Space and value in the primate amygdala and basal forebrain

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ABSTRACT

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A stimulus predicting reinforcement can trigger emotional responses, such as arousal, as well as cognitive ones, such as increasing attention towards that stimulus. Neuroscientists have long appreciated that the amygdala mediates spatially non-specific emotional responses, but it remains unclear whether the amygdala links motivational and spatial representations in a way that may be important for the emotional-guidance of attention. To test whether amygdala neurons encode spatial and motivational information, we presented reward-predictive cues in different spatial configurations while assessing whether these cues influenced spatial attention. Cue configuration and predicted reward magnitude modulated amygdala neural activity in a coordinated fashion. Moreover, fluctuations in activity were correlated with trial-to-trial variability in spatial attention. Thus the amygdala integrates spatial and motivational information, which may influence the spatial allocation of cognitive resources.

When surveying the environment, animals must be acutely aware of associations between stimuli and aversive outcomes in addition to those resulting in appetitive outcomes. This involves attending to appetitive stimuli in order to obtain positive outcomes, and aversive stimuli in order to avoid negative outcomes. While we first demonstrated that amygdala might play a role in influencing spatial attention towards appetitive stimuli, it is unclear whether the activity of individual amygdala neurons are modulated in a similar way by aversive stimuli that also attract attention. Recording from
amygdala neurons while monkeys allocated attention both towards appetitive and aversive stimuli revealed that firing rates reflected where attention was allocated irrespective of valence. We also found that amygdala neurons preferentially encode appetitive and aversive stimuli relative to those of little motivational significance in a conditioning paradigm where spatial characteristics were irrelevant. Thus, amygdala neurons respond with respect to the motivational significance of stimuli, which is tied to spatial attention in contexts involving multiple stimuli.

While the amygdala might be involved in guiding attention towards motivationally significant stimuli, this process is likely dependent on its interactions with anatomically linked brain areas. The basal forebrain is a candidate brain area for interacting with the amygdala in influencing emotionally-guided attention given its anatomical connectivity and influence over attentional processes. Here, we analyzed data from amygdala and basal forebrain neurons recorded while spatial attention was captured by appetitive and aversive stimuli. Neurons in the basal forebrain were spatial selective for appetitive and aversive stimuli much like the amygdala. We also found that the timing of value signals differed across brain areas in a manner dependent on the spatial configuration of stimuli. Together, these results demonstrate how the amygdala and basal forebrain may participate in coordinating cognitive and emotional processes and are suggestive of how dysfunction within this pathway might contribute to disorders where emotionally-guided attention is impaired.
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CHAPTER 1. Introduction

When presented with the vast array of stimuli encountered on a daily basis, animals must be able to identify those that might have either a beneficial or detrimental effect on their survival. For example, when in the wild, a carnivore identifies other animals that may serve as prey as well as those that are predators. Identifying stimuli as predator or prey is only one step in the set of complicated neural processes that are necessary to promote survival. The animal must also register the spatial location of the predator/prey to act accordingly. This entails localizing the prey in order to mount an attack or localizing the predator in order to effectively guide fight or flight behavioral responses.

How is the brain able to evaluate individual stimuli that predict particular reinforcement outcomes, prioritize the magnitude of each’s relevance, and deploy attention appropriately? A substantial amount of research has examined the neural systems that assign value to stimuli as well as those that focus attention; however, it is unclear how these two systems work together to guide attention towards behaviorally relevant stimuli. In my research, I have strived to understand how the brain uses associations between stimuli and outcomes to guide attentional resources. Particularly, I have focused on two brain areas in the primate brain that I believe to be vital in mediating this process: the amygdala and basal forebrain.

While evidence exists implicating both brain areas in processes related to attention and value, research has fallen short of establishing a functional relationship between the amygdala and basal forebrain in the context of emotionally-guided attention.
The amygdala has typically been thought of as an emotional processing center within the brain, but it has generally not been investigated in relation to attention. On the other hand, cholinergic input from the basal forebrain has been repeatedly cited as a likely substrate for attentional modulation of neural activity; however, little is known about how the basal forebrain might integrate value information in order to augment attention in the presence of motivationally significant stimuli. In other words, if the basal forebrain is important for regulating attention, what inputs and computational steps allow the basal forebrain neurons to ‘know’ what stimuli are deserved of attention? Here, I address these questions by recording the activity of individual neurons in the amygdala and basal forebrain while challenging animals to invoke evaluative and attentional processes.

*Emotional influence on behavior*

Stimuli of emotional value can have a profound effect on behavior. Perhaps the quintessential example of this is in the case of classical conditioning (Pavlov, 1927). By taking an inherently unemotional stimuli (a simple ring of a bell) and consistently pairing with the highly likeable outcome (food), Pavlov’s dog began to exhibit a response to the bell would not have occurred without this pairing – salivation. Another example of conditioning, which has been studied at great length in the field of neuroscience, is fear conditioning (LeDoux, 2000). Here, a rodent learns to associate a particular stimulus, often an auditory tone, with an impending electric shock. While the tone (the conditioned stimulus, or CS) has no inherent emotional value, the shock (the unconditioned stimulus, or US) is an emotional event that instills fear. After learning the association between the
tone and the shock, rodents exhibit freezing behavior (the conditioned response, or CR) following the presentation of that tone.

In many cases, the spatial characteristics of outcome-predictive stimuli influence behavioral responses as well. In the case of visual stimuli, the spatial location of the stimulus is often as important of a characteristic as the visual properties that define it (e.g. color, shape). Visual stimuli that promote or threaten survival tend to attract cognitive and behavioral resources, partly by prioritizing stimuli associated with highly positive or negative outcomes (Lang and Davis, 2006). For example, humans find negative images faster than emotionally neutral images (Ohman and Wiens, 2003), and more readily detect arousing words (Anderson, 2005) or angry faces (Pinkham et al., 2010). Attention can also spread to neutral stimuli nearby arousing ones (Armony and Dolan, 2002; Brosch et al., 2008; Phelps et al., 2006), and to locations directed by emotionally relevant cues such as gaze direction (Klein et al., 2009) or learned associations with monetary outcomes (Anderson et al., 2011). Experimental animals also show a number of spatial benefits for stimuli that are emotional, typically due to their association with rewarding outcomes. Monkeys exhibit an increase in the reaction times and peak velocity of saccadic eye movements towards locations associated with rewarding outcomes (Kobayashi et al., 2002). Similarly, a decrease in reaction time and increase in saccade accuracy towards reward-predicting stimulus occurs even when the direction of the saccade is not predicted by the location of that stimulus (Peck et al., 2009).
The amygdala and emotion

The neural underpinnings of emotion and its influence on behavior have been oft studied, and the amygdala is a brain area that has been repeatedly cited in meditating emotional processing (Kluver and Bucy, 1937; LeDoux, 2000; Weiskrantz, 1956). Anatomically, the amygdala is a subcortical brain structure consisting of distinct nuclei that vary in their function and anatomical connectivity. In a coarse scheme, the amygdala can be divided into the lateral, basal, accessory basal, central, cortical, and medial nuclei; while the connectivity of each nucleus is complex, and often overlapping with each other, the general trend of their connections differs considerably. The lateral nucleus is typically thought of as the sensory input region of the amygdala and receives input from brain areas encoding high-level sensory and polysensory information (Amaral et al., 1992). The basal, accessory basal, and cortical nuclei receive input from the lateral nucleus (Pitkanen and Amaral, 1998) and are anatomically interconnected with the prefrontal cortices, both directly (Amaral and Price, 1984; Ghashghaei et al., 2007) and indirectly via the mediodorsal thalamus (Amaral et al., 1992). The basal and accessory basal nuclei also send feedback projections to visual sensory cortices (Amaral and Price, 1984). The central nucleus projects to the brainstem and, along with the medial nucleus, to the hypothalamus (Amaral et al., 1992). Although other small nuclei make important behavioral contributions (e.g. (Likhtik et al., 2008)) and the structure of these major nuclei can be further divided (e.g. (Pitkanen and Amaral, 1998)), this simpler scheme has guided much of the research on the amygdala thus far (LeDoux, 2000).

The amygdala is a key part of the neural network important for learning, updating and maintaining the value of sensory events (Baxter and Murray, 2002; Morrison and
Salzman, 2010; Phelps and LeDoux, 2005). Early work suggested that the amygdala was primarily involved in forming associations between stimuli and aversive outcomes. Lesions of the amygdala basolateral or central nucleus disrupt the augmented startle responses for stimuli previously paired with shock (Campeau and Davis, 1995). Single unit recordings from the lateral nucleus in rats demonstrated that these neurons are increasingly responsive to tones after being paired with foot shocks (Quirk et al., 1995). Additionally, the amygdala is a likely site for regulating the autonomic responses to emotional stimuli such as the changes in skin conductance and heart rate that occur while human subjects observe emotional faces (Lang et al., 1993). Stimulation of the amygdala results in modulation of the skin conductance response (Lang et al., 1964), and the activity of individual amygdala neurons predicts spontaneous skin conductance responses (Laine et al., 2009).

Further research has demonstrated that the amygdala also influences associations between stimuli and appetitive outcomes. According to the Pearce-Hall model of learning (Pearce and Hall, 1980), the amount of attention paid to a stimulus (its associability) is a function of the consistency with which it predicts a particular outcome; when a stimulus is inconsistently paired with an outcome or an association is suddenly violated, that stimulus acquire high levels of associability and animals are more readily able to learn new associations. While rats learn associations between highly associable stimuli and appetitive outcomes more rapidly than associations with less associable stimuli, this benefit is abolished following lesions to the amygdala central nucleus (Holland and Gallagher, 1993). The amygdala has also been found to be important in mediating devaluation of appetitive reinforcing. Rats with basolateral amygdala lesions fail to
update changes in reinforcement value brought upon by experimenter manipulation (Hatfield et al., 1996), and monkeys with complete amygdala lesions do not exhibit devaluation of oft-sampled reinforcers (Malkova et al., 1997).

The emotional properties of stimuli have a profound influence on the firing rates of individual amygdala neurons. In practice, the concept of value is often referred to since researchers typically manipulate the relationship between stimuli and either rewards or punishments; thus, value and emotion are highly related since the value of a stimulus-outcome association can induce either positive or negative emotional states. Early studies looking at the firing rates of individual primate amygdala neurons have shown that subsets of neurons respond differentially for inherently motivational stimuli, such as food items (Nishijo et al., 1988), and those that obtain motivational value through learning, such as visual stimuli that predict food rewards (Sanghera et al., 1979); however, neurons typically responded to some, but not all, motivationally significant stimuli, and there was no attempt to determine how much of the signal was driven by the visual properties of the stimuli. Firing rates of amygdala neurons are also modulated by the temporal delay of the reward, which is linked to the monkeys’ motivational state (Sugase-Miyamoto and Richmond, 2005). Electrophysiological recordings have demonstrated that distinct populations of neurons respond preferentially to either appetitive or aversive stimulus-outcome associations (Paton et al., 2006) and that these populations may constitute distinct functional networks (Zhang et al., 2013). Amygdala neurons are also selective for inherent emotion properties such as the facial expression of other monkeys (Gothard et al., 2007) as well as appetitive and aversive unconditioned stimuli that occur unpredictably (Belova et al., 2007).
The amygdala and attention

Is the amygdala’s role limited to the forming stimulus-outcome associations and expressing conditioned responses or might it influence more complex processes such as the guidance of attention towards behaviorally-relevant stimuli? The idea that the amygdala might also regulate attention is supported by early experiments showing that electrical stimulation of the amygdala induces behavioral responses indicative of ‘orienting’ and ‘searching’ (Ursin and Kaada, 1960b). Additionally, the amygdala appears to be important in mediating orienting responses specific to conditioned stimuli. While rats typically exhibit an orienting response towards novel stimuli, this response is quickly attenuated after repeated presentation of that stimulus; however, if that stimulus is paired with reward, the orienting response can be renewed. Rats with lesions of the amygdala central nucleus orient normally to novel stimuli but fail to acquire conditioned orienting (McDannald et al., 2004) suggesting a deficit in using stimulus-outcome information to guide attention.

Data from rare patients with isolated amygdala damage also support the idea that the amygdala plays a role in guiding attention when viewing emotionally arousing stimuli (Pessoa, 2010; Phelps and LeDoux, 2005; Vuilleumier, 2005). Humans with amygdala damage are less efficient at detecting negative words (Anderson and Phelps, 2001), and examinations of SM, a woman with bilateral amygdala lesions, show that she is impaired at recognizing fear from facial expressions due to a marked failure to look at the eyes (Adolphs et al., 2005; Adolphs et al., 1994). Remarkably, this impairment disappears when she is instructed to fixate the eyes, suggesting that her impairment results from a failure to direct gaze and attention towards emotionally relevant parts of faces, a process
that requires linking emotional recognition with spatial processing. Additionally, humans with amygdala damage show deficits in using cartoon eyes to focus attention elsewhere (Akiyama et al., 2007); however, these experiments indicate that the amygdala is not involved in all attentional processes, since attention deficits were not observed when the experimenters used arrows to guide attention instead. Thus, the amygdala may be important for guiding attention towards the emotionally relevant aspects of the environment (Adolphs, 2010; Spezio et al., 2007).

Despite the insights gained from human studies, it remains unclear whether the amygdala actively modulates attention, and, if it does, what form of attention is modulated. We considered two possible effects that the amygdala may have on attention. First, the amygdala might have a direct influence on spatial attention. In the sensory domain, when multiple stimuli compete for limited processing resources, attention biases competition in favor of relevant stimuli (Desimone and Duncan, 1995), allowing one to prioritize specific spatial locations in the visual environment. Stimulus relevance may be determined by a wide variety of factors. In the simplest case of orienting the sensory receptors towards some stimuli and away from others, attention can operate in an exogenous (stimulus-driven or bottom-up) or endogenous (goal-directed or top-down) manner (Carrasco, 2011; Corbetta and Shulman, 2002). The former refers to reflexive processes by which organisms direct their sensory receptors towards abrupt or intense stimuli that violate expectations, while the latter refers to a more typically voluntary orientation towards an object or a location in space based on expectancy about an environmental event. The association between rewarding outcomes and a cue indicating spatial location is one such expectation (Maunsell, 2004).
A second means by which the amygdala might modulate attention could involve inducing a state of arousal that promotes vigilance (Davis and Whalen, 2001). Vigilance refers to the ability to sustain attention for prolonged periods of time that depends in part on an organism’s state of arousal. Arousal is thought to be a spatially non-specific way to allocate more processing resources (Boudreau et al., 2006; Davis and Whalen, 2001), and scientists have typically thought of the amygdala as augmenting vigilance by modulating arousal (Kapp et al., 1994), or as augmenting a form of attention described as attention for learning which is also spatially non-specific (Holland and Gallagher, 1999). Indeed, several prior studies have suggested that the amygdala could mediate arousal and/or attention for learning (Belova et al., 2007; Cain et al., 2002; Calu et al., 2010; Holland and Gallagher, 1999; Kapp et al., 1994; Roesch et al., 2010; Silvestri and Kapp, 1998). These prior experiments did not test whether the amygdala also participates in directing spatial attention to the location of motivationally significant cues.

**Amygdala-prefrontal interactions in processing value and attention**

The amygdala is nested within a network of prefrontal brain areas that are important in emotional and cognitive processes; however, it is unclear whether this connectivity may be important in influencing attention. One pathway that has received a considerable amount of focus in terms of value processing is that between the amygdala and the orbitofrontal cortex (OFC). Strong, bidirectional projections connect the amygdala and OFC (Ghashghaei et al., 2007), and the physiological properties of neurons in these two areas are similar along many dimensions. For example, neurons in the both the amygdala and OFC are modulated by the value associated with abstract stimuli, and
both include populations of neurons that respond more or less in proportion to stimulus value (Kobayashi et al., 2010; Morrison and Salzman, 2009; Paton et al., 2006). Additionally, neurons in both areas are responsive to the delivery of unconditioned stimuli and reflect their value (Belova et al., 2007; Morrison and Salzman, 2009). While the physiological responses of neurons in these two brain areas appear similar, recent results suggest a complex sequence of information flow between the amygdala and OFC during learning (Morrison et al., 2011). Unlike the robust coding of value, OFC neurons do not exhibit spatial selectivity for visual stimuli (Padoa-Schioppa and Assad, 2006; Wallis and Miller, 2003) casting doubt on whether connectivity between the amygdala and OFC might have anything to do with spatial attention.

The ventral anterior cingulate cortex (vACC) is another brain area that is likely to participate in computing value information along with the amygdala and OFC given its strong anatomical connectivity with both (Carmichael and Price, 1996; Ghashghaei et al., 2007). Neurons in vACC are selective for variables related to the value of stimulus-outcome associations (Cai and Padoa-Schioppa, 2012); however, like the OFC, vACC neurons are insensitive to spatial properties of stimuli and actions. Thus, vACC and OFC neurons appear to provide a generalized assessment of value that isn’t influenced by the specific properties of stimuli, actions, or outcomes that may be important in weighing possible outcomes and guiding economic choices.

While a number of brain areas within the prefrontal cortex encode value and spatial information that may be important for influencing attention, their connectivity with the amygdala is generally lacking. For example, neurons in the dorsal ACC (dACC) respond differentially depending on whether monkeys make a leftward or rightward
saccade in a free-choice task (Cai and Padoa-Schioppa, 2012), but, unlike vACC, dACC does not have strong connectivity with the amygdala (Ghashghaei et al., 2007). Neurons in the dorsolateral and ventrolateral prefrontal cortices (DLPFC & VLPFC) also encode spatial information that can be enhanced by reward (Kennerley and Wallis, 2009), and VLPFC is also part of a network important for orienting attention towards behaviorally relevant objects (Corbetta et al., 2008). Interactions with the amygdala may allow this network to orient attention based on emotional significance; however, direct projection from the DLPFC and VLPFC to the amygdala are weak (Ghashghaei et al., 2007) suggesting that such an interaction would likely occur indirectly. Thus, while interactions between the prefrontal cortex and the amygdala may play an important role in value processing, it does not appear that these interactions are involved in modulating spatial attention.

**Neural control of spatial attention**

Before concentrating on the functional pathways through which the amygdala might influence attention, we first consider the roles that the dorsal and ventral pathways play in spatial attention and, more generally, visual processing. Decades of work on visual processing has revealed that the ventral visual stream, spanning visual cortex and the temporal lobe, is involved in processing the fine details of stimuli needed for object recognition while brain areas in the dorsal visual stream, including areas in the frontal and parietal cortices, are primarily involved in coordinating the spatial aspects of perception and action (Mishkin et al., 1983).
Brain areas in the dorsal visual stream have been studied often in the context of spatial attention. Neurons in the lateral intraparietal area (LIP), for example, selectively fire in response to salient stimuli (Gottlieb et al., 1998) and prior to saccades directed at valuable stimuli (Peck et al., 2009; Platt and Glimcher, 1999; Sugrue et al., 2004). The frontal eye fields (FEF), which are bidirectionally connected to LIP (Schall et al., 1995; Stanton et al., 1995), share a number of similar properties. Individual FEF neurons are responsive to visual stimuli in a particular region of space and fire in anticipation of saccades to this receptive field (Bruce and Goldberg, 1985). Additionally, low current stimulation of FEF evokes short-latency saccades that are directed towards the receptive field of neurons near the site of stimulation (Bruce et al., 1985). FEF is involved in the allocation of spatial attention as well; stimulation at currents less than that necessary to induce saccades increases the behavioral benefits of attention for stimuli in these neurons’ receptive fields (Moore and Fallah, 2001) and sharpens the selectivity of visual cortex neurons that share the same receptive fields (Armstrong and Moore, 2007).

The influence of spatial attention has also been studied in brain areas in the ventral visual stream. In addition to responding selectively to the spatial location, color, and orientation of stimuli (Desimone et al., 1985), the firing rates of V4 neurons are modulated according to where spatial attention is directed (Moran and Desimone, 1985). In the terminus of the ventral visual stream, the inferotemporal cortex (IT), neurons are highly selectivity for complex objects over a large range of stimulus locations (Desimone et al., 1984). While responses are typically strongest to stimuli presented near the fovea, IT neurons have a preference for stimuli in the contralateral visual field relative to those in the ipsilateral visual field (DiCarlo and Maunsell, 2003; Op De Beeck and Vogels,
The nature of IT receptive fields is still unclear, however, as IT neurons can be very spatially selective for small stimuli (DiCarlo and Maunsell, 2003) suggesting that receptive field size may scale with stimulus dimensions. A more striking spatial influence on IT firing rates has been observed when preferred and non-preferred stimuli are presented simultaneously and in opposite hemifields; firing rates when the preferred stimulus appears contralaterally are considerably greater than when the preferred stimulus is positioned ipsilaterally (Chelazzi et al., 1998). Thus, it appears the visual stimuli appearing in the contralateral visual hemifield receive priority treatment in terms of coding by IT neurons.

**Anatomical interplay between the amygdala and visual areas**

In the visual domain, the amygdala receives input solely from the ventral visual stream, particularly the inferotemporal cortex (Stefanacci and Amaral, 2000); projections from lower levels of visual processing in occipital cortex are absent, as are projections from parietal cortex (Stefanacci and Amaral, 2000). Thus, it is clear that the amygdala receives object identity information that might be vital in forming associations between stimuli and outcome; however, it is unclear what degree of spatial information the amygdala might have access to and how these representations could be used to influence attention.

The idea that amygdala might influence spatial attention is suggested by the projections it sends throughout all levels of processing in the ventral visual stream (Amaral and Price, 1984; Iwai and Yukie, 1987). Evidence for a functional role of these projections has been obtained in fMRI studies; amygdala lesions, both in humans
(Vuilleumier et al., 2004) and monkeys (Hadj-Bouziane et al., 2012), attenuate selectivity for facial emotion within areas of the ventral visual stream. Similar to the pattern of visual inputs to the amygdala, projections from the amygdala to brain areas in the dorsal visual stream are extremely sparse (Baizer et al., 1993; Barbas and De Olmos, 1990). Nonetheless, the amygdala may still exert influence on these areas indirectly due to the connectivity between the dorsal and ventral visual streams (Ungerleider et al., 2008).

**Value and salience coding**

One question that has not been resolved is whether amygdala neurons reflect the value or salience of outcomes. Neurons that encode value should respond to either the ‘goodness’ or ‘badness’ of stimulus-outcomes while those encoding salience should respond to both aversive and appetitive outcomes since they both command attention and have a similar influence on certain autonomic responses (Lang and Davis, 2006). Again, we first consider examples of value and salience coding throughout the primate brain and then assess the available evidence for disseminating between these two types of coding in the amygdala.

Despite a large body of research characterizing the response properties of individual neurons in the primate brain, there are relatively few cases in which the activity of individual neurons in any brain area can be confidently described as encoding either value or salience. In one example, Kobayashi et al. (2006) trained monkeys to associate stimuli with either appetitive, neutral, or aversive outcomes. During task performance, distinct neural populations in the lateral prefrontal cortex discriminated between the stimulus predicting a neutral outcome and those predicting (1) appetitive
outcomes only, (2) aversive outcomes only, or (3) both appetitive and aversive outcomes. It is unclear from these results whether the appetitive-only and aversive-only neurons might simply be classified as such simply because the behavioral salience of the appetitive and aversive outcomes differed. For example, if the monkey places a higher weight on appetitive outcomes, then the difference in firing rates between appetitive and neutral trials might be larger, and therefore, more likely to be significant. This is a likely explanation given that appetitive outcomes had a stronger influence on performance metrics than did aversive outcomes and that the appetitive-only neural population was considerably larger than the aversive-only population. Despite this, the neurons responsive to both types of outcomes provide strong support for the existence of salience coding within the lateral prefrontal cortex.

Neurons in the dopamine-producing ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) have been implicated in mediating stimulus-outcome associations, particularly in computing reward-prediction error signals when associations are first learned (Schultz et al., 1997). Using a conditioning paradigm in which rewards and punishments were delivered with varying probability, Matsumoto & Hikosaka (2009) identified unique response properties amongst putative dopaminergic neurons. While firing rates generally increased in proportion to the probability of reward, distinct populations’ firing rates correlated positively and negatively with probability of punishment, therefore suggesting distinct coding of stimulus value and salience. In the mouse ventral tegmental areas, some diversity of punishment responses have also been identified; some neurons were excited by a punishment CS or US while others were inhibited, consistent with a mix of value and salience coding (Cohen et al., 2012). These
results are especially informative given the projection from the amygdala to VTA/SNc (Price and Amaral, 1981).

In a recent example, Leathers and Olson (2012) recorded from LIP neurons while monkeys chose between stimuli associated with rewards and punishment that were small or large in magnitude. Consistent with previous experiments (Sugrue et al., 2004), LIP neurons fired more when large reward stimuli appeared in their receptive fields as compared to small reward stimuli. These results were novel in that the experimenters also found that LIP neurons fired more for big punishment stimuli relative to small punishment stimuli. Although it has been argued that these neurons are distinct from those typically recorded in LIP given differences in their fundamental response properties (Newsome et al., 2013), the results are sound in depicting an example of salience coding within the primate brain.

**Evidence of attention processing by individual amygdala neurons**

If amygdala neuron responses reflect salience, then stimuli that attract attention should have a similar influence on firing rates, regardless of their valence (i.e. appetitive or aversive), leading to a straightforward explanation of how projections from these areas to cortex could influence attention. On the other hand, it’s possible that these neurons may code a quantity like reinforcement value where appetitive and aversive stimuli would have an *opposing* influence on firing rates; this result would suggest that amygdala may not have a direct influence on attention, although these signals might be summed at later neural processing stages to create a generalized attention signal.
Though Paton et al. (2006) termed amygdala neurons as encoding the ‘positive’ or ‘negative’ value of outcomes, their experiment did not test conditions in which one could definitively determine whether amygdala neurons reflect value or salience. While the predictive value of the stimuli that initially predicted reward and punishment were reversed at a random time during each session, the association between the third stimulus and a lack of outcome was held static; thus, there was no control for the image properties of the ‘neutral’ stimulus, and amygdala neurons might simply represent those image properties irrespective of the stimulus’ predictive value. Secondly, there was no means for comparing the subjective magnitude of the aversive and appetitive outcomes given that there wasn’t a common conditioned response relevant to both types of outcomes. Finally, the animals had to fixate only briefly before receiving an outcome 1.5 seconds later, so responses following the neutral stimulus may simply indicate the monkey’s disengagement from the task. In rats, Shabel and Janak (2009) claimed that a larger proportion of amygdala neurons responded in accordance with the motivational significance of conditioned stimuli. However, this study also failed to properly control for the sensory properties of the stimuli or the lack of task engagement following the presentation of a stimulus that predict no outcome. Thus, it is still unclear whether amygdala neurons reflect the value or salience of stimuli, or whether distinct populations within the amygdala are consistent with each.

The basal forebrain

In addition to its potential for regulating attention via projections to the ventral visual stream (Amaral and Price, 1984), the amygdala may exert its influence via other
brain areas. One candidate brain area that may serve as such a relay is the basal forebrain. The basal forebrain is a subcortical structure composed of distinct nuclei and serves as the main source of the neurotransmitter acetylcholine for the rest of the brain (Mesulam et al., 1983). In the primate brain, the basal forebrain consists of (1) the medial septum, (2) the vertical nucleus of the diagonal band, (3) the horizontal nucleus of the diagonal band, and (4) the nucleus basalis of Meynert. While the basal forebrain as a whole project to most of the brain, projection patterns of the individual nuclei differ systematically (Mesulam et al., 1983) with (a) the medial septum and the vertical nucleus of the diagonal band providing substantial input to the hippocampus, (b) the horizontal nucleus of the diagonal band projecting primarily to the olfactory bulb, and (c) the nucleus basalis projecting to cortex and the amygdala. Projections from within the nucleus basalis are organized in a topographic fashion (Ghashghaei and Barbas, 2001; Mesulam et al., 1983) suggesting that the response properties of nucleus basalis neurons may be specialized according to the brain area that they project to.

A number of results point towards the role of the basal forebrain as an intermediary between the amygdala and the dorsal stream attention network. The basal forebrain is anatomically well-placed to convey signals from the amygdala to the cortex; the basal and central nuclei of the amygdala project to the basal forebrain, particularly the ACh-rich nucleus basalis region (Russchen et al., 1985), and the basal forebrain sends projections throughout the cortex and back to the amygdala (Mesulam et al., 1983). Given that the basal forebrain projects to frontoparietal areas implicated in attentional control such as FEF and LIP (Ghashghaei and Barbas, 2001; Mesulam et al., 1983), this
input may be important for guiding attention towards the emotional stimuli identified by the amygdala.

**Evidence for attention modulation by the basal forebrain**

In terms of behavioral deficits, McGaughy et al. (1996) used the immunotoxin 192 IgG-saporin to specifically lesion cholinergic neurons in the rat basal forebrain and showed that rats were significantly impaired in detecting short-duration visual stimuli following the lesions, indicative of a vigilance impairment. Monkeys with cell-type non-specific lesions of the basal forebrain were slower to detect visual signals, especially when a shift in spatial attention was required (Voytko et al., 1994). Basal forebrain activity has also been shown to influence the neural signatures of attention observed in cortex. Specific cholinergic deafferentation of the rat posterior parietal cortex (PPC) attenuates the neural correlates of attention (Broussard et al., 2009); when lesioned rats were required to detect a target stimulus in the context of a visual distractor, PPC neurons tended to fire more to the distractor and less to the target (relative to controls), decreasing the neural signal-to-noise ratio. The basal forebrain may thereby be involved in sharpening the response profile of downstream neurons by increasing responses to behaviorally relevant stimuli and decreasing responses to distractors, which is characteristic of neurons in the primate parietal cortex (Ipata et al., 2006). While recording from populations of visual cortex neurons in rat, Goard & Dan (2009) found that simultaneous electrical microstimulation of the basal forebrain increased the trial-to-trial reliability of individual neuron responses and decreased the correlation of responses amongst populations of neurons. Both the increase in reliability (Mitchell et al., 2007)
and decrease in across-neuron correlations (Cohen and Maunsell, 2009; Mitchell et al., 2009) are consistent with what occurs when primates direct attention towards the receptive fields of individual visual neurons.

Evidence for the basal forebrain’s role in attention is also apparent from experiments using pharmacological manipulation to study the behavioral and neural effects of acetylcholine (ACh). Similar to the attention-like effects in visual cortex following microstimulation (Goard and Dan, 2009), direct ACh application decreased interneuronal correlations amongst neurons in the middle temporal lobe (area MT) during motion discrimination (Thiele et al., 2012) although it did not induce a decrease in firing rate variability as during microstimulation (Goard and Dan, 2009). Herrero et al. (Herrero et al., 2008) recorded from individual neurons in primary visual cortex (V1) while iontophoretically manipulating ACh levels in the immediate vicinity of the recording site and found an increased influence of attention on firing rates during ACh application. Even though iontophoretic injections affect a relatively small neural population, monkeys still exhibited a greater behavioral effect of attention (reaction times towards versus away the receptive field of the recorded neuron) in the presence of ACh.

Cholinergic input from the basal forebrain may influence downstream activity through either the ionotropic nicotinic receptors (nAChR) or metabotropic muscarinic receptors (mAChR) of postsynaptic targets. Suggesting that ACh influences attention primarily through its influence on mAChRs, attention modulation of firing rates was attenuated in presence of the muscarinic antagonist scopolamine, but unaffected in the presence of the nicotinic receptor antagonist mecamylamine (Herrero et al., 2008). The attention-like effects observed in visual cortex following microstimulation of the basal
forebrain were also mediated by muscarinic receptors (Goard and Dan, 2009). Additionally, Davidson & Marrocco (2000) found that local infusion of scopolamine into monkey intraparietal cortex (including LIP and area 7a) decreased the validity effect in a cued-target detection task, which is a typical measure of attention (Posner, 1980). On the other hand, nAChRs might be more important in influencing the gain of responses in visual cortex (Disney et al., 2007) as opposed to sharpening the selectivity between attended and unattended stimuli.

**Cholinergic and GABAergic neurons in the basal forebrain**

Due to the role that acetylcholine plays in many neural processes, the basal forebrain has typically been studies in the context of its ACh projections; in rodents however, the basal forebrain consists of a heterogeneous neural population. Approximately equal proportions of cholinergic and GABAergic basal forebrain neurons project to the cortex, together representing ~45% of these projections, while the rest originate from other non-cholinergic neurons (Gritti et al., 1997) that are likely glutamatergic (Gritti et al., 2006). By combining physiological recordings and juxtacellular labeling of neurons, Barbara Jones and colleagues have characterized the response properties of immunohistochemically-identified cholinergic and GABAergic neurons as well as putative glutamatergic neurons. While non-cholinergic neurons showed various types of modulation across the sleep-wake cycle, the activity of cholinergic neurons was consistently greatest during paradoxical sleep and awake states and suppressed during slow-wave sleep (Lee et al., 2004), thus suggesting a role for basal forebrain cholinergic neurons in regulating cortical arousal. Additionally, most
cholinergic neurons (4/5) fired rhythmically in correlation with cortical theta rhythms; of the non-cholinergic neurons that showed similar sleep-state modulation as ACh neurons, only a small proportion (2/17) exhibited this property (Lee et al., 2005). These results suggest that basal forebrain ACh neurons may be important for regulating neural theta oscillations and offers a physiological criterion for discriminating between ACh and non-ACh neurons.

Despite a large body of evidence characterizing the basal forebrain’s role in attention, only recently have researchers begun to address the contributions of the non-cholinergic population (Sarter and Bruno, 2002). In line with a role in attention, basal forebrain GABA neurons synapse onto inhibitory neurons in the cortex (Freund and Gulyas, 1991), representing a potential mechanism for disinhibiting the activity of cortical excitatory neurons. Consistent with these results, putative non-cholinergic basal forebrain neurons respond to conditioned stimuli in a manner consistent with a role in attention (Lin and Nicolelis, 2008). In lesions studies, differential performance deficits were observed in identical behavioral contexts following lesions targeting only ACh+ basal forebrain neurons as compared to those that are not specific to cell type (Burk and Sarter, 2001). One attractive hypothesis regarding this heterogeneity is that the non-cholinergic population specifically acts on the ‘fast’ mechanisms of attention (e.g. shifting spatial attention rapidly through the visual environment) while cholinergic projections may be more important for processes such as maintaining vigilance (McGaughy et al., 1996) and learning about stimulus-outcome associations (Chiba et al., 1995). This hypothesis is likely over-simplified, however, since cholinergic manipulation
can influence neural and behavioral correlates of attention on fast time scales (Herrero et al., 2008).

Despite the potentially distinct roles of GABAergic and cholinergic neurons in the rodent basal forebrain, little evidence has been found to suggest the GABAergic neurons represent a substantial population in the primate basal forebrain. In fact, it has been estimated that ~95% of neurons in the nucleus basalis of Meynert, the origin of most cortical projections from the basal forebrain, are cholinergic (Mesulam et al., 1983). The distinction between the proportions of cholinergic neurons in the rodent and primate could be due to differences in experimental technique, and may need to be reevaluated in the primate. On the other hand, evolutionary differences between the rodent and primate basal forebrain may explain the difference and preclude our ability to generalize results across species.

**Basal forebrain physiology**

Few studies have examined the physiological response properties of individual basal forebrain neurons, however, the work done so far has implicated the basal forebrain in processes related to value and attention. In the primate, Rolls and colleagues have demonstrated that basal forebrain neurons respond differentially to stimuli associated with appetitive and aversive outcomes (Wilson and Rolls, 1990); much like the amygdala (Paton et al., 2006), some neurons responded more to stimuli associated with appetitive outcomes while other responded more to those associated with aversive outcomes. Additionally, work from the Delong Lab has implicated the primate basal forebrain in attention-like processes. In a delayed motor responses task, the firing rate of basal
forebrain neurons was more likely to be modulated in a later epoch of the trial where monkeys had to make a motor choice (‘choice epoch’) as compared to an earlier epoch where they were required to make a fixed motor response (‘cue epoch’), even though visual stimuli were similar in each epoch (Richardson and DeLong, 1986, 1990). Alternatively, these neurons may in fact have responded to the impending reward since (1) reward delivery followed the choice epoch, not the cue epoch, and (2) neurons were also likely to be modulated by the reward itself. Neural responses were unlikely to be directly related to the monkeys’ motor response itself, since in a go/no-go task, firing rates were modulated for both go and no-go responses (Richardson and DeLong, 1990), thus suggesting a role in attention as opposed to motor preparation and/or output. Also consistent with a role in attention, primate basal forebrain neurons have been found to respond to unconditioned stimuli in a manner consistent with salience; many individual neurons responded similarly to the delivery of appetitive and aversive outcomes (Richardson and DeLong, 1991). Additionally, these experiments indicated that neurons were insensitive to the spatial location of stimuli (Richardson and DeLong, 1986, 1990); however, this analysis was limited to the delay epoch of the task where the monkey simply awaited the onset of the choice epoch, and the lack of spatial selectivity might have been due to the lack of peripheral stimuli and/or demands on spatial attention.

While responses to unconditioned stimuli don’t necessarily implicate a role in attention and may have more to do with autonomic responses, responses in proportion to the salience of conditioned stimuli would offer a stronger argument. Lin et al. (2008) found that rat basal forebrain neurons responded strongly to conditioned stimuli that predicted either appetitive or aversive outcomes; neurons responded little to the same
stimuli when the stimulus-outcome association had not yet been learned or had been previously extinguished. Rats were also tested in a simple detection task where they had to respond to a near-threshold tone in order to obtain reward; further supporting a role in attention, they found that firing rates accurately reflected whether the rats detected or failed to detect the tone. The neurons recorded in this experiment were likely GABAergic, and since GABAergic neurons are not prevalent in the primate basal forebrain (Mesulam et al., 1983), it is unclear what conclusions can be derived from this data.

**The amygdala & basal forebrain**

Unlike its projections which span much of the brain, a relatively few number of areas provide input to the basal forebrain (Ghashghaei and Barbas, 2001; Mesulam and Mufson, 1984; Mesulam et al., 1983). Regulation of attention by the basal forebrain with respect to stimulus-outcome associations is likely to be dependent on the amygdala since the amygdala is one of only a few input sources that might convey information regarding the emotional value of visual stimuli (Mesulam and Mufson, 1984). In terms of direct visual input, there is no evidence for inputs from occipital and parietal areas that could provide visual information (Mesulam and Mufson, 1984). Direct visual input from the inferotemporal cortex is a possibility; however, the presence of this projection is based on the result of a single anterograde tracing experiment in which the injection included portions of the perirhinal cortices as well (Mesulam and Mufson, 1984).

A few brain areas that project to the basal forebrain may provide information regarding stimulus value. The basal forebrain receives strong input from OFC
(Ghashghaei and Barbas, 2001), which is selective for value, but not spatial parameters (Padoa-Schioppa and Assad, 2006; Wallis and Miller, 2003). Inputs from the ventromedial PFC (vmPFC; area 32) to the basal forebrain were also observed (Ghashghaei and Barbas, 2001), and small clusters of neurons within vmPFC were found to encode spatial attention and value information (Kaping et al., 2011). Value and attention information appeared relatively late amongst these neurons (300-450 ms after the stimuli’s appearance), although one neural population spanning vmPFC and neighboring area 24 (ACC) jointly encoded value and attention at a relatively short latency (~150 ms). Whether this population of neurons might project to the basal forebrain is unclear given that not all sub-regions of vmPFC project to the basal forebrain (Ghashghaei and Barbas, 2001) and because the existence of ACC to basal forebrain projections are questionable at best (Mesulam and Mufson, 1984).

Projections from the amygdala to the basal forebrain originate most strongly in the parvicellular basal, magnoacellular accessory basal, and central nuclei (Russchen et al., 1985). These sites also appear to correspond to those in the cat amygdala basal nucleus for which electrical stimulation induces attention-like orienting responses (Ursin and Kaada, 1960a, b). Additionally, there is an apparent separation between those areas within the amygdala that either send input to or receive input from the basal forebrain; anterograde label from the basal forebrain, along with acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) labeling indicative of cholinergic input, was mainly restricted magnoacellular division of the basal nucleus, which is largely non-overlapping with those areas projecting strongly to the basal forebrain. Interestingly, projections from the amygdala to occipital and temporal visual areas (Amaral and Price, 1984; Freese and
Amaral, 2005; Iwai and Yukie, 1987) are largely overlapping with the sites receiving input from the basal forebrain suggesting an additional route for regulating cortical processing of attention.

**Evidence for spatial processing in the amygdala and basal forebrain**

If the amygdala and basal forebrain do have a role in spatial attention, then responses of neurons in these areas may have some form of spatial selectivity, at least in terms of the contralateral versus ipsilateral visual hemifields. The idea that the ‘pool’ of attentional resources within each brain hemisphere is independent is evidence by a number of behavioral and physiological results. Buschman et al. (2011) trained monkeys on a visual working memory task in which they had to remember a varying number of simultaneously presented stimuli and then report which stimulus had changed when an altered display was later presented. Monkeys’ behavioral performance declined with increasing numbers of stimuli in the same visual hemifield as the changed stimulus, but was insensitive to the number of the stimuli that had appeared in the opposite hemifield. A similar degradation of neural information was apparent in both prefrontal and parietal neurons; stimulus information contained in firing rates decreased with increasing numbers of stimuli ipsilateral to the changed stimulus but was unaffected by the number of contralateral stimuli. Additionally, Cohen and Maunsell (2010) demonstrated that the response of simultaneously recorded neural populations in V4 accurately predicted monkeys’ attention fluctuations from trial-to-trial; however, these fluctuations were uncorrelated across neural hemispheres suggesting that each hemisphere draws from its own pool of attention as opposed to competing with each other.
Evidence for distinct processing of contralateral and ipsilateral stimuli in the amgydala and basal forebrain is primarily indirect, as few studies have addressed this topic at the level of single neuron firing rates. Hinting at that idea that the amygdala might play a role in spatial processing, early studies found that electrical stimulation of the amygdala produced attention-like orienting responses that tended to be directed in the contralateral direction (Ursin and Kaada, 1960b). Additionally, feedback projections from the amygdala to visual cortices primarily terminate in ipsilateral areas (Iwai and Yukie, 1987). Consistent with this finding, amygdala lesions in humans decrease BOLD selectivity for emotional stimuli in ipsilateral, but not contralateral, visual areas (Vuilleumier et al., 2004). Although, a similar study in monkeys found that unilateral amygdala were sufficient to reduce emotional selectivity in both hemispheres (Hadj-Bouziane et al., 2012), the results may differ due to the use of central stimuli (Hadj-Bouziane et al., 2012) as opposed to peripheral stimuli (Vuilleumier et al., 2004). Finally, direct projections between the two amygdalae are not apparent in monkey (Demeter et al., 1990) suggesting that each amygdala may be specialized for encoding the value of the stimuli that appear contralateral to it. This specialization is also apparent in fear conditioning; unilateral inactivation of the amygdala lateral nucleus blocks learning and expression of conditioned freezing when a tone was paired with an electrical shock to the contralateral eyelid, but not when it was paired with an ipsilateral shock (Blair et al., 2005).

The response properties of neurons providing visual input to the amygdala are also informative. In a direct examination of visual information sharing between the two hemispheres, Tomita et al. (1999) recorded from single neurons in the inferotemporal
cortex following an experimental split-brain procedure; transection of the posterior corpus callosum and the anterior commissure affected cross-hemispheric projections of the temporal lobe and the amygdala while leaving those of the prefrontal cortex intact. Neurons in the inferotemporal cortex continued to encode ipsilateral visual information after the transection, although this information was considerably delayed relative to contralateral visual information, more so than in intact monkeys (DiCarlo and Maunsell, 2003). Following an additional transection of the anterior corpus callosum, however, inferotemporal neurons no longer were responsive to ipsilateral stimuli suggesting that these cross-hemispheric prefrontal projections mediate transfer of this information across hemispheres. Integration of value information for contralateral and ipsilateral may therefore occur in the prefrontal cortex, where, for example, OFC firing rates reflect stimulus value but are devoid of spatial selectivity (Padoa-Schioppa and Assad, 2006; Wallis and Miller, 2003).

Like the amygdala, projections from the basal forebrain to cortex also appear to be primarily ipsilateral (Walker et al., 1985); however, the hemispheric-specificity of the basal forebrain’s influence on downstream areas has not been tested to our knowledge. Given their connectivity (Russchen et al., 1985), it stands to reason that the basal forebrain may exhibit a similar degree of spatial selectivity as the amygdala, although it is unclear whether the projections between the amygdala and basal forebrain are exclusively ipsilateral.
Relation to translation research

The investigation of the amygdala and basal forebrain’s role in emotionally-guided attention promises to have a large impact on our understanding of the fundamental biological mechanisms that may become dysfunctional in autism spectrum disorders. When healthy individuals interpret the emotions of others, they typically bias their gaze towards emotionally-informative features such as the eyes and mouth. In autism spectrum disorders however, individuals are deficient in their ability to read others’ emotions, and this deficit is likely to be in part because their gaze is not preferentially biased towards these emotionally-informative features (Pelphrey et al., 2002). Amygdala dysfunction is thought to play a role in autism (Baron-Cohen et al., 2000), and subjects with amygdala lesions exhibit similar symptoms as those with autism (Adolphs et al., 2005). Deficits in emotion and attention are not specific to autism however, and amygdalar dysfunction is suspected to play a role in many neuropsychiatric disorders. For example, imaging data from schizophrenic patients has revealed amygdalar overactivation in response to neutral faces (Hall et al., 2008); this overactivation could account for why these patients are often unable to suppress gaze following upon viewing these faces, even when gaze following is disadvantageous (Langdon et al., 2006). Overactivation of the amygdala and basal forebrain might also explain the generalized deficits schizophrenic patients exhibit in disengaging attention during symbolically cued attention tasks (Maruff et al., 1996). Thus, by improving our understanding of how these brain areas process emotion and attention, we strive to elucidate the biological underpinnings of a wide array of neuropsychiatric disorders.
Experimental approach

The macaque monkey is an ideal model system in which to investigate the emotional-guidance of attention. First of all, its visual system is extremely similar to that of humans, and its visual attention abilities and neural substrates have been well-characterized (Reynolds and Chelazzi, 2004). Secondly, macaques are keenly able to form stimulus-outcome associations, and a number of easily quantifiable behaviors such as conditioned responses (Paton et al., 2006) and performance metrics (Kobayashi et al., 2002) serve as experimental measures for these learned associations. Again, the neural structures that are responsible for the formation of these associations are well-characterized (Schultz, 2006).

The experimental paradigm that we will describe offers a number of benefits. Monkeys will perform a task that will directly address the question of how the brain can guide attention based on the emotional importance of stimuli. Extracellular recording from individual amygdala and basal forebrain neurons during task performance will offer a number of advances in understanding the physiology of these brain areas for a number of reasons: (1) few single unit recording experiments have been done in the primate amygdala and even fewer have been done in the basal forebrain, (2) studies that have focused on the activity of these brain areas have examined them in the context of simple conditioning or operant tasks that do not challenge the primate’s visual attention abilities, and (3) despite the known anatomical connectivity between these areas (Russchen et al., 1985), no studies have sought to understand their functional relationship.

In Chapter 2, we will show that individual neurons in the amygdala respond in a manner consistent with spatial attention while also integrating information about
expected reward. Here, neurons accurately reflect the spatial location of those stimuli associated with large rewards, which are the target of spatial attention, and fluctuations in firing rates predict the degree of attention allocation on a trial-by-trial basis.

In Chapter 3, we will demonstrate that attention modulation of amygdala activity is consistent for stimuli predicting appetitive and aversive outcomes. Further, we show that preferential encoding of both appetitive and aversive stimuli is similar in a trace-conditioning task suggesting that the amygdala reflects the salience of stimuli both in attentionally-demanding and passive contexts.

In Chapter 4, we will address how the amygdala and basal forebrain may interact to influence attention towards motivationally significant stimuli. While neurons in both brain areas responded in agreement with spatial attention, the latency of these signals appears differed across brain areas and depended on the stimuli’s spatial configuration suggesting a bi-directional route of information flow between the amygdala and basal forebrain.
CHAPTER 2. The primate amygdala combines information about space and value

INTRODUCTION

The neural mechanisms linking our emotional world to spatial cognition remain poorly understood. The amygdala is important for learning, updating and maintaining the value of sensory events and mediates many aspects of non-spatial emotional responses (Phelps and LeDoux, 2005). Prior work implicates the amygdala in encoding the motivational significance, or value, of stimuli (Belova et al., 2008; Paton et al., 2006), but has not explored whether the amygdala is important for determining the spatial location of motivationally significant stimuli. Indeed, the amygdala is heavily interconnected (Ghashghaei et al., 2007) with the orbitofrontal cortex (OFC) and ventral anterior cingulate cortex (vACC), where neurons lack spatial selectivity (Cai and Padoa-Schioppa, 2012; Padoa-Schioppa and Assad, 2006), suggesting that information processed at the level of the amygdala may be largely non-spatial.

The amygdala could be involved in directing cognitive and behavioral resources towards stimuli in at least two ways. First, the amygdala may induce a vigilant or aroused state (Davis and Whalen, 2001), perhaps enhancing processing globally but leaving the representation of spatial information to other brain structures. Alternatively, the amygdala may register both the motivational significance and location of stimuli, allowing it to influence cognitive and behavioral functions in space. To distinguish between these possibilities, we trained monkeys to perform a task where we presented reward-predictive visual cues in different spatial configurations. We found that during task performance individual amygdala neurons encoded both the motivational
significance of visual stimuli as well as their spatial configuration. Furthermore, neuronal activity was correlated with the trial-by-trial allocation of attention, suggesting that the representation of value and space in the amygdala could influence the direction of spatial attention towards motivationally relevant stimuli.

**RESULTS**

*Monkeys use stimulus-outcome associations to allocate spatial attention.* To evaluate how the spatial configuration of reward-predictive visual cues influences the allocation of cognitive resources, we trained each of three monkeys to perform one of two tasks (Fig. 2.1). The basic structure of both tasks was the same in that cues associated with different amounts of reward briefly appeared near spatial locations where monkeys subsequently performed a perceptual task. In both tasks, monkeys initiated trials by fixating a central point and then held fixation during the brief presentation of two visual cues (appearing in opposite hemifields) and a subsequent delay. The delay terminated at a random time when a target stimulus appeared near one of the two cue locations. The monkey then reported the location of the target (task A; monkeys O & L) or the orientation of the patch revealed to be the target (task B; monkey C).
Figure 2.1 Sequence of events in the two attention tasks. After monkeys achieved central fixation, two cues appeared at either side of the fixation point for 300 (task A) or 350 ms (task B). In task A (top), the cues were followed by a delay where no peripheral stimuli were present. The brief appearance of a near-threshold oriented patch (50 ms) at one of the two locations served as the target and the monkey correctly detected it by saccading to its location. In task B (bottom), two randomly oriented patches appeared on either side of the fixation point 250 ms after the cues were extinguished. At a random time, both patches changed orientation simultaneously (in independent directions). A pair of choice targets was subsequently presented at one location, indicating which oriented patch was the target, and the monkey judged whether the target at the indicated location was closer to vertical or horizontal.

Each visual cue indicated how much liquid reward the monkey would receive for correct performance when the target appeared nearest the location of that cue; cues predicted either a high value or a low value outcome and were chosen from one of two cue sets in order to distinguish between effects related to the cue-outcome associations and those related to the physical characteristics of the cues. On each trial, we presented either two low value cues (high value absent) or one high value cue and one low value cue (high value present; spatial configuration randomized with equal probability). We used performance and reaction time to assay the effects of the cues on behavior (Posner, 1980). Since the target location was chosen randomly on each trial, we tabulated performance and reaction time at each location to determine how monkeys used cue-outcome associations to allocate cognitive resources (Fig. 2.2). When the target appeared near the high value cue, monkeys exhibited faster reaction times (218 ms vs. 285 ms;
Paired Wilcoxon, \( P < 10^{-19} \) and improved performance (83% vs. 46%; Paired Wilcoxon, \( P < 10^{-20} \)); on high value absent trials, performance improved and reaction times quickened relative to when the target appeared near the low value cue location on high value present trials (Paired Wilcoxon, \( P < 10^{-15} \)). When two low value cues were presented, attention was roughly split between the two locations (69%/66% hit rate for targets ipsilateral/contralateral to the recording location).

**Figure 2.2 Motivational cues bias spatial attention.** (a) Performance (top) and saccadic reaction times (bottom). On trials when one high value cue and one low value cue appeared (High value present), reaction times were faster when the target appeared near the location where the high value cue had appeared. Performance (100 x [# of correct trials / # of trials where target appeared]) was better when the target appeared near where the high value cue had appeared as well. When two low value cues appeared (high value absent trials), monkeys performed at a level that was significantly greater than when that low value cue was paired with a high value cue, and the target appeared near the low value cue location (compare grey & white bars). Thus, monkeys did not inherently perform poorly for the low value outcomes; rather, performance for low value outcomes suffered when a high value outcome biased attention away from the low value location. Asterisks indicates a significant difference between cue types (Paired Wilcoxon, \( P < 10^{-6} \)). (b,c) Behavioral results were similar for the two tasks.
The motivational significance and spatial configuration of stimuli influence amygdala neural activity. To determine if the amygdala represents spatial as well as motivational information, we recorded from 359 neurons in the amygdalae of three monkeys performing the two tasks (monkey O: 146, left amygdala; monkey L: 59, left amygdala; monkey C: 154, right amygdala; Fig. 2.3). Of these, 326 (91%) neurons were responsive during the task (see methods), and we restricted all further analyses to this data set.

Figure 2.3 MRI reconstruction of recording locations. (a) 3D reconstruction of the whole brain and the amygdala for Monkey O. (b) 3D reconstructions of the amygdala recorded from in each monkey overlaid on a single coronal MRI slice from that monkey. Each coronal slice has been tilted to enable visualization of all electrode tracks. Arrows provide the orientation of the slice after tilting. Each data point represents the location of one neuron recorded during the task and the selectivity of that neuron (see legend; green, REW+ cell; yellow, REW- cell).

We found that amygdala neural responses frequently encoded information about the both the value and spatial configuration of the cues (Fig. 2.4a). The activity of each example neuron is strikingly dependent on the location of the high value cue. Notably, the activity of these neurons is also modulated by the overall expected value of the cues. Consistent with prior studies (Belova et al., 2008; Paton et al., 2006), some neurons
responded most strongly on trials where no high value cues appeared (left example panels, “REW-“ neurons) and other neurons responded most strongly when a high value cue appeared (right example panels, “REW+” neurons)

The neurons depicted in Figure 2.4a are representative of the population we recorded in two ways. First, both expected reward and spatial configuration frequently had strong effects on neural responses. Second, there was a systematic relationship between value selectivity and spatial configuration selectivity. Neurons that signaled the presence of a high value cue with an increase (decrease) in firing also tended to fire more (less) when the high value cue was contralateral. We quantified these data by estimating selectivity indices (d’) for each neuron based on the firing rates 100 to 800 ms after cue onset. To calculate a spatial configuration selectivity index, we compared trials when the high value cue appeared contralaterally to when it appeared ipsilaterally. Surprisingly, 45% (148/326) of neurons were significantly modulated by spatial configuration (bootstrap, P < 0.05), of which 84 responded most and 64 responded least when the high value cue was contralateral.

To calculate a value selectivity index, we analyzed the same time window as for the spatial selectivity index but now compared trials in which a high value cue appeared to trials when a high value cue did not appear. We identified many neurons that significantly increased (REW+ neurons, n = 122) or decreased (REW- neurons, n = 71) activity when the overall value increased (bootstrap, P < 0.05).
Figure 2.4. Amygdala neurons encode the value and spatial configuration of cues.

(a) Peristimulus time histograms showing average firing rate plotted as a function of time relative to cue onset for four amygdala neurons (30 ms bins shifted by 2 ms; shading indicates standard error). Value selectivity indices and spatial selectivity indices (d’) were significantly (bootstrap, \( P < 0.05 \)) less than zero (REW−; left) or greater than zero (REW+; right) for each example neuron. (b) Scatterplot of spatial selectivity vs. value selectivity indices for each individual neuron (n = 326). Value selectivity indices greater than zero indicate higher activity when a high value cue was present, and indices less than zero indicate higher activity when the high value cue was absent. Spatial selectivity indices greater than zero indicate higher activity when the high value cue was contralateral; values less than zero indicate higher activity when the high value cue was ipsilateral. Symbol style indicates the significance of selectivity for each neuron (see legend, same conventions as Fig. 2.3b); black line represents the weighted least squares regression fit (\( \beta = 0.53, \ P < 10^{-6} \)). One neuron with a value selectivity index greater than 3.5 is excluded from this plot and from the plots in Figure 2.9a. Numbers indicate data points corresponding to the example neurons in (a).
If amygdala neurons indiscriminately combine spatial and value selectivity, these two measures would not be associated. This would be consistent with, for example, amygdala neurons combining value-related and space-related information in a random manner. Contrary to this hypothesis, we found a strong positive relationship between value and spatial selectivity indices (Fig. 2.4b; weighted least squares regression and bootstrap, $\beta = 0.53, P < 10^{-6}$). Thus, individual amygdala neurons selectively combined information about space and value to signal the location of reward-predictive stimuli with both negative and positive excursions in firing rate. REW+ neurons signal the presence of a more valuable cue in contralateral visual space with increases in firing rate, whereas REW- neurons do the same with decreases in firing rate.

**The timing of value information varies according to spatial configuration.** The combined representation of space and value in the amygdala indicates that individual neurons encode information about the location of a high value cue while also registering the value of both contralateral and ipsilateral stimuli. It is possible that the spatial configuration selectivity we observe could result from weaker visual inputs representing the ipsilateral visual field, which may also carry value information or interact with value information arriving to the amygdala from other brain areas. To gain insight into these possibilities, we examined the latency with which amygdala neurons encode value in each visual hemifield.

First, we characterized how the location of a highly valued cue affects the latency that neurons began to encode reward value. We determined when each neuron signaled the presence of a contralateral or ipsilateral high value cue by comparing trials in which no high value cue appeared to trials in which a high value cue appeared ipsilaterally or
contralaterally. For example, inspecting Figure 2.4a reveals that neural responses to a high value cue diverge more quickly when it appears contralaterally (the cyan and black curves separate earlier than the magenta and black curves). We quantified these latencies for each neuron (see methods) and found that the value latency for contralateral high value cues was shorter than that for ipsilateral high value cues for 97 (83%) of the 117 neurons for which we could estimate both latencies (Fig. 2.5a). The mean contralateral and ipsilateral value latencies were 177 and 240 ms, which differed significantly (Wilcoxon, $P < 10^{-11}$); this effect did not differ between REW- and REW+ neurons (Wilcoxon, $P = 0.73$). Normalizing and averaging activity across all value-modulated neurons ($n = 193$; Fig. 2.5b) revealed that the population signaled the presence of a contralateral high value cue 44 ms earlier than an ipsilateral high value cue (119 ms compared to 163 ms; bootstrap, $P = 0.001$). Thus, while amygdala neural activity is modulated by the value of both contralateral and ipsilateral stimuli, this modulation occurs on different timescales, suggesting the possibility of different neural sources for contralateral and ipsilateral value information.
Figure 2.5. Latency of value discrimination by amygdala neurons depends upon cue spatial configuration. (a) Timecourse of signals discriminating the value of cues. Color indicates the degree of differential firing on high value contralateral versus high value absent trials (top, cyan) and on high value ipsilateral versus high value absent trials (bottom, magenta). For each neuron and within each comparison, firing rate differences within each condition were normalized by their maximum (unsigned) deviation from zero. Only cells with a measurable latency for each comparison were included; green circles indicate latency estimates (2 contralateral and 9 ipsilateral value latencies fall outside the plot). (b) Timing of value discrimination for the population of value selective neurons (n = 193). Average firing rate differences (shading indicates standard error) are plotted along with population value latencies for each comparison. For each neuron, we took the average of the two firing rate difference curves, found the signed peak deviation from zero (to enable averaging across REW- and REW+ neurons), and normalized the curve for each comparison by this value. This normalization maintains the difference in magnitude between the two comparisons.

If the value latency differences we observed could be explained by delays already present in the feed-forward inputs to the amygdala, we would expect a corresponding
delay in the arrival of basic visual information from the ipsilateral visual field (Fig. 2.6a, same source). Alternatively, ipsilateral value information may be even more delayed than basic ipsilateral visual information, indicating that ipsilateral value information is not simply inherited from a delayed feed-forward signal (Fig. 2.6a, different sources). To address this possibility, we recorded the activity of amygdala neurons during a fixation task (Fig. 2.6b) where a single peripheral stimulus appeared either contralaterally or ipsilaterally (7° eccentricity). The fixation task allowed us to determine how visual onset latencies were influenced by spatial location, since a single stimulus, rather than two cues, appeared on every trial. Visual onset latencies for the population of visually-responsive neurons \((n = 32/141, \text{significant response modulation following presentation of contralateral and ipsilateral stimuli})\) did not differ significantly across spatial locations (Fig. 2.6c; bootstrap, \(P = 0.73\)), nor did they differ for those neurons with a measurable latency at both stimulus locations \((n = 19, \text{Paired Wilcoxon, } P = 0.8\)). A direct comparison of individual value latency delays in the operant tasks \((n = 117)\) with individual visual onset latency delays in the fixation task \((n = 19)\) confirmed that the delay in value latencies was larger than that of visual onset latencies (Fig. 2.6d; Wilcoxon, \(P = 0.0008\)). For the 68 neurons recorded in both the fixation tasks and one of the operant tasks (when visual onset latencies and value latencies could be computed for each neuron), the delay in value in ipsilateral value latencies was significantly longer than the delay in ipsilateral visual latencies \((n = 11/68; \mu = 63 \text{ ms}, \text{Paired Wilcoxon, } P = 0.03)\). Despite the dissociation of latencies between the tasks, we did find that spatial location selectivity in the fixation task was a strong predictor of spatial configuration selectivity in the attention task (Fig. 2.7). Thus, amygdala neurons integrated value information across
the visual field, albeit more slowly than expected if this information was simply due to
timing differences in feed-forward inputs.

![Diagram](image)

**Figure 2.6. Latency of visual information is insensitive to spatial location.** (a) Hypothetical latencies assuming that the delay in ipsilateral value information is already present in the feed-forward signal (delay derives from the *same source*) and that ipsilateral visual information and value information come from different sources (delay derives from *different sources*). Visual onset latencies and value latencies are plotted as a function of time relative to cue onset. (b) Fixation task. Monkeys were rewarded for maintaining fixation during the 350 ms cue presentation and for 1000 ms thereafter; the reward magnitude was not dependent on the stimulus location. (c) Population visual onset latencies in the fixation task for contralateral and ipsilateral stimuli. Firing rates were normalized in the same manner as in Figure 2.5b. (d) Mean latencies for the set of cells where the contralateral and ipsilateral visual onset latencies (*n* = 19) and/or the contralateral and ipsilateral value latencies (*n* = 116) could be estimated. Latencies are plotted as in (a); horizontal bars indicate the standard error for the distribution of single cell latencies.
Figure 2.7 Spatial configuration preferences in the attention task are predictive of spatial location preferences in the fixation task. Spatial configuration selectivity in the attention task and spatial location selectivity in the fixation task are plotted for neurons recorded in both tasks (n = 68). We calculated d’ values based on firing rates 100 to 500 ms after cue onset in each task. For the attention task, we compared responses on high value cue contralateral trials with responses on high value cue ipsilateral trials (as in Fig. 2.4b). For the fixation task, we compared responses when the stimulus appeared contralateral to those when the stimulus appeared ipsilateral. We used a weighted least squares regression to assess the relationship between the two sets of d’ values, using the inverse standard error of d’ (determined by bootstrapping) in the fixation task as the weights. The black line indicates the significant relationship between spatial location d’ and spatial configuration d’ (β = 0.44, C.I. 0.28 – 0.59; P < 10^-3, bootstrap analysis). The fact that the regression slope is less than 1 suggests that spatial encoding is weaker in the fixation task than in the attention tasks. The plot style of the data points indicates the significance of d’ values for individual neurons (as indicated by the legend).

Timecourse of signals carried by amygdala neurons. If the conjoint spatial and value selectivity we observed is relevant for influencing behavior, it should be present not only during the presentation of the cues, but also when the monkey makes a discrimination, i.e. when the target appears. We examined this by determining how different signal properties evolved during a trial. For each neuron, we estimated the influence of the experimentally manipulated factors on neural firing rates using a multiple regression carried out in a sliding window relative to cue onset (Fig. 2.8a). In each window, we determined how three factors influenced neural activity: (1) the presence of a
high value cue irrespective of space (spatially non-specific value), (2) the presence of a high value cue contralaterally (spatially specific value), and (3) the use of the different cue sets (stimulus identity). We focused our analysis on the time periods around cue presentation (cue period, 150-450 ms) and in the portions of the delay before (pre-target delay, 500-800 ms) and after (post-target delay, 850-1150 ms) the earliest possible time of target onset (Fig. 2.8b). For both the cue period and pre-target delay, there was a significant influence of all three factors on firing rates (compared to 300 ms prior to cue onset; bootstrap, $P < 0.005$). During the post-target delay, a different pattern emerged; while spatially specific and nonspecific value signals were maintained ($P < 10^{-4}$), the encoding of stimulus identity disappeared ($P = 0.17$). This analysis reveals that both spatially specific and spatially non-specific value signals are sustained into the time period when the target could appear, and could influence how the monkey performs the visual tasks.
Figure 2.8 The encoding of space, value and stimulus identity by amygdala neurons evolves according to task demands. (a) Timecourse of amygdala signals representing spatially specific and non-specific value, as well as stimulus identity, for individual value-selective neurons (n = 193). Color indicates effect size for each factor ($\omega^2$) for individual neurons at times relative to cue onset (100 ms bins shifted by 10 ms). Neurons were sorted according to the onset of spatially-specific value coding, and this ordering was the same in all three plots. White dashed line indicates the time of cue onset; yellow and red arrows indicate the average time of cue offset and first target onset, respectively. (b) Timecourse of signals averaged over the population. Curves depict the mean and standard error (shaded region) of effect size ($\omega^2$) measures across the population. Black bars indicate the time bins used for statistical analysis, and asterisks indicate that the distribution of $\omega^2$ for that time bin was significantly greater than during the baseline period ($P < 0.05$). Red arrow indicates the average time of first target onset.

**Activity correlates with trial-to-trial fluctuations in spatial attention.** Attention waxes and wanes from trial to trial throughout an experimental session and this presumably underlies some of the variability in behavioral measures such as performance and reaction time across trials (Parasuraman and Davies, 1984). If the combined representation of space and value in the amygdala influences the online guidance of spatial attention, trial-to-trial measures of amygdala activity and attentional allocation...
should co-vary. For example, consider a neuron that responds more when the high value
cue is contralateral (REW+ neuron). Individual trials in which this neuron responds more
than average should coincide with those trials in which the animal performs faster than
average contralateral saccades (a classic measure of attention). This pattern of activity
would result in a negative correlation between neural activity and reaction times to the
contralateral field. Moreover, a positive correlation for ipsilateral saccades (increased
activity coinciding with slower saccades away from the contralateral side) would support
a role in spatial attention. By contrast, correlations that are the same sign (both negative)
for ipsilateral and contralateral saccades would suggest a role in non-spatial attention like
alerting or vigilance that could be modulated by changes in arousal level (Davis and
Whalen, 2001; Parasuraman and Davies, 1984).

We examined the trial-by-trial relationship between saccadic reaction times and
amygdala activity around target onset (900 ms before to 100 ms after), focusing on
contralateral and ipsilateral saccade data separately. To determine whether the magnitude
of correlations depended on the value and/or spatial configuration of cues, we analyzed
each trial type separately, yielding a total of 6 conditions (3 trial types x 2 saccade
directions). Finally, just as the sign of value selectivity predicted that of spatial
configuration selectivity, we expected it would also predict the sign of the correlations
between firing rate and reaction time. Therefore, we used a linear regression to
characterize the relationship between neurons’ value selectivity (d’) and their trial-by-
trial relationship with reaction times (Fisher-Z transformed correlation coefficient).
Consistent with spatial attention, but not alerting or vigilance, value selectivity had a
negative relationship with contralateral correlation coefficients (bootstrap, $P = 0.0078$)
and a positive relationship with ipsilateral correlation coefficients ($P = 0.0048$) when the high value cue had appeared contralaterally (Fig. 2.9a). These relationships were not significant when the high value cue was ipsilateral or absent, and the magnitude of the regression slopes on these trial types were significantly smaller than those observed on high value contralateral trials (ANCOVA, $P < 0.005$ for each saccade direction). The relationship between firing rate and reaction time was robust, and remained even after accounting for differences in recent reinforcement outcome history and satiation (Appendix A, Fig. 1). In addition, the distance between eye position at the end of fixation and the target did not explain these results; this distance was not correlated with reaction times ($P = 0.16$), and distances between eye position at target onset and target location did not predict whether the monkey would perform the trial correctly (Wilcoxon, $P = 0.23$). We note that firing rates also predicted whether the monkey performed the trial correctly; however, differences in saccade behavior between hit and miss trials in the two tasks may have influenced these performance results (Appendix A, Fig. 2).
Figure 2.9 Trial-to-trial variations in firing rates are correlated with reaction times.
(a) Relationship between value selectivity and correlations between firing rate and reaction time. Fisher Z-transformed correlation coefficients are plotted as a function of the value selectivity indices for each trial type and saccade direction. The size of the data points indicates the reliability (inverse standard error) of the correlation coefficient. Regression lines are plotted in instances where a significant relationship was observed ($P < 0.05$). (b) Histograms of correlation coefficients on high value contralateral trials for the 25% most value selective neurons (based on the magnitude of $d'$), split according to sign of selectivity (positive: blue histograms; negative: red histograms) and the direction of the saccade (contralateral: left, ipsilateral: right). Filled bars, individual neurons with correlation coefficients significantly different from 0 ($P < 0.05$); inset scatterplots, example cells with significant correlation coefficients (dotted arrow indicate their respective position in the histograms). The solid blue and red arrows indicate the mean of the distributions for positive value and negative value neurons, respectively, and asterisks denote that the mean correlation coefficients for these two groups were significantly different from each other (bootstrap, $P < 0.05$). (c) The difference in correlation coefficients (for contralateral saccades minus ipsilateral saccades) is plotted for cells grouped into quartiles according to the absolute value and sign of value selectivity indices. Vertical bars indicate the standard error. Blue and red filled circles indicate that the distribution was significantly different from zero while green stars indicate that the distributions were significantly different across groups ($P < 0.05$).

The significant relationship between value selectivity and correlation coefficients on high value contralateral trials suggests that neurons with stronger value selectivity may play a more important role in influencing attention. To examine this more closely, we split neurons into quartiles based on their value selectivity magnitude (absolute value
of \( d' \) and then partitioned each group according to the sign of value selectivity. Focusing on the 25% of cells with the strongest value selectivity, Figure 2.9b shows the distribution of correlation coefficients for neurons with positive value selectivity (\( d' > 0 \), blue histograms) and negative value selectivity (\( d' < 0 \), red histograms), with contralateral and ipsilateral saccade data presented separately. Correlation coefficients were significantly different between neurons with positive selectivity and neurons with negative selectivity for both directions of saccades (bootstrap, \( P < 0.005 \)). Additionally, the distributions for ipsilateral and contralateral saccades were significantly different for positive value neurons (bootstrap, \( P = 0.001 \); \( \mu(\text{contra}) = -0.04, \mu(\text{ipsi}) = 0.16 \)), as were the distributions for negative value neurons under the same comparison (\( P = 0.004 \); \( \mu(\text{contra}) = 0.09, \mu(\text{ipsi}) = -0.09 \)). Thus the relationship between firing rate and reaction time differs depending upon the locus of spatial attention as measured by reaction time. Overall, these results support the notion that amygdala firing is correlated with spatial attention and not arousal-related processes, since, unlike arousal effects, spatially specific effects predict differential relationships with reaction time depending upon saccade direction.

The relationship between trial-by-trial firing rate of amygdala neurons and saccadic reaction time was not present for neurons that did not have strong value selectivity, and it was not present unless a motivationally significant cue appeared in the contralateral field. To demonstrate this, we combined data across saccade directions, taking the difference in the Z-transformed correlation coefficients for each neuron (subtracting ipsilateral from contralateral), which, based on the previous analysis, was negative for neurons with strong positive selectivity and positive for neurons with strong
negative selectivity. For high value contralateral trials, correlation coefficient differences were significantly different from zero (bootstrap, \( P < 0.05 \)) only for neurons with the greatest value selectivity; the sign of this effect was opposite for the positive value and negative value groups (Fig. 2.9c). No significant effects were observed for any other groups of neurons on high value contralateral trials or for any group on either high value ipsilateral or absent trials. Interestingly, neurons with the highest value selectivity magnitude also tended to have the widest spike waveforms (Appendix A, Fig. 3), suggesting that these neurons may be projection neurons that influence attentional processing; this effect was not different for neurons with positive and negative value selectivity, and the overall distributions of waveforms widths for these two populations did not differ (Wilcoxon, \( P = 0.66 \)). Together these results suggest that the representation of space and value provided by the amygdala may play a role in spatial attention when highly valuable stimuli appear in the contralateral field and that this influence is mediated by the most value-selective amygdala neurons.

**DISCUSSION**

Motivationally salient stimuli trigger a range of cognitive and emotional responses. These include spatially non-specific processes like arousal or freezing induced by fear, as well as spatially specific responses like orienting attention. Traditionally, the amygdala has been understood as participating in spatially non-specific responses, but not spatially specific ones. Here we show that amygdala neurons encode information about both the spatial configuration of visual stimuli, as well as the rewards predicted by stimuli. Moreover, fluctuations in activity correlated with fluctuations of spatial attention
on a trial-by-trial basis. These results suggest that the amygdala not only participates in spatially non-specific emotional responses, but it may also influence spatially specific cognitive processes, such as the allocation of enhanced cognitive resources to more valuable locations.

Amygdala neurons combine information about space and value such that activity changes to stimuli associated with increasing reward are associated with activity changes related to the spatial configuration of these stimuli. Previous studies showed that amygdala neural responses are sensitive to the reinforcement contingencies of CSs presented over the fovea, with some neurons responding more strongly when a CS predicts a reward as compared to an aversive stimulus, and other neurons having the opposite response profile (“positive” and “negative” value-coding neurons, respectively) (Belova et al., 2008; Paton et al., 2006). In the present experiments, positive (REW+) amygdala neurons, which responded more strongly when a highly valuable cue appeared, also responded most when this cue appeared contralaterally. By contrast, negative (REW-) amygdala neurons responded less to the presence of a highly valuable cue and also responded most weakly when this cue appeared contralaterally. Thus, there is a systematic relationship between spatial and value selectivity in the amygdala. The data suggest that although stimuli presented ipsilaterally or contralaterally may both drive amygdala neural responses, neurons can exhibit either positive or negative excursions in activity to signal the presence of a valuable cue in the contralateral field.

The discovery of a representation of space and value in the amygdala immediately leads one to wonder about the degree of spatial selectivity encoded by amygdala neurons. In the current experiments, we tested for spatial selectivity at the level of the visual
hemifield. We observed that the onset latency of value information was strongly dependent on the locations of outcome-predictive stimuli while visual response latencies were relatively insensitive to spatial location, suggesting that the spatial properties of amygdala neurons may have dynamic features that change depending upon task demands. Indeed, when monkeys directed attention, a signal representing space and value is sustained throughout a trial in the amygdala, long after the visual cues were extinguished. Although spatial selectivity at the level of the hemifield observed during the fixation task predicts spatial selectivity during the operant tasks, these observations indicate that it will be necessary to assess spatial properties in a variety of task contexts. The sheer number of trials required to map spatial properties in different tasks poses a significant experimental challenge for the future. Given the current data, the amygdala may at least be essential for quickly shifting attention to the left or right visual field based on the value of stimuli.

**How does the amygdala’s representation of space and value differ from other brain structures?** The response properties we describe suggest a distinct and perhaps unique role for the amygdala compared to other brain areas that integrate information about space and value. Neurons encoding various aspects of rewarding and punishing outcomes have been discovered throughout the brain (Schultz, 2006), and have been studied extensively by presenting single visual cues associated with different outcomes. Although these studies have shown how outcome-sensitive neurons may be relevant for computing the value associated with specific objects or actions (Schultz, 2006), it is less clear how these neurons respond when there are multiple objects in the environment, particularly in situations where resources must often be divided and allocated.
Experiments using two or more simultaneously presented stimuli associated with different values suggest that some brain areas combine space and value information to encode “action values” (Kable and Glimcher, 2009), the value associated with an available action. Two of the most studied brain structures in this regard are the lateral intraparietal area (LIP) and the dorsal striatum. During the performance of choice tasks both LIP and the dorsal striatum have been described as encoding the value of a choice in space (Kable and Glimcher, 2009; Sugrue et al., 2005). Notably, neurons in these brain structures encode value differently than amygdala neurons. First, neither LIP nor dorsal striatum contain large populations of neurons with a sustained preference for opposite reinforcement valences (like REW+ and REW- neurons) that is systematically related to spatial selectivity preference. Second, unlike in the amygdala, neural responses in LIP are inhibited according to the value associated with competing actions for targets appearing in the opposite hemifield (Louie et al., 2011; Sugrue et al., 2004). Third, dorsal striatal neurons most frequently encode the value of a single action (Lau and Glimcher, 2008; Samejima et al., 2005) or, similar to LIP, a quantity approximating the difference between the values of the two actions (Cai et al., 2011).

The encoding of action value in LIP and the dorsal striatum often coincides with the locus of attention or preparation of an action whose endpoint is the locus of attention (Maunsell, 2004), but some data indicates action value and attention can be dissociated (e.g., (Peck et al., 2009)). In our experiments, the response profiles of amygdala neurons do not simply represent the monkeys’ locus of spatial attention. If they did, we would have observed intermediate neural responses for high value absent trials (where attention is split approximately equally between the two hemifields) relative to trials where the
high value cue appeared contralaterally or ipsilaterally (where attention is heavily biased towards one hemifield or the other). Instead, valuable stimuli in either hemifield modulate activity in the same direction for individual amygdala neurons, indicating that these neurons integrate value information across the visual field in addition to encoding information about the locus of spatial attention.

**How is the amygdala’s representation of space and value created?** In order to allocate cognitive resources to valuable stimuli, “where” and “what” information must converge with stimulus value information. The amygdala is a potential site for this convergence; it receives direct inputs from the ventral visual stream (Freese and Amaral, 2009) and single neurons in the amygdala encode stimulus value (Belova et al., 2008; Paton et al., 2006). The source of spatial information in the amygdala is less clear. Direct projections to the amygdala from spatially selective areas such as the frontal eye fields or parietal cortex or the dorsal striatum are sparse or nonexistent in the primate (Freese and Amaral, 2009), suggesting that the dorsal visual pathway does not contribute directly to the spatial selectivity we observed. A superior colliculus to pulvinar pathway has been proposed as a source of direct subcortical input to the amygdala that would allow rapid processing of emotional information that bypasses slower cortical pathways (Tamietto and de Gelder, 2010). However, we suspect that it does not play a special role in our experiments since the visual onset latencies (> 100 ms) we observed in the amygdala are consistent with visual information arriving through cortical pathways (Pessoa and Adolphs, 2010).

Another possibility is that neurons in the inferotemporal cortex, although not encoding value information (Liu and Richmond, 2000; Rolls et al., 1977), may provide
enough spatial information for amygdala neurons to build the selectivity we observe (DiCarlo and Maunsell, 2003). However, the disassociation between the timing of visual information and value information in the amygdala suggests that these signals arise from different sources. Since the transfer of visual information between hemispheres is fast (~7-20 ms (DiCarlo and Maunsell, 2003; Swadlow et al., 1978)), the relatively long delay (~50 ms) that we observed in ipsilateral value information suggests that it arises from a source other than feed-forward visual pathways. One possibility is that value information is established in the contralateral amygdala and then passed to the ipsilateral amygdala, which would be indirect, since the amygdalae do not project to each other in primates (Demeter et al., 1990). Furthermore, a potential path through prefrontal cortices is likely slow, since amygdala-prefrontal connections are exclusively ipsilateral (Ghashghaei and Barbas, 2002), adding at least one additional synapse between the amygdalae.

Finally, it is also possible that the amygdala inherits its representation of space and value from other brain structures like frontal cortical areas many of which project to the amygdala with varying fiber density (Freese and Amaral, 2009; Ghashghaei and Barbas, 2002; Ghashghaei et al., 2007). It was recently shown that a small subset of neurons in ACC conjointly encodes spatial attention and reward value (Kaping et al., 2011). Projections from this area may carry space and value information to the amygdala; however, it is unclear from this data whether these neural sites overlap with those that connect strongly to the amygdala since projections to/from the amygdala are largely confined to the ventral portion of ACC (Ghashghaei et al., 2007). Additionally, these authors did not report that representations of space and value were combined systematically. Finally, joint selectivity for space and value was generally slower than we
observed in the amygdala, suggesting the possibility that this information in ACC may in fact depend on direct/indirect inputs from the amygdala.

**How might the amygdala influence spatial attention?** Our data demonstrate that amygdala neurons can encode relevant locations in space defined by arbitrary stimulus-outcome associations. This combined representation of space and value could influence a number of processes, including decision-making based on the location and value of choice options, as well as the allocation of cognitive resources, such as the enhancement of attention. The operant tasks we employed allowed us to evaluate how amygdala activity is related to the engagement of attention.

If the amygdala influences spatial attention, then the relationship between amygdala neural responses and quantitative measures of spatial attention would depend upon the spatial location of the attended stimuli. Using reaction time as a measure of spatial attention, we found that for REW+ neurons, higher activity during the delay period predicted shorter reaction times to targets in the contralateral visual field, and longer reaction times to targets in the ipsilateral field. REW- neurons’ neural activity made the opposite predictions with the same measures of spatial attention. If amygdala activity simply relates to spatially non-specific arousal, then higher activity should have had the same relationship with reaction time regardless of the saccade direction. Instead, our results suggest that the combined representation of space and value in the amygdala is related to the allocation of spatial attention, and the amygdala may therefore play a role in spatially-specific responses, as well as spatially non-specific responses, to motivational stimuli. Moreover, the fact that this correlation between neural activity and reaction time is present during the delay period, long after cues have disappeared from view, suggests
that the amygdala represents cognitive information, and not merely perceptual information. This could be used for movement preparation, but amygdala neural responses related to saccadic execution have not been reported.

Of course, signals representing space and value from the amygdala may not actually influence spatial attention, as signals from other brain structures representing spatial attention could modulate the representation of space and value we describe in the amygdala. However, our suggestion that amygdala neural activity may itself influence the allocation of spatial attention may explain why electrical stimulation of the amygdala elicits orienting responses (Ursin and Kaada, 1960a) similar to those observed during attentive states and why amygdala lesions result in conditioned orienting deficits (Holland and Gallagher, 1999). We suspect that in our tasks the amygdala is not directly producing movements per se because the monkeys were extensively trained to covertly attend while fixating. Instead, we believe that the amygdala could influence a number of brain systems involved with enhancing sensory processing. The amygdala could influence attention via direct projections to cortex, including lower level sensory areas (Freese and Amaral, 2009), as well as subcortical areas involved with attention, including the basal forebrain (Holland and Gallagher, 1999). These connections could help explain the increased activation of visual cortex to stimuli associated with reward or punishment (Armony and Dolan, 2002; Padmala and Pessoa, 2008), and the observation that amygdala damage reduces activation to fearful faces in a hemisphere-specific manner (Vuilleumier et al., 2004). Without knowing the topography of the projections from the amygdala back to the sensory cortices, it remains unclear whether amygdala enhancement of cortical processing occurs at the hemifield level, constrained by the predominantly
ipsilateral projections to the visual cortices (Freese and Amaral, 2009), or in more spatially-specific manner.

**Conclusion.** We showed that the amygdala links stimulus-outcome associations with their spatial relevance. Our results suggest that the amygdala may influence how a subject attends to valuable stimuli, with the two amygdalae competing to influence spatial attention when location is relevant and working in concert to increase vigilance when location is irrelevant or uncertain. Our findings may provide insights into neuropsychiatric disorders such as autism and schizophrenia where amygdala dysfunction is believed to underlie deficits in orienting attention according to emotionally relevant stimuli (Baron-Cohen et al., 2000; Pinkham et al., 2008). Thus the amygdala, long recognized as a critical coordinator of emotion, may also play a key role in representing emotional information in space, allowing it to influence spatially-specific cognitive responses to the emotional world.

**METHODS**

Three rhesus monkeys (*Macaca mulatta*, 8-13 kg) were used in these experiments. All experimental procedures complied with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committees at the New York State Psychiatric Institute and Columbia University. Prior to training, each animal was surgically implanted with a plastic headpost secured to the skull using ceramic bone screws. Surgery was conducted using aseptic techniques under isoflurane anesthesia, and analgesics and antibiotics were administered postsurgically. After behavioral training, we acquired T1-weighted MRIs with fiducial markers fixed to the
headpost. In a second surgery, the MRI and fiducial markers were registered intra-surgically (Brainsight, Rogue Research, Quebec, Canada), allowing us to accurately implant a plastic recording chamber over the amygdala based on the MRI for each monkey. We recorded the final position of the recording chambers and used these coordinates to the guide electrode placement during experiments. We logged the inferior/superior, anterior/posterior, and medial/lateral position of each recorded neuron to generate a 3D reconstruction using Brainsight software (Fig. 2). Although precise localization of all neurons to particular nuclei is not possible, using the MRIs and atlases, we estimated that 297 neurons in our study were located in the basolateral complex, and 29 neurons were located in the central nucleus.

**Behavioral task.** Monkeys were seated and head-restrained in darkened sound-attenuating booths during experiments and were operantly conditioned using liquid rewards. The monkeys performed one of two tasks designed to assess how reward expectations influenced attention. In both tasks, trials began with the monkeys fixating a central spot; the fixation window had a radius of 1.88° ± 0.03° in task A and 1.35° ± 0.03° in task B. In task A (monkeys L & O), a Gabor patch (sinusoidal grating windowed by a Gaussian) served as the target and then appeared at one of the two locations between 400 and 4000 ms after cue offset (truncated exponential distribution) for 50 ms. The monkeys were required to saccade to the location of the target between 100-600 ms after its onset. Because the interval during which the target could appear was long and the reaction time window was relatively short, chance performance was about 23%. In task B (Monkey C), the orientation of two gabor patches changed at a random time 350 to 1350 ms after their appearance (chosen from an truncated exponential distribution). Following
a target duration of 80-120 ms (adjusted online according to the monkey’s overall performance), the Gabor patches were then masked for 60 ms. Finally, two choice targets appeared around one of the two locations (4.2° away), indicating which Gabor patch was the target stimulus. The monkey was then required to saccade to one of the choice targets to indicate whether the target Gabor patch was more horizontal or more vertical (50% chance performance). The monkey had been trained that choosing the counterclockwise target indicates that the stimulus was more horizontal, and that choosing the clockwise target indicates that the stimulus was more vertical. In both tasks, trials were repeated when the monkeys made premature saccades. When the monkey failed to make a correct discrimination after the target appeared, the trial type was chosen at random on the next trial, just as we did when the monkey correctly performed the discrimination. Cues were colored rectangles (Task A, 2.25 deg.² at 7° eccentricity) or circles (Task B, 0.5° diameter at ~3° eccentricity), and we randomly interleaved two distinct sets of cues associated with the same outcomes.

We also trained two of the three monkeys (monkey O and C) to perform a simple fixation task. After fixating a central point for 500 ms, a plaid grating (1.5° SD Gaussian window) appeared either to the left or right of the fixation point (7° eccentricity) for 350 ms. The monkeys were required to maintain fixation for an additional 1000 ms to complete the trial and obtain a reward (delivered after an additional 500 ms delay).

Eye position was monitored using an infrared camera and digitized at 1000 Hz (SR Research, Ontario, Canada). Reaction times were defined as the beginning of the saccade detected by using a velocity/acceleration-based algorithm. Visual stimuli were
generated using EXPO (Center for Neural Science, New York University), and were displayed on a CRT monitor positioned 61 cm away from the monkey.

**Electrophysiology.** Recordings from single amygdala neurons were made through a surgically implanted plastic cylinder affixed to the skull. Three to eight electrodes were individually lowered into the left (monkeys O and L) or right (monkey C) amygdala using a multiple electrode microdrive (NaN Instruments, Nazareth, Israel). Extracellular activity was recorded using tungsten electrodes (2MΩ impedance at 1000 Hz; FHC Inc., Bowdoinham, ME). Analog signals were amplified, bandpass filtered (250-7500 Hz), and digitized (30000 Hz) for unit isolation (Blackrock Microsystems, Salt Lake City, Utah). Single-units were isolated offline using waveform principal components (Plexon Offline Sorter, Plexon, Dallas, TX).

**General data analysis.** We used two-tailed statistical tests in all instances. For all bootstrap analyses, we randomly re-sampled with replacement to obtain replications with the same size as the original data set; this involved choosing a random subset of cells for population analyses, or a random subset of trials for analyses on individual neurons. Random sampling was repeated at least 10000 times for each bootstrap analysis. Comparisons were significant if > 97.5% of the bootstrap distribution fell on the same side of the null hypothesis or if the test statistics in one condition were greater than those in the other in > 97.5% cases (both equivalent to a two-tailed test at \( \alpha = 0.05 \)). Non-parametric Wilcoxon tests were performed on unpaired data (rank-sum test) unless specified otherwise (sign-rank test). Neurons were defined as task-responsive if firing rates around the onset of the fixation point (100-600 ms), the cue (100-800 ms), the target (100-300 ms), or the reward (0-400 ms) were differed significantly from baseline (1000
ms before fixation point onset; Paired Wilcoxon, \( P < 0.05 \)). For selectivity indices, we computed:

\[
d' = \frac{\mu_1 - \mu_2}{\sqrt{\frac{(SS_1 + SS_2)}{(df_1 + df_2)}}}
\]

where \( \mu_X \) is the mean firing rates, \( SS_X \) is the sum of squares, and \( df_X \) is the degrees of freedom (number of trials minus one) for each condition. Behavioral and neural data was similar across cue sets, so the data were combined except where noted.

**Reaction time correlation analysis.** We calculated correlation coefficients between amygdala activity around target onset (900 ms before to 100 ms after) and saccadic reaction times. Before calculating each correlation coefficient, we subtracted the mean firing rate and reaction time for each cue set individually to ensure that any neural and/or behavioral differences between each cue set did not produce any across-group correlations. After mean subtraction, we calculated the correlation coefficients and applied the Fisher-Z transformation. To assess the relationship between value selectivity (\( d' \); same values as in Fig. 3B) and the correlation coefficients, we used a least squares regression weighted by the inverse standard errors of the Z-transformed correlation coefficients; significance was determined by a bootstrap analysis in which the set of cells for each resample was chosen randomly with replacement. Only correlation coefficients based on at least 15 trials were included for analysis; this resulted in a set of 274, 105, and 228 cells for contralateral saccades and 165, 215, and 208 cells for ipsilateral saccades (for high value cue contralateral, ipsilateral, and absent trials, respectively). We obtained similar results when we restricted the analysis of each trial type to the same set of cells (\( n = 83 \); those with 15 trials for each trial type and saccade direction).

**Timecourse analyses.** We sought to determine how three factors influenced neural activity as a function of time: (1) the inclusion of an high value cue irrespective of
space (spatial non-specific value), (2) the inclusion of an high value cue contralaterally (spatially specific value), and (3) the use of the different cue sets (stimulus identity). For each factor, we computed the effect size, $\omega^2 = (SS_A - df_A \times MSE) / (SS + MSE)$, where $SS_A$ is the across group sum of squares for factor A, $df_A$ is the degrees of freedom for factor A ($df_A = 1$ for all factors in our model), SS is the total sum of squares, and MSE is the mean squared error of the model. The spatially non-specific value component of this multifactor analysis differs from the $d'$ value selectivity index: a neuron that responds only on high value contralateral trials would result in $\omega^2 > 0$ only for the spatial specific value signal but would have $d' > 0$ for both spatial selectivity and value selectivity (with the spatial selectivity index roughly double in magnitude). The significance of values in each time bin was determined by comparing against those in the baseline interval (200 ms before cue onset; bootstrap). In comparison to the effect size measure $\eta^2$ (proportion of total variance explained), $\omega^2$ tends towards zero when the explanatory power of the factor is weak and does not exhibit a positive bias for increasingly small sample sizes (Olejnik and Algina, 2003). This was essential for analyzing cue-triggered responses because individual trials were truncated at the time of target onset, which occurred at a random time and resulted in progressively fewer trials available for analysis at increasingly later time times in the trial. Replotting Figure 6 using the $\eta^2$ measure yields a similar result except the values are biased upwards at later time points.

**Latency analyses.** For all instances where we computed value or visual onset latencies, we defined the latency as the first of 25 (population latencies) or 15 (individual neurons latencies) consecutive bins (30 ms bins slid by 2 ms) for which the comparison
of interest was significant \((P < 0.05)\). We used a bootstrap analysis to test for differences in population latencies.

For population value latencies, we first computed the difference in firing rates between high value contralateral trials OR high value cue ipsilateral trials with firing rates on high value absent trials for each value-selective neuron \((n = 186)\). Firing rate differences across were combined across REW- and REW+ by (1) subtracting any baseline firing rate differences (500 ms before cue onset) and (2) dividing by the signed peak deviation from zero during the signal period. The peak deviation from zero was based on the average of the two difference curves in order to illustrate the difference in their magnitude; the same results were obtained when the difference curves were normalized to reach the same asymptotic value. Neural discrimination in each post-cue time bin was tested against zero (Wilcoxon).

We used an analogous analysis to determine visual onset latencies in the fixation task for the population of stimulus-responsive neurons (compare firing rates 100-300 ms after stimulus onset versus 500 ms before stimulus onset; Paired Wilcoxon, \(P < 0.05\) for both locations). This included 32 neurons of which 19 had excitatory responses, 11 had inhibitory responses, and 2 had responses of opposite sign for the two cue locations. Again, we baseline subtracted, peak normalized, and sign-corrected the raw firing rates in order to average over neurons with excitatory and inhibitory responses; here, the peak response was based on the average of the responses to contralateral and ipsilateral stimuli. Visual onset latencies for contralateral and ipsilateral stimuli were estimated in the same way as for value latencies.
For individual visual onset latencies, we compared firing rate distributions at each time bin (50-500 ms after cue onset) against the distribution of all baseline time bins (500 ms before cue onset, Wilcoxon). For individual value latencies, we compared firing rate distribution on trials where the high value cue was contralateral or trials where the high value cue was ipsilateral with trials when the high value cue was absent. The analyses of visual onset latencies and value latencies were limited to set of stimulus-responsive and value-selective neurons, respectively, as in the population analyses. We used a fairly stringent criterion to ensure that the measured latencies were accurate; as a result, value latencies and visual onset latencies could only be computed for a subset of value-selective ($n = 116/186$) and stimulus-responsive ($n = 19/32$) neurons, respectively.

**AUTHOR CONTRIBUTIONS**

Brian Lau devised one version of the attention tasks (Fig. 2.1, Task B) and collaborated in designing the fixation task (Fig. 2.6b). Brian collected all data from Monkey C and advised in analyzing the complete data set. He initially suggested writing a manuscript regarding the spatial properties of amygdala neurons that we had identified and co-wrote the manuscript (Peck et al., 2013) that is reproduced here.
CHAPTER 3. Amygdala neurons reflect spatial attention biases towards both appetitive and aversive stimuli

INTRODUCTION

In every day life, we routinely encounter stimuli associated with appetitive and aversive outcomes. Optimal behavior depends on our ability to use these stimulus-outcome associations to guide our allocation of attention, which subsequently improves sensory processing of behaviorally relevant stimuli (Anderson et al., 2011) and quickens the purposeful actions that may be necessary in responding them (Posner, 1980).

Despite the plethora of research regarding the systems controlling visual attention and value processing, relatively little has been done to investigate how these two systems interact, especially in the primate brain. Research on this matter has been often complicated by an inability to separate stimulus value and the amount of attention paid to that stimulus (Maunsell, 2004). Recent work suggests that emotion can have a significant effect on visual processing (Phelps et al., 2006) and that conditioned stimuli can bias spatial attention (Peck et al., 2009). Thus, it stands to reason that value and attention networks in the brain must interact in some way such that visual attention can be deployed to stimuli of motivational significance.

One potential site for this integration of value and spatial information is the amygdala. A substantial body of literature has documented the amygdala's importance in processing both aversive (Campeau and Davis, 1995) and appetitive (Holland and Gallagher, 1993) associations between stimuli and outcomes, and individual amygdala neurons have been to shown to encode both types of associations (Belova et al., 2007;
More recent work has shown that an intact amygdala is vital for guiding gaze towards emotionally-relevant stimuli (Adolphs et al., 2005) and mediating preferential BOLD responses to emotionally-charged stimuli (Vuilleumier et al., 2004). This latter result is in line with the presence of projections from the amygdala to areas in the ventral visual stream (Amaral and Price, 1984; Iwai and Yukie, 1987) that have been well studied in the context of attention (Reynolds and Chelazzi, 2004). Further suggesting a role in attention, we have recently demonstrated that, in addition to being modulated by the overall value of stimuli, firing rates of individual amygdala neurons reflect the location of the more valuable stimulus, which is inescapably tied to where monkeys allocate spatial attention (Peck et al., 2013).

In our previous report (Peck et al., 2013), we analyzed how spatial factors influenced amygdala firing rates when attention was governed only by the amount of reward a stimulus predicted; here, we sought to determine whether amygdala spatial selectivity was similar for aversive stimuli that also attracted attention. We found that neurons whose firing rates were greater when an appetitive stimulus appeared in the visual field contralateral to the recording site also tended to fire more when a aversive stimulus appeared in the contralateral field; a separate population of neurons showed the same consistency, albeit with decreases in firing rate. This preferentially coding of both appetitive and aversive stimuli, relative to neutral stimuli, was also apparent in a reanalysis of data obtained while monkey performed a trace-conditioning paradigm (Paton et al., 2006). Together, these results present a unified framework of coding for appetitive and aversive stimuli in the context of both spatial and non-spatial forms of attention.
RESULTS

Appetitive and aversive stimuli attract attention. To determine how spatial attention deployment to both aversive and appetitive stimuli influenced the firing rates of amygdala neurons, we trained two monkeys on a value-biased detection task (Fig. 3.1a). While monkeys maintained fixation, they were briefly presented (300 ms) with two visual cues that appeared on either side of the fixation point. Following cue offset, a delay period ensued, and at a random time, a barely-perceptible target appeared (50 ms) at the same location as one of the two cues. The monkeys completed the trial correctly (a 'hit') by saccading to the location of the target within 600 ms; on 'miss' trials, the monkey either failed to make a saccade at all (61% of miss trials), made a saccade to the opposite location (28%), or made a saccade elsewhere (11%).

We interleaved three types of cues in our experiments (Fig. 3.1b): (1) a reward (R) cue which indicated an opportunity to obtain a drop of juice, (2) a punishment (P) cue which indicated an opportunity to avoid an air puff, and (3) a neutral (N) cue which predicted no outcome for either hit or miss trials; the location of the target was selected randomly and indicated which cue-outcome association was 'in play'. Two different cues were randomly chosen to appear on each trial, resulting in 3 trial types (Fig. 3.1c; R/P, R/N, & P/N), and two distinct cue sets were interleaved to control for any neural or behavioral preferences specific to a given cue's appearance, and this same set of 6 cues was used throughout data collection. The spatial configuration of the cues was also chosen at a random on each trial.
Figure 3.1 Detection task design. (a) Task schematic. After fixating, a pair of cues appeared at either side of the fixation point. Following a variable delay, a target appeared at one of the two locations; trials were scored a ‘hit’ if monkeys saccaded to the target’s location and a ‘miss’ if they failed to do so. (b) Association between cues and outcomes. The table illustrates the outcomes associated with each cue type (given that the target appeared at that cue’s locations) on hit and miss trials. (c) Trial types. On a given trial, monkeys viewed a reward and punishment cue (R/P), a reward and neutral cue (R/N), or a punishment and neutral cue (P/N).

The monkeys understanding of these associations was clear from behavioral metrics and indicated that the monkey paid more attention to cues that predicted an opportunity to obtain a reward or avoid a punishment as compared to a performance-independent neutral outcome; hit rate (Fig. 3.2a) was greater, reaction time was shorter (Fig. 3.2b), and false alarm frequency was greater (Fig. 3.2c; frequency of saccades to a cue location before the target appeared) when the target appeared at the R-cue location relative to either the P-cue or N-cue location (R/P & R/N trials) as well as when the target appeared at the P-cue location relative to the N-cue location (P/N trials). Thus, obtaining a reward was clearly of the greatest importance for the monkeys, but they also preferred
to avoid an air puff rather than responding to a target that resulted in no outcome either way.

![Figure 3.2](image)

**Figure 3.2 Monkeys allocate attention according to stimulus-outcome associations in a detection task.** (a) Hit rate varied according to the cue-outcome associations. The hit rate for each trial type (separated by the dotted lines) and target condition (connected by the solid lines) are plotted; green asterisks indicate a significant difference between each pair of target conditions (Paired Wilcoxon, $P < 10^{-3}$; 73 sessions). (b) Reaction times. (c) False alarm frequency.

**Consistent spatial selectivity for appetitive and aversive stimuli.** During task performance, we recorded the extracellular action potential of 185 single units (SUA) and 160 multi-unit sites (MUA) from the left amygdala of two monkeys (monkey L: 48 SUA, 44 MUA; monkey O: 137 SUA, 116 MUA). Here, we focused on firing rates 400 to 700 milliseconds after cue onset in order to (1) isolate attention signals that were not dependent on the presence of the cues and less likely to be influence by the cue’s visual properties (see Fig 2.8), and (2) avoid any response modulation do the appearance of the target, which always occurred later than 700 milliseconds. For all forms of selectivity, we used a receiver-operator characteristic analysis (ROC) to compare firing rate distributions across trial conditions.

To assess neurons’ spatial selectivity for the reward cue we combined data from R/P and R/N trials (Fig 3.3a) and focused on the comparison between when the R-cue
appeared contralaterally (R-contra) to the recording site and when it appeared ipsilaterally (R-ipsi). The location of the reward cue has a strong influence on firing rates such that some neurons fired more when R-cue was contralateral and other fired more when it was ipsilateral (Fig. 3.3b). Overall, many sites responded differentially on R- contra and R-ipsi trials (130 spatial-reward selective sites; bootstrap, \( P < 0.05 \)) and included those that had significantly greater (ROC > 0.5, 68 sites) or lesser (ROC < 0.5, 62 sites) firing rates when the R-cue appeared contralaterally. From these example neurons (Fig. 3.3b), it’s clear that the overall reward predicted by the cues influenced firing rates as well; firing rates were significantly greater for 82 sites (reward selectivity index, ROC > 0.5) when the R-cue was presented (R-present trials) than when it was absent (R-absent trials), and 87 sites showed significant selectivity in the opposite direction (ROC < 0.5). Consistent with our previous results (Peck et al., 2013), we found a strong, positive relationship between reward selectivity and spatial-reward selectivity (Figure 3.4a; weighted least squares regression and bootstrap, \( \beta = 0.60, P < 10^{-3} \)).

To quantify spatial selectivity for the punishment predicting cues, we compared firing rates on P/N trials according to whether the P-cue appeared contralaterally (P-contra) or ipsilaterally (P-ipsi). Neurons fired differentially for P-contra and P-ipsi trials (29 spatial-punishment selective sites; bootstrap, \( P < 0.05 \)) and included those that fired significantly more (ROC > 0.5, 12 sites) or less (ROC < 0.5, 17 sites) on P-contra trials. While the population of spatial-punishment selective sites was significantly smaller than the population of spatial-reward selective sites (\( \chi^2 \)-test, \( P < 10^{-4} \)), the relative frequency of sites with positive- and negative-selectivity was similar (\( \chi^2 \)-test, \( P = 0.29 \)).
Figure 3.3 Amygdala firing rates are modulated by the spatial location of appetitive and aversive cues. (a) Grouping of trial types for the purpose of neural analyses. (b) Example neuron firing rates as a function of time relative to cue onset. Firing rates are plotted for the four trial types (illustrated in diagram). Spatial-reward selectivity and spatial-punishment selectivity were significant (bootstrap, \( P < 0.05 \)) for each example neuron (ROC > 0.5, left; ROC < 0.5, right).

The critical comparison for our data was to determine whether a neuron’s spatial selectivity for reward cues was predictive of its spatial selectivity for punishment cues. To this end, we looked at the linear relationship between spatial-reward selectivity indices and spatial-punishment selectivity indices. A positive relationship between these indices would suggest that spatial attention has the predominant influence over firing rates such that neurons that fire more (less) on R-contra trials should also fire more (less) on P-contra trials, given that attention was biased contralaterally in each case. On the other hand, a negative relationship would imply that the value of the contralateral stimulus (on a 'good-to-bad' scale) has a stronger influence, where firing rates on R-contra and P-contra trials would be on the opposite ends of the spectrum. In line with the first hypothesis, we observed a clear positive relationship between spatial selectivity for appetitive and aversive cues (Fig. 3.4b; weighted least squares regression and bootstrap, \( \beta = 0.10, P = 0.002 \)). This result was true for both SUA and MUA, as well as for each monkey (Appendix B, Fig. 1). This was also apparent for individual recording sites; of those sites with significant spatial-reward and spatial punishment selectivity (\( n = 19 \)), the
sign of selectivity was the same for 15 (79%; Binomial-test, $P = 0.0192$). Thus, amygdala neurons signal where attention is deployed in a manner irrespective of valence and consistent with attention.

**Figure 3.4** Amygdala neurons exhibit consistency between reward selectivity, spatial-reward selectivity, and spatial-punishment selectivity. (a) Reward selectivity and spatial-reward selectivity are positively correlated ($P < 10^{-3}$). Selectivity indices are plotted for each recording site ($n = 345$), and plot style indicates the significance of each (see legend). (b) Spatial-reward selectivity and spatial-punishment selectivity are positively correlated ($P = 0.002$).

Given a particular spatial configuration, we found that the cue that appeared along with the R-cue on R/P & R/N trials had little influence on firing rate. Only 22 (6.4%) & 18 (5.2%) sites discriminated between R/P & R/N trials when the R-cue was either contralateral or ipsilateral, respectively (bootstrap, $P < 0.05$). This may be because the strong bias in attention towards the R-cue on these trials (see Fig. 3.2) essentially trumped any neural discrimination between the P-cue and N-cue. Despite the weak selectivity for these comparisons, we did find that sites that tended to fire more (less) on
P-contra trials relative to P-ipsi trials (spatial-punishment selectivity) also tended to fire more (less) on R/P trials relative to R/N trials when the R-cue was ipsilateral (Fig. 3.5; weighted least squares regression and bootstrap, $\beta = 0.10, P = 0.027$). Each comparison is consistent with more attention be drawn towards the contralateral hemifields by the P-cue and explains the positive correlation we observe. Given the weakness of these effects, we combined data from R/P and R/N for the remaining analyses of neural data (as in Fig 3.3).

**Figure 3.5** Contralateral punishment cues have a similar effect on firing rates for R-absent (x-axis) and R-present trials (y-axis). Selectivity indices on the x-axis are the same as those on the y-axis of Figure 3.4b. For selectivity indices on the y-axis, we compared R/P and R/N trials where the R cue appeared ipsilaterally. Plot style indicates the significance of selectivity indices (see legend), and the significant regression line is plotted ($P < 0.05$).

*Amygdala neurons reflect the salience of cue-outcome associations.* The results outlaid so far are also relevant in determining whether neurons in the amygdala respond to the ‘value’ or ‘salience’ of the associations between stimuli and outcomes. While it is
clear the neural responses are consistent with attention, the fact that attention is drawn towards the punishment cue (relative to the neutral cue) might be (1) because it is aversive, and the monkey wants to avoid an air puff, or (2) because the monkey finds the act of avoiding a punishment to be inherently rewarding. If the monkey truly does find the P-cue aversive, then the results would unequivocally argue for salience coding in the amygdala; however, if the second possibility were true, it would be impossible to reach a conclusion from this data since the P-cue would exceed the N-cue in terms of both salience and value.

To determine whether monkeys might prefer the chance to avoid an air puff rather than working for a behaviorally-irrelevant outcome, we looked at the rate at which monkeys’ aborted trials for each trial type. Amongst those trials where the monkey left the fixation window before the target appeared, some saccades were directed at one of the two cue locations (false alarms; Fig. 3.2c) while others were directed elsewhere (aborts). While we suggest that false alarms reflect a monkey’s desire to detect a target at a given location, aborts likely reflect that monkey’s willingness to complete a particular trial type. By this logic, we expected that abort rate should be highest on the least valuable trials (P/N) and lowest on the most valuable trials (R/N). For each monkeys (Fig. 3.6), we found that the inclusion of the R-cue tended to decrease the frequency of aborts (compare R/P vs. P/N trials; Bonferroni-corrected $\chi^2$-test, $P < 10^{-4}$ each for monkey) while the inclusion of the P-cue tended to increase the frequency of aborts (compare R/P vs. R/N trials; $P < 0.05$ for each monkey). This behavior was apparent during distinct portions of the trial for each monkey; monkey O exhibited this pattern for aborts around the time that the cue was on (0 - 300 ms after cue onset), while monkey L exhibited it for aborts during
the subsequent delay (300 - 1000 ms after cue onset). Abort frequency therefore correlated with the overall reinforcement value of the cues where R/N trials are the most valuable and P/N trials are the least valuable. Thus, these behavioral results suggest that monkeys do in fact find the punishment cue aversive.

Figure 3.6 Monkeys break fixation in proportion to the reinforcement value of the cues. Fixation break frequency is plotted for each trial type for monkey L (left) and monkey O (right). Time windows are relative to cue onset, and green asterisks indicate the significance of comparisons (\( P < 0.05 \)).

While the act of saccading to a target in order to avoid an airpuff might be rewarding in itself, the data the we have analyzed are at times before that appearance of the target; therefore, it was uncertain whether the monkey would successfully or unsuccessfully avoid the air puff at these time points. Supporting this argument, we found that the relationship between spatial-reward selectivity and spatial-punishment selectivity was similar for trials on which the monkey missed the target appearing at the P-cue location as compared to when he successfully detected it. In each case, we observed a positive relationship (Fig. 3.7; weighted least squares regression and bootstrap, \( P < 0.05 \) for each) that was not significantly different between successful avoidance and
unsuccessful avoidance trials ($\beta$(successful) = 0.16 vs. $\beta$(unsuccessful) = 0.15; bootstrap, $P = 0.96$).

**Figure 3.7 Spatial-punishment selectivity does not depend on performance.** Spatial-reward selectivity and spatial-punishment selectivity indices are plotted for successful (left) and unsuccessful (right) punishment avoidance trials. Selectivity indices were positively correlated in each case ($P < 0.05$) and the slope of the regression line did not differ between hit and miss trials ($P = 0.96$).

**Preferential coding of appetitive and aversive stimuli during trace conditioning.**

To determine whether amygdala neurons encode salience when punishment cues indicated an unavoidable air puff, we reanalyzed data from a trace-conditioning paradigm (Paton et al., 2006). In the trace task (Fig. 3.8), an individual cue was presented on each trial (300 or 350 ms) and predicted whether the monkey would get a liquid reward, and air puff, or no outcome at all (1500 ms after cue offset). At a random time during each experimental session, the associations for the cues that initially predicted reward and punishment were reversed in order to control for the image properties of the stimuli; no
such control was included for the cue predicting no outcome, the implications of which we discuss below.

![Figure 3.8 Trace conditioning task.](image)

**Figure 3.8 Trace conditioning task.** Monkeys initiated trials by fixating after which a single cue appeared centrally. Monkeys were then free to move their eyes and later received the outcome predicted by the cue. At a random trial within the session, the associations of the reward-predicting and punishment-predicting cues were reversed. Figure from (Paton et al., 2006).

Here, we made two critical comparisons: the difference in firing rates in response to a reward cue as compared to a neutral cue (reward selectivity) and the difference in response to a punishment cue as compared to a neutral cue (punishment selectivity). We quantified the magnitude of selectivity using an ROC analysis on firing rates in the cue period (90 after cue onset to 90 ms after cue offset) and the trace period (90 ms after cue offset to outcome onset) as in (Paton et al., 2006) and determined the significance of selectivity for each individual neuron ($n = 218$; bootstrap, $P < 0.05$). For both forms of selectivity, ROC values greater than 0.5 indicate greater firing rates in response to the reward/punishment cue relative to the neutral cue, and ROC values less than 0.5 indicate greater firing rates for the neutral cue. Neurons for which the sign of selectivity (greater or less than 0.5) was the same for these two comparisons are indicative of salience coding.
(Fig. 3.9a), while those with opposite signs of selectivity are consistent with value coding (Fig. 3.9b). Overall, we found salience neurons to be significantly more prevalent in the population than value neurons (49, 48 salience neurons and 8, 13 value neurons in the cue and trace epochs, respectively; $\chi^2$-test, $P < 10^{-5}$ for each epoch), and the proportion of value neurons did not exceed chance levels ($P > 0.29$ for each epoch). The population of salience neurons included those that fired more ($n = 32, 28$ in the cue and trace epochs, respectively) or less ($n = 17, 20$) in proportion to cue salience. On the population level, we found a strong positive relationship between reward and punishment selectivity indices that was significant both during the cue and trace periods (Fig. 3.10a; weighted least squares regression and bootstrap, $P < 10^{-3}$), thus confirming our individual neuron results.

**Figure 3.9 Example neurons firing rates during trace conditioning.** (a) Firing rate as a function of time relative to cue onset. Firing rates were either positively related to (top) or negatively related to (bottom) cue salience. (b) Same as (a) for neurons that reflected the value of the cues. For all example neurons (a,b), reward selectivity and punishment selectivity were significant in either the cue epoch, trace epoch, or both (bootstrap, $P < 0.05$).
These results could potentially be confounded by the fact that the neutral cue does not reverse in the task. If amygdala neurons track the associability of cues in terms of the Pearce-Hall model (Pearce and Hall, 1980), which has been suggested for rat amygdala neurons (Roesch et al., 2010), then firing rates should be modulated both when cue-outcomes are learned initially and when associations are violated at the time of the reversal. Since associations are violated only for the reward and punishment cues, we reasoned that an artifactual salience signal might appear after the reversal simply because the neutral cue wasn’t reversed (Fig. 3.10b). However, when we considered the data from pre- and post-reversals trials separately, we found a positive correlation between reward selectivity and punishment selectivity in each case (Fig. 3.10c; $P < 10^{-3}$) suggesting that differences in cue associability could not account for our results.
Figure 3.10 Amygdala firing rates are preferentially modulated by appetitive and aversive cues during trace conditioning. (a) Reward selectivity and punishment selectivity in the trace-conditioning task are positively correlated. Selectivity indices are plotted for each neuron ($n = 218$), and plot style indicates their significance (see legend). Regression lines were significant and positive for firing rates during the cue epoch (top) and trace epoch (bottom; $P < 10^{-3}$). (b) Hypothetical evolution of cue associability as a function of trial number. (c) Same as (a) for data before (left) and after (right) the reversal.

**DISCUSSION**

In our task, attention was allocated according to the importance of cue-outcome associations; monkeys exhibited a clear bias towards spatial locations that represented the possibility to obtain reward or avoid punishment relative to those that predicted no outcome (Fig. 3.2). Amygdala neurons reliably reflected which hemifield attention was
allocated towards regardless of whether attention was pulled there by appetitive or aversive stimuli (Fig 3.3, 3.4).

These results are consistent with the idea of 'salience coding' in that neural responses are preferentially modulated by stimuli associated with either positive or negative outcomes relative to neutral (or less-relevant) outcomes. In our task, it was possible that monkeys found punishment avoidance to be rewarding, thereby making it impossible to determine whether the neural responses outlined here reflect value, salience, or both; however, we find this explanation to be lacking for a number of reasons. Firstly, it is unlikely that the monkey would choose to have the punishment cue appear on a given trial; given the frequency with which they aborted trials (Fig. 3.6), it is apparent that monkeys preferred to work on R/N trials as compared to R/P trials suggesting that they do find the punishment cue aversive. In terms of the unconditioned stimulus itself, monkeys clearly prefer to avoid air puffs (Amemori and Graybiel, 2012), leaving little doubt that it is aversive. Secondly, even if the monkey does find successful avoidance of the punishment to be rewarding, this would not be apparent until later in the trial, particularly, after the monkey successfully perceives the target. Confirming that activity following cue onset was not related to the monkeys’ hit/miss performance, we found no evidence to suggest that that spatial selectivity for the punishment cue differed between trials where the monkey either successfully or unsuccessfully avoided the punishment (Fig. 3.7). Finally, we demonstrated that amygdala neurons fire preferentially for appetitive and aversive stimuli in a trace-conditioning task where punishments were unavoidable (Fig. 3.9, 3.10).
This consistency of attention effects for appetitive and aversive stimuli in the responses of individual amygdala neurons is in line with the amygdala’s suggested role in guiding attention. Projections from the amygdala to ventral visual areas in the primate (Amaral and Price, 1984; Iwai and Yukie, 1987) are likely an anatomical substrate for the amygdala’s influence on attention. The firing rate of neurons in the ventral visual stream areas such as the inferotemporal cortex (Chelazzi et al., 1993) and V4 (Desimone and Duncan, 1995) are modulated according to where attention is allocated. Although the response properties of ventral visual area neurons have not been investigated in an aversive context, we would expect that in a generalized attention scheme, these neurons would exhibit similar modulations when attention was allocated towards aversive stimuli. This idea is supported by the finding that unilateral amygdala lesions attenuate the preferential BOLD response for negative-valence stimuli that is observed within the ventral visual stream of amygdalae-intact subjects (Vuilleumier et al., 2004).

While amygdala neurons have been previously described as encoding positive and negative value (Paton et al., 2006), their response properties may be better described as ‘attention’ and ‘anti-attention’. The large population of neurons reflecting attention deployment with decreases in firing rates is distinct from most brain areas typically studied in the context of attention. Attention modulation in brain areas such as V4 (Mitchell et al., 2007) and the lateral intraparietal area (Peck et al., 2009; Sugrue et al., 2004) typically comes in the form of increased firing rates when attention is directed towards the contralateral hemifield and seems to be the case for both excitatory and inhibitory neurons (Mitchell et al., 2007). In our data, we see no evidence to suggest that the sign of selectivity with respect to attention is predictive of whether neurons are
excitatory or inhibitory (Peck et al., 2013). Further complicating the matter, the amygdala projects mainly to ipsilateral visual cortices (Iwai and Yukie, 1987), and amygdala projections to primary visual cortex and the inferotemporal cortex appear to be primarily excitatory in nature (Freese and Amaral, 2006). Thus, it may be that the sign of spatial selectivity is predictive of whether these projections target downstream excitatory or inhibitory neurons.

Another possibility is that the amygdala influences attention via a neural relay point, such as the basal forebrain. The basal forebrain is anatomically linked to the amygdala (Russchen et al., 1985), as well as the frontoparietal attention areas (Mesulam et al., 1983), and plays a functional role in attentional processing (Goard and Dan, 2009; Voytko et al., 1994). Like the amygdala, the basal forebrain consists both of neurons that increase and decrease firing rates in response to experimental manipulations, such as the value of stimulus-outcome associations (Wilson and Rolls, 1990). This pathway may influence the activity in areas including LIP (Leathers and Olson, 2012) and DLPFC (Kobayashi et al., 2006) where some neurons respond to the salience of stimuli. Cholinergic basal forebrain neurons project to both inhibitory and excitatory neurons in cortex indicating that they have both an inhibitory and excitatory influence (Sarter and Bruno, 2002); thus, this projection pattern may be related to the sign of response modulation within the amygdala and basal forebrain.

Conclusion. Here, we have shown that individual amygdala neurons exhibit spatial selectivity that is consistent for appetitive and aversive stimuli. Since stimuli associated with appetitive and aversive outcomes both can attract attention, these results shed light on how the amygdala might play a role in spatial attention. This influence may
be realized directly through projections to visual cortices or indirectly via projections to other brain areas such as the basal forebrain.

**METHODS**

**General methods.** Four rhesus monkeys (*Macaca mulatta*, 4-10 kg) were used in these experiments. All experimental procedures complied with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committees at the New York State Psychiatric Institute and Columbia University. Monkeys L & O performed the detection task (see (Peck et al., 2013) for general methods), and Monkeys V & P performed the trace-conditioning task (see (Paton et al., 2006) for general methods).

**Behavioral task.** Monkeys performed a detection task designed to assess how reward expectations influenced attention. Each trial began with the presentation of a central fixation point (0.25° x 0.25°); the monkey was required to moved its gaze within a window of 2° of the fixation point. After a fixation period of 500 to 1500 ms (exponential distribution, \( \lambda = 170 \text{ ms} \)), two cue appeared at either side of the fixation point along the horizontal axis (7° eccentricity) for 300 ms. Following the offset of the cues, the monkeys continued to fixate during a delay period where no peripheral stimuli were present. At a randomly chosen time 400-4000 ms (exponential distribution, \( \lambda = 390 \text{ ms} \)) later, a target appeared (50 ms) at one of the two locations at which the cues had appeared. Monkeys were required to make a direct saccade to within 3° of the target between 100-600 ms after its onset (‘hit’ trial). ‘Miss’ trials occurred when the target appeared but monkeys (1) failed to make a saccade, (2) saccaded to the opposite cue
location, or (3) saccaded elsewhere. Both hit and miss trials were considered to be ‘completed’ trials. Outcomes were delivered 1000 ms (monkey L) or 400 ms (monkey O) after trials were completed.

All trials in which the monkeys’ eye position left the fixation window before the appearance of the target were repeated such that the they weren’t able to avoid selected trial types; cue configuration, target position, delay length were re-randomized on repeated trials. We measured the proportion of false alarms to the cue locations offline by capturing all saccades that ended with 3° of one of the cue locations (Fig. 3.2c); all other premature fixation window exits were defined as aborts.

Monkeys learned to associate abstract visual cues with three possible outcomes – reward (R-cues), punishment (P-cues), or no outcome (N-cues); rewards occurred only on hit trials where the target appeared at the same location as the R-cue, and punishments occurred only on miss trials where the target was at the same locations as a P-cue. Cues were colored rectangles (Task A, 2.25 deg.² at 7° eccentricity), and we randomly interleaved two distinct sets of cues associated with the same outcomes (6 cues total). Targets were Gabor patches; we adjusted the contrast and size of the Gabors online to maintain an overall performance level of ~70% correct. Because the interval during which the target could appear was long and the reaction time window was relatively short, chance performance was about 23%.

In the trace-conditioning task, monkeys learned to associate 3 fractal images with either a reward (drop of liquid), punishment (air puff), or no outcome. A novel set of images was chosen for each experimental session, and at a random point during the session, the associations between the reward-predicting stimulus and the punishment-
predicting stimulus were reversed. In order to successfully complete trials, monkeys were required fixate a central point for 1000 ms and then maintain fixation during cue presentation (300 or 350 ms); following cue offset, monkeys were free to move their eyes and received an outcome following a 1500 ms trace period. For a detailed description of the trace-conditioning task, see (Paton et al., 2006).

**General data analysis.** All general statistical methods were identical to those outlined in Chapter 2. For selectivity indices, we used a receiver-operator characteristic (ROC) analysis to compare firing rate distributions between conditions. We used a weighted least-squares regression to assess the relationship between selectivity indices, using the inverse standard error (determined by bootstrapping) of the selectivity indices as weights. For the trace conditioning data, we removed the first 5 presentations of each stimulus (initially and after the reversal) in order to restrict analysis to trials after the monkeys had learned the stimulus-outcome associations.
CHAPTER 4. The amygdala and basal forebrain as a pathway for emotionally-guided attention

INTRODUCTION

Stimuli that are associated with motivationally significant outcomes demand attentional resources. The amygdala is a brain area known to be involved in forming associations between stimuli and outcomes, and recent results have shown that it might have a role in influencing attention as well (Peck et al., 2013; Vuilleumier et al., 2004). However, given that the amygdala does not project to many brain areas thought to be important in attentional control (Baizer et al., 1993; Barbas and De Olmos, 1990), another neural step may be required for some forms of emotionally-guided attention. The basal forebrain is likely candidate for this given that it is bidirectionally connected to the amygdala (Russchen et al., 1985) and projects throughout the cortex (Mesulam et al., 1983), including to the frontoparietal areas implicated in attention control that don’t receive direct input from the amygdala.

The basal forebrain’s role in influencing attention has been well documented, particularly with reference to the cholinergic input it provides to the rest of the brain. Attentional impairments due to lesions of the basal forebrain are apparent both behaviorally (McGaughy et al., 1996; Voytko et al., 1994) and at the level of individual cortical neurons (Broussard et al., 2009). In visual cortex, microstimulation of the basal forebrain improves the fidelity of responses (Goard and Dan, 2009), and direct pharmacological application of acetylcholine agonists augment attentional modulation of firing rates (Herrero et al., 2008).
To directly address the functional properties of amygdala and basal forebrain neurons during emotionally-guided attention, we recorded simultaneously from individual neurons in both brain areas while monkeys allocated spatial attention based on the importance of stimulus-outcome associations. Much like neurons in the amygdala, basal forebrain neurons were spatially-selective for stimuli that attracted attention, regardless of whether the stimulus was associated with an appetitive or aversive outcome. Additionally, we found that amygdala neurons more rapidly detected the presence of both appetitive and aversive stimuli appearing contralaterally, while basal forebrain neurons were first to detect valuable stimuli appearing ipsilaterally.

RESULTS

Monkeys attend to appetitive and aversive stimuli. Monkeys performed the same detection task described in Chapter 3. In short, monkeys showed an attentional bias towards those cues that represented the opportunity to obtain a reward or avoid a punishment, which was quantified by their hit rate, reaction times, and false alarm frequency.

Basal forebrain neuron firing rates reflect attention allocation. We recorded a total of 121 SUA and 117 MUA sites while monkeys performed the detection task. Unlike the amygdala, we found that spatial selectivity for punishment cues differed between basal forebrain SUA and MUA (Appendix C, Fig. 1), and therefore did not include MUA from either brain areas in any analysis of neural punishment selectivity (see methods). For assessing the selectivity of basal forebrain sites, we used the same analysis as for the amygdala; firing rates 400 to 700 ms after cue onset were compared
across condition using an ROC analysis, and the significance of individual ROC values were determined by bootstrapping ($P < 0.05$).

Amongst basal forebrain neurons, we found widespread selectivity for expected reward and the spatial configuration of the cues (Figure 4.1). Reward selectivity was determined by comparing firing rates on R-present and R-absent trials and was significant for a total of 145 sites, of which 102 (42.9%) fired more on R-present trials and 43 (18.1%) fired more on R-absent trials. Spatial-reward selectivity was similarly apparent with 79 (33.2%) sites firing more on R-contra trials relative to R-ipsi trials, and 25 (10.5%) sites exhibiting the opposite relationship. Amongst those sites with significant reward and/or spatial-reward selectivity, the proportion with positive selectivity was significantly greater for the basal forebrain in each case ($\chi^2$-test, $P < 0.001$ for reward and spatial-reward selectivity). Finally, basal forebrain neurons were also selective for the location of the punishment cue; 10 (8.3%) neurons fired more on P-contra trials than on P-ipsi trials, while 11 (9.1%) neurons fired less on P-contra trials.

**Figure 4.1** Firing rates as a function of time relative to cue onset for two example basal forebrain neurons. Each example neuron (SUA) exhibited spatial-reward selectivity and spatial-punishment selectivity (ROC > 0.5, left; ROC < 0.5, right) that was significant (bootstrap, $P < 0.05$).
Like the amygdala, we found a strong correspondence between reward and spatial selectivity in the basal forebrain. Reward selectivity indices and spatial-reward selectivity indices were positively correlated (Fig. 4.2a; weighted least squares regression and bootstrap, $\beta = 0.40, P < 10^{-3}$) indicating that sites with greater firing rates in the presence of the reward cue also tended to fire more when the reward cue was contralateral. Additionally, we found a positive relationship between spatial-reward selectivity indices and spatial-punishment selectivity indices (Fig. 4.2b; $\beta = 0.19, P < 10^{-3}$); therefore, neurons that fire more when the reward cue was contralateral (R-contra vs. R-ipsi) also fire more when the punishment cue was contralateral (P-contra vs. P-ipsi). This result was also apparent from the sign agreement of selectivity for individual neurons; 15 of 19 basal forebrain neurons with significant spatial-reward and spatial-reward punishment selectivity had the same sign of selectivity for the two (Binomial-test, $P = 0.0192$). Finally, the correspondence between spatial-reward and spatial-punishment selectivity did not differ across successful and unsuccessful punishment avoidance trials (bootstrap, $P = 0.65$) or across monkey ($P = 0.55$). Thus, basal forebrain neurons accurately reflect spatial attention towards salient stimuli.
Figure 4.2 Coordinate reward, spatial-reward, and spatial-punishment selectivity for basal forebrain neurons. (a) Reward selectivity and spatial-reward selectivity are positively correlated ($P < 10^{-3}$). Selectivity indices are plotted for each recording site ($n = 238$), and plot style indicates the significance of each (see legend). (b) Spatial-reward selectivity and spatial-punishment selectivity are positively correlated ($P < 10^{-3}$). Selectivity indices are plotted for each neuron ($n = 121$).

Latency of value information in the amygdala and basal forebrain. To assess the flow of information between the amygdala and basal forebrain, we looked at the latency at which neurons in these areas conveyed value information. Given our previous results showing that the latency of reward information depended greatly on the spatial position of the rewarding cue (Peck et al., 2013), we wished to determine whether (1) contralateral reward information preceded ipsilateral reward information in the basal forebrain as well, (2) whether the latency of reward information differed across brain areas, and (3) whether a similar latency difference was apparent for punishment information.

As a first step in quantifying the latency of reward information, we identified recording sites that exhibited selectivity for contralateral and/or ipsilateral reward cues in an early period after cue onset (50 - 350 ms) by comparing firing rates on either R-contralateral...
or R-ipsi trials against those on R-absent trials (Wilcoxon, \( P < 0.05 \)); this analysis therefore ignored the influence of spatial-punishment selectivity (P-contra vs. P-ipsi) which was generally weaker than that of spatial-reward selectivity (see Fig. 4.2). We included both SUA and MUA for both brain areas given the similarity in spatial-reward selectivity between the two (Appendix C, Fig. 1a). The mean firing rates difference between R-contra and R-absent trials (contralateral-reward comparison) and between R-ipsi and R-absent trials (ipsilateral-reward comparison) for each site was then peak-normalized and sign-corrected (see methods) before averaging across the population. To quantify latency differences, we found the optimal time-shift between the population-level discrimination curves and assessed its significance with a bootstrap analysis.

To compare the timing of reward information across cue configuration, we used the sites for which firing rates were significantly different in both the contralateral-reward and ipsilateral-reward comparisons (106 amygdala sites, 104 basal forebrain sites). Like the amygdala (Fig. 4.3a & Chapter 2), contralateral reward information appeared significantly earlier than ipsilateral reward information in the basal forebrain (Fig. 4.3b; ipsilateral lag of 33 ms; bootstrap, \( P < 10^{-3} \)). This result did not differ across monkeys (bootstrap, \( P = 0.48 \)) or across SUA and MUA (\( P = 0.91 \)). While this latency analysis differed from that used in Chapter 2, the amygdala results were highly similar (ipsilateral lag of 40 ms; \( P < 10^{-3} \)).
Figure 4.3 Contralateral reward information appears earlier than ipsilateral reward information in both brain areas. (a) Contralateral-reward comparison (cyan) and ipsilateral-reward comparison (magenta) as function of time relative to cue onset for amygdala sites. Firing rates differences were peak-normalized and sign-corrected before averaging over recording sites. (b) Same as (a) for the basal forebrain sites.

To compare the latency of reward information across brain areas, we used all cells that exhibited significant reward selectivity for that given comparison (173, 132 amygdala sites and 142, 112 basal forebrain sites for the contralateral-reward and ipsilateral-reward comparisons, respectively). In this case, there was a trend for contralateral-reward information appearing earlier in the amygdala (Fig. 4.4a; basal forebrain lag of 11 ms; bootstrap, \( P = 0.0860 \)). Surprisingly, this analysis suggested the opposite relationship for ipsilateral-reward information, which appeared 10 ms earlier in the basal forebrain (Fig. 4.4b; \( P = 0.0640 \)). Given the small magnitude of these temporal shifts, it was difficult to obtain strong statistical support; however, when we directly compared the latency differences across brain area between the contralateral-reward and ipsilateral-reward comparison, there was significant evidence to suggest that they differed across cue configuration (Fig. 4.4c; bootstrap, \( P = 0.0180 \)). These results suggest a bi-direction flow of reward information within these brain areas where the value of contralateral stimuli appears earlier in the amygdala while ipsilateral value is processed first in the basal forebrain.
Figure 4.4 Latency of reward information differs across brain area. (a) Contralateral-reward comparison as function of time relative to cue onset for amygdala sites (yellow) and basal forebrain sites (green). Firing rates differences were peak-normalized and sign-corrected before averaging over recording sites. (b) Same as (a) for the ipsilateral-reward comparison. (c) Latency difference across brain area for each cue configuration. Error bars indicate the standard error of the latency differences as determined by bootstrapping. Green star indicates the significance of comparing latency differences across cue configuration ($P < 0.05$); crosses indicate trend effects ($P < 0.1$) for comparisons against zero.

We note that our task design did not allow for an analogous analysis comparing the latency of punishment-related signals across brain areas; in order to calculate ‘contralateral-punishment’ and ‘ipsilaterial-punishment’ comparisons, it would be necessary to have included a ‘baseline’ neutral/neutral condition that was not present in our task. We did attempt to compare the dynamics of punishment related signals across brain areas in a different way by comparing P-contra and P-ipsi trials (as in Fig. 4.2.b, y-axis). We limited the analysis to SUA in both brain areas given the discrepancy between punishment selectivity for basal forebrain SUA and MUA (Appendix C, Fig. 1b) and used the same inclusion criterion as for the reward comparisons, resulting in 36 amygdala and 20 basal forebrain neurons. While the presence of punishment information was clearly not as robust as reward information, we did find that preferential coding of contralateral punishments cues appeared 13 ms earlier in the amygdala (Fig. 4.5); this latency difference was marginally significant (bootstrap, $P = 0.0480$) and did not differ
between monkeys (bootstrap, $P = 0.2850$). Thus, it appears that both reward and punishment information appear earlier in the amygdala for contralateral stimuli. Additionally, we compared R/P and R/N trials when the reward cue was ipsilateral (as in Chapter 3, Fig. 3.5) and again when the reward cue was contralateral; here, we found that neural discrimination of these conditions was too weak to effectively compare latencies ($P > 0.4$ for each comparison across brain areas, as in Fig. 4.4a,b).

**Figure 4.5** Punishment information appears earlier in the amygdala. Punishment comparison (P-contra minus P-ipsi) as function of time relative to cue onset for amygdala sites (yellow) and basal forebrain neurons (green).

**DISCUSSION**

*Similarity in spatial selectivity of the amygdala and basal forebrain.* For both amygdala and basal forebrain neurons, we found that firing rates were consistent with the allocation of spatial attention. ‘Positive’ neurons fired more to both appetitive and aversive stimuli that appeared in the contralateral visual hemifields, while ‘negative’ neurons did the opposite (Fig. 4.2). These results were consistent with monkeys’ behavioral performance, which indicated that both appetitive and aversive cues attracted spatial attention (Fig. 3.2). While we observed subtle differences in neural selectivity across brains (e.g. a higher proportion of positive selectivity in the basal forebrain),
response characteristics were largely similar for amygdala and basal forebrain neurons. Further research will be necessary to tease out whether there are any important differences in how the amygdala and basal forebrain encode space and attention. Nonetheless, these results points towards the amygdala and basal forebrain as potential neural substrate for influence attention based on the salience of stimuli-outcome associations.

**Latency of reward information suggests distinct neural pathways.** Our results demonstrate that the relative latency of stimulus-outcome information in the amygdala and basal forebrain is spatially dependent. Projections from high-level visual areas to the amygdala in the same hemisphere have been characterized (Stefanacci and Amaral, 2000), but evidence of direct visual projections to the basal forebrain is lacking (Mesulam and Mufson, 1984) suggesting that the amygdala may have earlier access to visual information, at least for contralateral stimuli; this evidence is consistent with out finding that contralateral value information appears earlier in the amygdala (Fig. 4.4a). Conversely, we found that the value of ipsilateral stimuli is apparent earlier in the basal forebrain (Fig. 4.4b). We can only speculate at the nature of this pathway for ipsilateral value information, but one clue is provided by the lack of direct projections between the two amygdalae (Demeter et al., 1990). The amygdala may therefore receive ipsilateral value information either from other brain areas known to encode value such as the prefrontal cortex (Kaping et al., 2011; Padoa-Schioppa and Assad, 2006; Wallis and Miller, 2003) or indirectly from the amygdala in the opposite hemisphere. Given that ipsilateral visual information in the inferotemporal cortex is dependent on cross-hemispheric projections between the prefrontal cortices (Tomita et al., 1999), ipsilateral
value information is likely to be conveyed across hemispheres at the level of the prefrontal cortex as well. In either case, it appears that such a route first passes through the basal forebrain, which receives prefrontal inputs (Ghashghaei and Barbas, 2001), and projects to the amygdala (Russchen et al., 1985).

**Conclusion.** Here, we show that neurons in the amygdala and basal forebrain show similar spatial selectivity for motivationally significant stimuli in a manner consistent with spatial attention. The latencies of these signals differed between the two brain areas in a complex manner suggestive of different neural pathways for the evaluation of contralateral and ipsilateral stimuli. While the specialization of each brain area and the importance of functional connectivity between the two must be further addressed, our results provide strong evidence supporting the amygdala and basal forebrain’s role in mediating emotionally-guided attention.

**METHODS**

**General Methods.** Two monkeys (L & O) were used in these experiments and performed the detection task described in Chapter 3. All other general methods are outlined in Chapters 2 & 3.

**Physiology.** Neural recording were conducted as described in Chapter 2. For basal forebrain recordings, we targeted the nucleus basalis of Meynert region given its projections to/from the amygdala and cortex (Mesulam and Mufson, 1984; Mesulam et al., 1983; Russchen et al., 1985).

**Neural data analysis.** We used the following criterion for including MUA in the analysis: (1) MUA from both brain areas was included when analyzing reward selectivity
and/or latencies (Figs. 4.2a, 4.3, 4.4), (2) MUA was not included from either brain areas when analyzing punishment selectivity and/or latencies (Figs. 4.2b, 4.5), and (3) MUA was not included if it was not valid for all cases in a particular comparison (across brain areas or forms of selectivity). Neural selectivity indices were computed as in Chapter 3.

**Latency analysis.** We determined the peak firing rate differences for each neuron within the same window used for determining early selectivity (50 – 350 ms after cue onset) and used this value to peak-normalize the firing rate differences. Firing rate differences were then sign-corrected based on the sign of selectivity in the 50 to 350 millisecond time period. A cross-correlation was used to determine the optimal shift between population firing rate difference curves, which was defined as the shift that resulted in the minimum squared-error between the two curves. Significance of the shift was determined by bootstrapping where a random set of cells was chosen, with replacement, on each iteration.
CHAPTER 5. Conclusions

In summary, we have investigated the properties of individual neurons in the primate amygdala and basal forebrain during the evaluation of stimulus-outcome associations and subsequent allocation of spatial attention. Our results are surprising in the sense that very little previous evidence has suggested that either brain area might be involved in some form of spatial processing, and none of these experiments characterized the physiological responses of individual neurons.

While the amygdala has been proposed to have a role in guiding attention and/or gaze towards emotionally-relevant stimuli (Adolphs et al., 2005), there has not yet been physiological evidence showing that the amygdala might be involved in attentional processes. Given our observations that individual amygdala neurons firing rates are spatial selectivity and consistent with attention, we are beginning to gain insight into how amygdala pathology, either due to disorder or lesion, might impact subjects’ ability to assign gaze based on the emotional content of stimuli. Similarly, amygdala dysfunction may disrupt the basal forebrain’s access to value and attention information, resulting in the observed attentional impairments.

Degeneration of cholinergic neurons in the basal forebrain, particular the nucleus basalis of Meynert, has also been proposed to be a contributor in Alzheimer’s disease (AD) (Coyle et al., 1983). Interestingly, one of the major nodes of pathology in the brains of AD patients spans the accessory basal nucleus of the amygdala and the nucleus basalis (Arnold et al., 1991), which are input/output sites, respectively, for amygdala to basal forebrain projections (Russchen et al., 1985). Thus pathology within this pathway may in
some part explain the attention deficits often observed in AD patients (Perry and Hodges, 1999).

An obvious target for future research will be establishing a causal role for activity in the amygdala and basal forebrain in influencing the behavioral expression of attention. Utilizing optogenetic techniques it may be possible to determine the specific influences on emotionally-guided attention that these brain areas have, as well as the isolating the neural pathways through which they act. For example, the influence of the amygdala and basal forebrain on attention might be realized through direct projections from the amygdala to ventral visual cortex, projections from the amygdala to basal forebrain to both dorsal and ventral visual areas, or projections from the basal forebrain to the amygdala and then to ventral visual areas. Using optogenetics, it would be possible to separately activate or silence the activity of neurons contributing to each of these candidate pathways. Future experiments such as this have the potential for greatly improving our understanding of the amygdala and basal forebrain in the context of emotionally-guided attention.
REFERENCES


Figure A.1 Satiation and recent reinforcement outcome history do not explain correlations between firing rates and reaction times. In principle, correlations between firing rates and reaction times (Fig. 2.9) could have resulted from a drift in satiation level (which would cause performance to suffer as the session progresses) or a dependence of behavior on outcome history (where rewards on previous trials may cause a systematic change in performance). Here, we present behavioral and neural analyses showing that neither factor could explain our results. (a,b) Behavioral effects are similar between the first and second half of experimental sessions and between trials following low value and high value outcomes. For these analyses, we plot the difference in hit rate/reaction time when the target appeared near the high value cue location minus when it appeared near the low value cue location (high value present trials only). We were primarily interested in this behavioral difference since changes in performance that applied to both target locations could not explain the opposite signs of correlation in Figure 2.9. For example, if satiation caused a non-spatial increase in reaction times over the course of the session (with a corresponding drift in firing rates), then the correlations for both saccade directions would have the same sign; a similar argument applies to a non-spatial change in behavior due to outcome history. Instead, we see opposite-signed correlations for contralateral and ipsilateral saccades suggesting that these results cannot be explained by a change in how the monkey allocates spatial attention due to satiation and/or outcome history. (a) Comparison of behavioral effects of between the first and second half of experimental sessions. The differences in hit rate (top) and reaction time (bottom) were not different between the first and second half of the session ($P > 0.18$). (b) Same as in (a) but comparing behavior on trials following either a low value or high value outcome. Here we found that the hit rate effect was greater following trials without a big reward ($P = 0.03$), although there was no difference in the reaction time effect ($P = 0.54$). Because the reaction time effect was not different, it is unlikely that differential behavior following large rewards could explain the reaction time correlations we observed (Fig. 2.9). (c) Replication of the analysis in Figure 2.9a using partial correlation to remove the...
influence of trial number and outcome history. This was accomplished using the matlab function ‘partialcorr’, where the controlled variables were the number of each trial within the session and whether the previous trial had resulted in a large reward (1) or not (0). The relationship between value selectivity and partial correlation coefficients was significant only for high value contralateral trials ($P < 0.05$ for contralateral and ipsilateral saccades).

![Graph](image.png)

**Figure A.2 Firing rate fluctuations predict correct performance.** Here, we perform the same analysis as in **Figure 7a** with the exception that we compare firing rates between correct and error trials, as opposed to correlations between firing rates and reaction times. On the y-axis, we use an ROC analysis to compare firing rate distribution on correct and error trials (performance selectivity index), as opposed to the correlation coefficients used to assess reaction time correlations (as in Fig. 2.9). The relationship between value selectivity and performance selectivity indices was significant only on high value contralateral trials for trials where the target appeared contralaterally ($P = 0.04$) and ipsilaterally ($P = 0.01$); no significant relationships were observed on other trial types ($P > 0.16$). Note that here the signs of effects are opposite those of Figure 2.9 indicating that increases in firing rate are associated with improved performance. To test whether these results differed between tasks, we repeated the analysis including a task factor (1 for task A, 0 for task B) and did not observe a significant interaction effect of task by reward selectivity for any condition (ANOVA, $P > 0.14$).

Fundamental differences between the tasks make us cautious in drawing strong conclusions for the performance results. In task A, the majority of error trials were those in which the monkey failed to make any saccade after the target’s appearance (67% of all errors); the other error trials consisted of saccades to the opposite hemifield as the target (28%) and saccades to the same hemifield that were not directed at the target (5%). On the other hand, correct trials always consisted of a saccade towards the same hemifield as the target. Thus, a difference in activity between correct and error trials may in part reflect a difference in saccade behavior on these trials related to motor preparation or the cognitive processes that accompany purposeful saccades. Limiting the data set to the 5%
of error trials where saccades were directed to the same hemifield as in correct trials would at least control for the direction of the saccade but does not yield sufficient data for analysis. In task B, the monkey always made a saccade after the appearance of the choice targets; error trials were those in which he saccaded to the wrong choice target, indicating the wrong direction of orientation change. Although it is encouraging that we found similar results in the two tasks, we present these results with caveats given the differences in behavior on error trials. These issues do not affect the reaction time correlations (Fig. 2.9a), since all data used for that analysis included a movement.

Figure A.3 Neural waveforms do not differ for REW+ and REW− neurons. (a) Average spike waveforms for individual neurons (left) and the distributions of spike waveform widths (right). Data are plotted for the populations of REW− neurons (top, red) and REW+ neurons (bottom, blue). Average waveforms were spline interpolated yielding a timing precision of 2.5 µs and the spike waveform width was defined as the trough to peak duration. A total of 10 cells were excluded from this analysis because either the trough or peak time could not be determined given the small time window around the spike saved during data acquisition. The distributions of spike waveform widths did not differ between REW− and REW+ neurons (Wilcoxon, P = 0.66). (b) Spike waveform width as a function of value selectivity magnitude. Neurons were split into quartiles based on the absolute value of the value selectivity d’; this was done separately for neurons with d’ < 0 and with d’ > 0. Vertical bars indicate the standard error of spike waveform widths for each group. Using a 2-way ANOVA, we verified that there was a significant (positive) relationship between the magnitude of value selectivity and waveform width (P = 0.0381); no significant effect was observed for the sign of selectivity (P = 0.3020) or an interaction between sign and magnitude (P = 0.4464).
Figure B.1 Consistent relationship between amygdala spatial-reward & spatial-punishment selectivity for single-unit and multi-unit activity, and across monkeys. Spatial-reward selectivity indices were positively correlated with spatial-punishment selectivity indices (weighted least squares regression and bootstrap, $P < 0.05$) for each monkey (left) and for both SUA and MUA (right); regression slopes did not differ significantly across monkeys (bootstrap; $P = 0.37$) or across activity types ($P = 0.55$).
APPENDIX C

Figure C.1 Spatial-punishment selectivity of basal forebrain firing rates differs between single-unit and multi-unit activity. (a) Spatial-reward selectivity as a function of reward selectivity. For both SUA and MUA, we observed a significant positive relationship (weighted least squares regression and bootstrap, $P < 0.05$) that did not differ across activity types (bootstrap, $P = 0.59$). (b) Spatial-punishment selectivity as a function of spatial-reward selectivity. A significant positive relationship was observed for SUA (bootstrap, $P < 10^{-3}$) but not for MUA ($P = 0.46$) and the regression slopes were significantly different across activity types ($P = 0.01$).