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Neuronal activity in the amygdala during appetitive and aversive emotional arousal

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Neuronal activity in the amygdala during appetitive and aversive emotional arousal

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For my family

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ABSTRACT

Several lines of evidence suggest that the amygdala is important for emotional arousal. However, whether overlapping or segregated populations of cells in the amygdala contribute to appetitive and aversive emotional arousal remains unclear. Here we test two predictions of the hypothesis that overlapping populations of cells in the amygdala contribute to appetitive and aversive emotional arousal: neuronal activity in the amygdala during appetitive and aversive arousal should tend to be similar, and the activity of cells with similar changes in activity during appetitive and aversive emotional arousal should be correlated with measures of arousal.

Consistent with the hypothesis that overlapping populations of cells contribute to appetitive and aversive emotional arousal, we show that a large proportion of amygdala cells (26%) has qualitatively similar changes in activity during conditioned appetitive and aversive emotional arousal, despite the different sensory modalities of the eliciting stimuli. Furthermore, when the entire population of recorded cells is considered together, neuronal activity is more similar than expected by chance during conditioned appetitive and aversive emotional arousal. The basolateral region (BLA) and central nucleus (CeN) of the amygdala had similar proportions of "Same" cells, suggesting that overlapping populations of cells contribute to appetitive and aversive emotional arousal in both regions. Interestingly, however, the CeN had a greater proportion of cells with opposite changes in activity during the conditioned appetitive and aversive stimuli than the BLA, suggesting that neuronal activity in the CeN has a greater degree of affective specificity.

We also found that a large proportion of cells in the amygdala, including over half of the "Same" cells, had activity that was correlated with blood pressure—a measure of emotional arousal that is sensitive to lesions of the amygdala in both appetitive and aversive settings.

Together, our results suggest that the amygdala contributes to appetitive and aversive emotional arousal through the recruitment of overlapping populations of cells that respond similarly to appetitive and aversive stimuli.

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CHAPTER 1

INTRODUCTION

The decline of behaviorism and the concomitant "cognitive revolution" in psychology in the 1960's and 1970's was associated with renewed interest in the study of affect. Since this time, there has been much theorizing (and little consensus) about the nature of affect and emotion by psychologists as well as philosophers and neuroscientists. Although there are multiple ways of classifying the literature, much research on emotion can be divided according to whether it was guided by a discrete or a dimensional approach.

Discrete vs. Dimensional Approaches to the Study of Emotion

Discrete emotion theory posits that there is some number of distinct emotions that can be activated independently of one another (Ekman, 1993) (though most theorists also assert that multiple basic emotions can be activated simultaneously and that activation of one emotion can lead to activation of another). Discrete emotion theorists view "basic" emotions as universal (among humans and some non-human animals) and innate, having been selected by evolution because of their adaptive value (Ekman, 1994). Thus, complexity in emotional expression is thought to be a "built in" feature of emotional systems. Evidence for the existence of discrete emotions comes largely from cross-cultural studies on the recognition of facial expressions of emotion (Ekman et al., 1969; Ekman and Friesen, 1971; Izard, 1977). These studies found that people from many cultures recognized the same prototypical facial displays of certain basic emotions (such as anger, fear, and happiness), providing strong evidence for the universality of emotional systems. Nevertheless, these studies have been criticized for using pictures of highly-stereotyped, posed expressions of emotion categories rather than non-scripted expressions

(Russell, 1994). The relationship between facial displays of emotion and emotional experience has also been questioned, since some facial expressions are associated with more than one emotion (e.g., smiling during happiness and embarrassment) and depend on the social context (Russell et al., 2003).

In contrast, dimensional theorists believe that emotion, and especially emotional experience, is better characterized by the activation of broad, continuous "dimensions" of emotion, which are activated to different degrees by different emotions (Russell, 1980). Dimensional theorists often differentiate between emotional experience, or "core affect", and other aspects of emotion, such as the appraisal of an emotion-eliciting object and one's behavioral response to it (Russell and Barrett, 1999). Thus, it is the proposal of a nonspecific "core affect" system which distinguishes dimensional from discrete theories of emotion. Evidence for the existence of such a system comes primarily from subjects' similarity ratings of affect labels. Factor analysis suggests that two factors, often called valence (which ranges from highly positive to highly negative) and arousal (which ranges from calm to excited or agitated), can account for a large amount of the variance in the ratings (~ 75%) (Russell and Barrett, 1999). More recently, some physiological measures of affect have been found to correlate with self-reported valence and arousal (Bradley and Lang, 2007). Specifically, valence correlates with startle, facial EMG, and prefrontal EEG asymmetries, while arousal correlates with skin conductance and cortical ERP/EEG (Bradley and Lang, 2007). Thus, valence and arousal are thought to be the two major dimensions of the "core affect" system. Note that for the sake of consistency, I

will substitute the phrase “emotional valence” for “affective value”, “positive and negative value”, and “affective valence” since I consider these labels to be synonymous.

Evidence for Neural Encoding of Dimensions of Emotion

Ultimately, the structure of “emotional space” will be greatly informed, if not determined, by how neural circuits responsible for emotion are organized in the brain. Much recent research in neuroscience has been devoted to this question, and there are several neuroimaging studies which have looked for neural correlates of valence and arousal (Lane et al., 1999; Garavan et al., 2001; Anderson et al., 2003; Anders et al., 2004; Dolcos et al., 2004; Kensinger, 2004; Kensinger and Corkin, 2004; Smith et al., 2004; Winston et al., 2005; Grimm et al., 2006; Kensinger and Schacter, 2006; Hurlemann et al., 2007; Lewis et al., 2007; Gerber et al., 2008; Posner et al., 2008; Straube et al., 2008). One common problem in the study of emotion is dissociating those effects (either neural or autonomic changes) that are due to emotion and those that are due to the context in which the emotion is induced. Two studies circumvented this problem by using simple stimuli — odors and tastes — that can be made more or less intense by simply changing their concentration (Anderson et al., 2003; Small et al., 2003). Both studies suggested that the valence of the stimuli was represented in the prefrontal cortex, with different areas being activated by either positively or negatively valenced stimuli, while the intensity of the stimuli was correlated with activation of the amygdala, independent of the valence of the stimuli. Interestingly, one study found that a particular odor which was rated similarly negative for both low and high intensities

produced different responses in the amygdala depending on the intensity (Anderson et al., 2003). Thus, these studies suggested that valence and intensity of emotional experience are represented in the brain by distinct neural circuits, although important caveats exist. One caveat is that both studies used ratings of intensity of the stimulus, rather than emotional intensity or arousal. Another caveat is that imaging technology does not have sufficient resolution to detect activation of distinct neural circuits within a given brain region. Therefore, it's possible that different circuits for the processing of positively and negatively valenced stimuli exist in the amygdala and could not be resolved in these studies.

The Amygdala and Emotional Arousal

Although the concept of "emotional arousal" is poorly defined, it can be roughly thought of as a set of changes in the body (including the brain) that facilitate responding to environmental events. The finding that activation of the amygdala correlates with intensity ratings is consistent with the results of many studies which indicate an important role for the amygdala in emotional arousal. As one would expect if the amygdala is important for emotional arousal, lesions of the amygdala blunt changes in autonomic arousal (Iwata et al., 1986; LeDoux et al., 1990; Kapp et al., 1992; Bechara et al., 1995; Braesicke et al., 2005), stimulation of the amygdala produces changes in autonomic arousal (Kapp et al., 1992; Davis, 2000), and neural activity in the amygdala correlates with multiple measures of arousal (Ben-Ari et al., 1973; Pascoe and Kapp, 1985; Zhang et al., 1986b; Lambertz et al., 1995; LaBar et al., 1998; Liberzon et al., 2000; Phelps et

al., 2001; Critchley et al., 2005; Dalton et al., 2005). The amygdala is also highly interconnected with areas of the brain that control changes in autonomic function and "cortical arousal" (EEG correlates of arousal) (Pitkanen, 2000) and also has a role in attention (Gallagher et al., 1990; Han et al., 1999; Holland and Gallagher, 1999; Holland et al., 2000), as well as the enhancement of memory consolidation by emotional arousal (McGaugh, 2004). One intriguing recent study found that war veterans with damage to their amygdala were much less likely to develop post-traumatic stress disorder and the associated symptom of hyperarousal than other war veterans (Koenigs et al., 2008). Thus, considerable evidence indicates that the amygdala is important for emotional arousal, although it remains unclear whether appetitive and aversive emotional arousal are controlled by distinct or overlapping neural circuits in the amygdala.

The Amygdala and Emotional Valence

Although the hypothesis that the amygdala encodes emotional arousal does not necessarily make predictions about whether appetitive and aversive stimuli are processed by distinct or overlapping neural circuits in the amygdala (the amygdala could encode appetitive and aversive arousal separately), the hypothesis that the amygdala encodes emotional valence clearly predicts that there should either be distinct neural circuits for the processing of appetitive and aversive stimuli in the amygdala or common neural circuits that have opposite changes in activity during appetitive and aversive stimuli. While several studies found mixed results (Sanghera et al., 1979; Nishijo et al., 1988; Muramoto et al., 1993), more recent recording studies have shown that the activity of

amygdala neurons during stimuli that predict either a positive or negative outcome is often affected by the associated outcome (Schoenbaum et al., 1998, 1999; Paton et al., 2006). These findings are clearly consistent with the hypothesis that the amygdala encodes emotional valence. Nevertheless, important alternative hypotheses remain. For example, it is possible that some outcome-selective neuronal activity is due to the different sensory properties of the outcomes (rather than their positive or negative valence) or specific affective responses induced by the conditioned stimuli (emotion or situation-specific reflexes). It is even possible that some of the outcome-selective neuronal activity is due to different levels of arousal during anticipation of the positive or negative outcome. Experiments that distinguish among these hypotheses are sorely needed.

The hypothesis that the amygdala encodes emotional valence also predicts that interruption of neuronal activity in the amygdala will affect behaviors that depend on valence encoding. Consistent with this prediction, lesions of the amygdala impair responding for cues that were previously paired with reward (Everitt et al., 1989; Robbins et al., 1989; Burns et al., 1999) and withholding responding for cues that were previously paired with punishment (Killcross et al., 1997). It is unclear, however, whether this is due to deficits in reinforcement, which does require valence encoding, or other processes, such as incentive salience or mnemonic processes that may not require valence encoding. Notably, amygdala lesions do not disrupt reinforcement in general, since amygdala-lesioned rats can be conditioned to respond for primary reinforcers at a similar rate as non-lesioned animals (e.g., Hatfield et al., 1996). Unfortunately, there are few behavioral

correlates of self-reported emotional valence that can be used to assess emotional valence in non-human animals. It has been demonstrated, however, that amygdala lesions do not alter affective reactions to "liked" or "disliked" fluids (Kiefer and Grijalva, 1980; Galaverna et al., 1993; Rana and Parker, 2008), nor do they affect the inhibition of startle by positive stimuli (Koch et al., 1996). Although amygdala lesions do impair fear-potentiated startle (Davis, 1986, 1989), this could be due to the amygdala's role in emotional arousal rather than valence (Bradley and Lang, 2007).

To my knowledge, only two studies have tested ratings of arousal and valence in amygdala-damaged humans (Glascher and Adolphs, 2003; Gosselin et al., 2007). Both studies found greater evidence for impaired recognition/report of arousal than valence, although conclusions were limited due to the small number of patients included. Thus, while there is substantial evidence for the amygdala's role in emotional arousal, its role in emotional valence is less clear. One possibility is that emotional valence is represented independently in multiple regions of the brain, so that damaging one region does not produce a measurable effect on behavior. Thus, recording of neuronal activity in the amygdala is vital to elucidating its role in emotional valence.

The Role of the Basolateral Region and Central Nucleus in Emotional Arousal

The amygdala is a heterogeneous structure comprised of thirteen cytoarchitectonically, chemoarchitectonically, and connectionally distinct regions (Pitkanen, 2000). The basolateral region (composed of the lateral, basal, and basomedial nuclei; BLA) and

central nucleus of the amygdala (CeN) have been the focus of much research due to their involvement in various forms of learning and memory (Kapp et al., 1992; Davis, 2000; Gallagher, 2000; LeDoux, 2000a; Parkinson et al., 2000; Baxter and Murray, 2002; McGaugh, 2004; Balleine and Killcross, 2006). Bruce Kapp and colleagues have suggested that the CeN plays an important role in conditioned arousal (e.g., changes in heart rate, blood pressure, EEG, and attention) (Kapp et al., 1992), consistent with its extensive reciprocal connections with cardiorespiratory and arousal systems in the brain stem (Pitkanen, 2000). However, the BLA also appears to mediate some aspects of arousal (e.g., memory enhancement, changes in heart rate and blood pressure) (LeDoux et al., 1990; Shekhar et al., 2003; McGaugh, 2004), perhaps through its projections to the CeN, hypothalamus, basal forebrain and cortex (LeDoux, 2000b; Pitkanen, 2000). Kapp and colleagues hypothesized that the CeN is important for conditioned changes in arousal in both appetitive and aversive settings (Kapp et al., 1992). Whether specific CeN lesions affect conditioned autonomic arousal in appetitive settings has not been tested, although lesions of the CeN do impair attention and orienting responses in appetitive settings (Gallagher et al., 1990; Han et al., 1999; Holland and Gallagher, 1999; Holland et al., 2000). It has also been shown that large amygdala lesions impair anticipatory appetitive increases in blood pressure in monkeys (Braesicke et al., 2005). Further experiments are needed to determine if the same nuclei that are involved in conditioned increases in blood pressure in aversive settings (BLA, CeN, and lateral hypothalamus) are also involved in conditioned increases in blood pressure in appetitive settings.

The Role of the Basolateral Region and Central Nucleus in Emotional Valence

Unlike some aspects of emotional arousal, the encoding of emotional valence within the amygdala may be nucleus-specific. Based in part on evidence that lesions of the BLA impair conditioned reinforcement, Everitt and colleagues have suggested that the BLA is important for encoding the emotional valence of stimuli (Everitt et al., 2003). However, Balleine and Killcross suggest that the BLA is important for representing sensory-specific aspects of reinforcers rather than their emotional valence and that the CeN is important for encoding the emotional valence of stimuli (Balleine and Killcross, 2006). Unfortunately, only two studies have compared neuronal activity in the BLA and CeN during the presentation of appetitive and aversive stimuli. One study found more cells with opposite responses to the appetitive and aversive conditioned stimuli in the BLA than CeN (Muramoto et al., 1993). Because appetitive and aversive systems are thought to reciprocally inhibit each other (Konorski, 1967; Dickinson and Dearing, 1979), this result clearly supports the Everitt hypothesis. However, the other study found similarities in neuronal activity during presentation of different types of aversive fluids in the CeN but not in the BLA (Yasoshima et al., 1995), consistent with the Balleine and Killcross hypothesis. Future studies, especially studies that use multiple types of affective stimuli, should help resolve this discrepancy.

Summary

Considerable evidence suggests that the amygdala contributes to emotional processing, especially emotional arousal. In this dissertation, we tested two predictions of the hypothesis that overlapping populations of cells in the amygdala contribute to appetitive and aversive emotional arousal: neuronal activity in the amygdala during conditioned appetitive and aversive arousal is similar, and the activity of cells with similar changes in activity during conditioned appetitive and aversive arousal is correlated with measures of arousal. We tested these predictions by recording the activity of single cells in the rat amygdala while simultaneously measuring blood pressure during a simple Pavlovian conditioning procedure.

CHAPTER 2

Similar patterns of neuronal activity in the amygdala during conditioned appetitive and aversive emotional arousal

ABSTRACT

The amygdala is important for determining the emotional significance of environmental stimuli. However, the degree to which appetitive and aversive stimuli are processed by the same or different cells in the amygdala remains unclear. Here we show that a large proportion (26%) of amygdala cells has qualitatively similar changes in activity during the expression of classically conditioned appetitive and aversive emotional responses ("Same" cells), despite the different sensory modalities of the eliciting stimuli. Furthermore, when considering the entire population of cells, neuronal activity during the conditioned appetitive and aversive stimuli is more similar than expected by chance. We also found that the activity of a large proportion of cells, including over half of the "Same" cells, was correlated with blood pressure. Together, our results suggest that the amygdala encodes the emotionally arousing properties of conditioned appetitive and aversive stimuli through the recruitment of overlapping populations of cells.

INTRODUCTION

The amygdala plays an important role in emotional processing (Gallagher and Chiba, 1996; Davis and Whalen, 2001; Zald, 2003; McGaugh, 2004; Phelps and LeDoux, 2005). Of particular interest is whether the amygdala encodes one or both of the most commonly described dimensions of emotion (Russell, 1980; Garavan et al., 2001; Anderson et al., 2003; Glascher and Adolphs, 2003; Small et al., 2003; Winston et al., 2005; Lang and Davis, 2006): valence, which ranges from highly positive to highly negative, and arousal, which ranges from calm to excited or agitated.

Consistent with the hypothesis that the amygdala encodes emotional arousal, neuroimaging studies in humans have found activation of the amygdala during the presentation of both positively and negatively valenced arousing stimuli (LaBar et al., 1998; Liberzon et al., 2000; Phelps et al., 2001; Anderson et al., 2003; Small et al., 2003; Zald, 2003; Critchley et al., 2005; Dalton et al., 2005; Winston et al., 2005). Several of these studies have also found that the degree of amygdala activation correlates with measures of autonomic arousal (LaBar et al., 1998; Liberzon et al., 2000; Phelps et al., 2001; Critchley et al., 2005; Dalton et al., 2005). Paralleling these findings, numerous non-human animal studies indicate that the amygdala is important for both appetitively and aversively motivated behaviors (Iwata et al., 1986; LeDoux et al., 1990; Kapp et al.,

1992; Davis, 2000; Baxter and Murray, 2002; Everitt et al., 2003) and corresponding changes in autonomic function (Iwata et al., 1986; LeDoux et al., 1990; Kapp et al., 1992; Braesicke et al., 2005). Thus, considerable evidence suggests that the amygdala is important for appetitive and aversive arousal. However, the degree to which overlapping or segregated populations of cells within the amygdala are involved in appetitive and aversive arousal remains unclear.

If overlapping populations of cells contribute to appetitive and aversive emotional arousal, then neuronal activity in the amygdala during conditioned appetitive and aversive stimuli should be similar. Here, we tested this prediction by using a novel experimental design which allowed us to quantify the degree of similarity between patterns of neuronal activity during conditioned appetitive and aversive stimuli. Rather than use two similar conditioned stimuli (i.e., stimuli from the same sensory modality) and look for differences in neuronal activity after pairing one with a rewarding outcome and the other with an aversive outcome (Muramoto et al., 1993; Schoenbaum et al., 1999; Paton et al., 2006), we used stimuli from different sensory modalities and looked for similarities in neuronal activity after pairing one with a rewarding outcome and the other with an aversive outcome. Hence, any observed similarity in neuronal activity can not be ascribed to encoding of similar sensory features (Fig. 1a). Our hypothesis also predicts that a substantial proportion of cells in the amygdala, and especially cells with similar changes in activity during conditioned appetitive and aversive stimuli, will be correlated with measures of arousal. We tested this prediction by simultaneously measuring single-cell activity and blood pressure – a component of autonomic arousal which is sensitive to

lesions of the amygdala in both appetitive (Braesicke et al., 2005) and aversive (Iwata et al., 1986; LeDoux et al., 1990) settings − and looking for systematic relationships between the two during appetitive and aversive conditioning sessions.

RESULTS

Sucrose and shock conditioned stimuli induce appetitive and aversive emotional arousal

Rats (n = 10) were first trained to discriminate between two tones of different frequency during appetitive Pavlovian conditioning. One tone (Sucrose CS+) was paired with sucrose delivery in a reward port, while the other tone (Sucrose CS-) was explicitly unpaired with sucrose delivery (Fig. 1b). After 12 conditioning sessions, rats approached and remained in the port more often during the Sucrose CS+ than the Sucrose CS- (t-test, $P<.0001$; Fig. 2a), and blood pressure was elevated during the Sucrose CS+ compared to the Sucrose CS- (t-test, $P< .01$; Fig. 2b, Supplementary Fig. 1). Thus, the rats clearly discriminated between the Sucrose CS+ and the Sucrose CS- after training. Interestingly, the mean increase in blood pressure during the Sucrose CS+ was larger during trials in which the rat was in the port at the end of the Sucrose CS+ ("IN" trials), presumably waiting for the delivery of sucrose, than during trials in which the rat was out of the port at the end of the CS+ ("OUT" trials) (t-test, $P<.01$, see Methods; Fig. 2c). This difference could not be explained by an increase in locomotor activity during "IN" trials, since the increase in blood pressure was actually larger on trials with a decrease, rather

than increase, in movement during the Sucrose CS+ (Supplementary Fig. 2). We also found that the blood pressure responses could not be explained simply by the sensory properties of the conditioned stimulus (Supplementary Data). Thus, changes in blood pressure during the CS+ reflect motivational, attentional, and/or emotional state.

After the initial discrimination acquisition sessions, animals continued daily conditioning sessions in which the appetitive conditioning session was followed immediately by an aversive conditioning session, forming joint appetitive/aversive conditioning sessions which are the source of the data presented here. During the first appetitive/aversive conditioning session only, rats were given 10 presentations of the light Shock CS+ without footshock ("Habituation" trials) to measure pre-conditioning changes produced by the Shock CS+. The habituation trials were followed by pairings of the Shock CS+ with footshock. Consistent with previous reports (Iwata et al., 1986; LeDoux et al., 1990; Pare and Collins, 2000), aversive conditioning increased blood pressure during the Shock CS+ relative to habituation trials (t-test, $P<.001$; Fig. 2d, Supplementary Fig. 1) and produced species-specific defense reactions during the CS (Bolles, 1970), i.e., escape/fleeing during the conditioning sessions (mean ± s.e.m.: pre-CS, 0.4 ± 0.1 counts/s; Shock CS+, 3.3 ± 0.8 counts/s, t-test, $P<.01$; Fig. 2e), and freezing during a test session without footshock (percentage of time freezing, mean ± s.e.m.: pre-CS, 0 ± 0; Shock CS+, 55 ± 12, one-sample t-test, $P<.001$). The changes in behavior and blood pressure we observed during the Sucrose CS+ and Shock CS+ indicate that these stimuli elicited appetitive and aversive emotional arousal, respectively.

Neuronal activity reflects the emotional significance of the conditioned stimuli

We recorded the activity of 518 cells in the amygdala (basal, lateral, basomedial, and central nuclei) across a total of 106 joint appetitive-aversive conditioning sessions in the 10 well-trained rats whose conditioned behavior is described above (see Supplementary Fig. 3,4 for location of cells). Most cells had low baseline firing rates, suggesting that we sampled predominantly from putative projection cells (Pare and Gaudreau, 1996; Rosenkranz and Grace, 1999; Likhtik et al., 2006) (Supplementary Fig. 5) .

Previous single-cell recording studies reported robust changes in neuronal activity in the amygdala to stimuli paired with positive or negative reinforcement, but not to stimuli presented repeatedly without consequence (Nishijo et al., 1988; Rolls, 1992; Muramoto et al., 1993; Quirk et al., 1995; Goosens et al., 2003; Tye and Janak, 2007), consistent with a role for the amygdala in assessing the motivational or emotional significance of stimuli. Similarly, we found more cells with significant changes in activity during the Sucrose CS+ and Shock CS+ than the Sucrose CS- (Sucrose CS+ vs. Sucrose CS-, χ^2=221.7, P<.0001; Shock CS+ vs. Sucrose CS-, χ^2=209.0, P< .0001; Sucrose CS+ vs. Shock CS+, χ^2=0.2, P=0.66; Fig. 3a). Additionally, the magnitude of the average normalized response across the entire population of recorded cells was larger in response to the Sucrose CS+ and Shock CS+ than the Sucrose CS- (ANOVA, main effect of CS type: $F_{2,1551}$ = 257.4, P<.0001; main effect of time: $F_{4,2585}$ = 7.8, P<.0001; CS type x time:

$F_{8,5170}$ = 1.9, P=.06; t-tests: Sucrose CS+ vs. Sucrose CS-, P<.0001; Shock CS+ vs. Sucrose CS-, P<.0001; Sucrose CS+ vs. Shock CS+, P=0.36; Fig. 3b).

When restricting our analysis to cells with statistically significant responses to the Sucrose CS+, we found that they had smaller responses to the Sucrose CS- (Increases in neuronal activity, t-test, P<.0001; Decreases in neuronal activity, t-test, P<.0001; Fig. 3c,d) and larger responses during "IN" Sucrose CS+ trials than "OUT" Sucrose CS+ trials (Increases in neuronal activity, t-test, P<.0001; Decreases in neuronal activity, t-test, P<.0001; Fig. 3e,f). These effects were independent of the sensory properties of the conditioned stimulus and movement (Supplementary Data, Supplementary Fig. 6). Together, these results indicate that neuronal activity in the amygdala, like changes in blood pressure, reflects the emotional significance of the conditioned stimuli.

Despite the parallels between neuronal activity and blood pressure, changes in neuronal activity during the Sucrose CS+ and Shock CS+ were not secondary to changes in blood pressure, since neuronal response latencies for the Sucrose CS+ and Shock CS+ tended to be shorter than blood pressure latencies (Fig. 3g,h). Thus, neuronal responses to the Sucrose CS+ and Shock CS+ tended to precede blood pressure responses, consistent with neuronal activity in the amygdala contributing to changes in blood pressure.

Similar changes in neuronal activity during conditioned appetitive and aversive stimuli

If overlapping populations of cells in the amygdala contribute to appetitive and aversive emotional arousal, then neuronal activity should be similar during conditioned appetitive and aversive stimuli. Consistent with prior studies (Schoenbaum et al., 1999; Paton et al., 2006), we found many cells that were selective for the appetitive or aversive conditioned stimulus ("Selective" cells; Sucrose CS+ selective, 23% of all cells; Shock CS+ selective, 19% of all cells; Fig. 4a) and a smaller group of cells that had opposite changes in activity during the Sucrose CS+ and Shock CS+ ("Opposite" cells; Fig. 4a). Notably, however, we also found a large population of cells (26%) which had qualitatively similar changes in activity during the Sucrose CS+ and Shock CS+ ("Same" cells), despite the different modalities of the stimuli, the different outcomes they predicted, and the different behaviors they elicited (Fig. 4a-c). "Same" cells tended to have larger responses to the Sucrose CS+ than the Sucrose CS-, larger responses during "IN" Sucrose CS+ trials than "OUT" Sucrose CS+ trials, and faster responses to the Sucrose CS+ and Shock CS+ than blood pressure (Supplementary Fig. 7), although these effects were not restricted to "Same" cells (Supplementary Data). Interestingly, "Same" cells were common in both the basolateral region (BLA—basal, lateral, and basomedial nuclei, 26% of cells) and central nucleus (CeN, 28% of cells) of the amygdala, although "Opposite" cells were more prevalent in the CeN than BLA (CeN, 11% of cells; BLA, 4% of cells; χ^2=9.0, P<.01; Supplementary Fig. 8).

To further characterize the patterns of neuronal activity during the appetitive and aversive conditioned stimuli, we investigated whether increases or decreases in activity during one CS+ predicted similar changes in activity during the other CS+. We found that, compared to the likelihood in the rest of the population, a cell was more likely to have a significant increase in activity during one CS+ if it had a significant increase in activity during the other CS+ (χ^2=24.9, *P*<.0001; Fig. 4d). The same was true for decreases in activity – a cell was more likely to have a significant decrease in activity during one CS+ if it had a significant decrease in activity during the other CS+ (χ^2=19.4, *P*<.0001; Fig. 4e). Importantly, the opposite relationships did not exist. Cells with increases or decreases in activity during one CS+ were not more likely to have the opposite change in activity; in fact, cells with decreases in activity during the Shock CS+ were less likely to have increases in activity during the Sucrose CS+ (P(Sucrose CS+ Increase | Shock CS+ Decrease), χ^2=8.3, *P*<.01; Fig. 4d), although the converse was not true (P(Sucrose CS+ Decrease | Shock CS+ Increase), χ^2=2.1, *P*=.15; Fig. 4e). While relationships between responses to the Sucrose CS+ and Shock CS+ were seen in both the BLA and CeN, they were not as prominent in the CeN (Supplementary Fig. 9).

We next tested the hypothesis that the Sucrose CS+ and Shock CS+ elicit quantitatively similar changes in neuronal activity. To compare neuronal activity during the Sucrose CS+ and Shock CS+, we computed for each cell the difference in normalized neuronal activity during the two conditioned stimuli and averaged over all the cells – the "Aligned" condition – and compared it to what one would expect if neuronal activity during the stimuli were independent – the "Shuffled" condition, obtained by finding the

difference in activity during the two stimuli in arbitrary cell pairs (Fig. 5a; see Methods). Thus, if neuronal activity during the Sucrose CS+ and Shock CS+ tends to be similar, the difference score in the "Aligned" condition should be less than the difference score in the "Shuffled" condition, and if neuronal activity during the Sucrose CS+ and Shock CS+ tends to be different, the difference score in the "Aligned" condition should be greater than that in the "Shuffled" condition. Consistent with the hypothesis that neuronal activity during the Sucrose CS+ and Shock CS+ tends to be similar, the difference score in the "Aligned" condition was indeed less than the difference score in the "Shuffled" condition (ANOVA, main effect of type of comparison: $F_{1,1034} = 27.2$, $P<.0001$; main effect of time: $F_{4,2585} = 3.7$, $P<.01$; type of comparison x time: $F_{4,5170} = .4$, $P=.79$; inset, t-test, $P<.0001$; Fig. 5b). These findings held true when we examined the BLA and CeN separately (Supplementary Fig. 10).

In contrast to the comparison between the Sucrose CS+ and the Shock CS+, there was no significant difference between the "Aligned" and "Shuffled" conditions for the comparison between the Sucrose CS- and the Shock CS+ (ANOVA, main effect of type of comparison: $F_{1,1034} = 1.7$, $P=0.19$; main effect of time: $F_{4,2585} = 4.5$, $P<.01$; type of comparison x time: $F_{4,5170} = .7$, $P=.62$; Fig. 5c, Supplementary Fig. 10). These results indicate that neuronal activity during the Sucrose CS+ and Shock CS+, but not during the Sucrose CS- and Shock CS+, was more similar than expected by chance. Additional analysis indicates that the similarity in neuronal activity during the Sucrose CS+ and Shock CS+ was not a function of baseline firing rates (Supplementary Data).

Activity of individual amygdala neurons is correlated with blood pressure and related to the direction of the neuronal response to the conditioned stimuli

If neuronal activity in the amygdala contributes to emotional arousal, then measures of arousal should covary with single-cell activity in the amygdala (Ben-Ari et al., 1973; Pascoe and Kapp, 1985; Zhang et al., 1986b; Lambertz et al., 1995). To determine if neuronal activity in the amygdala contributed to the changes in blood pressure we observed during the Sucrose CS+ and Shock CS+, we looked for relationships between single-cell activity and blood pressure using multiple linear regression. Remarkably, over 43% of cells (188/434 cells; BLA, 138/312, 44%; CeN, 41/101, 41%) were correlated with blood pressure despite accounting for possible coincident activation of neuronal activity and blood pressure by experimental and behavioral events (such as stimulus presentations; see Methods) in the analysis and despite a stringent statistical threshold ($\alpha = .0001$). In contrast, when the activity data for each cell was shuffled prior to the regressions, none of the cells was correlated with blood pressure.

Consistent with the hypothesis that "Same" cells are important for changes in arousal, "Same" cells were more likely to be correlated with blood pressure than the rest of the population (Same, 60/109, 55%; Rest, 128/325, 39%; $\chi^2=7.5$, $P<.01$). Furthermore, the direction of the correlations (positive or negative) tended to be congruent with the direction of the neuronal responses (increases or decreases in activity, respectively) to the conditioned stimuli (45/60 cells, 75%), as one would expect if the activity of "Same" cells contributes to increases in blood pressure during the conditioned stimuli. In fact,

"Same" cells were more likely to have congruent correlations with blood pressure than "Selective" cells (Same: 45/109, 41%; Selective: 52/187, 28%; χ^2=5.1, P<.05) but not more likely to have incongruent correlations with blood pressure (Same: 15/109, 14%; Selective: 28/187, 15%; χ^2=.01, P=.91). Interestingly, of those Opposite cells which were correlated with blood pressure (12 of 25 Opposite cells), the majority had correlations which were congruent with the Shock CS+ and incongruent with the Sucrose CS+ (10 of 12 cells).

DISCUSSION

In this study, we demonstrate striking similarities in the activity of amygdala neurons during conditioned appetitive and aversive emotional arousal and strong relationships between neuronal activity in the amygdala and blood pressure—a measure of arousal which is sensitive to lesions of the amygdala in both appetitive (Braesicke et al., 2005) and aversive(Iwata et al., 1986; LeDoux et al., 1990) settings. Additionally, we show that the activity of neurons with similar changes in activity during conditioned appetitive and aversive stimuli ("Same" cells) is especially likely to be correlated with blood pressure and to have correlations that are consistent with a contribution to conditioned blood pressure responses.

Importantly, we found similarities in activity despite the different sensory modalities of the conditioned stimuli, the different outcomes they predicted, and the different behaviors they elicited. We also found that the overall similarity in activity was specific to the

comparison between the conditioned appetitive and aversive stimuli, since neuronal activity during the control stimulus and the Shock CS+ was not more similar than expected by chance. In fact, the average change in activity during the control stimulus was much smaller than that during the conditioned appetitive and aversive stimuli. Thus, similarities in activity during conditioned appetitive and aversive stimuli are due to the learned significance of the stimuli rather than simple sensory stimulation.

Our results are consistent with single-cell recording studies which noted that some amygdala neurons respond to both appetitive and aversive stimuli (Sanghera et al., 1979; Nishijo et al., 1988; Muramoto et al., 1993). However, these studies were either descriptive in nature or were otherwise not designed to quantify the degree of similarity between appetitive and aversive stimuli. Furthermore, our finding that cells with similar responses to conditioned appetitive and aversive stimuli are especially likely to be correlated with blood pressure suggests that these cells are important for emotional arousal. These results also build on prior studies that found relationships between neuronal activity in the amygdala and measures of arousal but did not test single-cell responses to both appetitive and aversive stimuli (Ben-Ari et al., 1973; Pascoe and Kapp, 1985; Zhang et al., 1986b; Lambertz et al., 1995; LaBar et al., 1998; Liberzon et al., 2000; Phelps et al., 2001; Critchley et al., 2005; Dalton et al., 2005).

Although the activity of amygdala cells was more similar than expected by chance when the entire population of recorded cells was considered together, we nevertheless found many cells that were selective for either the appetitive or aversive conditioned stimulus.

This is consistent with studies which elegantly demonstrated that the activity of amygdala cells is often affected by the associated outcome (Schoenbaum et al., 1998, 1999; Paton et al., 2006). The precise meaning of this outcome-specificity remains to be determined, however. Outcome-specific cells could be encoding the sensory properties of the anticipated outcome, the positive or negative value (i.e., valence) of the conditioned stimuli or outcomes, or specific affective responses induced by the conditioned stimuli. Because of the different methods of data analysis from those used here, it is also possible that some of the outcome-specific encoding previously reported (Schoenbaum et al., 1998, 1999; Paton et al., 2006) is due to different levels of arousal generated by the anticipated outcomes. For example, cells with increases in activity during both the Sucrose CS+ and Shock CS+, which we classified as "Same" cells, could be classified as selective for one of the conditioned stimuli using these alternative methods of data analysis, as long as the magnitudes of the responses to the conditioned stimuli were different.

Indeed, it is important to note that emotional arousal is not a unitary process, and therefore, cannot be encoded exclusively by any particular population of cells(Robbins et al., 1998). Although our results suggest that "Same" cells are especially likely to contribute to increases in blood pressure during the conditioned stimuli, none of the relationships between single-cell activity and blood pressure were exclusive to "Same" cells. Interestingly, of those "Opposite" cells whose activity was correlated with blood pressure, the majority of the correlations were congruent with the direction of the cell's response to the Shock CS+ and incongruent with the direction of the cell's response to

the Sucrose CS+ (i.e., cells with positive correlations tended to have increases in activity during the Shock CS+ and decreases in activity during the Sucrose CS+ and cells with negative correlations tended to have decreases in activity during the Shock CS+ and increases in activity during the Sucrose CS+). This pattern is consistent with the possibility that "Opposite" cells contribute to larger increases in blood pressure during the Shock CS+ compared to the Sucrose CS+. Thus, it is possible that changes in blood pressure, and arousal in general, are produced by populations of amygdala cells with diverse response profiles.

Lesion studies suggest that both the BLA and CeN are important for conditioned increases in blood pressure during fear conditioning (Iwata et al., 1986; LeDoux et al., 1990). Consistent with these studies, we found that the activity of many cells in both regions was correlated with blood pressure. We also found substantial proportions of "Same" cells in both the BLA and CeN. Thus, both the BLA and CeN likely use overlapping populations of cells to produce changes in arousal in appetitive and aversive settings, although they may affect physiological, attentional, behavioral, and mnemonic processes differently via their distinct set of efferent projections (Pitkanen, 2000). It is also possible that projections from the BLA to the CeN coordinate or permit changes in aspects of emotional arousal (LeDoux, 2000a). The degree to which neuronal activity in the CeN depends on the BLA is an important unresolved issue (Pare et al., 2004).

Interestingly, although the BLA and CeN had similar proportions of "Same" cells, the CeN had a greater proportion of "Opposite" cells than the BLA. As noted above,

"Opposite" cells could contribute to differences in blood pressure or other affective responses during appetitive and aversive arousal. They are also good candidates for the representation of emotional valence, since it has been proposed that appetitive and aversive systems reciprocally inhibit each other (Konorski, 1967; Dickinson and Dearing, 1979). Thus, neuronal activity in the CeN may have a greater degree of affective specificity than the BLA, either because the CeN is representing specific affective responses or because it is representing emotional valence to a greater degree than the BLA.

Together, our findings indicate that the amygdala contributes to appetitive and aversive emotional arousal through the recruitment of overlapping populations of cells that respond similarly to appetitive and aversive stimuli. We propose that the similarities in neuronal activity reported here reflect the engagement of neuronal circuits whose function is to prepare the animal to respond to biologically important stimuli, independent of the valence of the stimuli.

Methods

Subjects. Ten male Long-Evans rats (325 - 400 g at the time of first surgery) were housed individually under a 12 hour light/dark cycle and given *ad libitum* access to

food throughout the experiment. Water access was restricted for ~ 19 hours before each behavioral session to motivate responding for the sucrose solution.

Surgery. Rats were anesthetized with isoflurane and implanted abdominally with a telemetric transmitter for measuring blood pressure and movement (Data Sciences International; St. Paul, MN). Seven of the rats were then implanted with 5-electrode driveable arrays unilaterally into the amygdala after 10 – 14 days of recovery from the first surgery. The other three rats were implanted after acquisition of appetitive conditioning (see below). Rats were given sulfatrim (an antibacterial) postoperatively to prevent infection and Tylenol to minimize pain. Stereotaxic coordinates were A-P: 2.3 - 2.9 mm posterior; M-L: 4.0 – 5.0 mm; D-V: 7.0 – 7.2 mm (ventral from the surface of the brain) relative to bregma. Training began/resumed after 10-15 days of recovery in the home cage.

Electrode Assemblies. The electrode assemblies consisted of five 50 μm Teflon-coated stainless steel wires (California Fine Wire; Grover Beach, CA) of equal lengths bundled into a 25 gauge cannula and attached to a driveable connector. The wires were advanced ventrally by turning a screw located in the connector. Before implantation, the wires were arranged into a single row ~ 1 mm wide and placed in brain so they extended primarily in the anterior-posterior direction. The stiffness and arrangement of the wires allowed the estimation of the position of each wire at the end of the experiment. Before each recording session, the wire with the least neuronal activity was used as an internal reference to minimize background activity

on the remaining four wires. A 200 μm Teflon-coated stainless steel wire (A-M Systems; Carlsborg, WA) stripped ~1 mm from the tip was placed it in the posterior cortex for use as an animal ground.

Behavioral procedures. The experimental chamber was a custom 31 x 33 x 29 cm (length x width x height) Plexiglas square encased in a sound-attenuating shell. Two recessed ports were located in the center of opposite walls, one of which was used to deliver sucrose. Entries into and exits from this port were monitored via an infrared beam. A high-frequency "tweeter" speaker (ENV-224BM) was located 1.5 cm above the top of the active port, 20 cm from the floor. A white noise speaker (ENV-225SM) located directly above the opposite port provided masking noise (65 dB) and a houselight (28V, 100 mA) directly above the white noise speaker provided constant illumination. The illumination of two leverlights (28V, 100 mA) located ~12 cm above the floor on opposite sides of the inactive port was used as the Shock CS+. Footshock was delivered through the grid floor by a constant current aversive stimulator (ENV-414S). All of the above were obtained from Med Associates, Inc. (St. Albans, VT). Movement and blood pressure data were sent to the neural data acquisition system from a receiver (Data Sciences International) located underneath the chamber floor. A video camera pointed through a small hole cut into the sound-attenuating shell monitored the animal's behavior.

Animals were first trained on a discriminative conditioned approach task. Training consisted of fifteen 90-minute sessions, one session per day. The first three sessions

were port training with sucrose (0.1 mL, 5% w/v) delivered randomly on a 2 minute interstimulus interval (ISI). During the next 12 sessions, auditory tones were played on a 1 minute ISI schedule (5 second duration, 3 or 11 kHz, 80-90 dB, 10 ms rise/fall). The stimulus for each trial was chosen randomly such that half of the presentations were the Sucrose CS+ and half were the Sucrose CS-. The Sucrose CS+ was immediately followed by sucrose delivery; the Sucrose CS- was never followed by sucrose. To keep the amount of sucrose consumed on each trial constant and to ensure that the rats were responsive during CS presentations, no further stimuli were presented until the rat entered the port for at least 500 ms since the last sucrose delivery. The 3 kHz tone was the Sucrose CS+ for six rats and the 11 kHz tone was the Sucrose CS+ for the other four rats. After acquisition of the discrimination (12 sessions), an aversive conditioning section was appended to the end of the appetitive conditioning session, which continued as before. During the first aversive conditioning section, we presented the Shock CS+ (illumination of the two leverlights for 5 seconds) 10 times without footshock ("Habituation" trials). These 10 trials were followed by 15 conditioning trials in which footshock (0.4 mA, 0.5 s) was administered immediately after termination of the Shock CS+. Shock CS+ trials were delivered on a 1 minute ISI schedule. The subsequent joint appetitive/aversive conditioning sessions were like the first except without the habituation trials and were the source of all the data presented here (except for the Shock CS+ habituation data). These sessions also included occasional probe Sucrose CS+ and Shock CS+ trials without reinforcement (30% of CS trials), as well as occasional probe sucrose deliveries not preceded by the CS+ (15% of all trials in appetitive section). These

probe trials were included to isolate neuronal activity and blood pressure responses that were due to the conditioned stimuli and unconditioned stimuli, respectively, although these results are not presented here. The appetitive conditioning section of the sessions lasted 2 hours 15 minutes and the aversive conditioning section lasted 1 hour. All trial types were delivered in a random order, except that no probe trials were presented in the first 15 and 5 minutes of the appetitive and aversive sections, respectively. We also interspersed shorter sessions without probe trials (100% reinforcement sessions), the results of which did not differ from those from the longer sessions with probe trials (Supplementary Fig. 11). After each session, rats were given ~90 minutes of access to water in their home cages. The data presented here were collected in 106 sessions from ten rats (median, 10.5 sessions/rat; range, 1-18 sessions/rat).

At the end of the experiment, rats were tested for freezing during the Shock CS+ in the same chamber but with novel contextual cues (visual, olfactory, and tactile). Three minutes after being placed in the chamber, the Shock CS+ was presented once for 8 minutes (Merino and Maren, 2006) and freezing was scored offline via videotape. Because most freezing occurred during the first ten seconds of the Shock CS+ during the test, we only compared the amount of freezing during the ten seconds prior to the start of the Shock CS+ and the ten seconds after the start of the Shock CS+. Rats were considered to be freezing if they were completely immobile (except for breathing) for one full second.

Blood pressure and movement. Mean arterial pressure was used in all blood pressure analyses, except for multiple linear regression, when systolic blood pressure was used to minimize variability due to the heart beat. Long-lasting artifactual drops or increases in the blood pressure signal were removed and treated as missing values in the dataset. If only one mean arterial pressure or one systolic blood pressure value was corrupted, then the value was replaced with the average of the immediately surrounding values. Because the catheter from one rat malfunctioned during the experiment, blood pressure data from these sessions (11 total sessions) were excluded from all analyses. Two animals with only one session of blood pressure data were excluded from Figure 2c because of the small number of "OUT" trials per session. Changes in blood pressure were calculated by subtracting the mean blood pressure during the five seconds preceding the onset of the CS from the interval of interest.

Movement was measured in "movement counts" generated by the blood pressure transmitter. Any movement of the rat which resulted in sufficient translocation of the transmitter produced a movement count. Since it was an arbitrary value, we used it as a relative measure. Sucrose CS+ trials with increases or decreases in movement were determined by comparing the number of movement counts during the CS to the number of movement counts during the five seconds preceding the CS.

Electrophysiological methods. At the beginning of each recording session, the wires were screened for identifiable single-cell activity. If none was found, the wires were advanced 80 μm and the animal was put back into its home cage. If identifiable

single-cell activity was present on any of the wires, a recording session was conducted. After the recording session, electrodes were advanced 80μm to acquire activity from new cells for the following day. Recording ceased for each rat when the estimated position of the array was ventral to the amygdala.

Neuronal activity was recorded with commercial hardware and software, including headstage amplifiers and programmable amplifiers, filters (0.4 and 5 KHz), and multichannel spike-sorting software (Plexon Inc.; Dallas, TX). Discrimination of individual units was performed offline using principal component analysis of waveform shape. Single cells were identified by constancy of waveform shape, cross-correlograms, and interspike intervals (Janak, 2002). Action potential durations were measured from the first trough to the first peak. Cells with peak-then-trough spike shapes (77 of 518 cells) were excluded from the spike width analysis because of difficulty measuring the time at peak.

Analysis of neural data. Neurons were considered to have significant increases or decreases in activity to the conditioned stimuli if their spiking activity during the CS interval was different than their activity during the 10 seconds preceding CS onset (Wilcoxon signed-rank test, $\alpha = .01$). "Same" cells were cells which had statistically significant increases or decreases in activity during both the Sucrose CS+ and Shock CS+. "Selective" cells were cells which had statistically significant increases or decreases in activity during one CS+ but no significant change in activity during the other CS+. "Opposite" cells were cells which had statistically significant increases in

activity during one CS+ and statistically significant decreases in activity during the other CS+. "No Response" cells were cells which did not have statistically significant changes in activity during either CS+. Z scores were calculated using the formula [(activity during time bin) – (mean activity of cell)]/[standard deviation of activity of cell], where the mean activity was computed during the 60 seconds preceding the ten second baseline period and the standard deviation was computed across the appetitive (for the Sucrose CSs) or aversive part of the session (for the Shock CS). Note that for most of the analyses described here, the particular interval used to compute mean activity is irrelevant since neuronal activity changes were computed by subtracting the mean neuronal activity during the five seconds preceding the onset of the CS from the interval of interest. Changes in Z scores rather than absolute Z scores were used to be consistent with the blood pressure analysis and to equate baselines for different trial types. For Figure 3a-b we equalized the number of trials for each of the conditioned stimuli for each session by only including the first x trials in the analysis, where x was the least number of trials among the three conditioned stimuli for a given session. This was done to ensure that any differences between the conditioned stimuli were not due to differences in variance caused by unequal numbers of trials.

For Figure 3b, we computed the mean change in Z score magnitude according to the following formula:

$$Z_{mag}(t) = \text{mean}\ (\text{abs}|(Z(t)_i - Z\_baseline_i|)$$

Where $Z_{mag}(t)$ = the mean change in Z score magnitude for time t; $Z(t)_i$ = Z score for cell i at time t; $Z\_baseline_i$ = the mean Z score for cell i during the five seconds preceding the CS; abs = absolute value.

Latencies of neuronal and blood pressure responses were calculated by first computing the mean and standard deviation of neuronal activity or blood pressure during the ten seconds preceding the CS. The first of three consecutive 100 ms time bins after the start of the CS that had values deviating by more than one standard deviation from the mean was considered to be the latency of the change in neuronal activity or blood pressure. We used relatively large time bins because of the difficulty in calculating a latency for cells with low spontaneous firing rates and decreases in firing rates during the conditioned stimuli and because the objective of the analysis (to compare the latencies of changes in neuronal activity and blood pressure) did not require smaller time bins. Cells and sessions (when measuring the latency of blood pressure increases) which did not meet the criterion of three consecutive bins with values greater or less than one standard deviation from the mean were excluded from further latency analysis (Sucrose CS+: Increases, Decreases, BP = 95.9, 85.8, 90.5 % of cells/sessions were included; Shock CS+: Increases, Decreases, BP = 97.0, 62.3, 100 % of cells/sessions were included).

To test whether a cell's response to one CS+ predicted its response to the other CS+, chi-square tests of association were performed as a comparison between the number of cells which had either similar or opposite responses to the Sucrose CS+ and Shock

CS+ and the number of cells which had a given type of response (increase or decrease in activity) to either the Sucrose CS+ or Shock CS+ in the rest of the population of recorded cells. Although all possible combinations of responses to the Sucrose CS+ and Shock CS+ are shown in Figure 4 and Supplementary Figure 8, note that half of the tests for significance are statistically redundant because the observed and expected numbers of cells used in the chi-square calculations are equivalent. For example, the number of cells that are expected to have increases in activity during the Shock CS+ out of the total number of cells that have increases in activity during the Sucrose CS+ is the same as the number of cells that are expected to have increases in activity during the Sucrose CS+ out of the total number of cells that have increases in activity during the Shock CS+. The Yates correction for continuity was used for all chi-square tests.

For the quantitative comparison of activity during the conditioned stimuli, the "Aligned" scores were computed by taking the difference in Z scores during the two conditioned stimuli being compared and averaging over all the cells. The "Shuffled" scores were computed by first shuffling the Z scores for one of the two conditioned stimuli 100 times and then taking the difference in Z scores for each "cell pair" (one Z score for one cell during one CS subtracted from one Z score for a different cell during the other CS) for each shuffle and averaging over all 100 shuffles for each cell. This resulted in one shuffled score (per time interval) for each cell; these scores were then averaged over all cells to produce the "Shuffled" scores as shown in Figure 5 and Supplementary Figures 9 and 10.

To investigate the relationship between single-cell activity and blood pressure, multiple linear regression was used. The dependent variable was systolic blood pressure and the independent variables were neuronal activity and other continuous and categorical regressors that we thought might be correlated with blood pressure. These other regressors were the Sucrose CS+, Sucrose CS-, Shock CS+, sucrose consumption, footshock, headpokes (when the rat was in the sucrose delivery port), sucrose and shock omission intervals, as well as, the first ten minutes of the session (habituation period), the appetitive section of the session, the aversive section of the session, and the movement of the animal. Blood pressure, neuronal activity, and movement were continuous regressors and the intervals were categorical regressors. Because there is likely a significant time lag between the effect of movement on blood pressure, before each multiple regression, we performed a crosscorrelation analysis on movement and blood pressure to determine the time lag with the maximal correlation (-10 to 10 seconds time lag range) for each session. The movement data was then shifted this amount of time so that the relationship between movement and blood pressure would be maximal. Because there would also likely be a time lag between the hypothesized effect of neuronal activity on blood pressure, we performed an initial crosscorrelation analysis on neuronal activity and blood pressure to determine the time lag with the maximal average correlation across all cells, which turned out to be neuronal activity preceded blood pressure by one second. We then shifted neuronal activity in time by this amount for each multiple linear regression

analysis (this shift was held constant for all cells to facilitate comparisons between different populations of cells).

Histology. Rats were deeply anesthetized with sodium pentobarbital and the final location of the wires was marked by passing current (19 μA; 10-15 s) to produce an iron deposit. Rats were then perfused transcardially with physiological saline, followed by 10% formalin, followed by 10% formalin-3% potassium ferrocyanide solution to react with the iron deposit, producing a blue reaction product. The brains were cut into 50 μM sections and stained with neutral red. To estimate the location of cells recorded on a given day, we followed the electrode tracks from the tips of the electrodes dorsally, using the known travel distance after each session (margin of error in each direction is ~ 300 μM). Using the atlas of Paxinos and Watson (1998) as a reference, 313 cells were determined to be in the BLA (basal, lateral, and basomedial nuclei), 178 cells were determined to be in the CeN, and 27 cells were close to the borders of the nuclei.

Figure 1, Janak

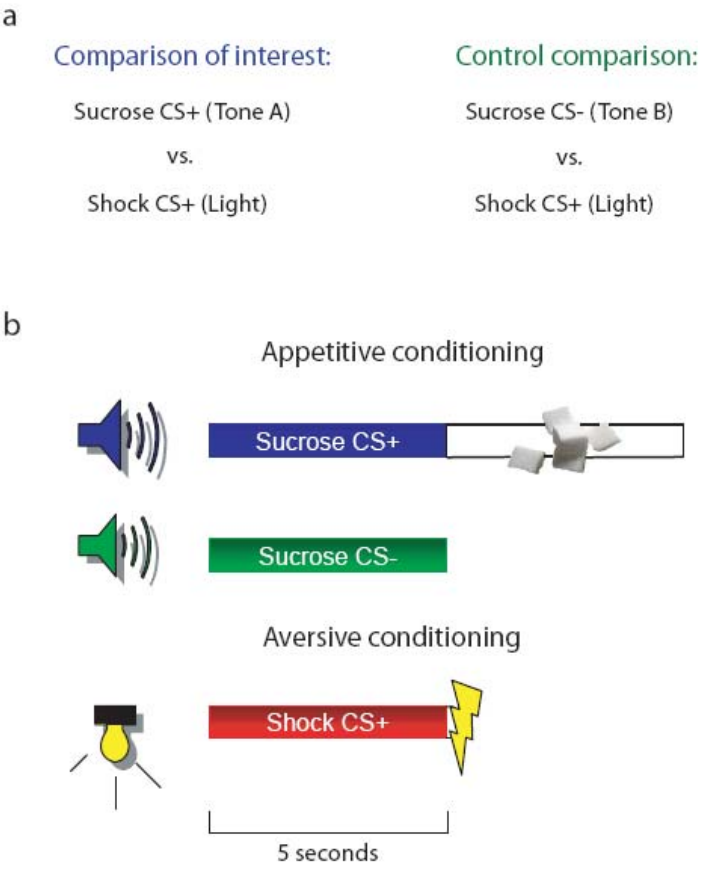

Figure 1. Experimental design and behavioral training. (**a**) Experimental design. (**b**) Behavioral training. All conditioned stimuli lasted five seconds. Sucrose (0.1 mL of a 5% solution, 5 s) was delivered into the reward port immediately after the termination of the Sucrose CS+. Footshock (0.4 mA, 0.5 s) was administered immediately after the termination of the Shock CS+.

Figure 2, Janak

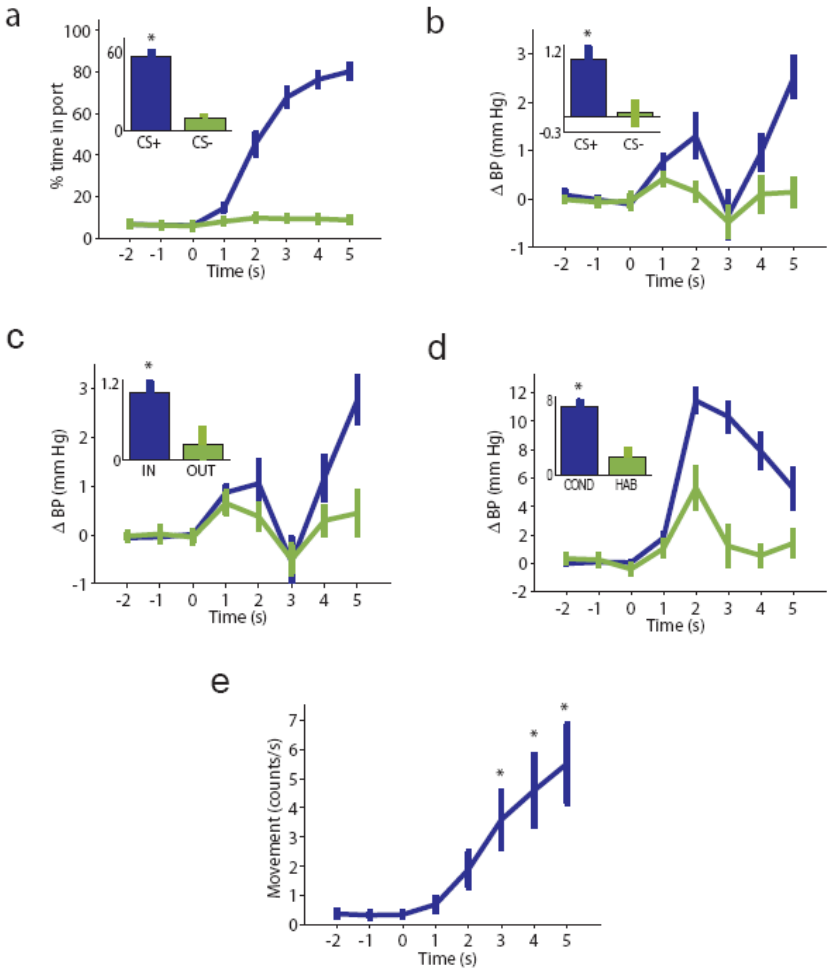

Figure 2. Behavior and blood pressure during Pavlovian conditioning. (**a-d**) Large panels depict mean behavior and blood pressure responses during the three seconds before (bins -2,-1,0) and five seconds after (bins 1-5) conditioned stimulus onset. Insets show the overall mean response during the conditioned stimulus (bins 1-5 together). (**a**) Rats approached and remained in the port longer during the Sucrose CS+ than Sucrose CS- (n = 10 rats). (**b**) Larger increases in blood pressure during the Sucrose CS+ than Sucrose CS- (n = 10 rats). (**c**) Larger increases in blood pressure during Sucrose CS+ trials in which rats were in the port at CS offset ("IN") compared to trials in which rats were out of the port at CS offset ("OUT"; n = 8 rats). (**d**) Larger increases in blood pressure during aversive conditioning than during habituation (n = 10 rats). (**e**) Increase in movement during the end of the Shock CS+ during aversive conditioning. Bins 3-5 are significantly different from bin 0 (n = 10 rats). * $P < .01$.

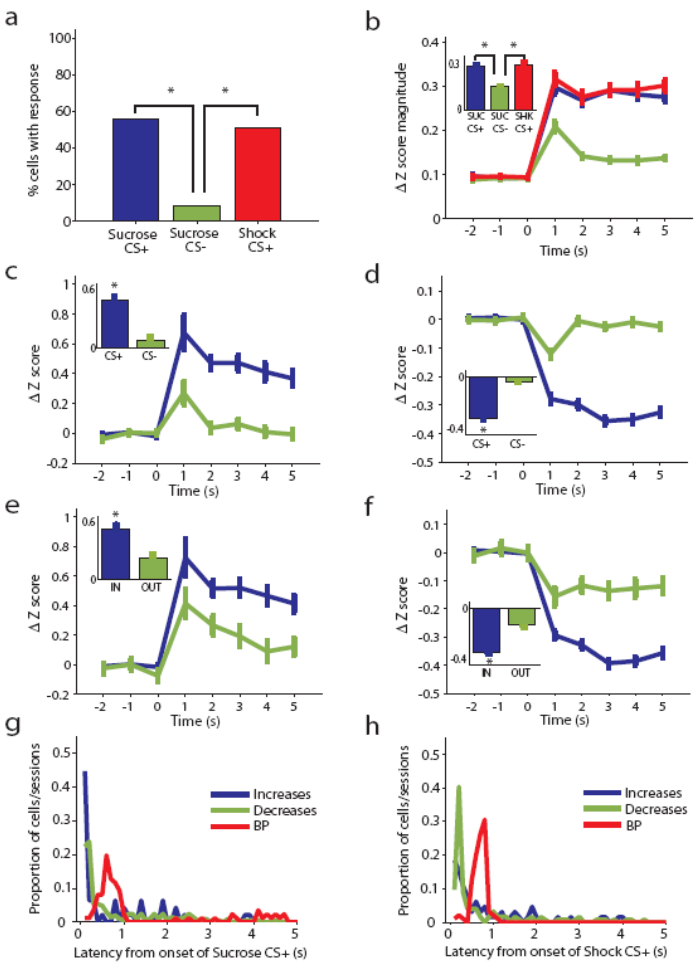

Figure 3. Neuronal activity during the conditioned stimuli. (**a**) Percentage of cells with statistically significant changes in activity during the conditioned stimuli (n = 518 cells). There were more cells with responses during the Sucrose CS+ and Shock CS+ than the Sucrose CS-. (**b-f**) Panels and insets as in Figure 2. (**b**) Mean magnitude change in neuronal activity during the conditioned stimuli. Mean activity of the population changed more during the Sucrose CS+ and Shock CS+ than during the Sucrose CS- (n = 518 cells). (**c**) Larger increases in neuronal activity during the Sucrose CS+ compared to the Sucrose CS- among cells with significant increases in activity during the Sucrose CS+ (n = 49 cells). (**d**) Larger decreases in neuronal activity during the Sucrose CS+ compared to the Sucrose CS- among cells with significant decreases in activity during the Sucrose CS+ (n = 240 cells). (**e**) Larger increases in neuronal activity during Sucrose CS+ "IN" trials compared to "OUT" trials among cells with significant increases in activity during the Sucrose CS+ (n = 49 cells). (**f**) Larger decreases in neuronal activity during Sucrose CS+ "IN" trials compared to "OUT" trials among cells with significant decreases in activity during the Sucrose CS+ (n = 240 cells). (**g**) The latencies of cells with significant increases or decreases in activity during the Sucrose CS+ are shorter than the latencies of increases in blood pressure during the Sucrose CS+. Bin size = 100 ms. (**h**) The latencies of cells with significant increases or decreases in activity during the Shock CS+ are shorter than the latencies of increases in blood pressure during the Shock CS+. Bin size = 100 ms. * $P < .0001$.

Figure 4, Janak

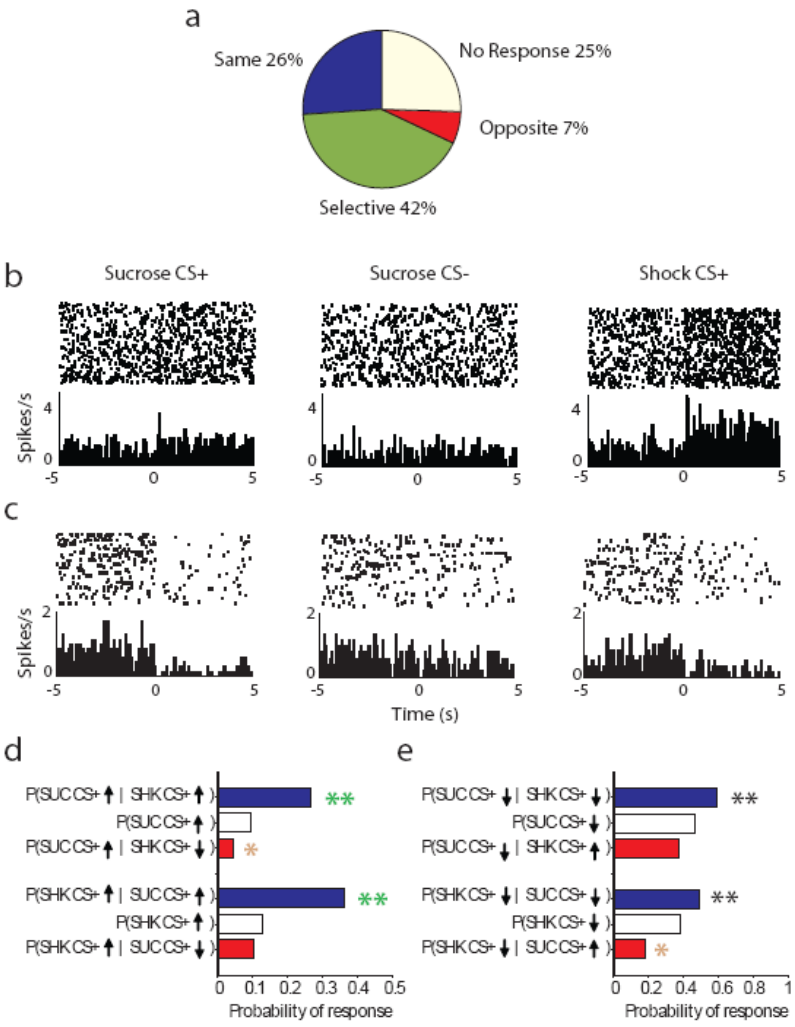

Figure 4. Neuronal activity during the Sucrose CS+ predicts neuronal activity during the Shock CS+ and vice versa. (**a**) Percentage of total population with a given neuronal response type during the Sucrose CS+ and Shock CS+ (n = 518 cells). (**b,c**) Perievent rasters and histograms of a single neuron response to each stimulus. Rasters depict spiking on individual trials; histograms depict mean firing rate across trials. Time on the x-axis is relative to stimulus onset (x = 0). Bin = 100 ms. (**b**) Example of a "Same" cell with increases in activity during the Sucrose CS+ and Shock CS+. This cell was located in the BLA. (**c**) Example of a "Same" cell with decreases in activity during the Sucrose CS+ and Shock CS+. This cell was located in the CeN. (**d**) Cells with increases in activity during one CS+ were more likely than the rest of the population to have increases in activity during the other CS+. Cells with decreases in activity during the Shock CS+ were less likely than the rest of the population to have increases in activity during the Sucrose CS+. (**e**) Cells with decreases in activity during one CS+ were more likely than the rest of the population to have decreases in activity during the other CS+. Cells with increases in activity during the Sucrose CS+ were less likely to have decreases in activity during the Shock CS+. Note that half of the statistical tests depicted in (**d**) and (**e**) are redundant (see Methods) and denoted as such by the same color asterisk. Also note the different scales on the x-axis for (**d**) and (**e**). * $P < .01$, ** $P < .0001$.

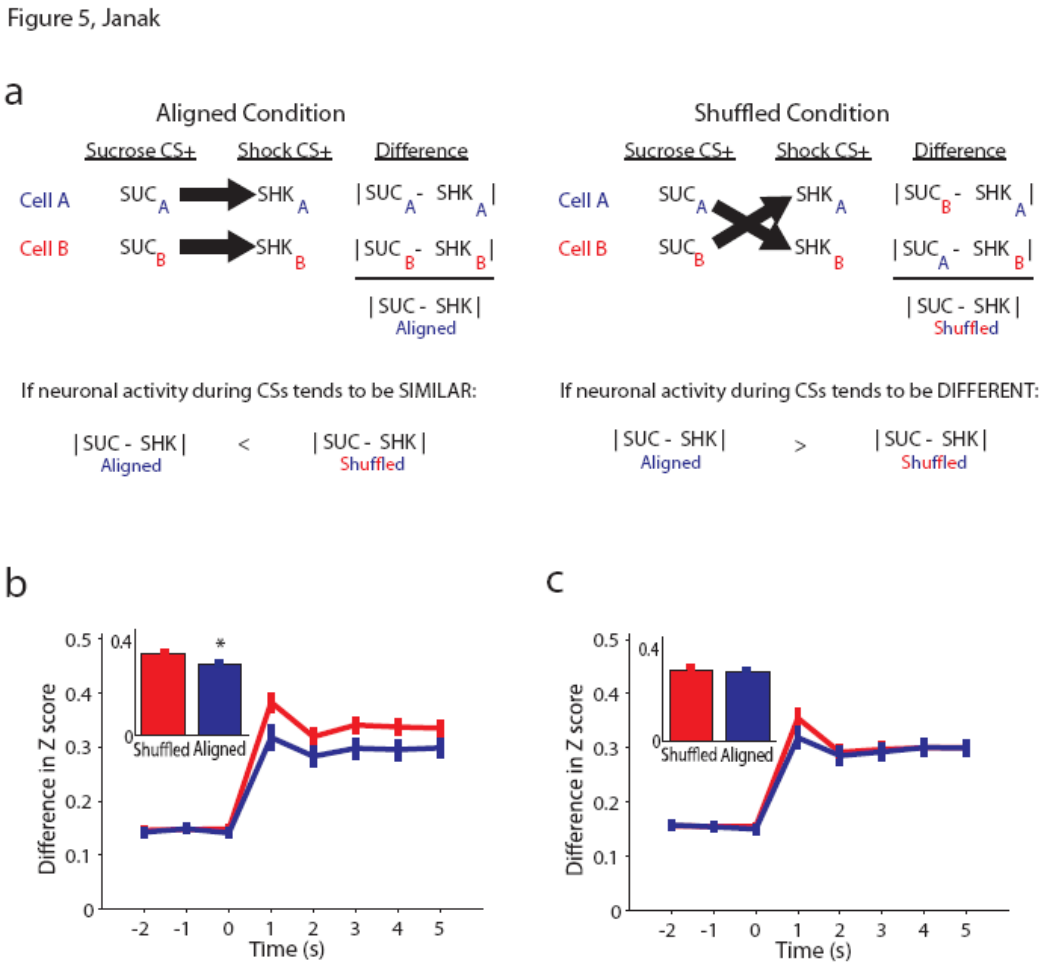

Figure 5. Quantitative comparisons of neuronal activity during the conditioned stimuli. (**a**) Design of aligned versus shuffled comparisons. If neuronal activity is similar during the conditioned stimuli, then the difference in the aligned condition should be less than the difference in the shuffled condition. If neuronal activity is different during the conditioned stimuli, then the difference in the aligned condition should be greater than the difference in the shuffled condition. (**b,c**) Large panels and insets as in Figure 2. (**b**) Comparison of neuronal activity during the Sucrose CS+ and Shock CS+. The difference in the aligned condition is less than the difference in the shuffled condition. (**c**) Comparison of neuronal activity during the Sucrose CS- and Shock CS+. No significant difference between aligned and shuffled conditions. n = 518 cells. * $P < .0001$.

SUPPLEMENTARY INFORMATION

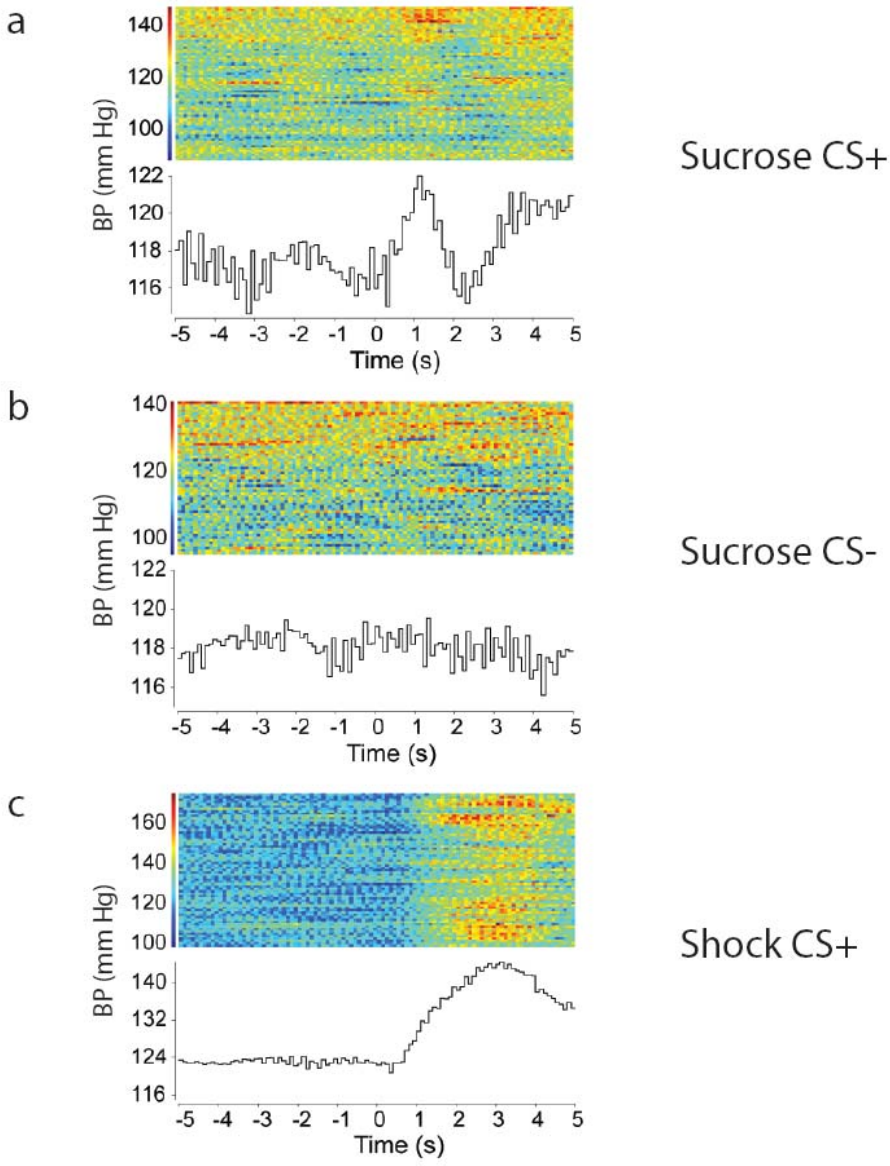

Figure S1. Example of blood pressure responses during one recording session. (a-c) Perievent rasters and histograms of blood pressure during each stimulus. Rasters depict blood pressure on individual trials; histograms depict mean blood pressure across stimulus presentations. Time on the x-axis is relative to stimulus onset (x = 0). Bin = 100 ms. (a) Blood pressure during the Sucrose CS+. (b) Blood pressure during the Sucrose CS-. (c) Blood pressure during the Shock CS+. Note the change in scale on the y-axis.

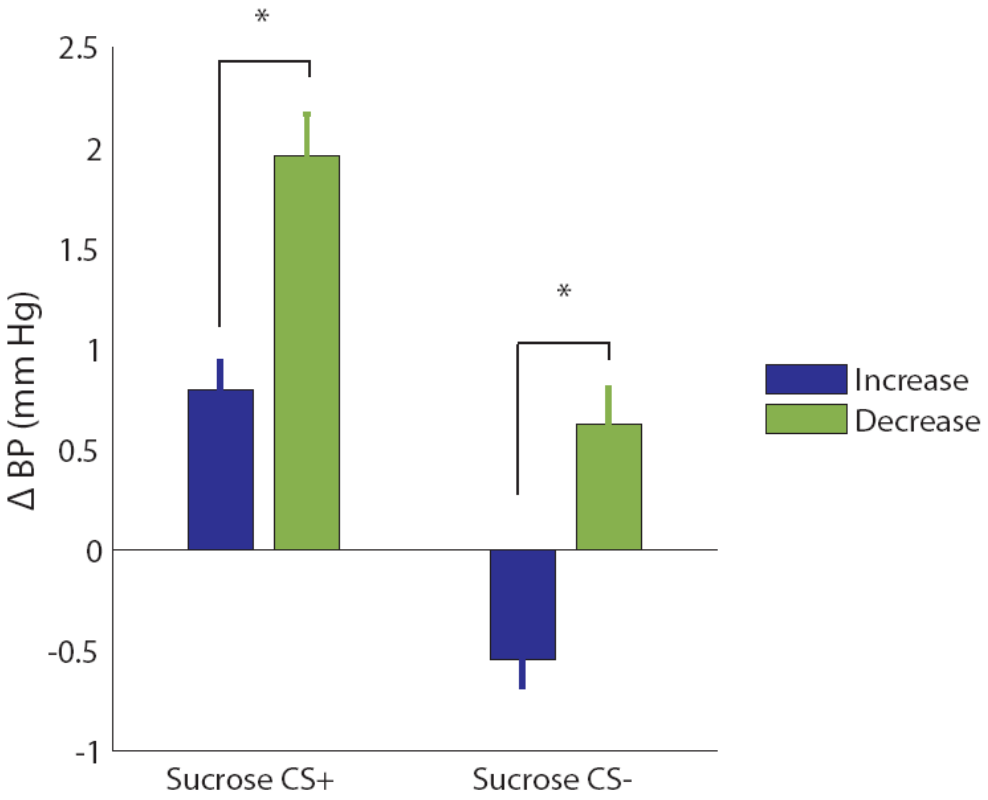

Figure S2. The effect of movement on blood pressure during the Sucrose CS+ and Sucrose CS-. Increases in movement during the conditioned stimuli tended to decrease blood pressure (Sucrose CS+, increase in movement vs. decrease in movement, t-test, n = 95 sessions, P < .0001; Sucrose CS-, increase in movement vs. decrease in movement, t-test, n = 95 sessions, P < .0001), suggesting peripheral vasodilation during the initiation of movement[1]. * P < .0001.

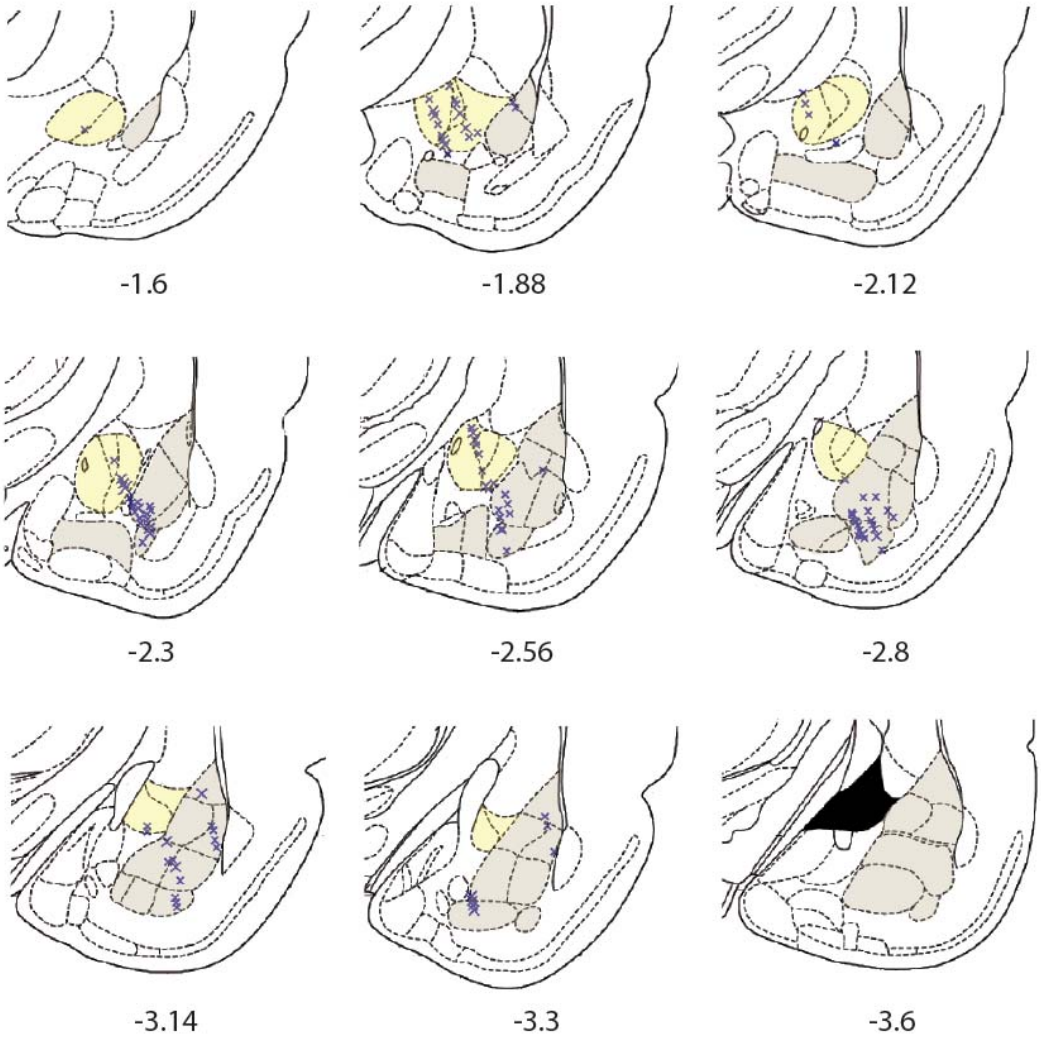

Figure S3. Location of "Same" cells. There was no clear anatomical clustering of "Same" cells relative to "Selective" and "Opposite" cells (see Supplementary Fig. 4). Numbers denote A-P distance from bregma. The size of the marks is proportional to the number of cells at that location as follows: 1 cell (×), 2 cells (×), 3 cells (×). BLA is shaded gray. CeN is shaded yellow. Atlas diagrams reprinted with permission from Paxinos and Watson (1998)[2].

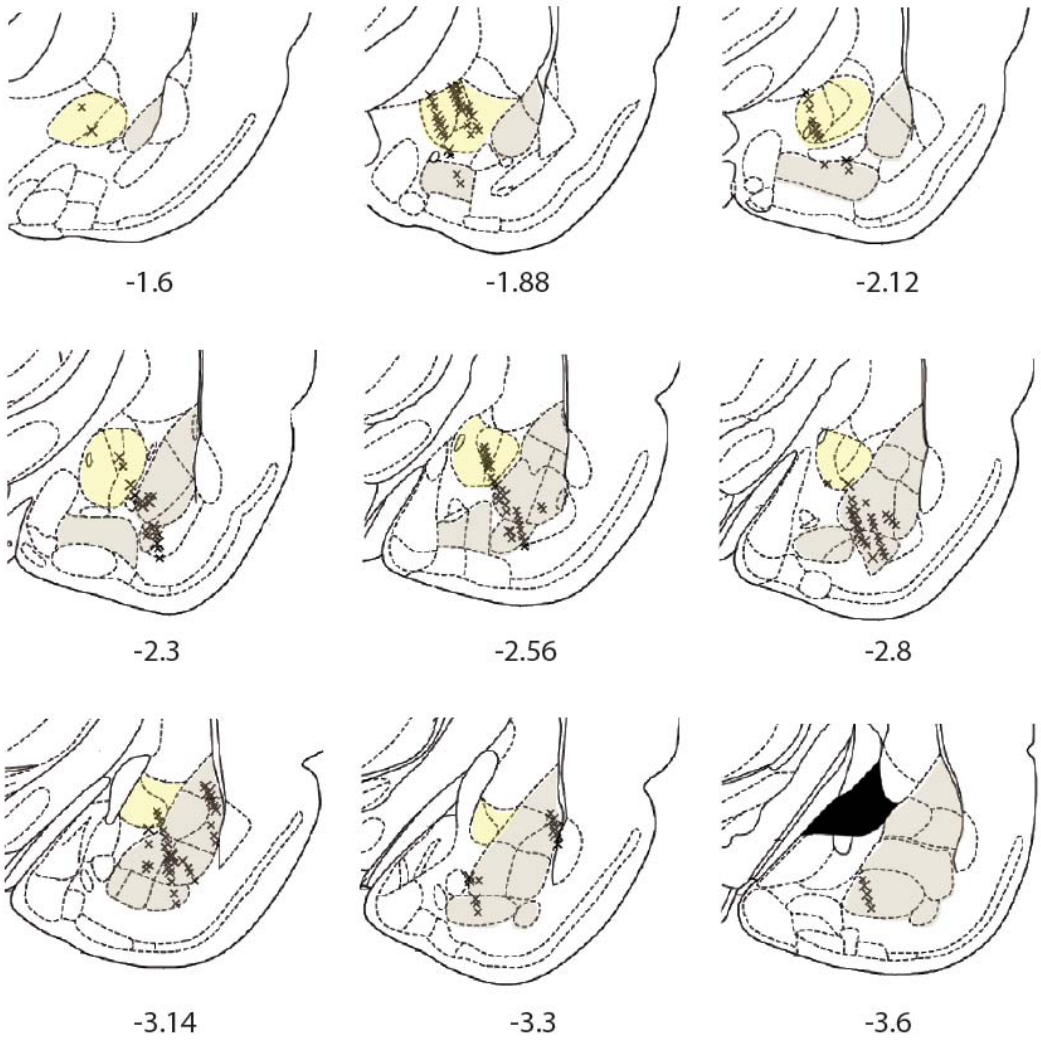

Figure S4. Location of "Selective" and "Opposite" cells. The location of these cells is representative of the entire population of cells. Numbers denote A-P distance from bregma. The size of the marks is proportional to the number of cells at that location as follows: 1 cell (×), 2 cells (×), 3 cells (×). BLA is shaded gray. CeN is shaded yellow. Atlas diagrams reprinted with permission from Paxinos and Watson (1998)[2].

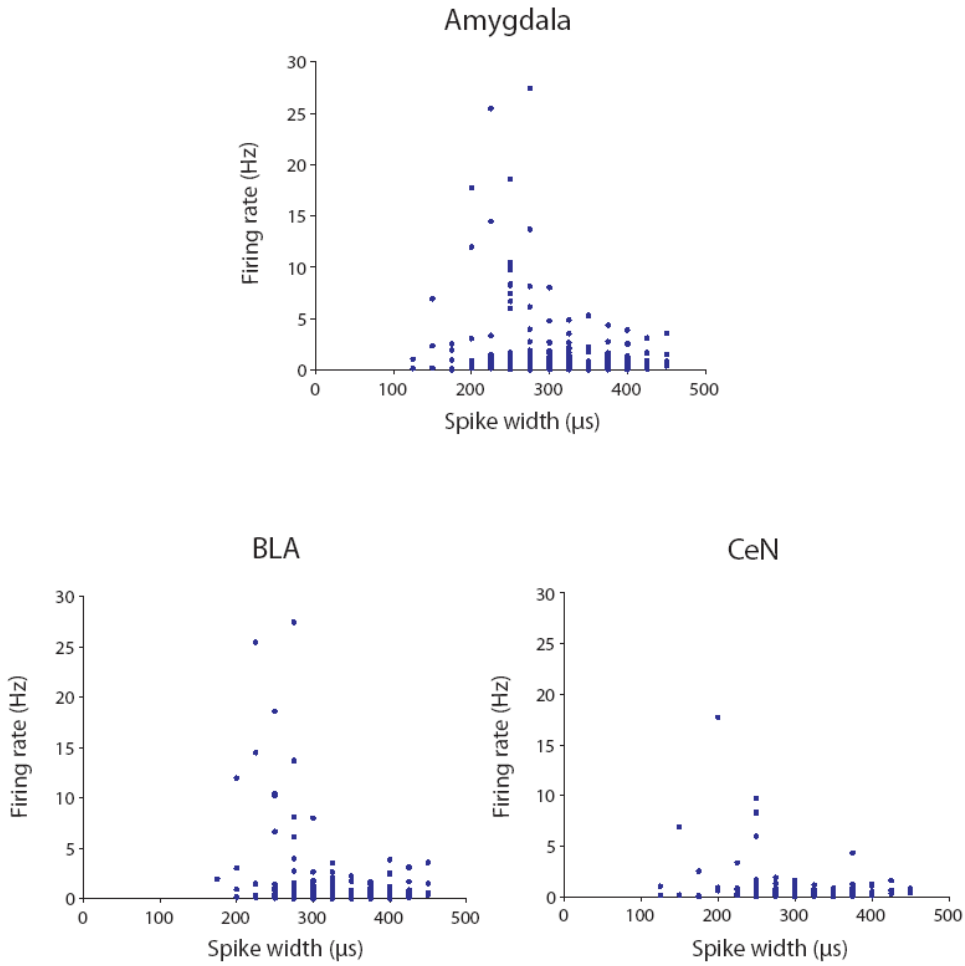

Figure S5. Firing rates and spike widths. (**a**) Twenty of 441 (4.5%) amygdala cells had baseline firing rates greater than 5 Hz (see Methods; median baseline firing rate = .44 Hz, mean baseline firing rate = 1.15 Hz; median spike width = 325 µs, mean spike width = 328 µs). (**b**) Twelve of 273 (4.4%) BLA cells had baseline firing rates greater than 5 Hz (median baseline firing rate = .48 Hz, mean baseline firing rate = 1.25 Hz; median spike width = 350 µs, mean spike width = 338 µs). (**c**) Six of 147 (4.1%) CeN cells had baseline firing rates greater than 5 Hz (median baseline firing rate = .37 Hz, mean baseline firing rate = .88 Hz; median spike width = 325 µs, mean spike width = 316 µs).

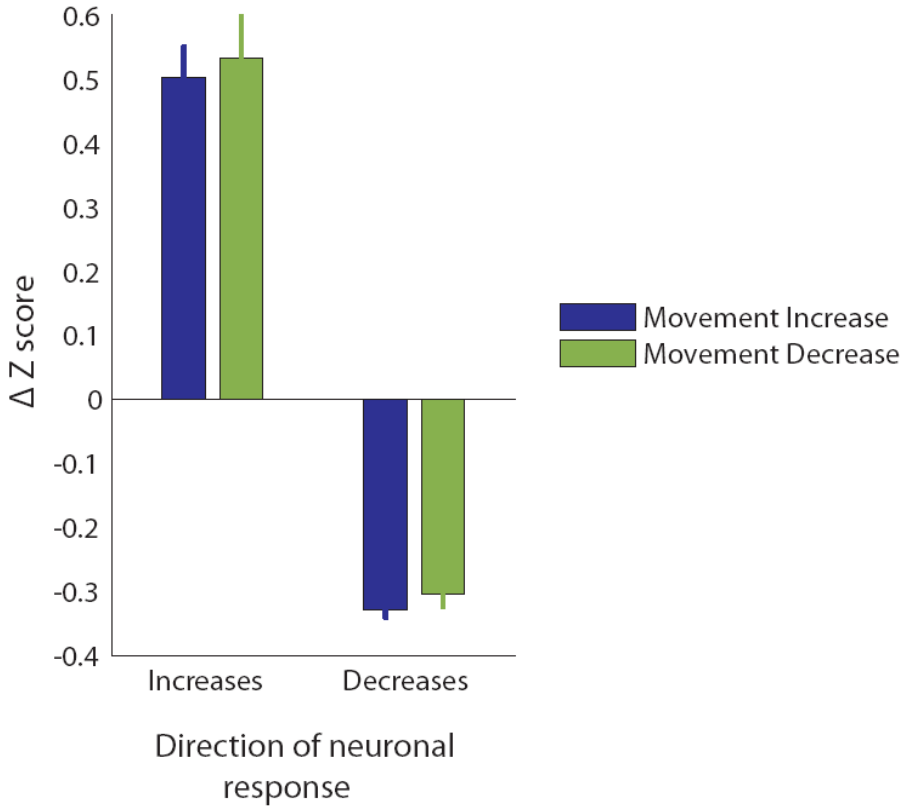

Figure S6. Movement does not affect neuronal responses to the Sucrose CS+. The average increase (left side of graph) and decrease (right side of graph) in neuronal activity during the Sucrose CS+ among cells with significant increases and decreases in activity, respectively, did not differ on trials with increases versus decreases in movement (Increases in neuronal activity, t-test, n = 49 cells, P = .61; Decreases in neuronal activity, t-test, n = 240 cells, P = .12).

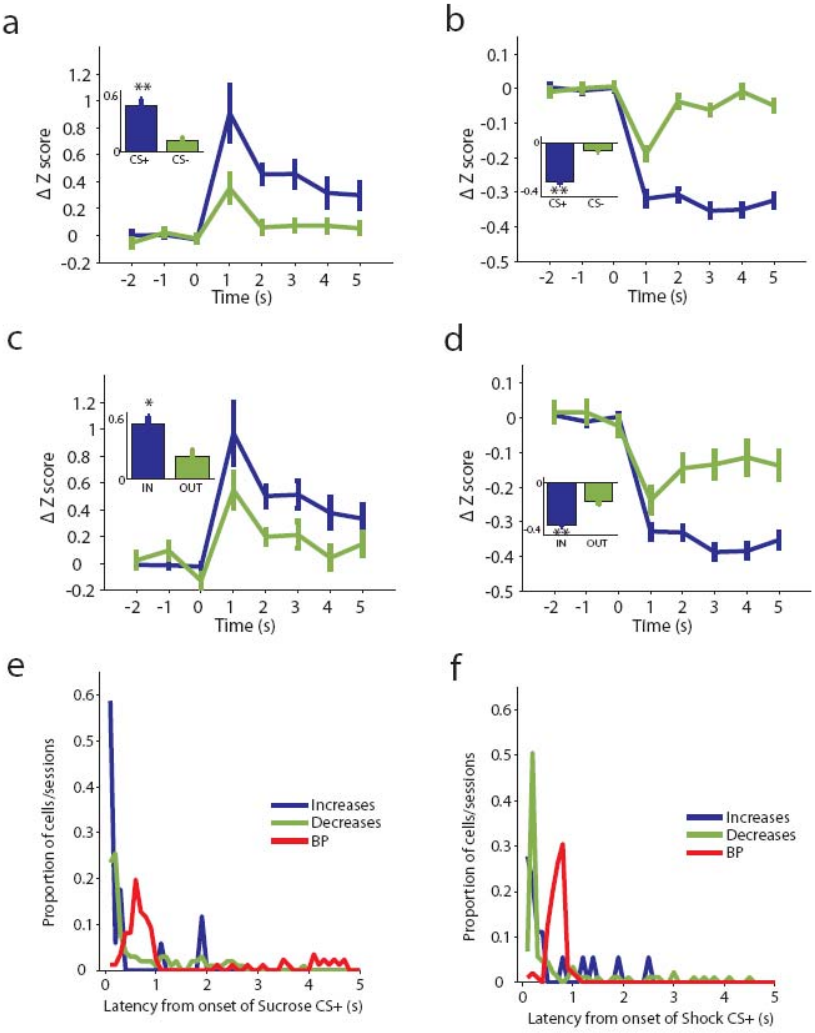

Figure S7. Neuronal activity of "Same" cells. (**a**-**d**) The large panels depict mean changes in neuronal activity during the three seconds before (bins -2,-1,0) and the five seconds after (bins 1-5) conditioned stimulus onset. Insets show mean activity across the cells during the conditioned stimuli (bins 1-5 together). (**a**) Larger increases in neuronal activity during the Sucrose CS+ compared to the Sucrose CS- (t-test, n = 18 cells, P < .0001). (**b**) Larger decreases in neuronal activity during the Sucrose CS+ compared to the Sucrose CS- (t-test, n = 117 cells, P < .0001). (**c**) Larger increases in neuronal activity during Sucrose CS+ "IN" trials compared to "OUT" trials (t-test, n = 18 cells, P < .01). (**d**) Larger decreases in neuronal activity during Sucrose CS+ "IN" trials compared to "OUT" trials (t-test, n = 117 cells, P < .0001). (**e**) The latencies of cells with significant increases or decreases in activity during the Sucrose CS+ are shorter than the latencies of increases in blood pressure during the Sucrose CS+. (**f**) The latencies of cells with significant increases or decreases in activity during the Shock CS+ are shorter than the latencies of increases in blood pressure during the Shock CS+. * P < .01, ** P < .0001.

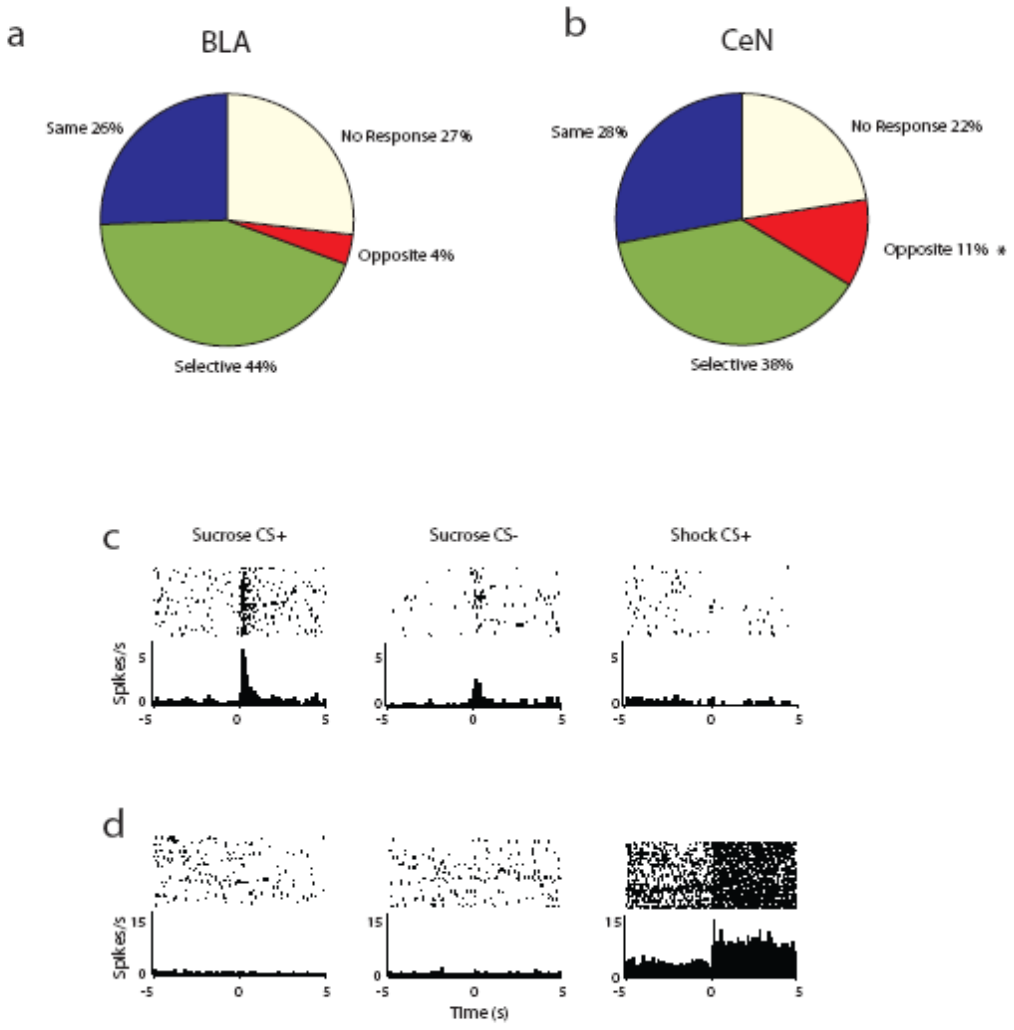

Figure S8. Neuronal responses to Sucrose CS+ and Shock CS+ in BLA and CeN. (a) BLA cells. (b) CeN cells. The CeN had a higher proportion of "Opposite" cells than the BLA. All other comparisons between the BLA and CeN were not significant ($p > .2$). (c) Opposite cell from the CeN with an increase in activity during the Sucrose CS+ and Sucrose CS- and a decrease in activity during the Shock CS+. (d) Opposite cell from the CeN with a decrease in activity during the Sucrose CS+ and an increase in activity during the Shock CS+. * $P < .01$.

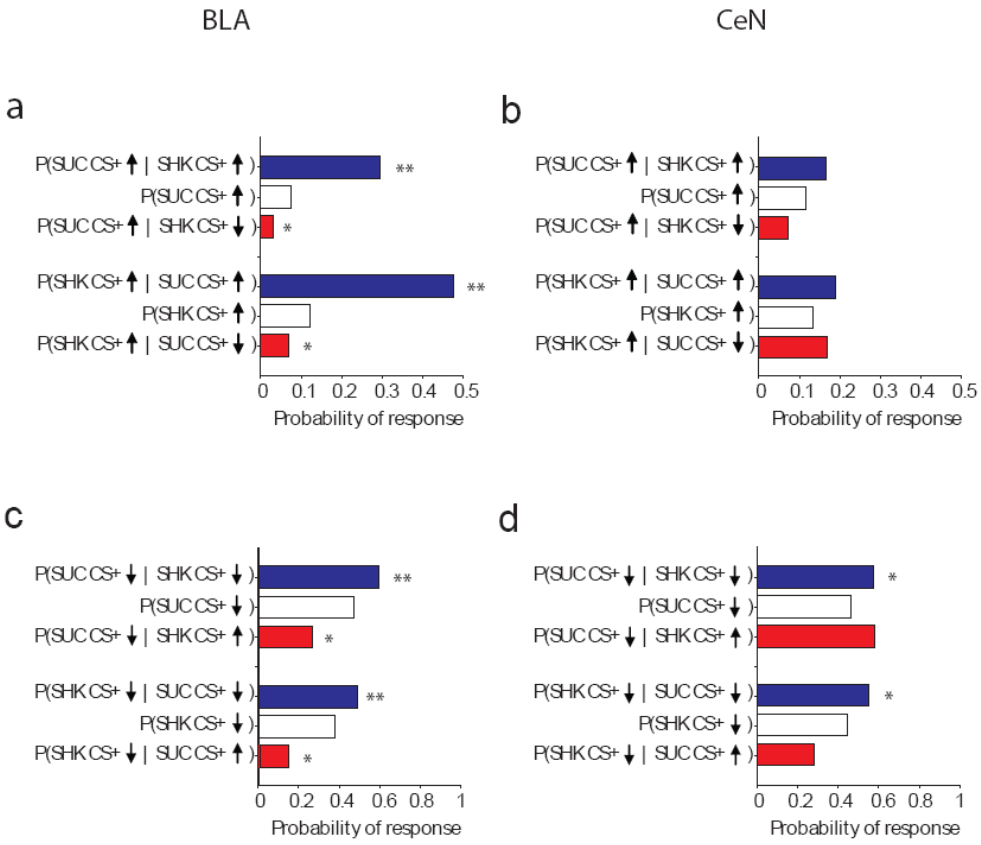

Figure S9. Relationships between responses to the Sucrose CS+ and Shock CS+ in the BLA and CeN. (**a,c**) BLA cells. (**b,d**) CeN cells. (**a**) Cells with increases in activity during one CS+ were more likely than the rest of the population to have increases in activity during the other CS+ (P(Sucrose CS+ Increase | Shock CS+ Increase), $\chi 2 = 26.0$, $P < .0001$). Cells with decreases in activity during one CS+ were less likely to have increases in activity during the other CS+ (P(Sucrose CS+ Increase | Shock CS+ Decrease), $\chi 2 = 5.9$, $P < .05$). (**c**) Cells with decreases in activity during one CS+ were more likely than the rest of the population to have decreases in activity during the other CS+ (P(Sucrose CS+ Decrease | Shock CS+ Decrease), $\chi 2 = 12.4$, $P < .0001$). Cells with increases in activity were less likely to have decreases in activity during the other CS+ (P(Sucrose CS+ Decrease | Shock CS+ Increase), $\chi 2 = 6.2$, $P < .05$). (**d**) Cells with decreases in activity during one CS+ were more likely than the rest of the population to have decreases in activity during the other CS+ (P(Sucrose CS+ Decrease | Shock CS+ Decrease), $\chi 2 = 6.1$, $P < .05$). Note that half of the statistical tests depicted are redundant (see Fig. 4 and Methods). Also note the change in scale on the x-axis for (**c**) and (**d**). * $P < .05$, ** $P < .01$.

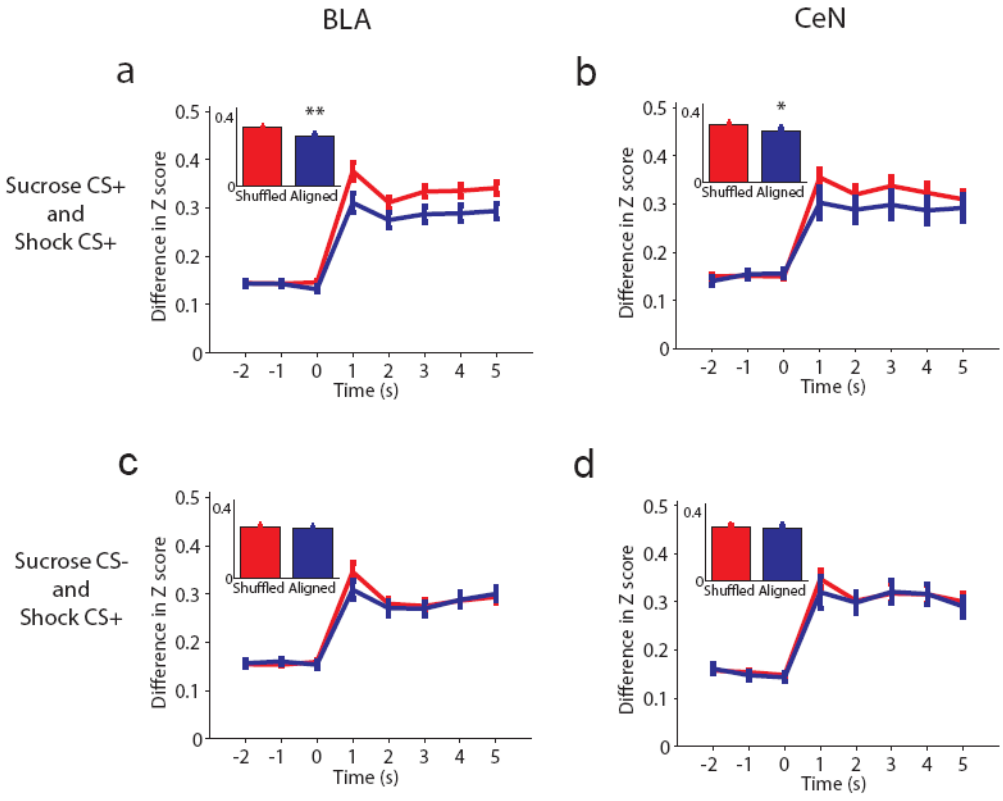

Figure S10. Quantitative comparisons of BLA and CeN neuronal activity during the conditioned stimuli. (**a-d**) Large panels show the mean aligned and shuffled scores during the three seconds before (bins -2,-1,0) and the five seconds after (bins 1-5) conditioned stimulus onset. Insets show the mean across all cells during the conditioned stimuli (bins 1-5 together). (**a,b**) Comparison of neuronal activity during the Sucrose CS+ and Shock CS+. (**c,d**) Comparison of neuronal activity during the Sucrose CS- and Shock CS+. (**a,c**) BLA cells. (**b,d**) CeN cells. (**a**) The difference in the aligned condition was less than the difference in the shuffled condition (ANOVA, main effect of type of comparison: $F_{1,626} = 25.1$, $P < .0001$; main effect of time: $F_{4,1565} = 2.8$, $P < .05$; type of comparison x time: $F_{4,3130} = .2$, $P = .92$; inset, t-test, n = 313 cells, $P<.0001$). (**b**) The difference in the aligned condition was less than the difference in the shuffled condition (ANOVA, main effect of type of comparison: $F_{1,356} = 5.4$, $P < .05$; main effect of time: $F_{4,890} = .5$, $P = .75$; type of comparison x time: $F_{4,1780} = .1$, $P = .97$; inset, t-test, n = 178 cells, $P < .05$). (**c**) There was no significant difference between the aligned and shuffled conditions (ANOVA, main effect of type of comparison: $F_{1,626} = .89$, $P = .3$; main effect of time: $F_{4,1565} = 4.71$, $P < .001$; type of comparison x time: $F_{4,3130} = .7$, $P = .61$; n = 313 cells). (**d**) There was no significant difference between the aligned and shuffled conditions (ANOVA, main effect of type of comparison: $F_{1,356} = .3$, $P = .59$; main effect of time: $F_{4,890} = 1.0$, $P = .40$; type of comparison x time: $F_{4,1780} = .2$, $P = .96$; n = 178 cells). * $P < .05$, ** $P < .0001$.

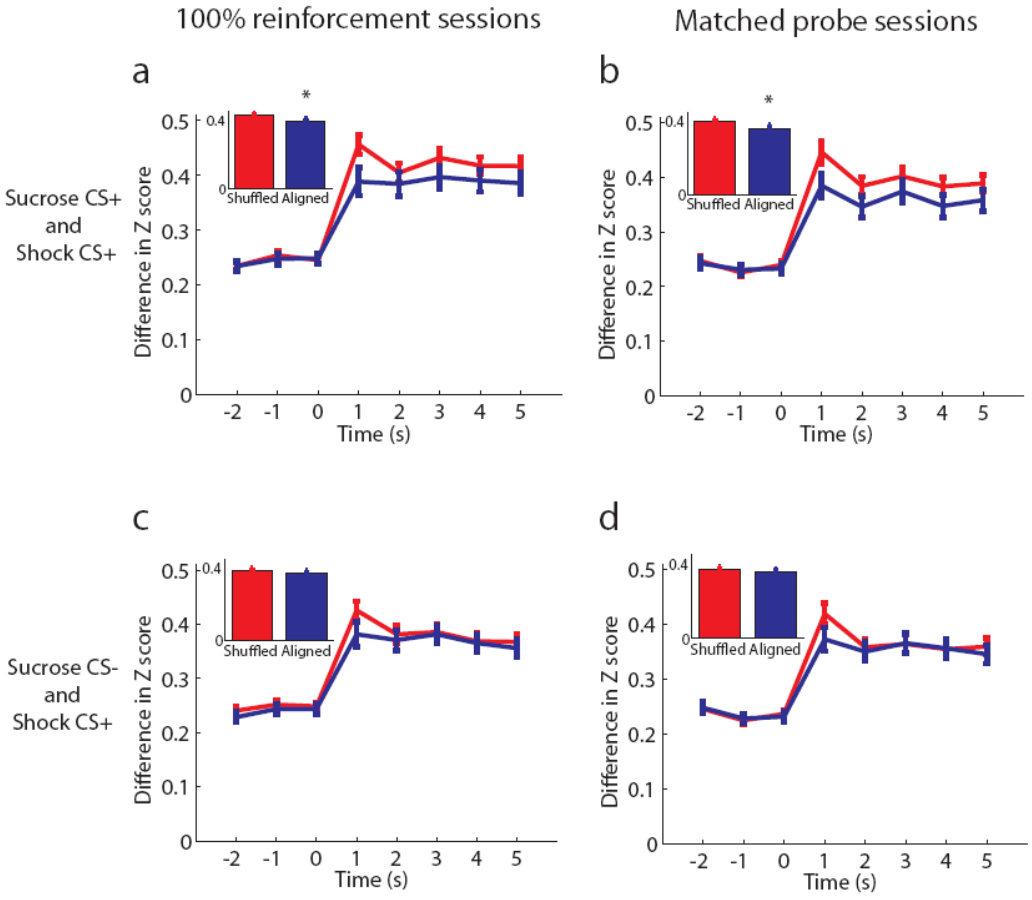

Figure S11. Comparisons of neuronal activity during conditioned stimuli in 100% reinforcement and matched probe conditioning sessions. (**a-d**) Panels and insets as in Figure S9. (**a,b**) Comparison of neuronal activity during the Sucrose CS+ and Shock CS+. (**c,d**) Comparison of neuronal activity during the Sucrose CS- and Shock CS+. (**a,c**) Neuronal activity during 100% reinforcement sessions. (**b,d**) Neuronal activity during matched probe sessions. (**a**) The difference in the aligned condition was less than the difference in the shuffled condition (ANOVA, main effect of type of comparison: $F_{1,776} = 8.71$, $P < .01$; main effect of time: $F_{4,1940} = .64$, $P = .63$; type of comparison x time: $F_{4,3880} = .44$, $P = .78$; inset, t-test, n = 388 cells, $P<.001$). (**b**) The difference in the aligned condition was less than the difference in the shuffled condition (ANOVA, main effect of type of comparison: $F_{1,800} = 10.18$, $P < .01$; main effect of time: $F_{4,2000} = 2.42$, $P < .05$; type of comparison x time: $F_{4,4000} = .24$, $P = .92$; inset, t-test, n = 400 cells, $P < .001$). (**c**) There was no significant difference between the aligned and shuffled conditions (ANOVA, main effect of type of comparison: $F_{1,776} = 1.78$, $P = .18$; main effect of time: $F_{4,1940} = 1.75$, $P = .14$; type of comparison x time: $F_{4,3880} = .46$, $P = .76$; n = 388 cells). (**d**) There was no significant difference between the aligned and shuffled conditions (ANOVA, main effect of type of comparison: $F_{1,800} = 1.36$, $P = .24$; main effect of time: $F_{4,2000} = 2.15$, $P = .07$; type of comparison x time: $F_{4,4000} = .64$, $P = .63$; n = 400 cells). * $P < .001$.

Supplementary Data

Differences in the magnitude of the blood pressure and neuronal activity responses to the Sucrose CS+ and Sucrose CS- were not due to incomplete counterbalancing of the two tones, because although there were large differences between the Sucrose CS+ and Sucrose CS-, there was no significant difference between the change in blood pressure when comparing the frequency of the tones (change in blood pressure, mean ± s.e.m.: 3 kHz tone, 0.26 ± 0 .10 mm Hg; 11 kHz tone, 0.56 ± 0.11 mm Hg; t-test, n=95 sessions, P=.08; note that the trend for a greater increase in blood pressure during the 11 kHz tone works against the effect of CS type, since more animals had the 3 kHz tone as the CS+), nor for the mean magnitude change in neuronal activity (change in Z score, mean ± s.e.m.: 3 kHz tone, .218 ± .008; 11 kHz tone, .215 ± .008; t-test, n = 518 cells, P = .78) .

Cells with different responses to the Sucrose CS+ and Shock CS+ ("Selective" and "Opposite" cells) also tended to have larger responses to the Sucrose CS+ than Sucrose CS- (cells with significant increases in activity during Sucrose CS+, t-test, n = 31 cells, P < .0001; cells with significant decreases in activity during Sucrose CS+, t-test, n = 123 cells, P < .0001), larger responses during "IN" than "OUT" Sucrose CS+ trials (cells with significant increases in activity during Sucrose CS+, t-test, n = 31 cells, P < .0001; cells with significant decreases in activity during Sucrose CS+, t-test, n = 123 cells, P < .0001), and faster responses to the Sucrose CS+ and Shock CS+ than blood pressure (data not shown).

Some single-cell recording studies have found a correlation between the baseline firing rate of a cell in the amygdala and the direction of its change in activity during sensory stimulation3.4. To test whether such a correlation could explain the similarity in activity we observed during the Sucrose CS+ and Shock CS+, we computed the correlation coefficients between the baseline firing rates of the cells (the mean firing rates during the 10 seconds preceding the Sucrose CS+ or Shock CS+; we found similar results no matter which 10 second period was used to compute baseline firing rate) and their change in activity (computed as a Z score) during the Sucrose CS+ and Shock CS+. We did indeed find correlations between the baseline firing rates and changes in neuronal activity during the Sucrose CS+ and Shock CS+, however the correlations were in opposite directions for the baseline – Sucrose CS+ comparison ($r = -.2288$, $P < .0001$) and the baseline – Shock CS+ comparison ($r = .10071$, $P < .05$). Therefore, the similarity in neuronal activity during the Sucrose CS+ and Shock CS+ occurs in spite of, rather than because of, correlations between the baseline firing rates and changes in neuronal activity during the Sucrose CS+ and Shock CS+.

1. Matsukawa, K., Komine, H., Nakamoto, T. & Murata, J. Central command blunts sensitivity of arterial baroreceptor-heart rate reflex at onset of voluntary static exercise. *Am J Physiol Heart Circ Physiol* **290**, H200-8 (2006).

2. Paxinos, G. & Watson, C. *The rat brain in stereotaxic coordinates, fourth edition*. Figures 25, 27-34 (1998).

3. Ben-Ari, Y. & Le Gal la Salle, G. Relationship between spontaneous and evoked unit activity in the amygdala of the cat. *Brain Res* **32**, 474-8 (1971).

4. Ben-Ari, Y., Le Gal le Salle, G. & Champagnat, J.C. Lateral amygdala unit activity: I. Relation ship between spontaneous and evoked activity. *Electroencephalogr Clin Neurophysiol* **37**, 449-61 (1974).

CHAPTER 3

GENERAL DISCUSSION

Predictions of Overlapping Model of Appetitive and Aversive Arousal

In this dissertation, we tested and confirmed two predictions of the hypothesis that overlapping populations of cells in the amygdala contribute to appetitive and aversive arousal: neuronal activity tends to be similar during appetitive and aversive arousal, and the activity of cells with similar changes in activity during appetitive and aversive arousal is correlated with measures of arousal. This hypothesis makes several other intriguing predictions, however. One untested prediction is that nuclei-specific lesions or inactivations will have similar effects on measures of appetitive and aversive arousal. The effect of such manipulations on appetitive and aversive arousal should be correlated — animals that are greatly affected by such manipulations during tests of appetitive arousal should also be affected during tests of aversive arousal. Lesion studies suggest that both the BLA and CeN are involved in conditioned increases in blood pressure during aversive conditioning (Iwata et al., 1986; LeDoux et al., 1990), but whether these same lesions affect conditioned increases in blood pressure during appetitive conditioning is unknown. The hypothesis also predicts that measures of appetitive and aversive arousal should be sensitive to the same pharmacological manipulations of the amygdala. It has been shown that infusions of opioid agonists and noradrenergic antagonists into the amygdala reduce conditioned changes in heart rate in rabbits during fear conditioning (Gallagher et al., 1980; Gallagher et al., 1981; Gallagher et al., 1982), but it is unknown how they affect other measures of arousal in other species.

Another prediction is that the same pharmacological manipulations of the amygdala that enhance aversive memories should also enhance appetitive memories. Interestingly,

systemically administered noradrenergic antagonists block the enhancement of memory for both positive and negative stimuli (Hurlemann et al., 2005). They also block valence-specific retrograde amnesia (negative valence effect) and hypermnesia (positive valence effect) (Hurlemann et al., 2005). These mnemonic effects are thought to be partly mediated by the amygdala (Kensinger and Corkin, 2004), but whether norepinephrine is acting in the amygdala to produce all of these effects is unknown. In particular, it is unclear whether norepinephrine in the amygdala is required for the enhancement of positively-valenced memories as it is for negatively-valenced memories (Liang et al., 1986). It would also be interesting to see if opioid antagonists in the amygdala enhance consolidation for positively-valenced memories as they do for negatively-valenced memories (Liang et al., 1983; Introini-Collison et al., 1989).

One caveat of the above discussion is that pharmacological studies of the amygdala suggest that excitation of cells in the BLA is critical for its effects on autonomic arousal (Sajdyk and Shekhar, 1997) and memory consolidation (McGaugh, 2004); whereas, most of the neuronal responses in our study in both the CeN and BLA during the conditioned appetitive and aversive stimuli were inhibitions. Notably, other recording studies in monkeys, cats, and rats report mostly excitations during presentation of affective stimuli in the BLA (Sanghera et al., 1979; Muramoto et al., 1993; Quirk et al., 1995; Pare and Collins, 2000; Repa et al., 2001; Goosens et al., 2003) but see (Ben-Ari et al., 1973; Ben-Ari et al., 1974). One possible explanation for the discrepancy is that our recording sessions were long and included many trials, thus facilitating measurement of inhibitions in slow-firing neurons. It's also possible that there are differences depending on the

precise location of the electrodes within the BLA. Most of our cells were located in the ventral part of the basal nucleus, whereas many studies recorded only cells in the lateral nucleus, often looking for cells with excitatory responses to stimuli before recording (Iwata et al., 1986; Quirk et al., 1995; Pare and Collins, 2000; Goosens et al., 2003). How neuronal responses to appetitive and aversive conditioned stimuli change with respect to the location within the BLA remains to be determined. One intriguing possibility is that region-specific inhibitions in the BLA permit the flow of neuronal activity through the BLA and CeN. This possibility is consistent with recent, nuanced views of information processing in the amygdala (Royer et al., 1999).

Possible functions of "Same" cells in the BLA and CeN

"Same" cells are obvious candidates for encoding aspects of arousal, since the conditioned appetitive and aversive stimuli both elicited increases in blood pressure and changes in behavior in our study. Given the different efferent projections of the BLA and CeN (Pitkanen, 2000), however, "Same" cells in the BLA and CeN may have different functions. For example, "Same" cells in the CeN may coordinate autonomic and attentional responses to the conditioned stimuli, while "Same" cells in the BLA may permit changes in blood pressure and heart rate via its connections with the CeN and/or hypothalamus. Unfortunately, it is difficult to explain why some responses, such as conditioned changes in blood pressure and heart rate, appear to require the function of both the BLA and CeN (Iwata et al., 1986; Zhang et al., 1986a; LeDoux et al., 1990; Sanders and Shekhar, 1991; Kapp et al., 1992), while others, such as conditioned

orienting (Gallagher et al., 1990) and memory consolidation (McGaugh, 2004), appear to require the function of only the CeN or BLA, respectively. Experiments that explore the functional anatomy of neural circuits within the amygdala (e.g., Petrovich et al., 2005) or that examine how neuronal activity in the BLA affects activity in the CeN during conditioning would address this issue.

Possible functions of "Opposite" cells in the CeN

Given that appetitive and aversive systems are thought to reciprocally inhibit each other (Konorski, 1967; Dickinson and Dearing, 1979), cells with opposite changes in activity during appetitive and aversive stimuli are good candidates for the representation of emotional valence. Interestingly, although we found similar proportions of "Same" cells in the BLA and CeN, there were about three times as many "Opposite" cells in the CeN than the BLA, suggesting that the CeN is more involved than the BLA in encoding emotional valence. This result contrasts with a previous study which found a greater proportion of "Opposite" cells in the *BLA* than CeN (Muramoto et al., 1993). The reason for this discrepancy is unclear, although it may be due to the use of different types reinforcers — intracranial stimulation in their study versus sucrose in ours. Our results are consistent with a different study, however, which found similar changes in activity among cells in the CeN, but not BLA, during the presentation of multiple types of aversive fluids (Yasoshima et al., 1995). Interestingly, lesions of the CeN do not affect EEG correlates of positive/negative emotionality in monkeys (Kalin et al., 2001), suggesting that the CeN does not play a prominent role in positive/negative temperament.

It is possible, however, that the CeN is important for reinforcement in certain situations, perhaps through its connections with the substantia nigra (Balleine and Killcross, 2006).

Although "Opposite" cells are good candidates for the encoding of emotional valence, they also might reflect a greater role for the CeN than BLA in encoding specific affective "reflexes" (Everitt et al., 2003) that are oppositely regulated in certain appetitive and aversive situations. For example, it is likely that preparation to eat and fleeing a predator, although both are arousing situations, differentially activate autonomic systems involved in digestion. Intriguingly, we found that of those "Opposite" cells that had activity that was correlated with blood pressure, most had correlations that were congruent with the aversive CS and incongruent with the appetitive CS, suggesting that these cells might contribute to the larger increase in blood pressure during the aversive CS. Determining where "Opposite" cells project would help constrain hypotheses about their function.

Possible functions of "Selective" cells in the BLA

Although we found few "Opposite" cells in the BLA, there were many cells which discriminated between the appetitive and aversive conditioned stimuli ("Selective" cells), consistent with previous reports (Schoenbaum et al., 1998, 1999; Paton et al., 2006). These cells could also contribute to the encoding of emotional valence (Paton et al., 2006). Indeed, it is likely that there is at least some information about emotional valence in the BLA, since it is interconnected with regions of the prefrontal cortex that are thought to process emotional valence (Davidson et al., 1990; Anderson et al., 2003; Small

et al., 2003). Most "Selective" cells probably reflect the different sensory properties of the conditioned stimuli and outcomes these stimuli predict, however. This would be consistent with evidence that the BLA is particularly important for the representation of the sensory properties of biologically important stimuli (Balleine and Killcross, 2006). Yet another possibility is that "Selective" cells encode specific affective programs (e.g., discrete emotions or appraisal components) induced by the conditioned appetitive and aversive stimuli.

Future Directions in Affective Neuroscience

Understanding the organization of emotion systems in the brain is a major challenge due to the brain's complexity. A better understanding of the logic of brain development and plasticity is crucial to making sense of this complexity. Fortunately, the neuroscientist's toolbox is growing rapidly (Marek and Davis, 2003; Miyawaki, 2003; Brecht et al., 2004; Miesenbock, 2004; Callaway, 2005; Deisseroth et al., 2006; Luo et al., 2008). Recently developed optogenetic tools will greatly facilitate the dissection and elucidation of neural circuits responsible for various brain functions (Deisseroth et al., 2006), including emotions, providing the foundation for a biologically-informed affective lexicon. Single-cell recording studies, such as the one described here, will surely continue to play an important role in generating and testing hypotheses about the functions of neural systems.

Current psychological theories of emotion focus on the importance of time-dependent processes in emotional expression (Ellsworth and Scherer, 2003). Advances in

neuroscience will continue to emphasize another important dimension — space (in the brain). Given that the brain processes information in parallel (Rumelhart and McClelland, 1986), it is possible that discrete and dimensional appraisal processes occur simultaneously in different parts of the brain. It is also possible that neuroscientific research will reveal a new structure of "emotional space" that does not fit any currently conceived framework (Barrett, 2006).

CONCLUSION

The major findings of this thesis are:

1. A large proportion of cells in the BLA and CeN has qualitatively similar changes in activity during conditioned appetitive and aversive arousal.

2. When considering the entire population of recorded cells, neuronal activity during the conditioned appetitive and aversive stimuli was more similar than expected by chance.
3. Over half of the cells with similar changes in activity during the conditioned appetitive and aversive stimuli have activity that is correlated with blood pressure.

Together, these results suggest that overlapping populations of cells in the amygdala contribute to appetitive and aversive emotional arousal.

CHAPTER 4

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