Uncertainty about aversive events is central to worry and an integral part of anxiety disorders. However, little is known about the neural instantiation of such uncertainty. In the neuroimaging literature, uncertainty has been explicitly examined only in the context of reward-related decision making (Critchley et al., 2001; Hsu et al., 2005). Also relevant is research implicating the amygdala in anticipation of aversive events (Davis & Whalen, 2001). Previously, we reported that, following the amygdala and insula activate during exposure to aversive pictures following certain warning cues that in- creased risk or probability. Our analyses showed heightened activation in the amygdala and insula responses to aversive pictures preceded by uncertain warning cues that may be part of a network that represents the influence of uncertainty on the anticipation and experience of aversion. Of relevance to anxiety disorders, these data suggest that uncertainty magnifies the impact of aversive events on threat deflection and response mechanisms in the amygdala (Whalen & Davis, 2001; Nitschke et al., 2006) and on the integration and body-state representation mechanisms of the insula (Critchley, 2004; Damasio, 2003; Craig, 2002, 2003; Nitschke et al., 2006).

![Fig. 1. Experimental Design.](image1)

**Methods and Materials**

**Participants**

39 right-handed undergraduates (16 female) free of any medical or neurological problems.

**Design and Stimuli**

Each functional run consisted of the presentation of a cue for 1 sec, followed by a black screen presented for 6-10 sec. followed by the presentation of a picture for 1 sec, and fol lowed by a 4-sec break. The amygdala was activated following aversive pictures. Uncertain warning cues were equally likely to be followed by an aversive or neutral picture. A circle was the neutral cue and was always followed by a neutral picture. The average trial length was 19 sec. All pictures were taken from the IAPS (Lang et al., 1999). Pictures were presented only once. There were 13 uncertain warning cues, 12 certain warning cues, 24 trials: 8 negative trials, 8 neutral trials, and 8 uncertain trials. Trial order was pseudorandomized with no condition being presented more than twice in a row. In each run, pictures were presented using a polyCGA (Lehmann, 1999) vi a pair of fiber optic driven stereo- copes. Participants re ceived instructions about the nature of the study and the type of stimuli to expect prior to scanning.

**fMRI Data Acquisition**

Anatomical and functional data were collected on GE 3.0 Tesla system (GE Medical Systems, Waukesha, WI) equipped with a quadrature head coil, with a 192-channel head coil, and an 11-channel head coil anterior sub- cingulum cortex during exposure to aversive pictures following uncertain (A) compared to certain (B) warning cues (C illustrates the uncertain-certain contrast). R = right. L = left.

**Data Analysis**

We employed a random effects, two-sample t-test to compare the parameter estimates associated with each condition. In the uncertain warning cue condition, we used a second-level random effects t-test to compare the functional connectivity of the amygdala and insula responses to aversive pictures preceded by uncertain warning cues and the amygdala and insula responses to aversive pictures preceded by certain warning cues. Our analyses showed heightened activation in the amygdala and insula responses to aversive pictures preceded by certain warning cues as compared to aversive pictures preceded by uncertain warning cues. Our analyses also showed greater synchrony between the right amygdala and dorsal anterior cingulate cortex (DACC) during exposure to aversive pictures preceded by uncertain warning cues and the dorsal ACC may play a modulatory role. The amygdala and DACC may be part of a network that represents the influence of uncertainty on the anticipation and experience of aversion. Of relevance to anxiety disorders, these data suggest that uncertainty magnifies the impact of aversive events on threat deflection and response mechanisms in the amygdala (Whalen & Davis, 2001; Nitschke et al., 2006) and on the integration and body-state representation mechanisms of the insula (Critchley, 2004; Damasio, 2003; Craig, 2002, 2003; Nitschke et al., 2006).

**Results**

1. The amygdala and insula should exhibit greater activation in response to aversive pictures preceded by un cer tain warning cues than neutral or aversive pictures preceded by certain warning cues.

2. Amygdala and insula activation should exhibit greater symmetry with prefrontal and anterior cingulate cortex (Sarinopoulos et al., 2006) for the aversive pictures preceded by uncertain warning cues than neutral or aversive pictures following certain warning cues that were equally likely to precede aversive or neutral pictures.

3. Participants in the right-handed undergraduates (16 female) free of any medical or neurological problems.

4. Our analyses showed heightened activation in the amygdala and insula responses to aversive pictures preceded by uncertain warning cues. The same regions have previously been implicated in our lab and elsewhere in anticipation of and response to aversion (Nitschke et al., 2006). This heightened response to aversive stimuli when preceded by un cer tain cues suggests the top-down modulation of these key brain areas. Exploratory connectivity analyses showed greater synchrony between the amygdala and dorsal anterior cingulate cortex (DACC) during exposure to aversive pictures preceded by uncertain warning cues and the dorsal ACC may play a modulatory role. The amygdala and DACC may be part of a network that represents the influence of uncertainty on the anticipation and experience of aversion. Of relevance to anxiety disorders, these data suggest that uncertainty magnifies the impact of aversive events on threat deflection and response mechanisms in the amygdala (Whalen & Davis, 2001; Nitschke et al., 2006) and on the integration and body-state representation mechanisms of the insula (Critchley, 2004; Damasio, 2003; Craig, 2002, 2003; Nitschke et al., 2006).