried out in this region, which could not be differentiated by BOLD signals. A third explanation may be that the sgACC

itself serves as the “emotion regulation executive” in our paradigm, a rather unlikely scenario given previous evidence regarding the function of this region (see above).

Based on the above, we propose a heuristic model wherein in the context of a fear-eliciting situation, goal-directed motivation to overcome fear leads to activation of the sgACC to reduce autonomic arousal and enable a display of courage. We suggest that because our paradigm allowed for anticipation of the upcoming choice (Rachman, 2004b), this activation was initiated already prior to the choice (in both *Advance* and *Retreat*, Figure 3B) so as to permit efficient dampening of somatic arousal upon the expected exposure of the snake. Following exposure to the fear-eliciting stimulus, sensory information relevant to both implicit and explicit assessments of the threat imposed by the stimulus (e.g., proximity, mobility, posture, gaze direction, etc. in the case of the snake) is conveyed to multiple brain systems. These include circuits that perform computations relevant to the specific object of fear (e.g., appraisal of menacing motion and intentionality, possibly by STS and rITG in the present paradigm) and circuits that are item invariant. In view of their previous implication in other types of emotion paradigms, amygdala, dorsal ACC, and insula are candidates for such a “global” role. To the extent that, following processing in these circuits, certain thresholds of both subjectively experienced, reportable fear and autonomic arousal (or a threshold derived from summation of the two) are surpassed, the mental effort invested in overcoming fear is aborted. This leads to a concomitant drop in sgACC activity and a further rise in autonomic arousal, culminating in succumbing to fear. If, however, either of these thresholds (or a combined one; see above) is not reached, the effort invested in overcoming fear and concomitant sgACC activity is sustained (and is proportional to the level of subjectively experienced, reported fear). This leads to dampening of autonomic arousal, i.e., a process of fear inhibition, which promotes a display of courage. Hence courage involves fear inhibition, yet is not identical to it, since fear inhibition may not necessarily result in voluntary action opposing that promoted by fear, whereas courage does. It is noteworthy that the equivalent subjective fear reported for *HFA* and *Retreat* choices indicates that under certain circumstances exceeding the above suggested autonomic arousal threshold might not be explicitly detected, i.e., may remain dissociated from the subjective, reported experience.

Our results thus propose an account for brain processes and mechanisms supporting an intriguing aspect of human behavior, i.e., the ability to carry out a voluntary action, namely courage, opposed to an action promoted by ongoing fear. Specifically, our results delineate the importance of maintaining high sgACC activity in successful efforts to overcome ongoing fear. They hence point to the possibility of manipulating sgACC activity in therapeutic intervention in disorders involving a failure to overcome fear. Such interventions may range from training in meditation techniques that lead to greater activity in this region (Tang et al., 2009) to transcranial magnetic stimulation (TMS) similar to that attempted to alleviate depression (Paus and Barrett, 2004). On top of its potential applied importance, further analyses of brain circuits that subserve instantaneous overcoming of fear could also contribute to better understanding of how the brain shifts swiftly between internal representations

(Eisenberg et al., 2003; Mobbs et al., 2007) to select one of several conflicting behavioral outcomes in other contexts.

EXPERIMENTAL PROCEDURES

Participants

Selection of potential high-fear participants was carried out using the SNAQ (score range: 0 – no fear, 30 – highly fearful; Klorman et al., 1974), translated in-house into Hebrew. Individuals scoring 11 or higher on the SNAQ, corresponding to the upper 20% in our screen, were considered candidate high-fear participants. The selection of potential low-fear participants was based in the first stage of the selection on self reports of extensive prior experience with snakes in the context of occupation or hobby. All candidates meeting the above criteria were then contacted by phone, given a detailed description of the outline of the experiment and asked whether they would be willing to participate. Those who agreed were summoned to a preliminary session in which they completed a STAI-T (Spielberger, 1983). Participants of the low-fear group also completed the SNAQ at this time. All participants were additionally interviewed by a certified MD to identify potential susceptibility to panic attacks. After that stage, all the participants signed a consent form. The aforementioned procedure resulted in two pools of participants, a high-fear pool ($n = 39$) and a low-fear pool ($n = 22$). All had normal or corrected-to-normal vision. Ultimately, the *FR*, *FNR*, and *FL* (see main text) were selected from these pools (see Inclusion Criteria in Supplemental Information). The experimental protocol was approved by the Institutional Review Board of the Geha Mental Health Center, Petah Tikva. The use of the snake was approved by the Institutional Animal Care Committee of the Weizmann Institute of Science.

Analysis of Choice-Related SCR

Skin conductance level (SCL) was recorded continuously throughout the experiment from the participant’s first phalanges of the second and third fingers in the left hand using a BIOPAC systems skin conductance module (Biopac Systems, Inc, Coleta, CA), shielded Ag-AgCl electrodes grounded through an RF filter panel, and AcqKnowledge software (Biopac Systems, Inc, Coleta, CA). A choice-related SCR was defined as the base-to-peak difference for the largest deflection in SCL (larger than $0.1 \mu\text{S}$) commencing in the time window between object exposure and choice button press (i.e., the same time window defined as the choice event and for which fear ratings were collected), analyzed using in-house MATLAB (The MathWorks, Natick, MA) software. Choice-related SCRs were averaged per participant per condition.

Statistical Analysis of the Behavioral and SCR Measures

Fear ratings determined on the Visual Analog Scale (VAS) were calculated separately for each condition type (*Snake* and *Toy Bear*) in each of the groups (*FF* and *FL*) by calculating the average fear ratings collapsed over all choices for each participant. The average fear ratings were analyzed with a mixed model ANOVA with condition (*Snake*, *Toy Bear*) as the within-subject factor and group (*FF*, *FL*) as the between-subject factor. STAI-S scores and decision-related SCRs (see above) were analyzed in the same manner. For the STAI-S scores each subject’s single score was used in the ANOVA. For all measures a Tukey HSD test was used for specific posthoc comparisons. Comparisons between STAI-T scores and SNAQ scores of the *FF* and *FL* were conducted using a Student’s *t* test. The two groups significantly differed in fear of snakes ($FF = 15.69 \pm 1.02$; $FL = 2.38 \pm 0.34$; $t_{30} = 12.39$, $p < 10^{-6}$). A comparison between fear ratings of the *FR* and *FNR* was conducted using a Student’s *t* test. Comparisons within the *FF* group between *Advance* and *Retreat* for fear rating, SCRs, number of choices, and choice response times were conducted using dependent *t* tests. Comparisons within the *FF* group between *LFA*, *HFA*, and *Retreat* choices for fear ratings, SCRs, proximity to the snake and chronology in the experiment were conducted using repeated-measures ANOVA for each of these measures separately. In all analyses pertaining to the *FR* involving the three choice bins (including those of fMRI data; see below), data of one participant that had only three choices in the *LFA* bin was excluded from the analysis. A comparison between choice response times of *Advance* choices in the *Toy Bear* condition of the three groups (*FR*, *FNR*, and *FL*) was conducted using a one-way ANOVA.

fMRI Acquisition

Imaging was performed on a 3T Trio Magnetom Siemens scanner at the Norman and Helen Asher Center for Brain Imaging, Weizmann Institute of Science. All images were acquired using a 12 channel head matrix coil. Three-dimensional T1-weighted anatomical scans were acquired with high resolution 1 mm slice thickness (3D MP-RAGE sequence, TR 2300 ms, TE 2.98 ms, 1 mm³ voxels). For BOLD scanning, T2*-weighted images were acquired using the following parameters: TR = 2000 ms, TE = 27 ms, Flip angle = 75°, 38 oblique slices without gap, 30° toward coronal plane from ACPC, 3 × 3 × 4 mm voxel size, covering the whole brain.

fMRI Analysis

Preprocessing

Preprocessing and data analyses of the fMRI data were performed using BrainVoyager QX version 1.10 (Brain Innovation, Maastricht, Netherlands). Images were corrected for slice-timing, head movements (three scans with head movements larger than 3 mm [translation] or 3° [rotation] were rejected), and linear drifts. Low frequencies were filtered out from the data. Images were spatially smoothed using a 6 mm full-width at half-maximum (FWHM) Gaussian kernel. The first four volumes (8 s) from the beginning of each scan were removed from the data set to account for signal equilibrium. Functional and anatomical scans were spatially normalized by extrapolation into a 3D volume in Talairach space (Talairach and Tournoux, 1988). Functional scans were resliced into an iso-voxel resolution of 3 mm³.

Statistical Analysis

All contrasts of interest were performed at the group level using a random-effects analysis. To correct for multiple comparisons in the whole-brain statistical maps (Figures 3, 4, and S1), we implemented a randomization technique to estimate a corrected cluster-level confidence for the entire volume (at $\alpha = 0.05$) based on a method described by Forman et al. (1995) and Goebel et al. (2006) using the Brain Voyager Cluster-level Statistical Threshold Estimator Plugin. The method uses a nonparametric Monte Carlo simulation that calculates the likelihood of obtaining a cluster of randomly generated voxels across the entire volume at the given individual voxel probability threshold. The correction takes into account the resolution of the sampling and the spatial smoothness applied to the data. Preprocessed time series data for scans of interest were analyzed using GLMs specified to investigate the conditions of interest.

GLMs Used

GLM1 included separate predictors for *Advance*, *Retreat*, and fear ratings. Black screen periods were considered as baseline (see Figure 1B). An additional predictor was used to model the few “No choice” events (i.e., choice events in which no choice button press was performed) that occurred in a small number of subjects. This GLM was used for comparing *Advance* and *Retreat* choices in the *FR* (Figure 3).

GLM2 included the same predictors as GLM1, albeit with an additional predictor, *PFA*, used to detect voxels in which BOLD activity was correlated with the level of subjective fear (assessed by VAS scores) experienced in *Advance*. To create the above predictor, each *FR* group participant's *Advance* choices were first binned into three groups (*Low-Fear*, *Medium-Fear*, and *High-Fear*) according to their corresponding fear levels using a K-means algorithm implemented in MATLAB. The predictor was assigned a negative weight (−1) for *Advance* choices within the *Low-Fear* cluster, zero weight for those within the *Medium-Fear* cluster, and a positive weight (1) for those within the *High-Fear* cluster. As mentioned above, this predictor was used to identify within the *FR* group ROIs in which BOLD activity correlated with the level of fear in *Advance* (Figure 4).

GLM3 included the same predictors as GLM1, albeit with the *Advance* predictor replaced by two separate predictors. The first, *HFA*, modeled (per *FR* participant) the *Advance* choices with the highest fear levels that were equal in number to *Retreat* choices of that participant. The second, *LFA*, modeled the remaining *Advance* choices. This GLM was used to control for the difference in average number of *Retreat* and *Advance* choices (Figure S1), and to extract separate BOLD activity measures for the *HFA*, *LFA*, and *Retreat* choice bins of the *FR* participants in the ROIs identified using GLM2 (Figures 5, 6, and S3). For calculation of these activity measures (beta values) in the different choice bins, the average beta for the 125 contiguous anatomical

voxels (125 mm³) displaying the peak activity in each of the group level ROIs derived from the *PFA* versus *Baseline* contrast (see GLM2 above, Figure 4, and Table S2) was first calculated separately per participant per bin. All participants' average betas were further averaged to create the group average for each bin. For the sgACC, the peak 125 contiguous anatomical voxels derived from the conjunction of the original [*Advance* > *Retreat*] and [*PFA* versus *Baseline*] contrasts (see Figure 4) were used. In all analyses pertaining to the *FR* involving the three choice bins, data of one participant that had only three choices in the *LFA* bin was excluded from the analysis. Similar GLMs were used to extract separate BOLD activity measures for *HFA* and *LFA* choices in the *Snake* condition of the *FNR* (the *FNR*'s *Advance* choices were separated into these two bins according to their corresponding fear levels using a K-means algorithm implemented in MATLAB) (inset in Figure 5A, top panel), for *Early* and *Late Advance* choices in the *Snake* condition of the *FL*, and in the *Toy Bear* condition of all groups (Figure S2). In these analyses, data of subjects for whom differential activity between the two choice bins was larger than two standard deviations from the group mean differential activity was excluded from the analysis. This resulted in exclusion of data of one participant in each of these analyses.

GLM4 included the same predictors as GLM1, albeit with the *Advance* and *Retreat* predictors modeling the whole time window between beginning of the prechoice black screen and the choice button press (see Figure 1B). Consequently, in this GLM only the prestarting black screen was considered as baseline. GLM4 was used to search for areas, in addition to the sgACC and rTP, that might have displayed choice related BOLD activity already prior to the choice in *FR* group participants (Figure S1).

Calculation of Percent of BOLD Signal Change

For calculation of average percent of BOLD signal change (PSC) (Figures 3 and S1), zero PSC was defined as the average BOLD signal in the two TRs prior to the onset of the choice event of interest (predefined as beginning of exposure to the object; see Figure 1B). Average PSC was first calculated separately for each participant for the group level ROIs derived from the *Advance* versus *Retreat* contrast in the *FR*. This was done by averaging the BOLD signal of all voxels in the original group ROI for each participant for each TR in the −5 to 12 TR window around time 0 (see above). A group average for each TR in each ROI for all choice events of interest was then calculated.

Statistical Analysis of Concomitant Changes in SCR and BOLD Activity in the sgACC and Amygdala ROIs, in the Transitions between Different Choice Bins of the FR

The difference in the value of each of these measures (SCR, sgACC beta, amygdala beta) between the different choices bins ([*HFA* − *LFA*], or Transition A, and [*Retreat* − *HFA*], or Transition B) were calculated for the *FR*. A positive difference was assigned a value of 1. A negative or nonexistent difference was assigned a value of 0. A combined score representing both differences per measure (Transition B − Transition A) per participant was then calculated. A Sign test was conducted on these combined scores for all comparisons of interest (sgACC beta versus SCR [Figure 6], amygdala beta versus SCR [Figure 6], and sgACC beta versus amygdala beta [Figure S3]). Thus, a significant p value indicates that changes in the two compared measures in the transitions between the different choices bins were opposite in direction (i.e., a rise in one measure was accompanied by a drop in the other and vice versa). Conversely, a nonsignificant p value indicates that changes in the two compared measures in the transitions between the different choices bins were similar in direction.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes three figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.neuron.2010.06.009.

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