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Contributions of orbitofrontal subregions to socioemotional processing in rhesus macaques

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Contributions of orbitofrontal subregions to socioemotional processing in rhesus macaques

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B.S., Florida State University, 2005

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An abstract of
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James T. Laney School of Graduate Studies of Emory University
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Abstract

Contributions of orbitofrontal subregions to socioemotional processing in rhesus macaques

By Lauren Murphy

The orbitofrontal cortex (OFC) is a heteromodal association area within the prefrontal cortex responsible for understanding and maintaining the flexible relationship between a stimulus and a subsequent outcome. As such, damage to the OFC impairs behaviors ranging from complex social interactions to Pavlovian fear conditioning. Despite extensive research to understand the complexities of the whole OFC, few studies have attempted the dissociate the separable contributions of OFC subregions to socioemotional processing and flexible behavioral modulation. Understanding the functional dissociation and organization of OFC subregions in the context of emotional processing will help elucidate the mechanisms underlying emotional dysregulation present in many mood disorders. Thus, this dissertation describes two studies comparing the effects of restricted cortical lesions to subregions of the OFC (Brodmann Areas BA12, BA13, and BA14) on emotional processing. In Study 1, data demonstrated that OFC areas 12 and 13, part of the lateral orbital network of the OFC, but not BA14, part of the medial OFC network, critically support attention to salient social cues and regulate arousal in socioemotional contexts. In Study 2, the results showed that this same lateral orbital OFC network, but not the medial OFC network, support the modulation of emotional behaviors in the presence of conflicting cues, though differences occurred in the magnitude of the impairment. Thus, while damage to BA12 yields impairments only in the modulation of fear response in the presence of a safe cue, damage to BA13 causes impairments in both the expression fear and safe associations and modulation of fear responses. Together, the two studies provide additional support to the view that the lateral orbital network of the OFC, but not the medial OFC network, is critical for emotional regulation in primates and suggest that emotional dysregulation may stem from impairments in flexibly maintaining and updating stimulus-outcome associations.

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General Introduction

The ability to flexibly control and update emotional response to a stimulus, known as emotional regulation, is critical for survival in many species, particularly in group-living, social mammals. Emotional regulation allows an individual to both rapidly respond to threats and to modulate the magnitude of an emotional response based on information provided by internal and external cues. Impaired emotional regulation manifests in two ways: decreased response to emotionally significant cues or deficient down-regulation of an unnecessary emotional response. Numerous neuropsychiatric disorders share impairments in emotional regulation, including depression (Drevets, 2007; Versace et al., 2010), anxiety disorders (Hahn et al., 2011; M. R. Milad & Rauch, 2007), post-traumatic stress disorders (PTSD; Jovanovic, Kazama, Bachevalier, & Davis, 2012), and Autism Spectrum Disorders (ASD; Bachevalier & Loveland, 2006; Dalton et al., 2005). Emotional regulation appears to be supported by a neural network that includes the amygdala, several regions of the prefrontal cortex (PFC), and the insula (LeDoux, 1995; Price, Carmichael, & Drevets, 1996; Rolls, Yaxley, & Sienkiewicz, 1990). Within the prefrontal cortex, the orbitofrontal cortex (OFC) represents a key area for the processing of socioemotional stimuli and the flexible modulation of emotional response based on its vast anatomical connections to multimodal association cortices and structures that regulate arousal and emotional response, such as the amygdala. Understanding the contributions of emotion processing regions to mood and behavior has implications for identifying risk and resilience in neuropsychiatric disorders and potential for disease prevention. This chapter will first provide a brief overview of the anatomical and functional organization of the OFC, with particular emphasis on the differences

between three architectonically distinct OFC subregions (Brodmann Area (BA) 12, 13, and 14) and the relationships between these OFC subregions and the amygdala. Next, we will review the specific contributions of the OFC in general, as well as each region specifically, to socioemotional processing and emotional modulation, focusing on gaps in the literature that led to the two comparative lesion studies described in this thesis. The goal of this thesis to is examine the contributions of three OFC subregions to social and emotional processing using translational paradigms relevant to both the human and rodent literature.

Features of the Orbitofrontal Cortex

Anatomical Organization of the Orbitofrontal Cortex

The OFC, located just above the orbit, consists of the most ventral regions of the PFC and has extensive connectivity with sensory and regulatory cortical and subcortical regions (Petrides & Mackey, 2006). The OFC features two connectional and functional networks (the orbital and medial networks), and is composed of anatomically and functionally distinct subregions (Fig. 1). Specifically, the primate OFC extends medially and laterally across the ventral surface of the PFC, bordered anteriorly by BA10, posteriorly by the insula and medially by the anterior cingulate cortices (ACC; Barbas, 2007b; Price, 2006; Saleem & Logothetis, 2012). The OFC has been subdivided into several architectonically-distinct subregions and for the purpose of this thesis, we will follow the subdivisions described by Barbas and her group (Barbas, 2007b): BA12 (in humans, BA47), extends from the principal sulcus on the lateral surface to the lateral orbital sulcus, BA11 (anterior) and BA13 (posterior) occupy the middle surface of the OFC between the lateral and medial orbital sulci, and BA14, located on the gyrus rectus,

extends from the medial orbital sulcus laterally to the rostral sulcus ventromedially (Barbas, 2007b; Carmichael & Price, 1995; Price, 2006). Each of these subregions have been further divided into 2 - 4 subfields based on architectonic divisions and regional connectivity (Öngür, Ferry, & Price, 2003).

Anatomical Connectivity Between the OFC and Other Structures

The OFC is a highly interconnected region, receiving sensory projections and reward-processing information from prefrontal, sensory, and limbic regions. The OFC has extensive connections across the OFC subregions, and this connectivity can be separated into two primary networks, the orbital and medial networks. The orbital network is comprised primarily of BA11, BA12, and BA13, whereas the medial network is comprised of the medial wall, including BA14 and BA25 (Öngür et al., 2003). In addition, BA12, BA13, and BA14 exhibit distinct patterns of connectivity within and beyond the OFC. Within the OFC, BA13 is reciprocally connected to both areas BA12 and BA14, and BA12 sends mostly unreciprocated projections to BA14 (Barbas, 1993; Price, 2006; Price et al., 1996). Beyond the OFC, both the medial and orbital networks have limited connectivity to other regions of the prefrontal cortex, especially to the dorsolateral prefrontal cortex (DLPFC), ACC and anterior PFC (Price et al., 1996). Outside of the PFC, the OFC has notable connections with important emotion processing regions in the limbic system, such as the amygdala, hippocampus, hypothalamus, and cingulate gyrus, though connectivity with these regions varies by subregion (Barbas, 1993; Carmichael & Price, 1995; Ghashghaei, Hilgetag, & Barbas, 2007).

The amygdala is a key region for processing emotion and arousal in the brain and shares significant connectivity with areas of the OFC (Fig. 2). Within the amygdala,

separate nuclei that have been implicated in different stages of emotional processing show distinct patterns of connectivity with OFC subregions. For example, the lateral nucleus of the amygdala (LA) receives significant sensory inputs (Phelps & LeDoux, 2005; Schiller & Delgado, 2010), the basolateral amygdala complex (BLA) is important for connecting an unconditioned stimulus (US) with a conditioned stimulus (CS; Schafe et al., 2001), and the central nucleus of the amygdala (CE) sends significant projections to areas that regulate arousal and homeostasis (Phelps & LeDoux, 2005; Schiller & Delgado, 2010). Within the OFC, areas BA12, BA13 and BA14 all receive projections from the BLA, however BA12 and BA13 send significant back-projections to the BLA and BA14 sends much fewer (Barbas & De Olmos, 1990; Barbas, 1993; Carmichael & Price, 1995). In addition, BA13 sends projections to the CE and has bidirectional connectivity with other amygdala areas such as the LA that are not found in BA12 and BA14 (Barbas & De Olmos, 1990; Barbas, 1993; Carmichael & Price, 1995). It has been proposed that the amygdala supplies the OFC with relevant information about emotional cues in the environment and the OFC then contextualizes these signals, returning inhibitory or excitatory signals to the amygdala depending on the optimal outcome (Banks, Eddy, Angstadt, Nathan, & Luan Phan, 2007; Barbas & De Olmos, 1990; Ghashghaei & Barbas, 2002).

In addition to the amygdala, the OFC has connections with regions in the medial temporal lobe associated with memory and perception, such as the hippocampus, entorhinal and perirhinal cortices (Barbas, 1993; Carmichael & Price, 1995). Areas BA13 and BA14 project to the hippocampus and entorhinal cortex, with return projections limited to BA13. Areas BA12, BA13, and BA14 all project to the perirhinal cortex, with

return projections again limited to BA13. Additionally, BA12, BA13, and BA14 are all connected to the hypothalamus, a region important for regulating mood and arousal, and OFC-hypothalamic connectivity differs for areas within the orbital and medial prefrontal networks. More orbital regions of the OFC have stronger connectivity with the lateral hypothalamus and more medial regions of the OFC have stronger connectivity with the medial hypothalamus (An & Price, 1998; Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003; Hirose, Osada, Ogawa, & Tanaka, 2016; Rempel-Clower & Barbas, 1998). Lastly, the ACC is associated with reward, decision making and emotional processing, and receives projections from BA12, BA13, and BA14 with back projections limited to BA13 (Barbas, 1993; Carmichael & Price, 1995). Together, these anatomical differences suggest separable roles for BA12, BA13, and BA14 in emotional processing and regulation.

Functions of the Orbitofrontal Cortex

The extensive anatomical connectivity of the OFC is reflected in the functional domains of the region. The OFC is broadly conceptualized as an association area important for emotion processing and arousal (Bechara, Tranel, & Damasio, 2000; Paton, Belova, Morrison, & Salzman, 2006), maintenance of stimulus-outcome associations (Rudebeck, Saunders, Prescott, Chau, & Murray, 2013), and the flexible use of internal and external cues to guide behavior (Bechara, Damasio, Damasio, & Lee, 1999; Rolls & Grabenhorst, 2008; Rolls, Hornak, Wade, & McGrath, 1994; Zald & Andreotti, 2010). Below, we will demonstrate that damage isolated within individual subregions of the OFC yields separable patterns of behavioral deficits in both emotional processing and

behavioral modulation, suggesting distinct and complementary contributions of each region.

The Role of the Orbitofrontal Cortex in Emotional Processing

Emotional perception is mediated by a series of cortical and subcortical structures and functions to prime an organism for efficient action in important survival situations. The amygdala has long been regarded as the seat of emotional processing, but the amygdala alone is not sufficient to support the complex social and emotional behaviors seen in primates and other social mammals (Bachevalier & Loveland, 2006; West, DesJardin, Gale, & Malkova, 2011). A growing number of studies have indicated that the interactions between the amygdala and the OFC are critical for the regulation of complex socioemotional function. Individuals with OFC lesions display aberrant social behavior, such as inappropriate feelings of intimacy, inappropriate teasing, and difficulty interpreting the emotional states of others (Anderson, Bechara, Damasio, Tranel, & Damasio, 1999; Bechara et al., 2000; Beer, Heerey, Keltner, Scabini, & Knight, 2003; Beer, John, Scabini, & Knight, 2006; Hornak, Rolls, & Wade, 1996). Similarly, macaques with OFC lesions behave inappropriately, and may give or receive more aggression than typical monkeys in social contexts (Bachevalier, Machado, & Kazama, 2011; Izquierdo, Suda, & Murray, 2005; Kazama, Davis, & Bachevalier, 2014). One reason for these difficulties may be due to an inability to perceive emotional valence or understand socioemotional cues, a process that may be mediated by OFC-amygdala interactions.

In social primates, discrete, ritualized facial expressions facilitate affective communication and a single facial expression offers a predictive cue to the viewer,

presumably reflecting the affective state of the expresser. Facial expressions have been shown to elicit behavioral and physiological changes across primate species (Leopold & Rhodes, 2010), and OFC neurons code for both facial identity expression (Azzi, Sirigu, & Duhamel, 2012) as well as social value (Watson & Platt, 2012), supporting the importance of this area for emotional processing. In humans, individuals with OFC lesions have difficulty differentiating facial expressions from neutral (Tsuchida & Fellows, 2012), and are less accurate than controls on matching facial expressions to emotional categories (Blair & Cipolotti, 2000; Hornak et al., 1996; Salas et al., 2016), especially negative emotions (Blair & Cipolotti, 2000; Marinkovic, Trebon, Chauvel, & Halgren, 2000; Monte et al., 2013). Additionally, poor facial emotion identification is found in neuropsychological disorders marked by decreased OFC activity, such as major depression (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005) and Autism Spectrum Disorders (Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008). However, some studies report no difference in emotion matching in patients with OFC damage (Hornak et al., 2003; Jenkins et al., 2014), and the emotions affected are not always consistent across studies. One reason for this discrepancy may be an interaction between the differential processing of emotional valence across the subregions of the OFC and variations in the extent of damage produced by natural lesions. In particular, more lateral regions, such as BA12, show increased activity during the processing of negative emotions (Blair, Morris, Frith, Perrett, & Dolan, 1999; Vuilleumier, Armony, Driver, & Dolan, 2001), and damage to these areas impairs emotional processing and identification of facial expressions (Blair, 2007; Vuilleumier et al., 2001). In contrast, medial areas, such as BA14, are preferentially involved in the processing of positive emotions

(Hampshire, Chaudhry, Owen, & Roberts, 2012; Winecoff et al., 2013), and damage to central OFC area BA13 leads to general impairments in facial emotion processing across valence (Tsuchida & Fellows, 2012), resulting in emotional behavior deficits (Izquierdo et al., 2005; Machado & Bachevalier, 2003). Thus, subregions of the OFC appear to show some functional specificity in the type of expressions they seem to process, but more research is needed to fully characterize the contributions of each region to socioemotional behavior.

Furthermore, emotional perception is not strictly a conscious phenomenon, and a significant amount of emotional processing occurs below the threshold of conscious perception, indicated by changes in autonomic processes (Laeng, Sirois, & Gredeback, 2012; Liddell et al., 2005; Tamietto & de Gelder, 2010). Changes in autonomic processes can be readily measured by noninvasive techniques, such as pupil dilation (Bradley, Miccoli, Escrig, & Lang, 2008) and heart rate variability (Appelhans & Luecken, 2006), and may help elucidate the mechanisms underlying deficits in emotional processing. Indeed, critical alterations in levels of arousal to social and nonsocial emotional stimuli are well documented in individuals with OFC damage (Bechara et al., 2000; Heberlein, Padon, Gillihan, Farah, & Fellows, 2008a; Manohar & Husain, 2016) and OFC activity in healthy humans is correlated with autonomic arousal during demanding tasks (Patterson, Ungerleider, & Bandettini, 2002). Specifically, ventromedial regions of the OFC, such as BA14, are strongly associated with changes in self-reported arousal and decreased arousal to reward (Heberlein et al., 2008a; Manohar & Husain, 2016) and are more strongly connected with hypothalamic areas responsible for autonomic regulation (Barbas, 2007b; Hirose et al., 2016; Rempel-Clower, 2007). More lateral areas of the

OFC, including BA12 and BA13, have been implicated in modulation of arousal through inhibitory and excitatory projections to the amygdala (Banks et al., 2007; Ghashghaei & Barbas, 2002).

To summarize, there exists ample evidence, especially in humans, that the OFC plays a key role in the perception of emotional stimuli. Furthermore, subregions of the OFC differ with respect to the processing of emotional valence and modulation of arousal, presumably due to variations in their functional connectivity with other key emotion regions, such as the amygdala and hypothalamus. Given that OFC area BA12 is involved in the processing of negative stimuli and emotional regulation through inhibition, we predict that damage to BA12 may yield deficits in social functioning due to changes in attention to highly salient emotional cues. Similarly, given that area BA13 is implicated in processing emotional stimuli generally and monitoring emotional cues from social signals, we predict that damage to BA13 may yield aberrant patterns of social attention. Finally, given that BA14 is often implicated in arousal and physiological integration of emotional states, we predict that damage to BA14 may yield deficits in arousal to salient cues. To test these hypotheses, Study 1 will contrast the effects of damage to specific OFC subregions (BA12, BA13, and BA14) on attention to dynamic emotional stimuli (both social and nonsocial) using eye-tracking technology to measure the changes induced by OFC damage in both the perception of (i.e. gaze duration) and autonomic arousal to (i.e. pupil dilation) socioemotional stimuli. Importantly, BA11 of the OFC is often associated with the processes described above due to the regions extensive interconnectivity with BA13 (Carmichael & Price, 1996). However, BA11 has limited direct connectivity with regions such as the amygdala and hypothalamus (Barbas,

1993; Carmichael & Price, 1995) and may have a limited role in direct emotional regulation (Carmichael & Price, 1996). Consistent with these anatomical differences, damage to BA11 in isolation appears to impact reward processing (Elliott, Agnew, & Deakin, 2008; Longe, Senior, & Rippon, 2009; Murray, Moylan, Saleem, Basile, & Turchi, 2015), but there is limited evidence that BA11 alone is important for socioemotional regulation (Beer et al., 2003; Bramham, Morris, Hornak, Bullock, & Polkey, 2009; Heberlein, Padon, Gillihan, Farah, & Fellows, 2008b; Winecoff et al., 2013; Wolf, Pujara, Baskaya, & Koenigs, 2016), and therefore the region was excluded in this study in favor of regions BA12, BA13, and BA14.

Orbitofrontal Contributions to Stimulus-Outcome Associations

Much of the early research on the functions of the OFC focused on the development and maintenance of stimuli-outcome associations. Early lesion studies in rhesus macaques emphasized the importance of the OFC in suppressing conditioned responses, as demonstrated by impaired ability to reverse stimulus-outcome associations (i.e. reversals) and prolonged perseverative responding to formerly reinforced stimuli (i.e. extinction failure) (Butter, 1969). These results have been replicated in multiple non-human primate species (Dias, Robbins, & Roberts, 1996; Iversen & Mishkin, 1970; Rudebeck, Bannerman, & Rushworth, 2008), in humans (Fellows & Farah, 2003; Hornak et al., 2004; Rolls et al., 1994) and in rodents (Geoffrey Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003). Further, these findings are supported by electrophysiological studies that demonstrate specific activation patterns in OFC neurons to primary reinforcers (Rolls, Critchley, Mason, & Wakeman, 1996), specificity to appetitive or aversive outcomes (Morrison & Salzman, 2009), during the first

reinforcement occasion (Hampshire et al., 2012; Noonan, Kolling, Walton, & Rushworth, 2012; Noonan, Mars, & Rushworth, 2011), and to changes in stimulus-outcome associations (Thorpe, Rolls, & Maddison, 1983), anticipation of outcomes (Rudebeck & Murray, 2011; Simmons, Ravel, Shidara, & Richmond, 2007; Tremblay & Schultz, 1999), and unexpected outcomes (Rolls, 1999; Schoenbaum, Chiba, & Gallagher, 2000; Thorpe et al., 1983), such as during extinction. These data suggest that the OFC, particularly BA13 where much of these data were collected, is critical for updating stimulus-outcome associations. However, later studies using refined techniques, such as specific fiber-sparing lesions, have demonstrated that key areas of the OFC are not critical for updating stimulus associations, such as in reversal learning, suggesting that the role of the OFC is more nuanced. Indeed, numerous recent studies demonstrate that BA13, either alone or with BA11 or BA12, is not critical for this ability (Bachevalier et al., 2011; Kazama et al., 2014; Rudebeck & Murray, 2011, 2014). These new findings suggest that perhaps more ventromedial areas not included in these recent reports are critical for this ability. Indeed, in some cases lesions including the vmPFC are more likely to report impairments in reversals (Butter, 1969; Dias et al., 1996; Fellows & Farah, 2003; Hornak et al., 2003; Rudebeck et al., 2008; Rygula, Walker, Clarke, Robbins, & Roberts, 2010), though not consistently (Rudebeck & Murray, 2011).

Instead, the role of the OFC may be related to the complex modulation of behaviors in the face of conflicting information and the ability to flexibly shift associations based on internal and external cues. Areas of the OFC show increased functional activity when presented with conflicting cues (Noonan et al., 2011) and when suppressing a preferred response to meet conflicting goals (Arana et al., 2003).

Furthermore, damage to the OFC consistently impairs the ability to inhibit a preferred response following a change in internal state (Bachevalier et al., 2011; Bradfield, Dezfouli, Van Holstein, Chieng, & Balleine, 2015; Murray et al., 2015; Rudebeck & Murray, 2011). The ability to modulate response based on internal cues is typically measured using the reinforcer devaluation task in which primary reinforcers acquire conditioned associations with secondary cues and the value of the primary reinforcer is degraded (i.e. devalued) through either aversive pairing or satiety. Individuals with a functional OFC will inhibit choosing the cue associated with the preferred treat after devaluation, but individuals with OFC damage are unable to adjust their choice preference based on the changed internal state. In monkeys, damage to the OFC, especially BA13, reduces the ability to suppress choice of the object representing the preferred, but devalued, treat both when lesions were created in infancy (Kazama et al., 2014) and adulthood (Bachevalier et al., 2011; Bradfield et al., 2015; Murray et al., 2015; Rudebeck & Murray, 2011). Indeed, reinforcer devaluation appears to be supported by the OFC-amygdala connections, as either unilateral OFC-amygdala lesions or OFC-amygdala disconnection are sufficient to interrupt reinforcer devaluation (Baxter, Parker, Lindner, Izquierdo, & Murray, 2000; Izquierdo & Murray, 2004) and temporary inactivation of the amygdala during devaluation blocks suppression of the preferred choice (West et al., 2011). More importantly, inactivation of the OFC blocks reinforcer devaluation either during selective devaluation or during the test phase, suggesting that the OFC is critical for the modulation of behavior both during initial updating of associations and during behavioral expression (West et al., 2011).

However, much of the research discussed above is limited to the positive (i.e. stimulus-reinforcer) domain, and as outlined in the previous section, we believe that the role of OFC subregions varies with regard to emotional valence, with lateral BA12 responding more strongly to negative outcomes (Gottfried, O'Doherty, & Dolan, 2002; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001), medial area BA14 showing stronger activity to positive outcomes (Gottfried et al., 2002; Monosov & Hikosaka, 2012; Morris & Dolan, 2004; O'Doherty et al., 2001), and BA13 intermediate to the two (Hampshire et al., 2012; Monosov & Hikosaka, 2012; Morrison & Salzman, 2009; Noonan et al., 2012; J. O'Doherty et al., 2001). Given the profound emotional dysregulation present following OFC lesions (Izquierdo & Murray, 2004; Izquierdo et al., 2005; Machado & Bachevalier, 2008), and the involvement of the OFC in stimulus-outcome association maintenance, the OFC is likely involved in the expression of learned fear. Although early (neonatal) lesions of the OFC do not disrupt fear learning and expression (Kazama et al., 2014), there is evidence of OFC-amygdala synchrony during fear-safety discrimination in rodents (Likhtik, Stujenske, Topiwala, Harris, & Gordon, 2014), suggesting that the system is necessary for fear expression in adults. Further, the strong bidirectional projections between the OFC and the amygdala (Barbas, 2007a, 2007b; Carmichael & Price, 1995) support the notion that the OFC may contribute to fear expression and modulation in dynamic contexts. Given that the OFC is critical for the flexible maintenance of stimulus-reinforcer associations, it is thus possible that the OFC may also be critical for fear expression and modulation. Together these patterns of results may represent the mechanism underlying social deficits caused by OFC damage.

To effectively measure the effects of lesions to subregions of the OFC on fear expression and modulation in Study 2, we have chosen to use the fear-potentiated startle (AX+/BX-) task (Davis, 2006; Jovanovic et al., 2012; Kazama, Schauder, McKinnon, Bachevalier, & Davis, 2013). The AX+/BX- paradigm has been widely used in rodents (Davis, 2006) and humans (Kong, Monje, Hirsch, & Pollak, 2014), and is increasingly used in clinical populations to study the specific cognitive processes affected in PTSD (Jovanovic et al., 2012). This paradigm assesses subject's ability to not only learn conditioned discriminations predicting fearful or safe conditions (i.e. fear and safety learning and expression) but also to use safe cues to modulate reactivity to fear stimuli (i.e. conditioned inhibition) and to extinguish their fear reactivity when aversive events are no longer associated with the fearful stimulus (i.e. extinction).

To summarize, there is substantial evidence that the OFC plays a key role in the flexible modulation of behavior, and that subregions of the OFC differ in respect to their contributions to stimulus-outcome association learning and flexible choice selection. Given the role of OFC area BA12 in the suppression of previously rewarded associations (Arana et al., 2003; Iversen & Mishkin, 1970) and in the rapid modulation of stimulus-outcome associations (Hampshire et al., 2012), we predict that damage to BA12 will impair emotional modulation, especially to fearful cues. The role of BA13 in the flexible modulation of stimulus-outcome associations (Bachevalier et al., 2011; Izquierdo et al., 2005; Rudebeck & Murray, 2011) as well as the general role of BA13 in emotional regulation (Bachevalier et al., 2011; Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin, Shelton, & Davidson, 2007; Machado & Bachevalier, 2008), suggests that damage to this region may be associated with the retention and modulation of stimulus

associations in fear-potentiated startle paradigm. As restricted lesions to BA13 do not consistently impair learning of stimulus-associations (Dias et al., 1996), we hypothesize that this region is not essential for the initial learning of associations, but rather the flexible updating required in dynamically changing contexts. Finally, given the role of BA14 in modulating emotional and physiological response to stimuli and the apparent importance of BA14 in processing reward, we predict that damage to BA14 may result in impaired arousal, particularly to positive (safe) cues. Thus, using the same animals as in Study 1, Study 2 will compare the effects of damage to OFC subregions (BA12, BA13, and BA14) on reinforcement learning for negative (fear) and positive (safe) nonsocial stimuli during acquisition of stimulus-reinforcer associations, as well as flexible use of the safe signal to dampen the fear reactivity (conditioned inhibition), and extinction of associations, using the AX+/BX- paradigm.

Summary

As outlined above, the OFC is an important region for the flexible modulation of emotional behavior in social and nonsocial contexts. However, differential damage to OFC subregions results in distinct behavioral outcomes, suggesting that the subregions, and perhaps their connectivity, make separable contributions to the functions of the OFC. Though many studies have addressed the impacts of broader OFC damage and others have investigated the contributions of individual subregions in isolation, no study to date has compared damage across subregions of the OFC and assessed the impacts of this damage on emotional function in multiple paradigms. As such, our collective understanding of OFC functions is lacking and the specific role of OFC subregions in emotional processing and modulation remains unknown. Thus, the goal of the present

experiments was to establish the contributions of three key regions of the OFC (BA12, BA13, and BA14) to socioemotional processing and emotional modulation in a model species, the rhesus macaque. Taken together, these studies highlight the importance of lesion studies to understanding the specific contributions of OFC subregions, and make a small but important step forward to identify the mechanisms by which OFC damage and maldevelopment may produce profound behavioral impairments.

A Note on Cross-Species Homology

The OFC is highly evolutionarily conserved in primates, as subregions in both macaques and humans appear to be both anatomically and functionally homologous (Öngür et al., 2003; Petrides & Pandya, 1999). The significant similarity between macaques and humans suggests that lesions in macaques can be reasonably expected to produce deficits similar to those reports in humans with damage to OFC. In addition, significant evolutionary homology in primate behavior and emotional processing support the widespread use of this model in OFC research (Leopold & Rhodes, 2010). Rodents, however, have a significantly smaller frontal cortex and dramatically reduced anatomical homology compared to that of primates, inciting controversy around the use of rodent OFC studies to model primate OFC function (Kolb, 1984). Yet, although the effects of lesions to different PFC areas in rodents may differ from those found in primates, lesions of the whole OFC in rodents produce cognitive deficits that mimic some of those documented in primates (Heilbronner, Rodriguez-Romaguera, Quirk, Groenewegen, & Haber, 2016; Wallis, 2013). Thus, nonhuman primates represent an excellent model species in which different aspects of OFC function can be explored and translated to human OFC functioning. These findings will contribute to broader knowledge of the

functions of the OFC, as well as specifically help understand how dysfunction within OFC subregions contributes to aberrant social behavior, and how OFC dysfunction could be the source of the behavioral and cognitive impairments reported in several neuropsychological disorders.

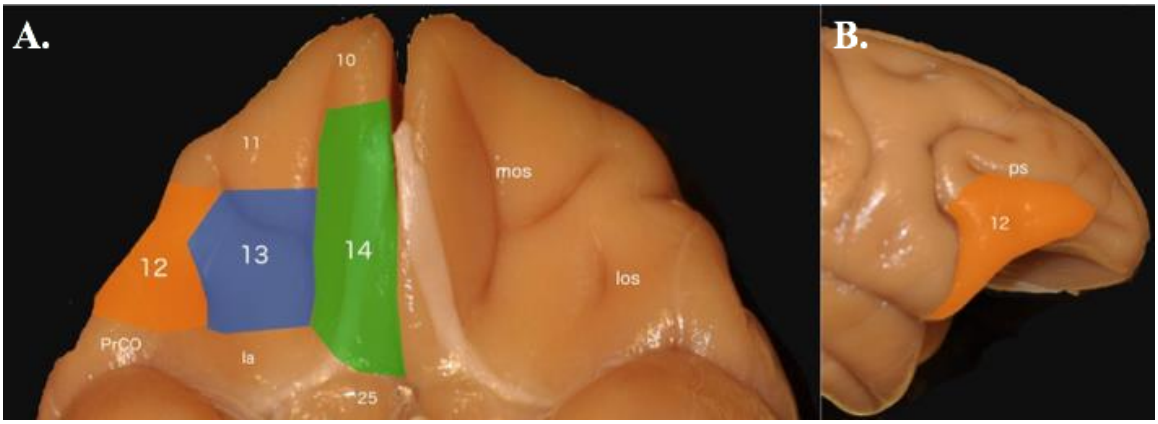

Figure 1: Subregions of the orbitofrontal cortex.
Ventral (A) and lateral (B) views of the macaque orbitofrontal cortex. Brodmann area (BA) 12 is the most lateral (orange), BA14 is the most medial (green) and BA13 (blue) lies intermediate to the lateral and medial regions. mos = medial orbital sulcus, los = lateral orbital sulcus, ps = principle sulcus, PrCO = posterior opercular cortex, Ia = insula.

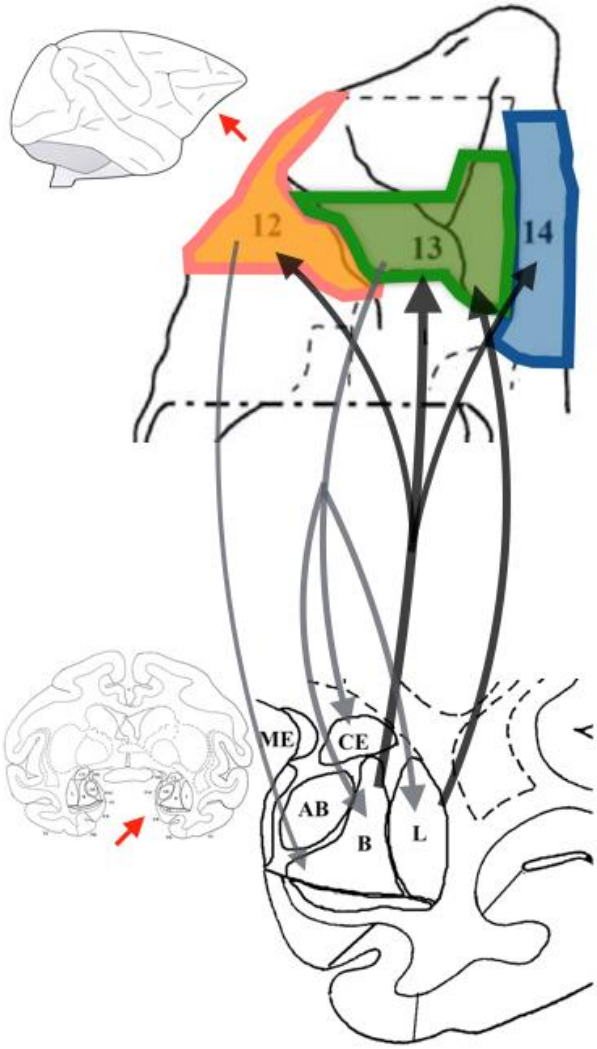

Figure 2: The orbitofrontal cortex shares significant connectivity with the amygdala that varies across subregions.
The top image depicts a ventral view of the left orbitofrontal cortex (as indicated by the red arrow in top inset figure of the lateral view of the macaque brain). The bottom image depicts a coronal view of the right amygdala (as indicated by the red arrow in the bottom inset figure of a coronal view of the macaque brain). Arrows indicate OFC projections to the amygdala (gray) and projections from the amygdala to the OFC (black). Note that BA12 and BA13 have reciprocal connectivity with the basolateral amygdala, but not BA14. In addition, BA13 has significant projections to the central and lateral nuclei of the amygdala that are not found in areas BA12 and BA13. ME = medial amygdala, CE = central amygdala, AB = accessory basal, B = basolateral amygdala, L = lateral amygdala.

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Impacts of orbitofrontal lesions on social attention and arousal in rhesus macaques

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Abstract

The orbitofrontal cortex (OFC) is a key region of the ventral prefrontal cortex that supports socioemotional interactions, as damage to this region produces profound impairments in social behavior and emotional perception (Hornak et al., 2003; Hornak, Rolls, & Wade, 1996; Rolls, 1999; Vuilleumier, Armony, Driver, & Dolan, 2001; Wolf, Philippi, Motzkin, Baskaya, & Koenigs, 2014). Anatomically, the OFC integrates signals from all sensory modalities, emotional and physiological arousal centers, and downstream social and emotional processing areas (Rolls, 2004, for review). Given the OFC's anatomy, as well as its well-documented role in updating stimulus-outcome associations (Holland & Gallagher, 2004; Murray, Moylan, Saleem, Basile, & Turchi, 2015; Rudebeck & Murray, 2011; West, DesJardin, Gale, & Malkova, 2011), the OFC may regulate socioemotional behavior through the monitoring of the value of social cues such as facial expressions. However, no study has examined the role of specific OFC subregions on attention to facial expressions. Thus, the present study compared attention and arousal to social and nonsocial scenes in three groups of rhesus macaques with restricted lesions to one OFC area (BA12, 13, or 14) and a control group using eye-tracking to capture pupil location and size. Animals with damage to the lateral OFC (BA12 and BA13) showed decreased attention specifically to the eyes of threatening social stimuli and increased arousal (increased pupil diameter), only to positive social scenes. In contrast, animals with damage to medial OFC (BA14) displayed no changes to attention or arousal in the presence of social stimuli. These findings support the notion that areas of the lateral OFC are critical for directing attention and modulating arousal to emotional social cues.

Introduction

The complex social structures of human and nonhuman primates mandate that individuals possess the capacity to recognize and interpret socially relevant signals and mount the appropriate, species-specific responses to those signals. The orbital frontal cortex (OFC), the most ventral area of the prefrontal cortex (PFC), has been implicated in these socioemotional abilities. In particular, the OFC appears to use social information to guide and adjust behaviors appropriately in accordance with changing contexts and values (Bechara, Damasio, & Damasio, 2003; Clark, Cools, & Robbins, 2004; Holland & Gallagher, 2004; Schultz, Tremblay, Hollerman, & Schultz, 2000). Human patients with discrete lesions to the OFC, including Brodmann Areas (BA) 11 and BA13, are characterized by an array of socioemotional deficits, ranging from behavioral changes, such as increased aggression, to cognitive changes, such as difficulties identifying voices and/or faces (Hornak et al., 2003, 1996; Rolls, 1999). Electrophysiological studies in nonhuman primates (Rolls, Critchley, Browning, & Inoue, 2006; Watson & Platt, 2012) and neuroimaging studies in humans (Blair et al., 1999; Dalton et al., 2005; Gorno-Tempini et al., 2001; Mitchell, Elliott, Barry, Cruttenden, & Woodruff, 2003; Morris, Ohman, & Dolan, 1998; Nakamura et al., 1999; Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998) support the role of the OFC in processing emotional content from faces and voices. Such reports are consistent with the neuroanatomical organization of the primate OFC, a heteromodal association area that receives converging inputs from multiple sensory systems, including visual (Barbas, 1988; Barbas & Pandya, 1989; Barbas, 1993; Barbas, 1995; Carmichael & Price, 1995; Morecraft, Geula, & Mesulam, 1992; Pandya & Kuypers, 1969; Seltzer & Pandya, 1989; Webster, Bachevalier, &

Ungerleider, 1994) and auditory inputs (Barbas, 1988; Barbas, 1993; Hackett, Stepniewska, & Kaas, 1998; Romanski et al., 1999; Romanski & Goldman-Rakic, 2002) as well as inputs from limbic structures, such as the amygdala critical for the regulation of emotion and arousal (Barbas & De Olmos, 1990; Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003; Carmichael & Price, 1995; Ghashghaei, Hilgetag, & Barbas, 2007; Rempel-Clower & Barbas, 1998). Despite substantial evidence that the OFC is poised to integrate and interpret socioemotional information broadcast over multiple sensory modalities, to date there have been few investigations directly assessing the role of the OFC subregions in subjects' abilities to detect social signals, especially those displayed by expressive faces. This knowledge is critical for a better understanding of the specific contributions of OFC subregions to emotional deficits associated with mood disorders, such as depression (Drevets, 2007; Keedwell et al., 2005; Versace et al., 2010), anxiety (Hahn et al., 2011; Milad & Rauch, 2007), post-traumatic stress disorders (PTSD; Jovanovic et al., 2012) as well as disorders of sociability, such as Autism Spectrum Disorder (Bachevalier & Loveland, 2006; Dalton et al., 2005).

One mechanism that could explain the widespread socioemotional deficits reported after OFC damage is associated with the critical contribution of the OFC to stimulus valuation. Social cues, such as facial expressions, are inherently positive or negative cues, providing critical information on how to proceed in social interactions. Both lesion studies and electrophysiology studies have indicated that a substantial proportion of OFC neurons encode the potential for, sensory attributes of, and subjective value of outcomes associated with external stimuli (Bradfield et al., 2015; Holland & Gallagher, 2004; Kazama et al., 2014; Murray et al., 2015; Rudebeck & Murray, 2011;

West et al., 2011), and neurons in the OFC also signal similar information when the predicted outcomes actually occur (Padoa-Schioppa & Assad, 2006). Thus, the OFC likely plays a key role in using stimulus-evoked signals to convey the subjective value of biologically significant outcomes because, unlike neurons in other parts of the brain, neurons in OFC distinguish between appetitive and aversive outcomes and discriminate between categories of relevant information (Watson & Platt, 2012). In addition, the OFC exerts control over emotional regulation and autonomic function through reciprocal connectivity with emotion regulation centers, such as the amygdala (LeDoux, 1995; Price et al., 1996; Rolls et al., 1990) and hypothalamus (Weddell, 1994) that vary across OFC subregions. Specifically, lesions to more lateral regions with greater amygdala connectivity (BA12 and BA13) are associated with reduced perception of emotions (Blair et al., 1999; Bramham et al., 2009; Hornak et al., 2003; Vuilleumier et al., 2001; Wolf et al., 2016), whereas lesions to more medial areas with greater hypothalamic connectivity (BA14) may dampen arousal in emotional contexts (Bechara, Damasio, & Damasio, 2000; Bechara et al., 1999; Heberlein et al., 2008; Manohar & Husain, 2016; Patterson et al., 2002). In addition, lateral and medial areas of the OFC show differential activation to differently valenced stimuli, with lateral regions (BA12 and BA13) showing specific activation to more negative and aversive stimuli and the medial areas (BA14) showing specific activation to positive or appetitive stimuli (Gottfried et al., 2002; Hampshire et al., 2012; Monosov & Hikosaka, 2012; Noonan et al., 2012, 2011; O'Doherty et al., 2001; Schiller & Delgado, 2010). However, much less is known on the role of the OFC on the encoding of cues provided by faces.

Humans and nonhuman primates process faces using a combination of mutually non-exclusive scanning strategies, including holistic, configural, and feature-based processing (Parr, 2011, for review). In addition, the eye region seems to attract the most attention even when the facial features have been altered (Ghazanfar & Santos, 2004; Gothard, Brooks, & Peterson, 2009; Gothard, Erickson, & Amaral, 2004; Guo, 2007; Guo, Robertson, Mahmoodi, Tadmor, & Young, 2003; Nahm, Perret, Amaral, & Albright, 1997). The neural circuit underlying these processes in nonhuman primates shares many similarities with that of humans and includes several structures within the occipital and temporal regions, such as the fusiform face area (FFA), superior temporal sulcus (STS) and amygdala (Dekowska, Kuniecki, & Jaśkowski, 2008; Rolls, 2007; Rossion & Gauthier, 2002; Tsao, Moeller, & Freiwald, 2008) and more recently, the orbital frontal cortex (OFC) has also been shown to play a regulatory role in face processing (Rolls, 2007; Tsao & Livingstone, 2008; Wright et al., 2008). This is not surprising given that this mesio-cortical area is reciprocally connected with the FFA, STS and amygdala as well as autonomic centers, such as the hypothalamus (Barbas, 1988; Barbas, Zikopoulos, & Timbie, 2011; Ghashghaei & Barbas, 2002; Morecraft et al., 1992; Rolls, 2004; Seltzer & Pandya, 1989). The OFC contains neurons that respond selectively to faces (O'Scalaidhe, Wilson, & Goldman-Rakic, 1997; Rolls et al., 2006; Thorpe, Rolls, & Maddison, 1983; Tsao & Livingstone, 2008) and, in humans, its activity is modulated when judging emotion in faces (Monk et al., 2003) and proportionately increases with heightened intensity of facial expressions of anger (Blair et al., 1999). Yet, the contribution of specific OFC subfields to the encoding of facial cues remains to be fully investigated.

Therefore, the present study examined the effects of damage to three subfields of the OFC (BA12, BA13, and BA14) on visual scanning patterns and processing of social cues (faces) and nonsocial cues (scenes) of different valences from short movies in monkeys. Furthermore, given that the processing of faces and emotional expressions in primates is associated with autonomic physiological changes, such as variations in heart rate, skin conductance and pupil size (Bradley, Codispoti, Cuthbert, & Lang, 2001; Bradley, Miccoli, Escrig, & Lang, 2008; Conway et al., 2007; Laine, Spitler, Mosher, & Gothard, 2009; Lang, Greenwald, Bradley, & Hamm, 1993; Skwerer et al., 2009), variations of pupil diameter, which index variations in physiological arousal, were also recorded while the monkeys were spontaneously scanning the same movies. As areas BA12 and BA13 appear to play a role in the processing of negative stimuli, we predicted that these regions will decrease attention to highly salient areas of negative stimuli, such as the eyes. Furthermore, due to the extensive connectivity between areas BA12 and BA13 and the amygdala, we also predicted that these areas will increase pupil diameter while viewing social stimuli, indicative of heightened arousal. In addition, as BA14 exerts control over arousal and skin conductance during risk-taking, we predicted that damage to BA14 will decrease pupil diameter, indicating decreased arousal to emotional stimuli.

Methods

All protocols and procedures have been reviewed and approved by the Institutional Animal Care and Use Committee at Emory University and align with the standards set by the NIH Guide for the Care and Use of Laboratory Animals.

Subjects

Seventeen rhesus macaques between the ages of 6 and 14 years of age (mean = 9.2) participated in this study. All subjects were born into large social groups at the Yerkes National Primate Center Field Station (Lawrenceville, GA). Subjects were moved to the Yerkes National Primate Center Main Station (Atlanta, GA) as adults and singly housed in appropriate cages allowing visual and auditory contacts with similarly aged macaques of both sexes. All subjects were fed a diet of nonhuman primate chow (Purina, St. Louis, MO), provided with water ad libitum and supplied additional fruit and vegetables daily. All animals had been trained for behavioral testing for related experiments (Kazama et al., 2013), but were naïve to the procedures and stimuli presented in this experiment. Subjects were pseudorandomly assigned into one of four groups: lesions of OFC BA12 (Group BA12, n = 4; 1 female), lesions of OFC BA13 (Group BA13, n=4, 1 female), lesions of OFC BA14 (Group BA14, n=5, 4 females), and sham-operations (Group C, n=4, 1 male).

Orbital Frontal Cortex Lesions Neuroimaging and Surgery

Neuroimaging procedures were performed the day of surgery and then again one week post-surgery for all animals in groups BA12, BA13 and BA14. Vital signs and hydration were monitored and managed as subjects were sedated using ketamine HCl (10mg/kg, 100 mg/ml), intubated, anesthetized with inhaled isoflurane (1.0 –2.0%, v/v, to effect), and placed into a stereotaxic apparatus using appropriate pain management treatment. Then, T1- (3D T1-weighted fast spoiled gradient (FSPRG)-echo sequence, TE=2.6ms, TR=10.2ms, 25º flip angle, contiguous 1mm sections, 12cm FOV, 256 x 256 matrix) and Fluid Attenuated Inversion Recovery (TE=140ms, TR=1000ms, inversion

time (TI)=2200ms, contiguous 3mm sections, 12cm FOV, 256 X 256 matrix, acquired in 3 series offset 1mm posterior) MR-images were acquired using a 3T Siemens Magnetom Trio system (Siemens Medical Solutions, Malvern, PA) to guide lesion placement and for later lesion estimation, respectively.

Following the pre-surgical neuroimaging session, all animals in the lesion groups were maintained under gas anesthesia, immediately transferred to the surgical suite, and prepared for surgical procedures using aseptic techniques. Following injection of Bupivicaine (0.25% concentration, 1.5 ml) along the location of the skin incision, the skin was cut from the mid-orbital ridge to the occiput and was thereafter retracted along with the temporal muscles below. A small craniotomy was made on the cranial bone at the level of the orbitofrontal cortex in each hemisphere. The dura was retracted and a 30-gauge needle attached to a 10µl Hamilton syringes was used to inject the neurotoxin. For injections of areas BA13 & BA14, the syringes were attached to stereotaxic arms and lowered based on stereotaxic coordinates estimated from the structural T1 images. Bilateral lesions were created simultaneously during a one-stage surgery in all subjects. For injections of BA12, the syringes were handheld by the surgeon and bilateral lesions were created one at a time for each hemisphere in a one-stage surgery, except for one subject (BA12-1), which received surgeries in the left and right hemispheres in a two-stage surgery one month apart. For all injections, ibotenic acid (0.8-1µl; Biosearch Technologies, Novato, CA, 10mg/ml in PBS, pH 7.4) was delivered at a rate of 0.2µl per minute at each injection site. At the end of each injection, the needle was maintained in place for one-minute before its retraction to reduce spread of the neurotoxin across other brain tissue.

The extent of each intended lesion is illustrated on the ventral view of the OFC in Figure 1 as well in red in the coronal sections of Figure 4. Lesions of BA12 (4-23 injections) extended from the principal sulcus laterally to the lateral orbital sulcus medially, and from BA11 anteriorly to opercular cortex (PrCO) posteriorly. Lesions of BA13 (7 injections) extended from the lateral orbital sulcus laterally to the medial orbital sulcus medially and from BA11 anteriorly to the insula (Ia) posteriorly. Lesions to BA14 (4-5 injections) extended from the medial orbital sulcus laterally to the rostral sulcus medially and from BA10 anteriorly to BA25 posteriorly.

Animals in Group C were sedated using ketamine HCl, intubated, anesthetized with inhaled isoflurane, and placed into a stereotaxic apparatus using appropriate pain management treatment in the surgical suite. As with lesioned subjects, control animals were maintained under gas anesthesia, and prepared using aseptic techniques. Then, the skin was cut and the skin and muscle retracted under Bupivicaine anesthesia, and a small craniotomy was made on the cranial bone at the level of the OFC in each hemisphere, but in contrasts to Groups BA12, BA13, and BA14, no needle was inserted and no injections were made.

For all subjects, once surgical procedures were complete, the wound was sutured in anatomical layers, and the subject was recovered from anesthesia. Post-surgical care included management of pain (acetaminophen, 10 mg/kg, p.o.), of infection (Cefazolin, 25 mg/kg i.m.), and of swelling due to immunoreactive process (dexamethasone sodium phosphate, 0.4 mg/kg i.m.). Recurrent monitoring of the animals was performed by veterinary and laboratory staff, for a minimum of one week post-surgery.

Eye-Tracking Procedures

Eye position was measured using a 60Hz infrared eye-tracker (ISCAN ETL400, Woburn, MA) and a specially configured desktop computer. Images were presented on a 20" monitor using a custom image presentation script in Presentation (v16.5; Neurobehavioral Systems, Inc, Berkeley, CA). Subject gaze behavior was monitored on a second computer monitor by the experimenter during testing. Subjects were seated in a primate chair and wore a thermoplastic helmet to reduce head movements and maintain animals' gaze toward the screen (Fig. 2), thus reducing the testing time (Machado & Nelson, 2011).

Stimuli

All stimuli consisted of digital recordings (movies) of dynamic stimuli. All movies were 720 x 480 pixel avi's and unedited 10 seconds in length.

Social stimuli depicted unknown adult rhesus macaques filmed at University of California, Davis, and generously provided by a colleague (Dr. Katalin Gothard). All monkeys were filmed through a clear panel in a holding cage. For videos of positive social stimuli, monkeys displayed periods of positive facial expressions (i.e. lipsmacks; Fig. 3A). For videos of neutral social stimuli, monkeys displayed neutral faces for the entire 10-seconds (Fig. 3B). For negative social stimuli, monkeys displayed periods of negative facial expressions (i.e. open mouth threat; Fig. 3C). Social videos displayed the specific valence intermixed with periods of no facial expressions, and varied in monkeys' movements within the cage and gaze direction.

Nonsocial stimuli were pulled from online locations (i.e. YouTube) or recorded in the lab as necessary. All nonsocial stimuli were judged to be absent of any face-like

configurations and absent of any human feature (i.e. arm, foot) by an experimenter. To ensure that nonsocial stimuli were comparable to social stimuli, all nonsocial movies consisted of an object or non-primate animal moving in front of a discernible background. Positive nonsocial stimuli consisted of familiar food items or toys that had gained positive valence (i.e. candy, Fig. 3D). Neutral nonsocial stimuli consisted of a wide variety of novel stimuli that had no apparent valence or relevance to rhesus macaques (i.e. train, Fig. 3E). Negative nonsocial stimuli consisted of familiar and novel items that are known to promote fear or threat response in monkeys. The familiar negative items were items used by veterinarians or animal care technicians in the facility that have been judged to have negative associations (i.e. syringes, capture net). Novel negative items were videos of reptiles and invertebrates that have been shown to elicit fearful responses in macaques (i.e. snake, Fig. 3F; Nelson, Shelton, & Kalin, 2003).

Behavioral Testing Procedures

Subjects were transported from their home cage to a familiar behavioral testing room and transferred to the primate chair. They were then brought to the eye-tracking room and positioned between 18- and 24-inches in front of the testing monitor. Thermoplastic helmets were then attached, and the subject was rewarded for cooperation. At the start of each testing session, eye location was calibrated by hand, using the presentation of an orientating GIF animation in one of five preset positions on the screen (top left, top right, bottom left, bottom right, center). When the subject made a visible saccade to the location of the stimulus, the experimenter pressed a key to record the eye location in the ISCAN program. Successful calibration was assessed by presenting calibration stimuli during a “data-out” visualization mode in which a white crosshair on

the screen represented the location of the monkey's gaze. If the crosshair matched the location of the calibration stimulus, calibration for that point was judged accurate. If the crosshair did not match, calibration procedures were repeated until all five calibration points appeared accurate. Eye-tracking calibration was completed within 10-minutes of arriving in the room. If an accurate calibration was obtained in less than 10-minutes, subjects sat calmly watching nature videos until 10-minutes had elapsed to ensure that all subjects were under the testing conditions for the same duration. If a calibration was not obtained within 10-minutes, testing was cancelled, the subject was returned to its home cage, and the session was re-run the next day. Ten-minutes following accurate calibration, the experimental stimuli were presented in a controlled, randomized order using Presentation software. All stimuli were presented for a total of five times, with a 30-second intertrial interval during which an orienting GIF animation was presented. At the completion of stimuli presentation, the experimenter removed the thermoplastic helmet and rewarded the subject with both verbal and food reinforcement. Subjects were then returned to their home cage.

Histological Lesion Assessment

Once all behavioral testing was complete, all subjects in the OFC groups were euthanized and perfused for post-mortem histological assessment of cell loss and quantification of lesion extent. Briefly, subjects were sedated and administered a lethal dose of sodium pentobarbital, and then perfused intracardially with saline and paraformaldehyde. Brains were then fixed in a 30% sucrose-formalin solution, followed by a cryoprotective solution, and then placed in a -80˚C freezer until processed. Brains were cut frozen in the coronal plane at 50 µm. Every five brain sections (250 µm)

throughout the extent of the OFC were stained with thionin (cell body) and every 20 brain sections (1 mm) were stained with gallyas (fiber). Each brain section was mounted on a glass slide, stained, and cover slipped. Slides were then magnified at 0.28x and digitized using a Leica Z6 Microscope (Leica Microsystems, Wetzlar, Germany) fixed with a Excelis HD Lite Camera (Accu-Scope Inc., Commack, NY). The digitized coronal sections were matched with those of an atlas of a normal monkey brain and areas of cell loss were drawn onto control sections using Photoshop (v8; Adobe Systems Inc., San Jose, CA). The resulting image of cortical damage in atlas space was then quantified using Image-J software (v1.46r; U.S. National Institutes of Health, Bethesda, MD), and damage to both intended and unintended regions was recorded and calculated as a percent of the normal control brain.

Data Analyses

Raw eye-tracking data and raw stimulus presentation data were combined in Excel (Microsoft, Redmond, WA). All data were then imported into the GazeTracker (v10.0; Eyetellect, Charlottesville, VA) program for analyses. Within GazeTracker, experimenters drew frame-by-frame regions-of-interest (ROIs) on predetermined salient regions of the stimuli. For social stimuli, ROIs were drawn on the body, eyes, mouth, and whole scene (see Fig. 3A-C for example social ROIs). For nonsocial stimuli, ROIs were drawn around the main object and the whole scene (see Fig. 3D-F for example nonsocial ROIs). Gaze points beyond the whole scene were not analyzed. To control for overall looking time, attention to ROIs was expressed as a proportion of total time attending to the ROI divided by total time attending to the whole scene of the same movie. To compare the effects of OFC lesions on arousal to emotional stimuli, average pupil

diameter (in pixels) were measured using an infrared eye-tracking camera of the eye-tracker while subjects viewed the 10-second long social and nonsocial emotional videos.

To compare the effects of treatment group (Control, BA12, BA13, and BA14) on attention or arousal to stimuli a three-way Social Context X Valence X Group RM-ANOVA was used for all ROIs with comparable social and nonsocial areas (i.e. Overall Attention, Focal Attention, and Pupil Diameter). For ROIs that only exist in social contexts (i.e. eyes, mouth), a two-way Valence X Group RM-ANOVA was used. Simple planned comparisons were used to compare each lesion group separately to controls in all conditions, and post-hoc Bonferroni tests were used to compare the effects of Valence when significant. Post-hoc t-tests were also conducted to determine whether attention to stimuli differed by sex and Pearson correlations were used to measure the effects of age on attention. Finally, we used Pearson correlations to measure the relationship between lesion extent and each measure listed above.

Results

Lesion Assessment

A full description of lesion extent (cell loss) can be found in Table 1 and a visualization of lesion extent for each area can be found in Figures 4. It is important to note that in some cortical areas the cell loss was restricted to either the upper or lower layers of the cortex, resulting in a dysfunction of the entire cortical region. Thus, the lesion extent for each area was most likely greater than what is displayed by only the cell loss in Table 1 and Figure 4.

For Group BA12, average cell loss varied between 15% and 41%, with three animals (BA12-1, BA12-2, BA12-3) showing bilateral lesions and animal BA12-4

showing a more unilateral lesion (Left hemisphere: 26%, Right hemisphere: 4%). As shown in Fig.4 (left column), the extent of cell loss covered the entire anteroposterior extent of area B12, especially on the left hemisphere. All BA12 cases received minor unintended damage to areas BA45 (range: 10%-31%; see Fig. 4, see orange arrows in +34, +32, +30, and +28), and cases BA12-1, BA12-3 and BA12-4 received minor damage to PrCO (range: 1%-9%; Fig. 4 see orange star at +28).

For Group BA13, average cell loss varied from 2% to 26% with two animals (BA13-2, BA13-5) receiving bilateral lesions, two animals BA13-3 and BA13-4 receiving more unilateral lesions (BA13-3 - L: 14%, R: 0%; BA13-4 - L: 12%, R: 27%), and one animal BA13-1 receiving only a small (2%) lesion. As shown in Fig. 4 (middle column), the cell loss was visible across the entire anteroposterior extent of area BA13 with some sparing of the most posterior portion of BA13. Cases BA13-2, BA13-3 and BA13-5 received unintended damage to BA11 (1%-20%, Fig. 4 see green arrow at +36 and +34 bilaterally).

For Group BA14 (Fig. 4, right column), average cell loss varied between 1% and 41%, with two animals (BA14-1 and BA14-2) receiving bilateral lesions, one animal (BA14-4) receiving a more unilateral lesion (L: 5%, R: 47%) and one animal BA14-3 receiving a small (<1%) lesion. This cell loss spanned the full anteroposterior extent of area B14. All animals received unintended damage to BA13 (4%-20%, Fig. 4 see blue arrows at +34, +32, +30 and +28) and BA25 (7%-16%, Fig 4 see blue star at +30 and +28) mostly restricted to the left hemisphere.

Removal of the two animals with the smallest lesions in Groups BA13 and BA14 (BA13-1 and BA14-3) did not result in a different pattern of results, thus the two animals were included for all statistical analyses reported below.

Differences in attention across social context and emotional valence

Effects of OFC lesions on overall attention to social and nonsocial movies (Figure 5)

First, to assess whether lesions to specific subregions of the OFC impacted attention to emotionally salient stimuli, overall gaze duration to social and nonsocial emotional movies was analyzed using a three-way Social Context X Valence X Group repeated-measure ANOVA. Overall attention to the movies was modulated by both their social context (Fig. 5A) and the valence displayed by the stimulus within the movies (Fig. 5B-C), but not by OFC damage (Fig. 5D and E). Thus, all subjects, irrespective to lesion groups, looked longer at social than nonsocial movies (Social context effect: $F_{1,13}$=85.29, $p<0.001$, η^2=0.84) and regardless of social context, subjects tended to look longer at movies containing negative stimuli than at movies containing neutral or positive stimuli (Valence effect: $F_{2,6}$=17.32, $p<0.001$, η^2=0.51; Social Context X Valence interaction: $F_{2,26}$=3.11, p=0.06, η^2=0.14). Follow-up analyses indicated that for social movies, animals looked the longest at negative compared to positive movies ($F_{2,26}$=6.60, $p<0.01$, η^2=0.27; Neg > Pos: p=0.02) and for nonsocial movies, animals looked the longest at negative and neutral movies compared to positive movies ($F_{2,26}$=43.75, $p<0.001$, η^2=0.74; Neg > Pos: $p<0.001$; Neu > Pos: $p<0.001$).

Although the main effect of Group did not reach significance for overall attention ($F_{3,13}$=0.79, p=0.52, $\eta^{2=}$0.15), there was a trend toward a Social Context X Valence X Group interaction ($F_{6,26}$=2.06, p=0.09, η^2=0.28) driven by greater attention to negative than positive movies, by control animals and animals in Group BA14, but not in animals in Groups BA12 and BA13 (Fig. 5D). However, none of the planned comparisons reached significance, likely due to the small sample size and no other interactions reached significance (Social Context X Group: $F_{3,13}$=1.15, p=0.37, $\eta^{2=}$0.03; Valence X Group: $F_{2,26}$=1.21, p=0.33, $\eta^{2=}$0.11).

Effects of OFC lesions on attention to focal object in videos (Figure 6)

Next, to determine whether OFC lesions impact attention to focal regions within social and nonsocial stimuli, percent gaze duration for the ROI containing the focal object was analyzed using a three-way Social Context X Valence X Group RM-ANOVA with Social Context and Valence as the within-subjects factors and Group as the between-subjects factor. For social movies, the focal object was the monkey, and for nonsocial movies the focal object was the primary object (i.e. train, snake, etc.).

As for the overall attention described above, Social Context (Fig. 6A) and Valence (Fig. 6B) significantly modulated attention to the focal object, but OFC lesions did not. Thus, all subjects looked longer at the focal objects in social scenes compared to nonsocial scenes ($F_{1,13}$=7.33, p=0.02, η^2=0.24) and tended to look longer at the focal object in neutral compared to negative stimuli ($F_{2,26}$=6.44, p<0.01, η^2=0.30; Neu > Neg: p<0.01). The overall Group factor did not reach significance ($F_{3,13}$=1.27, p=0.33, η^2=0.23), but there was a significant Social Context X Group interaction ($F_{3,13}$=3.43, p=0.05, η^2=0.34) driven by greater attention to nonsocial focal objects by Group BA14

compared to controls (Fig. 6C), though planned comparisons did not reach significance (Control < Group BA14: p=0.10). None of the other interactions reached significance (Social Context X Valence: $F_{2,26}$=2.52, p=0.10, η^2=0.13; Valence X Group: $F_{6,26}$=0.70, p=0.66, η^2=0.10; Social Context X Valence X Group: $F_{6,26}$=1.54, p=0.21, η^2=0.23).

Effects of OFC lesions on attention to salient regions of social stimuli (Figure 7)

Attention to specific cues within the faces of social movies was analyzed to determine whether OFC lesions impacted attention to specific cues in the faces, i.e. eyes or mouth, using a two-way Valence X Group RM-ANOVA for each face area.

For the eye region, all animals regardless of groups looked longer toward the eyes of positive and neutral social stimuli compared to negative social stimuli ($F_{2,26}$=16.00, p<0.001, η^2=0.47; Pos > Neg: p<0.001; Neu > Neg: p=0.02; see Fig. 7A). This pattern of viewing the eyes of expressive faces was greater in the controls and animals in Groups BA13 and BA14 than in animals in Group BA12 (Group effect: $F_{3,13}$=3.68, p=0.04, η^2=0.46; Control > Group BA12: p=0.03; see Fig. 7B). Although the Valence X Group interaction did not reach significance ($F_{6,26}$=1.72, p=0.16, η^2=0.15), post-hoc analyses (Fig. 7C) revealed group differences for attention to the eyes of negative ($F_{3,13}$=4.45, p=0.02, η^2=0.51) and positive ($F_{3,13}$=3.45, p=0.05, η^2=0.44) faces, but not to neutral faces ($F_{3,13}$=2.34, p=0.12, η^2=0.35). Specifically, as compared to controls, both Groups BA12 and BA13 looked less at the eyes of negative (Control > BA12: p<0.01; Control > BA13: p=0.01), and positive (Control > BA12: p=0.05; Control > BA13: p=0.08) stimuli.

As compared to attention to the eyes, attention to the mouth (Fig. 7F-E) was much shorter than attention to the eyes for all group, and did not differ across Valence or Group

(Valence: $F_{2,26}$=0.77, p=0.47, η^2=0.04; Group: $F_{3,13}$=0.13, p=0.94, η^2=0.03; Valence X Group: $F_{6,26}$=1.74, p=0.15, η^2=0.28).

Effects of OFC lesions on Pupil Diameter (Figure 8)

Pupil diameter was also used as a proxy to determine how OFC lesions impacted arousal to emotionally charged stimuli. Average pupil diameter was analyzed using a three-way Social Context X Valence X Group ANOVA. These analyses revealed that Social Context (Fig. 8A) and Valence (Fig. 8B) significantly modulated pupil diameter. Thus, subjects had greater pupil diameter to nonsocial than social stimuli ($F_{1,13}$=53.75, p<0.001, η^2=0.76) and had greater pupil diameter to neutral compared to emotional stimuli for the social videos (Valence: $F_{2,26}$=59.76, p<0.001, η^2=0.75; Social Context X Valence interaction: $F_{2,26}$=56.61, p<0.001, η^2=0.73; Neu > Pos: p<0.001; Neu > Neg: p=0.01). Although the group difference did not reach significance ($F_{3,13}$=0.48, p=0.70, η^2=0.10), there was a significant Social Context X Valence X Group interaction ($F_{6,26}$=2.58, p=0.04, η^2=0.10). Post-hoc analyses revealed that this three-factor interaction was driven by greater pupil diameter to positive social stimuli for animals in Groups BA12 and BA13 as compared to controls ($F_{3,13}$=10.94, p<0.001, η^2=0.72; BA12 > Controls: p<0.001; BA13 > Controls: p<0.01; see Fig. 8C). Such changes in pupil diameter were not seen, or less so, for nonsocial stimuli. No other interactions reached significance (Social Context X Group: $F_{3,13}$=1.46, p=0.27, η^2=0.06, Valence X Group: $F_{6,26}$=2.38, p=0.06, η^2=0.09).

The effects of age and sex on social attention

We used t-tests and Pearson correlations, respectively, to determine whether the sex or age of subjects impacted attention to social and nonsocial stimuli and found

significant effects of sex on attention to the scene of positive and negative social stimuli and significant effects of age on attention to social and nonsocial stimuli. Specifically, male monkeys looked longer at positive and negative scenes than female monkeys (Pos: $t(15)=2.23$, $p=0.04$; Neg: $t(15)=2.84$, $p=0.01$). Increased age was significantly correlated with decreased total attention to positive and negative stimuli (Pos: $R=-0.52$, $p=0.03$; Neg: $R=-0.77$, $p<0.001$), decreased focal attention to the body of neutral stimuli ($R=-0.53$, $p=0.03$), and decreased attention to all aspects of nonsocial neutral stimuli (Scene: $R=-0.68$, $p<0.01$; Focal Object: $R=-0.50$, $p=0.04$).

Correlations between looking time and lesion extent (Figures 9-11)

To determine whether the amount of intended or unintended damage to each OFC subregion modulated gaze duration, percent looking, or pupil diameter, Pearson correlations between lesion extent and all measures for each lesion group were calculated.

For Group BA12, percent cell loss to BA12 or BA45 was significantly associated with reduced time looking at the bodies of positive social stimuli, i.e. more extended damage to these areas resulting in greater reduction in looking time to the body (BA12: $R=-0.97$, $p=0.02$, $n=4$; BA45: $R=-0.97$, $p=0.03$, $n=4$; Fig 9A-B). Also, within Group BA12, greater damage to PrCO was associated with increased gaze toward neutral social scenes ($R=0.96$, $p=0.04$, $n=4$; Fig. 9C), decreased gaze toward the body in negative social scenes ($R=-0.95$, $p=0.05$, $n=4$; Fig. 9D) and decreased total gaze toward negative nonsocial scenes ($R=-0.96$, $p=0.04$, $n=4$; Fig. 9E).

For Group BA13, greater percent damage to BA11 was associated with increased attention to the body and the mouth of positive social stimuli (Body: $R=0.93$, $p=0.02$,

n=5, Fig. 10A; Mouth: R=0.95, p=0.01, n=5, Fig. 10B). For Group BA14, greater damage to BA14 was associated with decreased gaze to the bodies of positive and neutral social stimuli (Positive: R=-0.98, p=0.02, n=4; Fig. 11A; Neutral: R=-0.99, p<0.01, n=4; Fig. 11B). In addition, damage to BA25 produced widespread changes in animals with BA14 lesions, including decreased gaze toward positive and negative social scenes (Positive: R=-0.97, p=0.03, n=4; Fig. 11C; Negative: R=-0.95, p=0.05, n=4; Fig. 11D) and decreased attention to the eyes of positive and negative stimuli (Positive: R=-0.98, p=0.02, n=4; Fig. 11E; Negative: R=-0.95, p=0.05, n=4; Fig. 11F).

Discussion

This study indicated for the first time that subregions of the OFC make separable contributions to social attention and emotion regulation. Although none of the lesions impacted the differential looking to social versus nonsocial movies (greater looking at social than nonsocial movies), they did alter the pattern of looking across social contexts. As summarized in Table 2, both areas BA12 and BA13 appear critical for directing attention to the eyes of negative and positive social stimuli and for modulating arousal to emotional cues, more specifically positive social cues. In contrast, BA14 does not critically contribute to attention to social cues and has a more limited role in modulating arousal to social cues. These findings will be discussed in turn.

The role of BA12 in modulation of attention to salient social cues

Lesions to OFC BA12 yielded significant deficits in attention to social stimuli without producing any effects on nonsocial stimuli. Thus, across all valences of social movies, three of the four BA12 animals showed a reduced attention to the eyes as compared to controls, and this effect was particularly pronounced when viewing

emotional (positive and negative) stimuli as compared to neutral stimuli. Furthermore, the extent of BA12 lesions correlated negatively with attention to the body of positive stimuli within the movies, such that the larger the BA12 lesions, the greater reduction in attention to the stimuli within the positively valenced movies. Finally, compared to control animals, all animals with BA12 lesions showed greater pupil diameter, especially to positive social stimuli, indicating altered modulation of arousal when animals viewed positive social movies. Taken together, these findings suggest that dysfunction of area BA12 may alter the processing of emotionally charged stimuli as reflected by an avoidance of salient regions of positive and negative faces and an increase in arousal to positive social stimuli.

Despite the small number of animals and the variability in lesion extent, the findings are consistent with previous studies in humans and nonhuman primates suggesting that BA12 is important for processing social affective cues by introducing a negative bias when processing faces with emotional content. For example, in humans, BA12 shows specific functional activation to negatively valenced facial expressions compared to neutral ones in emotional face viewing tasks (Blair et al., 1999; Vuilleumier et al., 2001) and in macaques the lateral OFC exhibits specific functional activity when viewing social interactions (Sliwa & Freiwald, 2017). Furthermore, natural lesions to the OFC in humans, including area BA12, decreased eye gaze, especially to fearful faces (Wolf et al., 2016) and decreased recognition of negative emotion in faces (Dal Monte, Costa, Noble, Murray, & Averbeck, 2015). In marmosets, lesions to the lateral OFC that include area BA12 increases anxiety behaviors in social groups (Agustín-Pavón et al.,

2012), which may be a manifestation of the decreased eye attention seen in the present study following area BA12 lesions (see Fig. 7B).

Area BA12 lesions also increased monkey's pupil diameter when viewing faces of conspecifics depicting positive valence. In humans, pupil dilation during faces and/or emotional stimuli processing is thought to reflect levels of arousal (Bradley et al., 2008; Siegle, Granholm, Ingram, & Matt, 2001; Siegle, Steinhauer, Carter, Ramel, & Thase, 2003; Steinhauer & Hakerem, 1992), mediated by sympathetic autonomic system activation (Bradley et al., 2008). Accordingly, the present results suggest that area BA12 plays a role in the regulation of socioemotional-dependent autonomic arousal (emotional self-regulation). Further, although the sympathetic tone during emotion processing appears to be modulated by the amygdala (Laine et al., 2009), prefrontal cortex areas, including the OFC, exert a top-down regulation of the amygdala to enable cognitive reappraisal and effective emotion regulation (Bachevalier & Loveland, 2006; Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Roberts, 2006). For instance, in humans, down-regulation of negative emotion via reappraisal specifically activated the lateral orbitofrontal cortex (area BA12) and decreased amygdala activity (Ochsner et al., 2004). In addition, decreased amygdala activity was associated with effort-related pupil dilation (Johnstone et al., 2007; Urry et al., 2006; van Reekum et al., 2007). Thus, in the present study, the heightened pupil dilation in monkeys with area BA12 lesions may also reflect higher arousal resulting from an increased amygdala activity.

The present findings mirror also those reported in many studies examining the effects of amygdala lesions on social attention, perhaps indicative of the importance of the OFC-amygdala network for social attention. Specifically, amygdala lesions in humans

result in broad impairments of emotion processing, especially for negative valence (Adolphs, Baron-Cohen, & Tranel, 2002; Adolphs, Tranel, Damasio, & Damasio, 1994) and amygdala lesions in rhesus macaques yield decreased attention to the eyes of negative faces and increased pupil diameter to negative faces presented in still images (Dal Monte et al., 2015).

It is also interesting to note that changes in emotional regulation and modulation of arousal seen after area BA12 lesions may be the result of unintended damage to cortical areas adjacent to area BA12. Principally, damage to PrCO was found in three of the four BA12 animals and although small, this damage was associated with increased overall attention to neutral social stimuli, decreased attention to the body of negative social stimuli, and decreased overall attention to negative nonsocial stimuli. These deficits may reflect the broader role assumed to PrCO in reward and stimulus valuation (Kaskan et al., 2016) and may be associated to the strong connections between PrCO and the lateral OFC, including BA12 (Cavada et al., 2000). In addition, damage to BA45, which most likely resulted from leakage of ibotenic acid while removing the needles, was also correlated with decreased gaze toward the body of positive social stimuli, mirroring the effects found with damage to BA12 and highlighting the tight relationship between damage in these two regions. Overall, the findings support the important role of the lateral prefrontal area in attention to social cues, particularly cues with a negative valence, and the modulation of arousal.

The role of BA13 in attention to emotional stimuli

Compared to controls, all five animals with area BA13 lesions showed less attention to the eyes of negative stimuli and three of the five animals with BA13 lesions

also showed less attention to the eyes of positive stimuli. Furthermore, all animals with area BA13 lesions displayed greater pupil dilation to positive social stimuli. These results were consistent regardless of variations in lesion extent and suggest that damage to BA13 has broad impacts on the modulation of attention and arousal, by decreasing attention to the eyes of emotional stimuli and increasing arousal to positive social cues. Interestingly, these deficits parallel those obtained by damage to area BA12 (see above), suggesting a complementary role of areas BA13 and 12 in emotional modulation. These data paralleled those previously reported in the literature.

With regard to attention to facial cues, broad damage to the OFC in humans, including areas BA13, yields poorer abilities to distinguish emotional expressions from neutral faces (Tsuchida & Fellows, 2012), presumably due to a lack of attention to the eyes. In addition, monkeys with damage restricted to areas BA13 & BA11 display blunted response to social threats (Bachevalier et al., 2011; Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin et al., 2007; Kazama et al., 2014; Machado, Kazama, & Bachevalier, 2009) and receive increased aggression and threatening behaviors from their partners, likely due to impaired emotional behavior exhibited by the lesioned animals.

Furthermore, our results indicated that damage to area BA11, just anterior to area BA13, was significantly correlated with increased attention to the mouth and body of positive social stimuli, further supporting the complimentary role of area BA11 and its relation to area BA13. Several previous reports have also shown that OFC damage including area BA11 is associated with impaired socioemotional regulation (Beer et al., 2003; Bramham et al., 2009), impaired identification of emotional expressions (Hornak et al., 2003; Tsuchida & Fellows, 2012), and changes in attention to facial features (Wolf et

al., 2014) in humans. Such damage also reduced fear expression (Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin et al., 2007; Machado et al., 2009) and impaired social interactions (Kazama et al., 2014) in nonhuman primates.

Damage to BA13 also caused significant increases in pupil diameter to positive social stimuli, again mirroring the deficits caused by BA12. As discussed above, increased pupil diameter in humans is thought to represent increases in emotional arousal and cognitive effort and these effects may be caused, in part, by disinhibition of the amygdala. The role of BA13 in mediating arousal to social cues is well-supported in the literature, as damage to BA13 results in decreased fear response in social and nonsocial tasks (Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin et al., 2007; Machado & Bachevalier, 2008). Though these studies did not measure arousal, per se, it is clear that damage including BA13 disrupts normal behavior in highly arousing contexts. Our studies extended these findings by demonstrating that damage to BA13 yields an impaired and dysregulated emotional state during the processing of affective cues from a conspecific. Increased arousal during social interactions may contribute to the decreased eye gaze present with BA13 lesions to both positive and negative social stimuli and may result in disrupted social interactions found in previous reports. Overall, it appears that BA13, most likely in concert with areas BA11 and BA12, modulates attention and arousal to social information regardless of valence.

The role of BA14 in modulating attention

Finally, we have shown that damage to BA14 is not essential for modulating attention or arousal when viewing social stimuli. Compared to controls, animals with BA14 lesions did not differ significantly from controls on overall attention to nonsocial

stimuli or to the focal objects of neutral and negative nonsocial stimuli. In addition, animals with BA14 lesions did not differ from controls in any measure of attention to specific cues of faces and of arousal to social stimuli. The lack of significant deficits could be due to the limited damage of area BA14 in one animal (BA14-3; < 1%); although this possibility is unlikely given that an equal lack of emotion regulation was seen in another animal in the BA14 group with a more complete lesion (BA14-1, 41%).

The present findings are consistent with other reports in nonhuman primates (Noonan, Sallet, Rudebeck, Buckley, & Rushworth, 2010), indicating limited behavioral effects following BA14 lesions and suggesting that this area alone is not critical for the regulation of emotion, particularly to threatening cues. Though some studies suggest that BA14 is important for the processing of positive outcomes (Hampshire et al., 2012; Noonan et al., 2012, 2011), given that BA14 lesions in our studies yielded no changes in behavior to positive cues in social or nonsocial contexts, our findings support the notion that BA14 is not critical for this ability. Interestingly, within Group BA14, unintended damage to BA25 resulted in widespread effects on attention to social stimuli, including decreased gaze toward the scenes and eyes of emotional stimuli and decreased gaze toward negative nonsocial stimuli. These findings suggest that area BA25 may make a greater contribution to attention to social cues than area BA14. Area 25 is an area of the subgenual anterior cingulate cortex (sgACC) that plays a role in modulating arousal. Based on the broader literature on the importance of area BA14 in regulating arousal (Bechara et al., 2000; Patterson et al., 2002), we had hypothesized that BA14 would be involved in modulating arousal in the present study. However, our findings suggest that

levels of arousal are not regulated by area BA14, but rather by area BA25 and the sgACC. Overall, our study highlights the limited role of BA14 in emotional processing.

Conclusions and Limitations

Together, these data support the role of OFC areas BA12 and BA13 in modulating socioemotional processing by influencing attention to key regions of social stimuli and modulating arousal to positive social cues. In addition, these data elaborate on the proposed role of OFC area BA14 by demonstrating that this region is not critical for emotional modulation. These data should be considered in light of the experimental limitations of our study, including small sample sizes and variations in surgical lesion extent and unintended damage. In addition, though there were variations across our sample related to the age and sex of the subjects, these only impacted broad attention measures and did not impact the core findings of the study, demonstrating that subregions of the OFC are differentially involved in the processing of highly salient regions of socioemotional stimuli. Despite these limitations, we believe that the data presented here paralleled earlier reports in the literature and capture the critical role of the lateral OFC in socioemotional processing.

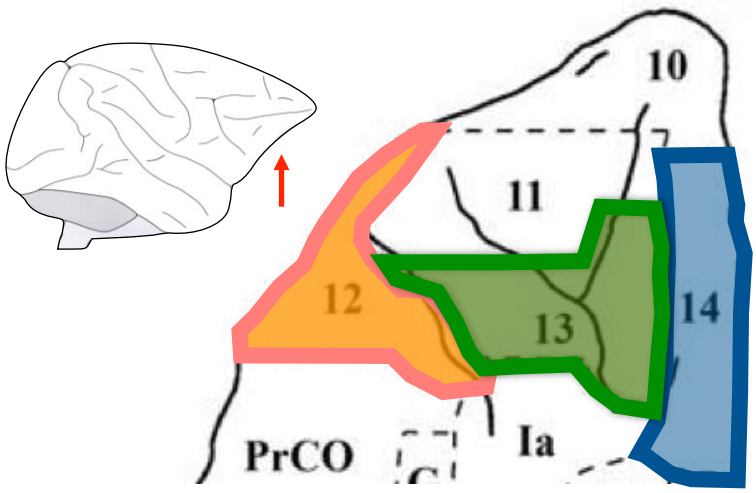

Figure 1. Subregions of the orbitofrontal cortex.
Illustration of the ventral view of the left orbitofrontal cortex (OFC), location indicated by the red arrow in the inset picture of a macaque brain. The intended orbitofrontal lesion areas in our study include BA12 (orange), BA13 (green), and BA14 (blue). Surrounding areas of the OFC include BA11, posterior opercular cortex (PrCA), insula (Ia).

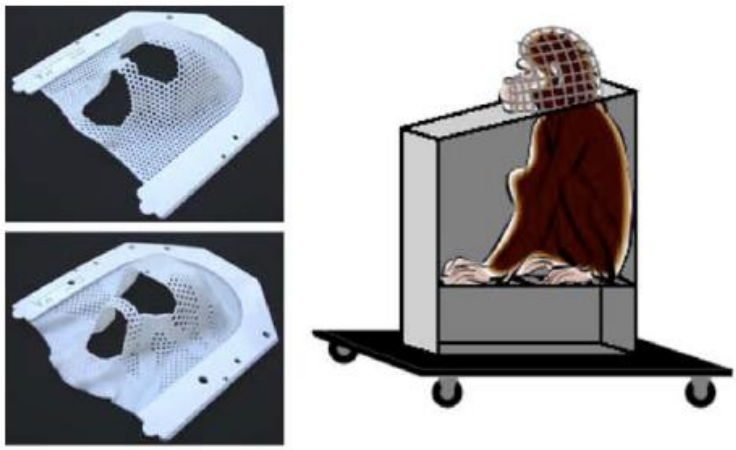

Figure 2. Depiction of head restraint.
Thermoplastic helmets (left) were custom fitted for each subject, and affixed to the primate chair (right) to reduce head movement during testing. From Machado and Nelson, 2011.

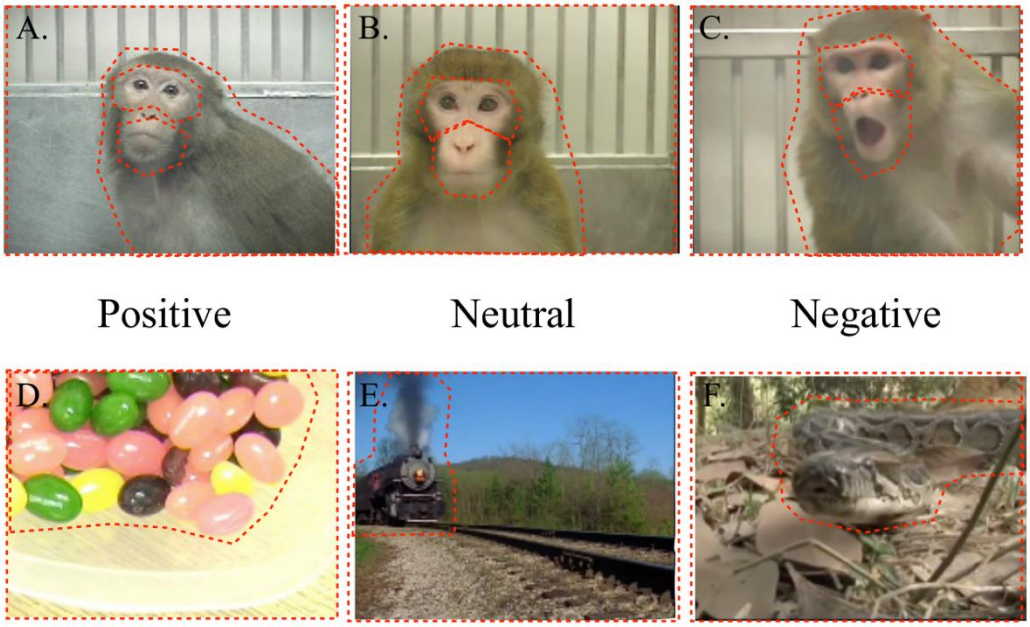

Figure 3. Examples of stimuli used in eye-tracking experiment.
Top row depicts exemplars of still images from social movies of positive (A.), neutral (B.), and negative (C.) valence. Bottom row depicts exemplars of still images from nonsocial movies of positive (D.), neutral (E.), and negative (F.) valence. Dotted red borders outline the region of interest (ROI) areas for each stimulus type. For social stimuli, ROIs covered the eyes, mouth, body, and whole scene. For nonsocial stimuli, ROIs covered the focal object and the whole scene.

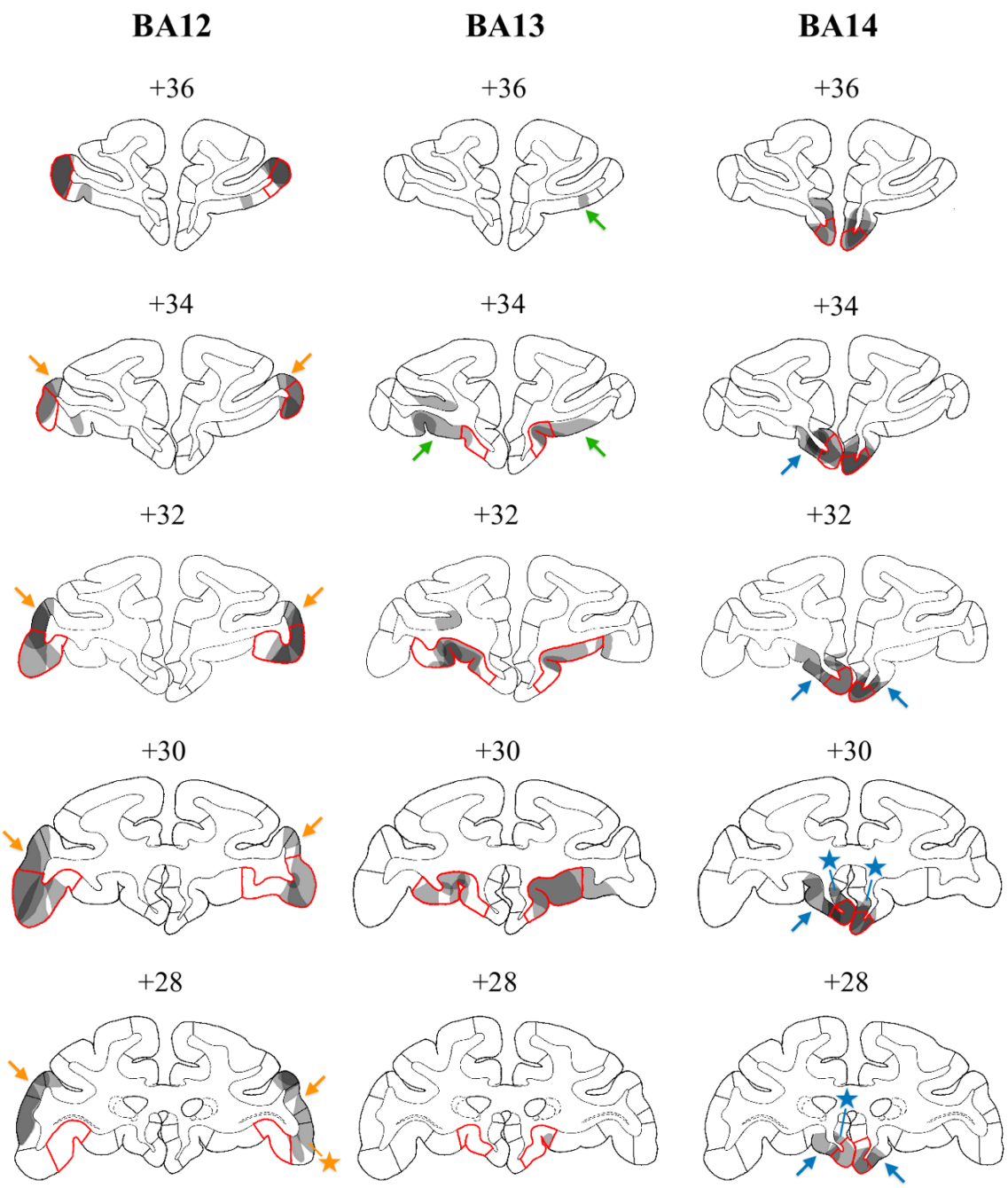

Figure 4. Depiction of lesion extent for each lesion group.
Coronal sections through the extent of area BA12 lesions (left column), area BA 13 lesions (middle column), and area BA14 lesions (right column). The red outlines on each section indicate the intended extent of the lesions and numerals above each section refer to the distance in millimeters from the interaural plane. Lesion extent for each subject was layered on each other, such that darker areas indicate area of damage common to all subjects, whereas lighter area indicate damage to only some subjects. Unintended damage included BA45 (orange arrows) and PrCO (orange stars) from Group BA12, BA11 for Group BA13 (green arrows), and BA25 (blue stars) and BA13 (blue arrows) for Group BA14.

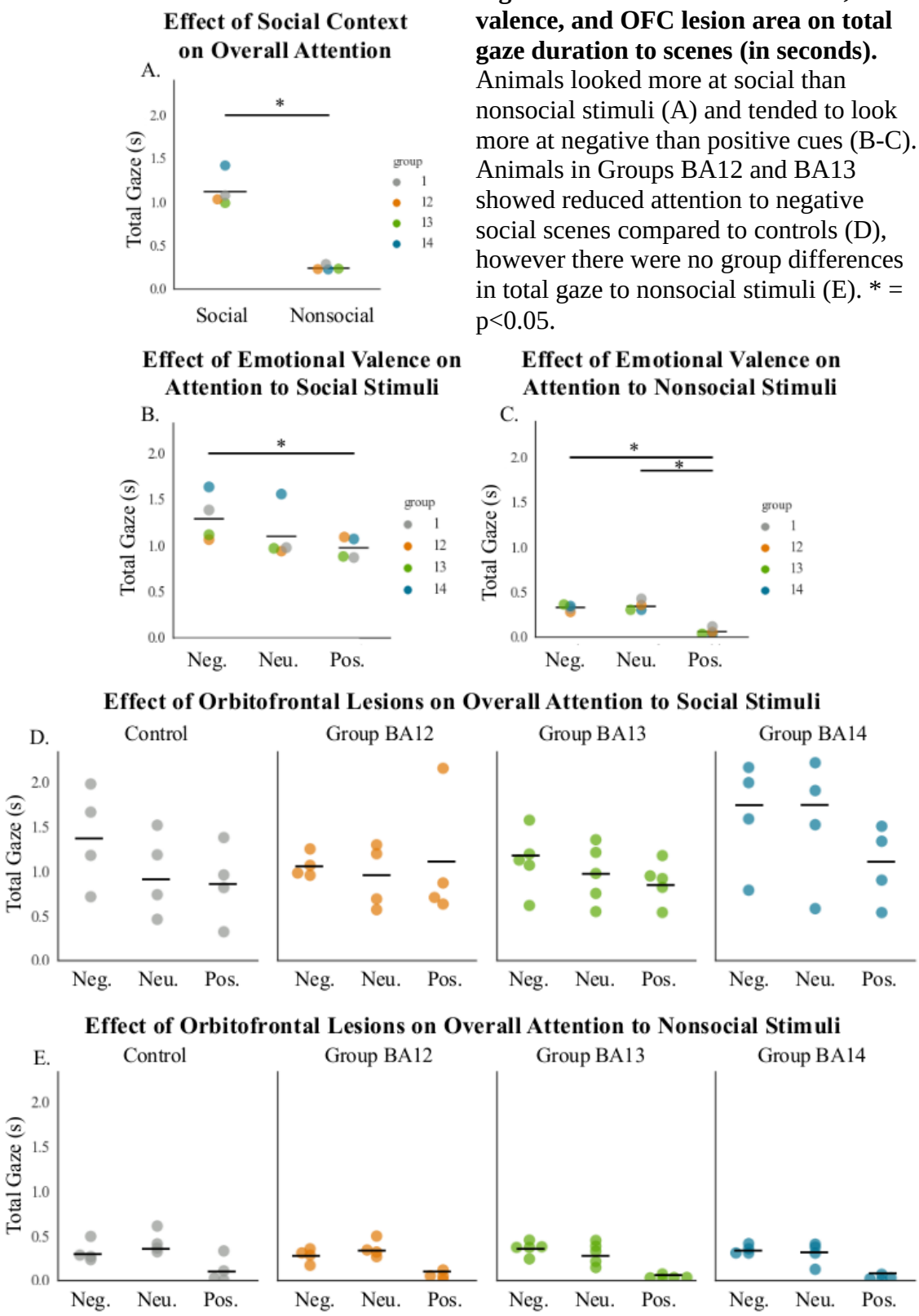

Figure 5. Effects of social context, valence, and OFC lesion area on total gaze duration to scenes (in seconds). Animals looked more at social than nonsocial stimuli (A) and tended to look more at negative than positive cues (B-C). Animals in Groups BA12 and BA13 showed reduced attention to negative social scenes compared to controls (D), however there were no group differences in total gaze to nonsocial stimuli (E). * = $p<0.05$.

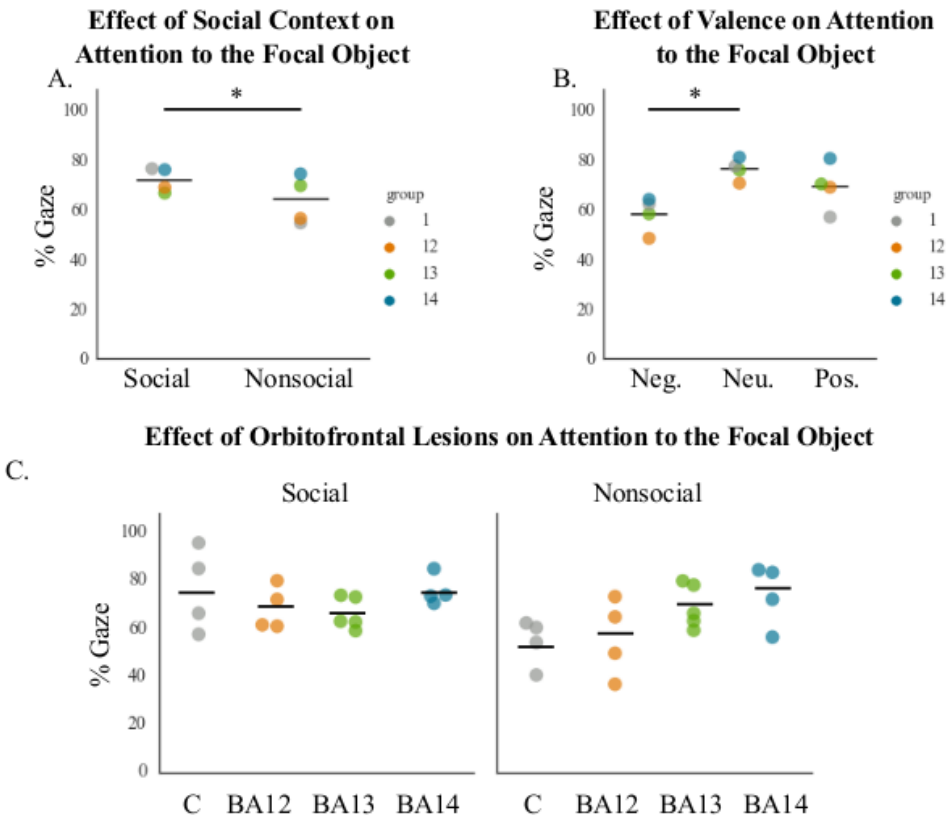

Figure 6. Effects of social context, valence, and OFC lesion area on percent gaze toward focal objects of scenes.
Animals tended to look more at the focal object in social compared to nonsocial scenes (A). Regardless of social context, all subjects looked more at the focal object of neutral compared to negative scenes (B). There was a significant interaction between OFC lesion group and social context, driven by increased attention to nonsocial stimuli by Group BA14, though this effect did not reach significance (C.). * = $p<0.05$.

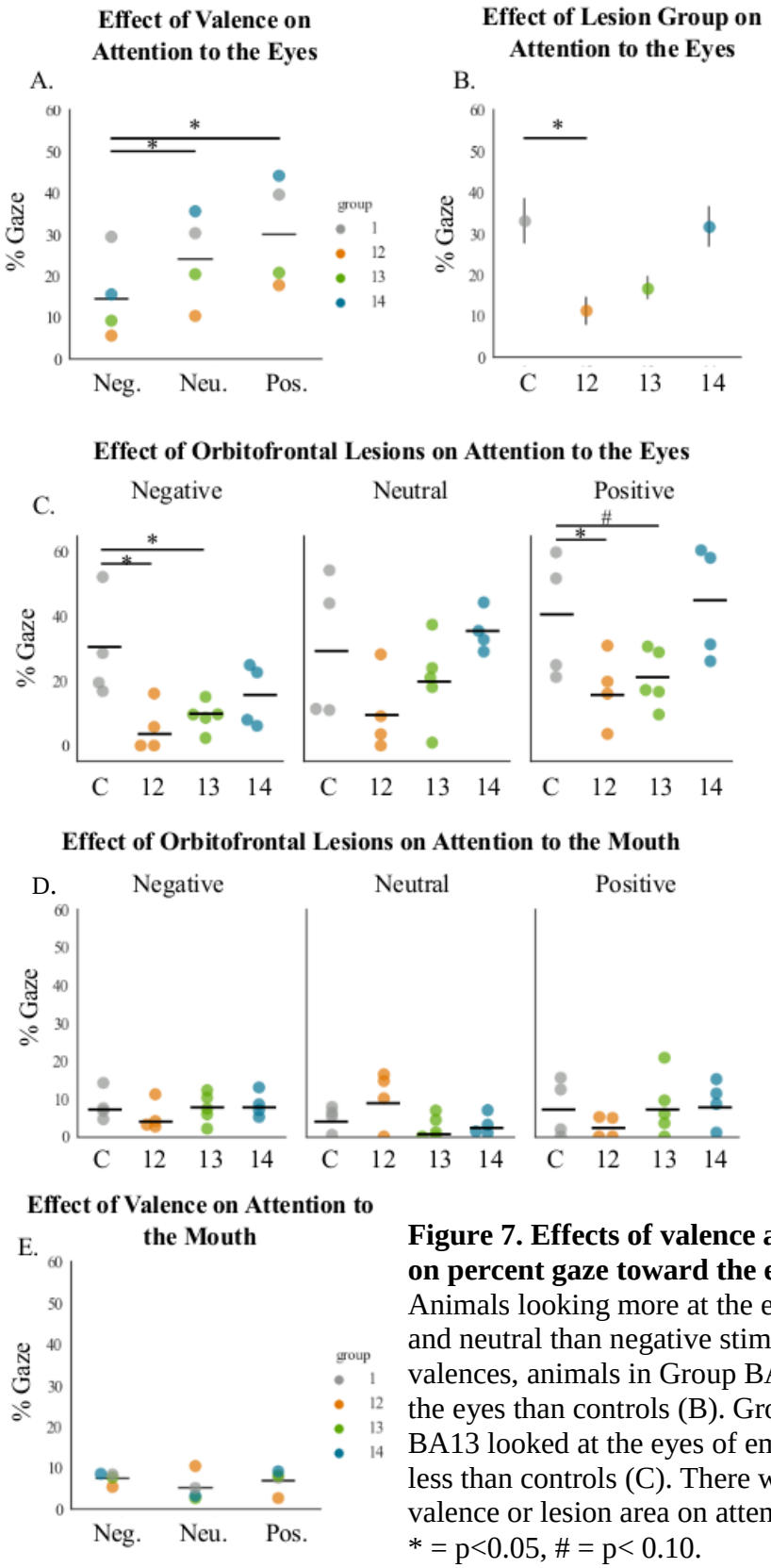

Figure 7. Effects of valence and OFC lesions on percent gaze toward the eyes and mouth. Animals looking more at the eyes of positive and neutral than negative stimuli (A). Across all valences, animals in Group BA12 looked less at the eyes than controls (B). Groups BA12 and BA13 looked at the eyes of emotional stimuli less than controls (C). There were no effects of valence or lesion area on attention to the mouth. * = $p<0.05$, # = $p< 0.10$.

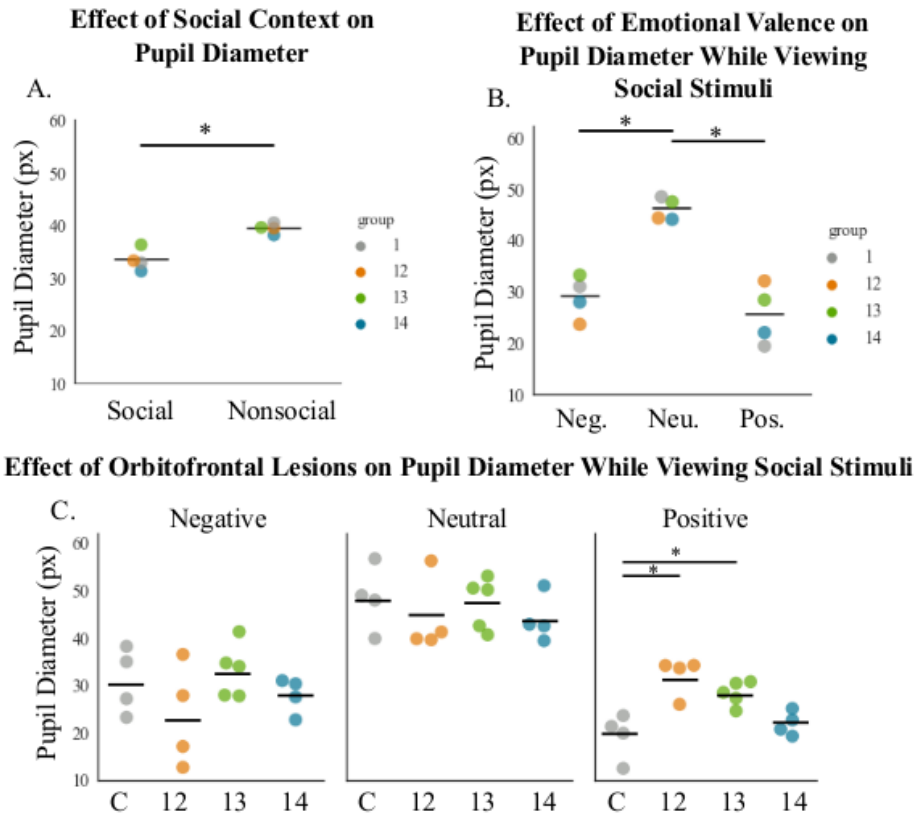

Figure 8. Effects of social context, valence, and OFC lesion area on pupil diameter (in pixels).
Animals showed significantly greater pupil diameter to nonsocial compared to social stimuli (A). Within social stimuli, animals showed greater pupil diameter to neutral compared to emotional stimuli (B). In addition, Groups BA12 and BA13 showed significantly greater pupil diameter to positive social stimuli compared to controls (C). * = $p<0.05$.

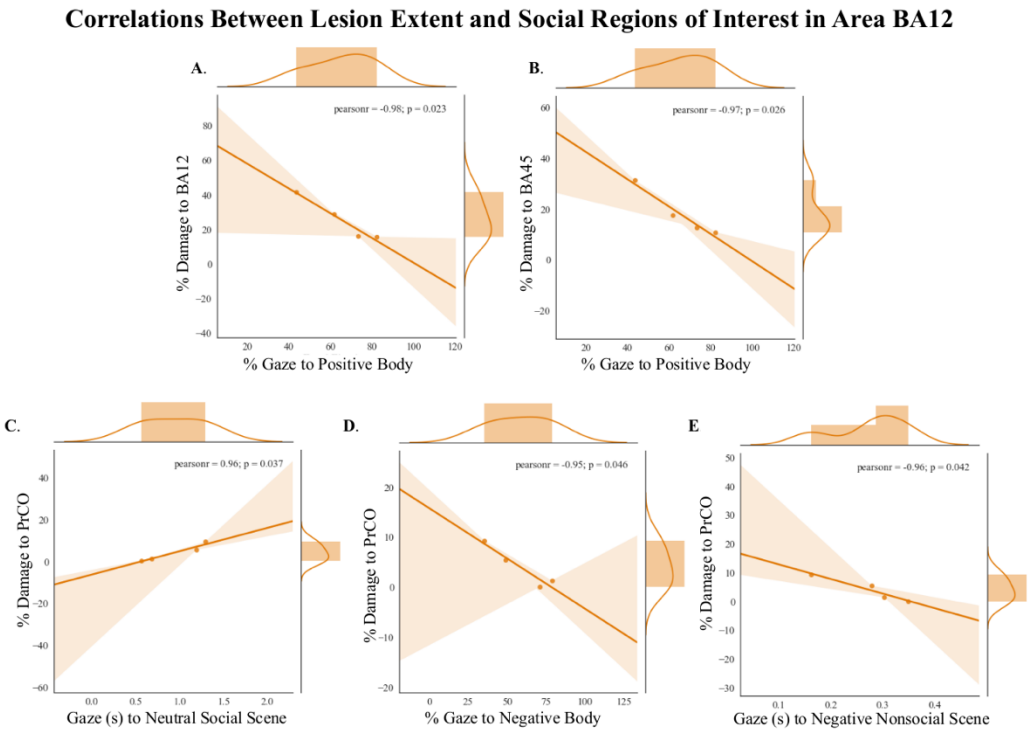

Figure 9. Correlations between lesion extent and regions of interest for Group BA12.

Damage to BA12 and BA45 was significantly correlated with decreased gaze to the body of positive social stimuli (A-B). Within Group BA12, damage to PrCO was correlated with increased gaze toward neutral social scene (C), decreased gaze toward the body of negative stimuli (D) and decreased gaze toward negative nonsocial scenes (E).

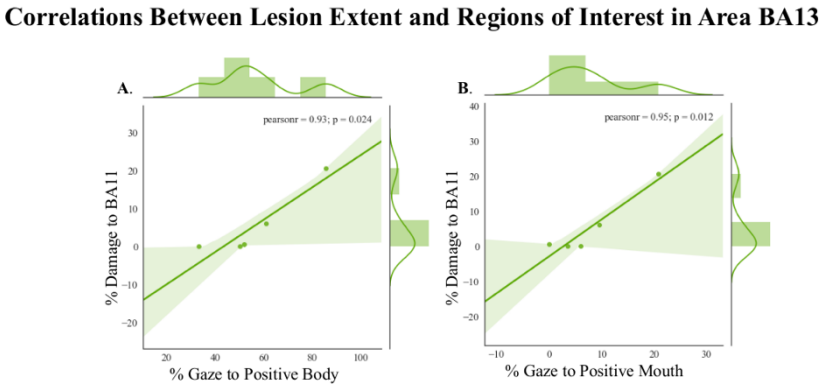

Figure 10. Correlations between lesion extent and regions of interest for Group BA13.
Within Group BA13, damage to BA11 was significantly correlated with increased gaze toward the mouth and the body of positive social stimuli (A-B).

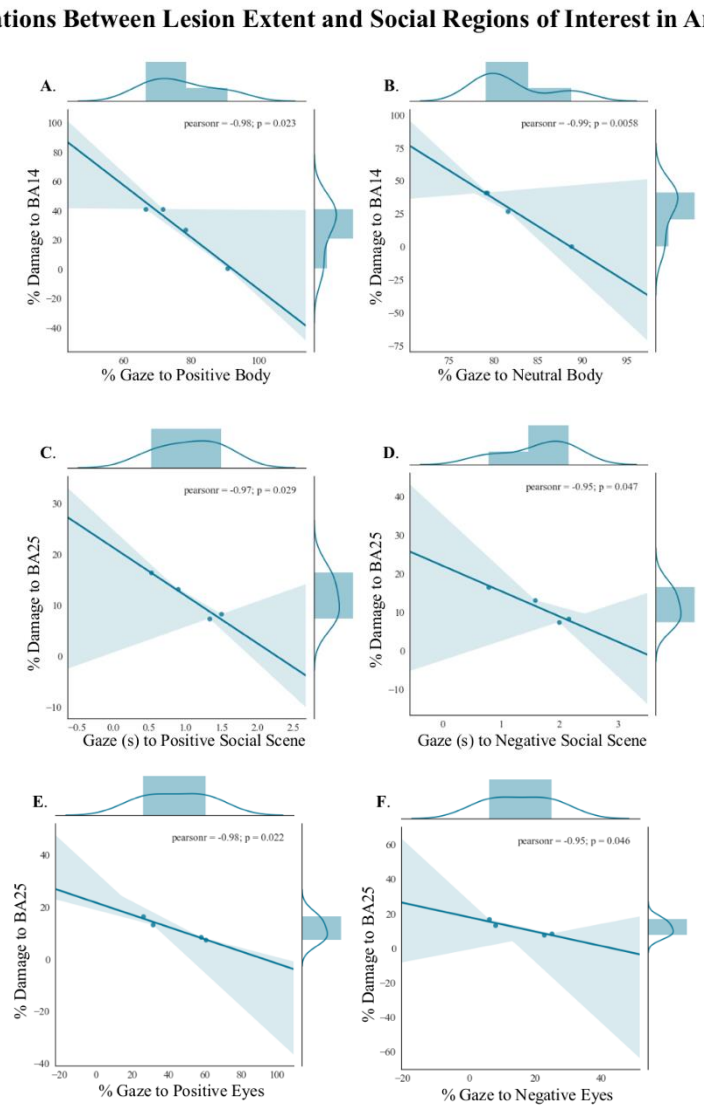

Figure 11. Correlations between lesion extent and regions of interest for Group BA14.

Damage to BA14 was inversely correlated with gaze toward the body of positive and neutral social stimuli (A-B). Within Group BA14, damage to BA25 was significantly correlated with decreased gaze toward the overall scene of emotional social stimuli (C-D) and also with decreased attention to the eyes of emotional social stimuli (E-F).

Cases	Area 12				Area 45				Area PrCO			
	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W
BA12-1	20.17	36.99	28.58	7.461	22.04	12.76	17.4	2.813	0	18.5	9.252	0
BA12-2	48.6	34.35	41.47	16.69	42.73	19.82	31.28	8.47	0	0	0	0
BA12-3	14.13	17.48	15.8	2.47	15.04	10.14	12.59	1.524	0	2.57	1.285	0
BA12-4	26.82	4.274	15.54	1.146	18.79	2.734	10.76	0.514	3.545	7.196	5.371	0.255
X	**27.43**	**23.27**	**25.35**	**6.943**	**24.65**	**11.36**	**18.01**	**3.33**	**0.886**	**7.068**	**3.977**	**0.064**

Cases	Area 13				Area 11			
	L	R	Avg	W	L	R	Avg	W
BA13-1	2.736	1.945	2.341	0.053	0	0	0	0
BA13-2	18.95	21.58	20.26	4.089	11.78	0.404	6.092	0.048
BA13-3	14.79	0	7.397	0	1.24	0	0.62	0
BA13-4	12.67	27.59	20.13	3.495	0	0	0	0
BA13-5	31.11	22.37	26.74	6.96	22.32	18.72	20.52	4.179
X	**16.05**	**14.7**	**15.37**	**2.919**	**7.068**	**3.825**	**5.446**	**0.845**

Cases	Area 14				Area 13				Area 25			
	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W
BA14-1	45.09	36.85	40.97	16.61	10.9	4.011	7.455	0.437	10.38	6.083	8.231	0.631
BA14-2	48.93	32.83	40.88	16.06	5.328	3	4.164	0.16	11.67	14.51	13.09	1.694
BA14-3	0.084	0.379	0.231	0.00	21.66	0	10.83	0	13.01	1.755	7.381	0.228
BA14-4	5.647	47.67	26.66	2.692	29.21	9.935	19.57	2.902	22.69	10.13	16.41	2.299
X	**24.94**	**29.43**	**27.18**	**8.843**	**16.78**	**4.236**	**10.51**	**0.875**	**14.44**	**8.12**	**11.28**	**1.213**

Table 1. Histological lesion extent across all impacted areas.
Percent damage to relevant orbitofrontal areas for animals in Group BA12 (top), BA13 (middle) and BA14 (bottom). For each case, damage is listed for the left (L) and right (R) hemispheres, and the average (Avg) and weighted average (W) are computed. The weighted average is calculated as W = (L*R)/100 as defined by Hodos & Bobko (1984). Below each group in bold is the average value for each parameter (X).

	Overall Attention	Focal Attention	Attention to the Eyes	Attention to the Mouth	Pupil Dilation
Group BA12	-	% damage associated with ⬇ to pos.	⬇ to pos. and neg.	-	⬆ to pos. social
Group BA13	-	-	⬇ to pos. and neg.	-	⬆ to pos. social
Group BA14	-	% damage associated with ⬇ to pos. and neu.	-	-	-

Table 2. Summary of the effects of OFC damage on attention to social and nonsocial stimuli.

Damage to BA12 and BA13 of the OFC results in similar deficits, specifically increased pupil dilation to positive social stimuli and decreased attention to the eyes of positive and negative social stimuli. In contrast, damage to BA14 produced no significant effects on attention or arousal.

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Impacts of orbitofrontal lesions on fear retention and expression in rhesus macaques

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Abstract

Damage to the orbitofrontal cortex (OFC) results in broad impairments in emotional regulation, perhaps due to impaired maintenance and valuation of stimulus-outcome associations. The subregions of the OFC have distinct connectivity across brain regions important for supporting these behaviors, suggesting a potential functional dissociation among OFC subregions in emotional regulation. However, few studies have examined OFC subregions in isolation. Thus, eight rhesus macaques with lesions to OFC areas BA12 (n=3), BA13 (n=3), and BA14 (n=2) and sham-operated controls (n=3) were tested on the AX+/BX- fear-potentiated startle paradigm (Kazama et al., 2013) to measure the impact of damage to OFC subregions on emotional regulation. Subjects were trained on an initial set of AX+/BX- cues prior to surgery and then tested following recovery to measure the impacts of OFC lesions on the retention of fear and safe cues and modulation of fear-potentiated startle. Following this initial testing, subjects were trained on novel AX+/BX- cues and retested to measure retention and modulation of cues learned after OFC lesion creation, followed by extinction training. Damage to BA12 resulted in specific deficits in the modulation of fear response without impacting the retention of cue values. In contrast, damage to BA13 yielded impairments in the retention of fear and safe cue value, and thus impaired modulation of fear response. Surprisingly, damage to BA14 did not result in any impairments to retention or modulation, and no lesion group was impaired on the acquisition or extinction of stimulus associations. Thus, subregions of the OFC make unique contributions to emotional regulation through moderating emotional stimulus-outcomes associations.

Introduction

Emotional dysregulation, the inability to modulate emotional response (Gross, 2014), is a marker of numerous neuropsychiatric disorders, such as anxiety, depression, and PTSD (Drevets, 2007; Jackowski et al., 2012; Jovanovic, Kazama, Bachevalier, & Davis, 2012; Milad et al., 2007). Both the amygdala and prefrontal cortex (PFC) are known to modulate emotional regulation (Myers & Davis, 2007; Phelps & LeDoux, 2005; Sabatinelli et al., 2011). Within the PFC, the orbitofrontal cortex (OFC) is a critical region for processing emotional stimuli and modulating emotional response (Bechara, Tranel, & Damasio, 2000; Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994; Hornak, Rolls, & Wade, 1996) and cortical damage that includes the OFC results in profound impairment in emotional regulation (Bechara et al., 2000; Bechara, Damasio, Tranel, & Damasio, 1997; Beer, Heerey, Keltner, Scabini, & Knight, 2003; Beer, John, Scabini, & Knight, 2006; Wolf, Pujara, Baskaya, & Koenigs, 2016).

The OFC contributes broadly to complex human behavior, impacting acquisition and maintenance of stimulus-outcome associations and changing the weights of those associations based on additional information, like context and memory. Most of our understanding of the OFC comes from reports of natural lesions in humans (Bechara et al., 2000; Beer et al., 2003, 2006; Wolf et al., 2016) and intentional lesion studies in nonhuman animals (Agustín-Pavón et al., 2012; Bachevalier et al., 2011; Dias et al., 1996; Izquierdo & Murray, 2004; Izquierdo et al., 2005; Machado & Bachevalier, 2008; Schoenbaum, Chiba, & Gallagher, 1998; Schoenbaum, Nugent, Saddoris, & Setlow, 2002; Schoenbaum et al., 2003). In humans, OFC damage is rarely limited to one area of the OFC and often impacts the surrounding structures. However, common features of

lesions that include the OFC is a decrease in the capacity to regulate emotional response (Bechara et al., 1997; Beer et al., 2003; Blair & Cipolotti, 2000; Saver & Damasio, 1991) and impaired modulation of stimulus value (Fellows & Farah, 2003; Hornak et al., 2004; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001; Rolls, Hornak, Wade, & McGrath, 1994). Intentional lesions in nonhuman primates and rodents also impair emotional behavior (Izquierdo et al., 2005; Kalin et al., 2007; Kazama et al., 2014; Machado & Bachevalier, 2008; Noonan et al., 2010) as well as the flexible modulation of stimulus-outcome associations (Butter, 1969; Dias, Robbins, & Roberts, 1996; Jones & Mishkin, 1972; Milad & Quirk, 2002; Schoenbaum, Nugent, Saddoris, & Setlow, 2002; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003). In addition, a large corpus of electrophysiological recording studies in rodents (Milad & Quirk, 2002; Schoenbaum, Chiba, & Gallagher, 1998; Schoenbaum, Chiba, & Gallagher, 2000), and neuroimaging studies in humans (Gottfried, O'Doherty, & Dolan, 2002; O'Doherty, Kringelbach, Rolls, Hornak, & Andrews, 2001; Phelps, Delgado, Nearing, & Ledoux, 2004) have shown that neurons in the OFC show distinct patterns of activation during all stages of stimulus-outcome association learning (i.e. acquisition, anticipation, errors, etc.) and rapidly change their firing patterns when reward contingencies shift (Arana et al., 2003; Bechara et al., 2000; Elliott, Dolan, & Frith, 2000; Hampshire, Chaudhry, Owen, & Roberts, 2012; Kaskan et al., 2016; Pears, Parkinson, Hopewell, Everitt, & Roberts, 2003; Roesch & Olson, 2005; Rudebeck & Murray, 2011; Schoenbaum et al., 2000; Strait, Blanchard, & Hayden, 2014; Thorpe, Rolls, & Maddison, 1983; Zald et al., 2014). There is also evidence indicating some functional dissociation of emotional regulation across the OFC subfields. For example, during stimulus-outcome association learning, lateral areas of the

OFC, such as Brodmann Area (BA) 12, show increased activity during changes in outcomes and suppression of conditioned response (Arana et al., 2003; Elliott et al., 2000; Hampshire et al., 2012). However, once contingencies are learned, central OFC areas, such as BA13, are particularly important for the flexible representation and updating of reward value (Elliott, Agnew, & Deakin, 2008; Kringelbach & Rolls, 2003; Murray, Moylan, Saleem, Basile, & Turchi, 2015; Noonan, Kolling, Walton, & Rushworth, 2012; Noonan, Mars, & Rushworth, 2011; Rolls & Deco, 2016; Rudebeck & Murray, 2011; West, DesJardin, Gale, & Malkova, 2011). Area BA13 and more medial OFC areas, such as BA14, are active during anticipation of a reward (Thorpe et al., 1983; Tremblay & Schultz, 1999). In addition, lateral OFC shows greater activation to negative stimuli, whereas medial OFC responds more to positive stimuli, and central OFC to both (Hampshire et al., 2012; Monosov & Hikosaka, 2012; Morrison & Salzman, 2009; Noonan et al., 2012; J. O'Doherty et al., 2001). Together, these findings suggest that the OFC is critically involved in associative learning and flexible goal-directed behavior in adult animals, and that subregions of the OFC may offer differential support for these functions.

The importance of the OFC in emotional regulation lies in part in the widespread inputs it receives from sensory cortical areas and autonomic signals from the hypothalamus as well as its extensive bi-directional connections with the amygdala (Barbas, 1993; Carmichael & Price, 1995). Thus, dysfunction of the OFC may lead to unchecked bottom-up influence of autonomic signals from the hypothalamus or emotionally-charged information provided by the amygdala (Milad & Rauch, 2007). The complex functional connectivity between the OFC and both the amygdala and

hypothalamus varies across OFC subregions. For example, both BA12 and BA13 have reciprocal connections with the basolateral nucleus of the amygdala (BLA), whereas BA14 sends unilateral projections to the BLA, and only BA13 projects to the central nucleus of the amygdala (Barbas, 1993; Carmichael & Price, 1995). In addition to these clear differences in anatomical projections (Barbas, 1993; Öngür et al., 2003), OFC subregions mediate different functional control of emotion regulation (Jenkins et al., 2014). Specifically, areas BA11, BA12, and BA13, together known as the orbital network of the OFC, are associated with the integration and modulation of sensory information and the representations of the value of stimuli, whereas the medial OFC network, including areas BA14, BA25, and BA10, is more strongly implicated in modulating arousal (Ongür & Price, 2000; Price, 2006). The OFC networks also vary in their connectivity with regions of the hypothalamus, as the orbital OFC network primarily connects to the lateral hypothalamus and the medial OFC network has extensive connections with the medial hypothalamus, two functionally distinct regions (Ongür et al., 1998), further supporting the notion that OFC regions may contribute differentially to arousal as well as emotional regulation. However, the specific functions of the OFC subregions remain understudied, and the distinct contributions of cortical fields BA12, BA13 and BA14 to emotional processing remain unclear. Thus, the present study assessed the effects of selective damage to OFC regions BA12, BA13, and BA14 on the acquisition and expression of fear and safety cues, as well as the modulation of the fear response in the presence of safety signals (conditioned inhibition), and extinction in a model system, the rhesus macaque.

Emotional modulation was measured using the fear-potentiated startle AX+/BX- paradigm (Kazama et al., 2013), a task that has been successfully used in rodents, nonhuman primates, and humans to measure fear expression and modulation (Jovanovic et al., 2012; Kazama et al., 2014; Myers & Davis, 2007; van Rooij et al., 2017). Using this task, subjects first learned to discriminate the fear cue from the safety cue to ensure they could successfully discriminate between the two cues and then immediately underwent surgical procedures. Following recovery, subjects were administered a probe test to measure expression of the fear and safe cues as well as to probe their ability to use the safe cue to modulate their emotional responses to the fear cue. Then, subjects underwent post-surgical learning using novel fear and safe stimuli, followed by a final probe test. Finally, using the most recently learned fear stimuli, animals were tested for extinction of emotional reactivity to that fear cue.

Given the role of BA12 in value prediction and stimulus-reward associations (Lopatina et al., 2015; Noonan et al., 2012), we predicted that damage to BA12 will impair the flexible modulation of fear expression by impairing response suppression after a change in contingencies. Given the broad involvement of BA13 during both the encoding and expectation of reward (Chang, Gariépy, & Platt, 2013; Tremblay & Schultz, 1999; West et al., 2011) and the behavioral changes resultant from BA13 damage in previous reports (Kazama et al., 2014), we predicted that damage to BA13 may impair emotional processing and thus impact the retention and modulation of cues predicting positive and negative outcomes. Finally, given that damage to BA14 impairs physiological arousal to emotional stimuli in humans, we hypothesized that damage to BA14 may result in decreased arousal.

Materials & Methods

All protocols and procedure have been reviewed and approved by the Institutional Animal Care and Use Committee at Emory University and align with the standards set by the NIH (Guide for the Care and Use of Laboratory Animals, 2011).

Subjects

Eleven rhesus macaques were assigned to the study. They ranged in age between 6 and 14 years (mean age = 9.0 years). Subjects were all born into large, naturalistic social groups at the Yerkes National Primate Center Field Station (Lawrenceville, GA) and moved to the Yerkes National Primate Center Main Station (Atlanta, GA) as adults. Subjects were singly housed in appropriate cages with visual and auditory contact with similarly aged macaques of both sexes in a housing room maintained on a 12-hour light/dark cycle. All subjects were maintained on a diet of nonhuman primate chow (Purina, St. Louis, MO), enriched with fruit and vegetables daily and provided water ad libitum. All animals maintained healthy weights through the course of the study. Subjects were pseudorandomly assigned into one of four groups to receive lesions of either OFC area BA12 (Group BA12: n=3, 1 female), OFC area BA13 (Group BA13: n=3, 2 females), OFC area BA14 (Group BA14: n=2, 1 female), or sham-operations (Group C: n=3, 1 female).

All animals had first participated in a study to assess whether the AX+/BX- paradigm could be given to monkeys using stimuli similar to those used in humans (i.e. visual images representing the CS+ and CS- stimuli) and whether the task could be administered repetitively using new stimuli each time. They underwent 2-5 rounds of training in the AX+/BX- task using new visual stimuli for each round (Kazama et al.,

2013). The last round of training preceding surgery was used to assess pre-surgical performance (Fig. 1A). Then, subjects underwent OFC surgery or sham-operations, as described below, corresponding to their group assignment. Following a 1-month recovery period, subjects were tested on post-surgical retention and modulation of the same cues learned prior to surgery (Fig. 1B), followed by acquisition of a novel set of cues to criterion (new learning; Fig. 1C) and then a probe test using the novel (newly learned) stimuli to assess post-surgical retention, conditioned inhibition (Fig. 1D), and finally, extinction.

Neuroimaging and Surgery

Neuroimaging and surgical procedures have been reported elsewhere (Murphy et al., *in prep*). In brief, all lesion subjects were sedated using ketamine HCl (10mg/kg, 100 mg/ml), intubated and anesthetized with inhaled isoflurane (1.0 –2.0%, v/v, to effect) and placed in stereotaxic head restraint. Subjects received an intravenous drip of 0.45% dextrose and sodium chloride to maintain hydration and were placed on a heating pad to prevent hypothermia during scanning and surgical procedures. Vital signs (heart rate, respirations, blood pressure, expired CO2) were monitored throughout the procedures. Magnetic resonance (MR) images were acquired using a 3T Siemens Magnetom Trio system (Siemens Medical Solutions, Malvern, PA). All subjects underwent two different MRI sequences prior to surgery and one-week post-surgery: 1) a high resolution structural T1-weighted MRI images used to determine stereotaxic coordinates of injection sites (3D T1-weighted fast spoiled gradient (FSPRG)-echo sequence, TE=2.6ms, TR=10.2ms, 25º flip angle, contiguous 1mm sections, 12cm FOV, 256 x 256 matrix) and 2) Fluid Attenuated Inversion Recovery (FLAIR) images to help estimate *in vivo* the

location and extent of lesions (TE=140ms, TR=1000ms, inversion time (TI)=2200ms, contiguous 3mm sections, 12cm FOV, 256 X 256 matrix, acquired in 3 series offset 1mm posterior).

Following the first imaging procedure, lesion subjects were immediately transferred to the surgical suite and prepared for aseptic surgical procedures. In all cases, the scalp was disinfected with Nolvasan solution and a local anesthetic (Bupivicaine 0.25% concentration, 1.5 ml) was injected subcutaneously along the midline to reduce the pain during skin incision. The skin was then cut from the orbital ridge to the occiput, and muscle and tissue were gently retracted. For all lesion groups, a craniotomy was made over the OFC of each hemisphere and the dura was retracted to expose the brain surface. Injections were made with a 30-gauge needle attached to a 10μl Hamilton syringe mounted on stereotaxic arms, except for injections of BA12, which were made manually. At each injection site, 0.8-1μl of ibotenic acid (Biosearch Technologies, Novato, CA, 10mg/ml in PBS, pH 7.4) was delivered at a rate of 0.2μl per minute and the injection needles remained in place for one minute before retraction.

Figure 2 illustrates the extent of intended cortical removals for each type of lesion. BA12 lesions (4-23 injections) were intended to include the lateral and orbital surface of the OFC from the ventral lip of the principal sulcus to the lateral lip of the lateral orbital sulcus, and from the posterior border of BA11 anteriorly to the opercular cortex (PrCO) posteriorly. BA13 lesions (7 injections) were intended to include the cortical area between the lateral orbital sulcus and the medial orbital sulcus, posterior to BA11 and anterior to the insula (Ia). BA14 lesions (4-5 injections) were intended to include the ventromedial cortex from the medial orbital sulcus laterally to the rostral

sulcus medially, and from BA10 anteriorly to BA25 posteriorly. All subjects received bilateral lesions during the same surgery, except for one animal (BA12-1) that received the surgery in two stages one-month apart. Sham-operations followed exactly the same procedures for the anesthesia and surgical preparation as the OFC lesions but no needles were lowered and no injections were made.

Following the intended lesions or sham procedures, the wound was sutured in anatomical layers and the animal was removed from isoflurane gas and recovered in the surgical facility until it could breathe on its own and maintained an SpO2 of 88% for one hour. Post-surgical care included a 7-day regimen of dexamethasone sodium phosphate (0.4 mg/kg i.m.) to reduce swelling, Cefazolin (25 mg/kg i.m.) to minimize infection, and acetaminophen (10 mg/kg, p.o.) for postoperative pain management, administered by veterinary staff. During surgery and recovery, all subjects were monitored by laboratory members and Yerkes Veterinary Staff for any signs of distress.

Behavioral Testing Materials

Startle Chamber

The startle chamber is a sound attenuated testing box (Industrial Acoustics Company, New York, NY) containing a stimulus presentation monitor and load cell. The load cell collects movement data and translates these data into an electrical signal that is used to measure startle response (Med Associates Inc., St. Albans, VT, v2.1). Stimuli were presented using Powerpoint (Microsoft, Redmond, WA) and animal behavior during testing was monitored by an experimenter using a webcam and Skype (Microsoft) video conferencing software.

Stimuli

Stimuli include the unconditioned stimulus (US), the auditory startle stimulus (startle), and the visual conditioned stimuli (CS). The US is a 500-ms burst of compressed air, which is directed toward the face of the monkey through four nozzles. The auditory startle stimulus is a 50-ms 95- or 105-db burst of white noise projected through speakers in the startle chamber (Med Associates Inc.). The CS (fear and safe stimuli) and nonpredictive stimulus consist of visual shapes of different colors (see Fig. 1) projected at a size of 8cm X 8cm onto a monitor facing the monkey. Cues indicate either the prediction of an aversive outcome (AX+; CY+) or the prediction of the presence of a safe outcome (BX-; DY-). Images used for the CS stimuli and nonpredictive stimulus were novel prior to each acquisition phase.

Behavioral Testing Procedures

Subjects were transported from their home cage to a familiar behavioral testing room and transferred to the primate chair. Subjects were then brought to the startle room and positioned in the startle box approximately 40cm away from the monitor. Once the chair was secured in place, the air delivery mechanism was secured, and stimulus presentation initiated. The experimenter then left the room and observed subject behavior across the daily experimental procedures on a remote computer via Skype.

Pre-surgical testing

Pre-training: To assess whether animals had preexisting associations with any of the chosen stimuli, all to-be-conditioned stimuli (Stimulus Set A, see Fig. 1A and Fig. 1B) were presented prior to conditioning in association with a 95-dB startle cue. Each individual stimulus (A, B, X) and stimulus combination (AX, BX, AB) were

presented with a startle noise five times during a 60-trial testing session, all pseudorandomly mixed. Criterion for pre-training was a difference in startle response between the to-be-conditioned safe cue (i.e. BX-) and the baseline startle that was less than 100%. Subjects were tested for a minimum of two sessions until criterion was reached. The number of testing days needed to reach criterion were compared to assess baseline reactivity.

Acquisition of Fear-Safety Discrimination (Fig. 1A): To test learning of fear-safety discriminations, subjects were conditioned to associate the fear cue (i.e. AX+) with an aversive air blast and the safe cue (i.e. BX-) with the absence of an air blast during 60-trial sessions. The fear cue was presented with the aversive air blast at the beginning (Trial 1), middle (Trial 30) and end (Trial 60) of the session. In the remaining trials, the AX+ cue, BX+ cue, and a blank screen were paired with no sound or the startle noise pseudorandomly intermixed with six repetitions for each condition. Percent fear-potentiated startle (%FPS) for the fear or safe cues was computed as: [(startle amplitude with cue − startle amplitude alone) / (startle amplitude alone)] × 100. Criterion for fear-safety discrimination was set at 100% difference between the %FPS to fear cue (i.e. AX+) compared to %FPS to safe cue (i.e. BX-). The number of testing days needed to reach criterion were used to measure learning of the fear-safety discrimination. Once subjects reached criterion, they underwent surgery as described above.

Post-surgical testing

Post-surgical Probe Test (Fig. 1B): After recovery from surgical procedures, all animals received a probe test to 1) measure retention of the fear and safety cues they learned pre-surgically and 2) test modulation of fear response to a novel

combined stimulus condition (AB) made up of the two previously conditioned fear and safe cues. This combined cue was expected to elicit lower %FPS than those elicited by the fear cues. Stimuli presented during the probe test included the previously associated fear and safe cues (i.e. AX, BX), a novel combined fear-safe cue (i.e. AB) as well as each cue component presented in isolation (i.e. A, B, X). During this 48-trial session, subjects experienced each type of stimulus alone and in combination with a startle cue, as well as five repetitions of the novel pairing of the fear and safe cues, all intermixed pseudorandomly within the session. Percent FPS was compared for the conditioned cues (AX, BX) and the novel combined cue (AB) to assess retention of fear learning and modulation of fear response.

Post-Surgery Learning of a New Set of Stimuli (Fig. 1C & 1D):

Procedures for post-surgical new learning are identical to those described above, except that novel stimuli were used for each cue (Stimulus Set B, see Fig. 1C and 1D). They included 1) pre-training with the new cues (CY, DY), 2) acquisition of new fear and safety cues (CY+ and DY-), followed by 3) probe test with newly learned cues (CY, DY) and a novel combined cue (CD). One subject in the Group C (C-13) and one in Group BA13 (BA13-5) did not participate in the novel post-surgery tests due to failure to pass pre-training with the new cues.

Extinction: After the final round of post-surgical testing was complete, animals underwent extinction testing. Extinction of stimulus associations was tested by presenting the aversive cue from Stimulus Set B (CY) in the absence of the aversive event and measuring changes in %FPS. Extinction criterion was set as two days with a less than 100% difference between %FPS to the fear cue (CY) compared to baseline

startle amplitude. The number of testing days needed to reach criterion were compared to measure extinction of fear associations. Only subjects that participated in the novel post-surgical tests were given extinction testing.

Histological Lesion Assessment

Following completion of behavioral testing, the operated subjects were sedated and given a lethal dose of sodium pentobarbital, then perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. Each brain was fixed in a 30% sucrose-formalin solution, cryoprotected, and placed in a -80^{0}C. They were then photographed and cut frozen at 50 µm in the coronal plane. Every fifth section was mounted and stained with thionin to visualize cell bodies and every 10th section was mounted and stained with a silver Gallyas stain to visualize fibers.

To assess lesion extent, each histological section through the prefrontal cortex was digitized at 0.28x using an Excelis HD Lite camera (Accu-Scope Inc., Commack, NY) and a Leica Z6 microscope (Leica Microsystems, Wetzlar, Germany), and saved using CaptaVision (Accu-Scope Inc., v3.9) software. Images were matched with digitized drawings of coronal sections at 1 mm intervals through a normal monkey brain and then imported into Photoshop (Adobe Systems Inc., San Jose, CA, v8). The extent of cell loss and gliosis on each section were drawn on each coronal section of a normal monkey. Using Image-J software (U.S. National Institutes of Health, Bethesda, MD, v1.46r), the extent of intended damage to the cortical areas, as well as of unintended damage to adjacent structures, was measured on each section, and the percentage of cell loss for each structure (compared with the normal brain) was calculated.

Data Analyses

Scores obtained during pre-training, acquisition training, and extinction criterion were based on the percent fear-potentiated startle (%FPS) calculated from the median startle value for each stimulus type (e.g. AX+, BX-, AB, etc.). During probe testing, the %FPS was calculated based on the startle value during the first exposure to each stimulus type (e.g. AX+, BX-, AB, etc.). All %FPS calculations were normalized using base 10 logarithmic transformation. For each testing condition, mean values were calculated for each lesion group.

To measure differences in trials to criterion for the pre-training and acquisition stages, we compared the average number of testing days required to reach criterion for each group using the non-parametric Kruskal-Wallis test to measure overall group differences and the Mann-Whitney U test to directly compare each lesion group to the control group in the following test conditions: pre-surgical pre-training, pre-surgical acquisition, novel post-surgical pre-training, novel post-surgical acquisition, and extinction.

For the post-surgical and novel post-surgical retention and modulation conditions, differences in %FPS to the fear and safe cues were analyzed with a two-way Cue X Group repeated measures ANOVA with Cue as the within-subjects measure and Group as the between-subjects measure. Paired t-tests for each group were performed separately to test whether %FPS to the fear cue was greater than %FPS to the safe cue. To measure modulation to the combined cue, difference scores were calculated for each cue combination (%FPS fear - %FPS combined; %FPS combined - %FPS safe) with higher values representing greater startle response in the expected direction (i.e. greater %FPS to

the fear cue than the combined cue or greater %FPS to combined versus safe cue). To determine whether the difference scores were greater than zero, one-sample t-tests were run separately for each group.

Post-hoc t-tests were conducted to determine whether fear-potentiated startle to all cues differed by sex and Pearson correlations were used to measure the effects of age on fear-potentiated startle. Finally, we used Pearson correlations to analyze the relationship between lesion extent and task performance in all conditions.

Results

Pre-Training to the Stimulus Set A Cues

To ensure that subjects had no preexisting associations with the to-be conditioned safety cues, we compared the average number of testing days required to reach pre-training criterion between groups. As shown in Figure 3, there were no significant group differences in the number of days to reach criterion for pre-training ($\chi^2(3)=0.89$, $p=0.83$) nor did any group significantly differ from controls (C vs. BA12: $U=4$, $p=0.80$; C vs. BA13: $U=4.5$, $p=0.99$; C vs. BA14: $U=2$, $p=0.41$).

Pre-Surgery Acquisition of Fear-Safety Discrimination

To ensure that animals of all groups learned the fear-safety discrimination at the same rate prior to surgery, we compared the average number of testing days required to reach criterion in pre-surgical acquisition. As shown in Figure 4, control animals learned to discriminate between the cues in an average of 2.3 days. Only one of the three animals in Group BA12, two of the three animals in Group BA13 and one of the two animals of Group BA14 learned as rapidly as the controls. By contrast, two animals in the BA12 group and one animal in the BA13 group required the maximum of 20 days. Despite

these group variations in the number of acquisition days, these differences did not reach significance ($\chi^2(3)=2.21$, p=0.53) nor did any group significantly differ from controls (C vs. BA12: U=2, p=0.24; C vs. BA13: U=2.5, p=0.35; C vs. BA14: U=3, p=0.99, see Fig 4).

Retention of Fear-Safety Discrimination Following OFC Surgery

Following the surgical procedures, animals were given the probe test to assess whether they showed good retention of the fear and safe cues. As shown in Figure 5, all three sham-operated controls displayed greater %FPS for the fear cue than for the safe cue (C: t(2)=2.45, p=0.07, *d*=1.42). Subjects in groups BA12 and BA14 showed a similar pattern to the controls, and though the difference was smaller and though the difference in the %FPS between to the two cues did not reach significance, the effect size was large (BA12: t(2)=1.94, p=0.10, *d*=1.12; BA14: t(1)=4.09, p=0.08, *d*=2.89). By contrast, Group BA13 failed to exhibit successful discrimination with most animals showing no difference in %FPS between the cues (BA13: t(2)=-1.24, p=0.83, *d*=-0.72). The Group factor did not reach significance ($F_{3,7}$=0.37, p=0.78, η^2=0.14) but there was a significant effect of Cue ($F_{1,7}$=13.28, p<0.01, η^2=0.41), with greater %FPS to AX+ than BX-. There was also a trend toward significance for the Cue X Group interaction with a large effect size ($F_{3,7}$=4.10, p=0.06, η^2=0.38), confirming that animals in groups Control, BA12 and BA14 displayed good retention of the fear and safe cues, whereas animals in Group BA13 did not.

Modulation of Fear Response to the Combined Cue Following Surgery

To determine whether damage to the orbitofrontal cortex impacted the modulation of fear response when the fear cue was presented together with the safe cue (AB), we used a two-way Cue X Group RM-ANOVA and also calculated difference scores in %FPS between the fear cue and the combined cue and between the combined cue and the safe cue. For difference scores, we expected that individuals with intact modulation will show positive difference scores for AX+ minus AB, whereas individuals lacking modulation will have scores that do not differ from zero for AX+ minus AB as measured by one sample t-tests.

As shown in Figure 6, control animals showed good modulation displaying greater %FPS to the fear cue than the combined cue and to the combined cue than the safe cue, although these differences were significantly greater than zero only for the AB minus BX- condition ($t(2)=8.91$, $p<0.01$, $d=5.14$) and not for the AX+ minus AB condition ($t(2)=1.58$, $p=0.13$, $d=0.91$). Similarly, the two animals with BA14 lesions successfully modulated fear response with greater %FPS for the AX+ than the combined AB cue. Though these difference scores did not reach significance due to individual variation, the effect size was large for the difference between AX+ and AB (AX+ minus AB: $t(1)=1.81$, $p=0.16$, $d=1.28$; AB minus BX-: $t(1)=0.00$, $p=0.5$, $d=0.00$). By contrast, two animals from Group BA12 and all three subjects from Group BA13 lack fear modulation, displaying difference scores close to zero for both AX+ minus AB (BA12: $t(2)=0.61$, $p=0.30$, $d=0.35$; BA13: $t(2)=-2.75$, $p=0.95$, $d=-1.59$) and AB minus BX- (BA12: $t(2)=0.55$, $p=0.32$, $d=0.32$; BA13: $t(2)=0.69$, $p=0.28$, $d=0.39$). As shown in Figure 6A, the effects described above are supported by an overall main effect of Cue ($F_{2,14}=6.66$, $p<0.01$, $\eta^2=0.32$, AX+>BX-: $p=0.09$). Although there was no main effect of

Group ($F_{3,7}$=0.46, p=0.72, η^2=0.17), the Cue X Group interaction showed a trend towards significance ($F_{6,14}$=2.48, p=0.08, η^2=0.35), indicating that animals in Groups C and BA14 showed good modulation to the combined cue, but those in Groups BA12 and BA13 did not.

Post-surgery Pre-Training with Novel Stimulus Set B Cues

To again ensure that subjects did not have pre-existing associations with the to-be conditioned safe cue, we compared the average number of test days required to reach pre-training criterion. As shown in Figure 7, the groups took the same number of days to reach criterion (χ^2(3)=3.84, p=0.28), and no lesion groups differed from the control group (C vs. 12: U=2.5, p=0.35; C vs. 13: U=1, p=0.18; C vs. 14: U=2, p=0.41).

Post-Surgery Acquisition of Novel Fear-Safety Discriminations

Next, to determine whether damage to OFC subregions impacted the ability to learn new fear-safety discriminations, we compared average number of test days to reach criterion. As shown in Figure 8, most animals acquired fear-safety discriminations as quickly as controls, with the exception of one animal in Group BA12 that required the maximum of 20 days to learn the associations. Overall, there were no significant group differences in the days to acquire the fear-safe discriminations (χ^2(3)=1.50, p=0.68) and none of the OFC groups differ significantly from controls (C vs. 12: U=2, p=0.54; C vs. 13: U=1.5, p=0.68; C vs. 14: U=0, p=0.10).

Retention of Fear-Safety Discrimination Learned After OFC Surgery

To determine whether OFC lesions impacted subjects ability to retain fear-safety discriminations, we compared %FPS to the fear and safety cues during the probe test. As shown in Figure 9, all subjects, with the exception of one BA13 animal, displayed higher

%FPS for the fear than the safe cues indicating retention of the cues, although differences only reached significance for BA12 and a trend for BA14 (C: t(2)=1.91, p=0.15, *d*=1.35; BA12: t(2)=3.51, p=0.04, *d*=2.03; BA13: t(2)=1.35, p=0.20, *d*=0.96; BA14: t(1)=3.52, p=0.09, *d*=2.49). This pattern of results was supported by an overall effect of Cue with greater %FPS to the fear than the safe cue ($F_{1,6}$=15.69, p=0.01, η^2=0.74). The Cue X Group ($F_{3,6}$=0.14, p=0.93, η^2=0.02) and Group factors ($F_{3,6}$=0.34, p=0.80, η^2=0.17) did not reach significance suggesting all groups exhibited the same pattern.

Modulation of Fear Response to Novel Cues After OFC Surgery

To determine whether damage to the OFC impacted the modulation of fear response to a novel combined (CD) stimulus when conditioned cues were learned after surgery, we used a two-way Cue X Group RM-ANOVA and also compared difference in %FPS between the fear cue and the combined cue and between the combined cue and the safe cue. We expected that individuals with intact modulation will show positive difference scores for CX+ minus CD, whereas individuals lacking modulation will have scores that do not differ from zero for CX+ minus CD as measured by one sample t-tests. As shown in Figure 10, control animals showed good modulation displaying greater %FPS to the fear cue than the combined cue, and although difference scores were not significantly greater than zero, there was a large effect for the CX+ minus CD condition (t(2)=1.33, p=0.21, *d*=0.94) that was not found for the CD minus DX- condition (t(2)=0.00, p=0.50, *d*=0.00). Group BA12 did not show the expected modulation, with one animal in each condition showing the inverse of the expected effect and both findings are supported by non-significant t-tests (CX+-CD: t(2)=0.65, p=0.29, *d*=0.38; CD-DX-: t(2)=0.91, p=0.32, *d*=0.52). Group BA13 performed similarly to Group BA12, and

though one animal did show modulation, Group BA13 overall did not show difference scores significantly greater than zero in the CY+ minus CD condition (t(2)=0.82, p=0.28, *d*=0.58) but both animals in Group BA13 showed modulation in the CD minus DY- condition (t(2)=5.89, p=0.05, *d*=4.16). Similar to controls, Group BA14 showed good modulation displaying greater %FPS to the fear cue than the combined cue, supported by a trend toward CX+ minus CD difference scores being greater than zero (t(1)=4.40, p=0.07, *d*=3.11) that was not found for the for the CD minus DX- condition (t(1)=-7.29, p=0.96, *d*=-5.15). Interestingly, difference scores for Group BA14 were significantly below zero for the combined minus safe condition (t(1)=-7.29, p=0.04, *d*=-5.15), indicating that subjects in this group show more startle response to the safe compared to the combined cue. As shown in Figure 10A, the finding described above were supported by an overall effect of Cue ($F_{2,10}$=8.42, p<0.01, η^2=0.55) with greater %FPS to the fear cue than both the safe and combined cues (CY+>CD: p<0.01; CY+>DX-: p=0.07). Though the Group factor did not reach significance, there was a large effect size ($F_{3,5}$=0.66, p=0.61, η^2=0.28). There was no interaction of Cue X Group ($F_{6,10}$=0.65, p=0.69, η^2=0.13).

Extinction of Fear-Safety Discrimination

Next, to determine whether damage to specific OFC regions impacted the extinction of fear associations, we compared the average number of days needed to reach criterion during the extinction condition. As shown in Figure 11, though there was some variation in time to extinguish fear response, all animals reached criterion for extinction in an average of 5.1 days, resulting in no significant main effect of Group (χ^2(3)=0.38,

p=0.95) and all OFC animals met extinction criterion within the same number of days as controls (C vs. 12: U=4.5, p=0.99; C vs. 13: U=2.5, p=0.77; C vs. 14: U=2.5, p=0.77).

Effects of sex and age on fear-potentiated startle

We used t-tests and Pearson correlations, respectively, to determine whether the sex or age of subjects impacted fear-potentiated startle and found that there was a positive correlation between increasing age and higher fear-potentiated startle to the post-surgical safe cue (R=0.67, p=0.02). However, there were no other associations between age and fear-potentiated startle, nor were there any effects of sex on fear-potentiated startle.

Lesion Assessment

A full description of lesion extent can be found in Table 1. For BA12 lesions (Fig. 12, left column), average lesion extent varied between 16% and 29%, with two animals (BA12-1, BA12-3) receiving bilateral lesions and animal BA12-4 receiving a more unilateral lesion (Left hemisphere: 26%, Right hemisphere: 4%). All BA12 cases received unintended damage to areas BA45 (10%-17%, Fig. 12, orange arrows) and PrCO (1%-9%, Fig. 12, orange stars). For BA13 lesions (Fig. 12, center column), average lesion extent varied between 2% and 26% with two animals (BA13-2, BA13-5) receiving bilateral lesions and case BA13-1 receiving a small (2%) lesion. Cases BA13-2 and BA13-5 received unintended damage to BA11 (6%-20%, Fig. 12, green arrows). For BA14 lesions (Fig. 12, right column), average lesion extent was consistent and bilateral across both animals at 41%. All animals received unintended damage to BA11 (3%-4%, Fig. 12, blue arrows) and BA25 (8%-13%, Fig. 12, blue stars).

Correlations of Fear-Potentiated Startle with Lesion Extent

Finally, to determine whether lesion extent impacted behavioral measures, for

each lesion group correlations were made between the percent lesion extent in all impacted areas and all behavioral measures where subject groups had greater than two subjects. Within Group BA12, damage to BA45 was significantly correlated with decreased fear-potentiated startle to the novel combined cue ($R=-1.00$, $p<0.01$, $n=3$, see Fig. 13). Importantly, BA12 follows the same pattern ($R=-0.98$, $p=0.12$, $n=3$), and the amount of damage to BA45 was tightly linked to BA12. Within Groups BA13 and BA14, there we no significant correlations between lesion extent and any behavioral measure.

Discussion

The study indicated for the first time differential effects of lesions to specific OFC subfields in fear potentiated startle (see Table 2). Although lesions of OFC BA14 did not alter discrimination of the fear and safety cues or modulation of the fear cue in the presence of the safe cues, lesions to OFC BA12 impacted the modulation of fear response in presence of safe cues, and lesions of OFC BA13 impacted both the expression (retention) of cues learned prior to surgery and the modulation of fear response. None of the lesions to OFC subfields impacted the acquisition or extinction of fear associations. These findings will be discussed in turn.

The role of BA12 in the modulation of fear

First, we demonstrated that lesions to OFC area BA12 yielded specific deficits in the modulation of learned fear as revealed by a failure to reduce fear-potentiated startle reactivity in the presence of a safe cue. Following surgery, animals in Group BA12 tended to discriminate the fear and safe cues learned prior to surgery as well as the new fear and safe cues, although their discrimination of the two cues was weaker than that

observed in control animals. Despite their ability to retain the different values of the cues, animals with BA12 lesions failed to modulate their fear response in the presence of a safe signal when using the cues learned prior to surgery. Although the strength of conditioned inhibition was slightly stronger in one animals (BA12-1) than in the other two (BA12-3, BA12-4), this difference was not driven by the extent of BA12 lesions since case BA12-1 showed conditioned inhibition and had larger lesions (29%) than the other two (16%).

When presented with a new set of stimuli, two of the three animals in Group BA12 quickly learned fear-safe associations and all animals were able to retain the fear-safe discrimination. However, in the presence of a safety signal, animals with BA12 lesions again showed weaker modulation of fear, although there was small variation within the three animals of this group (see Fig. 10A). These differences are not explained by extent of damage to BA12 or unintended damage to adjacent areas; although damage to BA45 in this group was associated with decreased %FPS to the novel combined cue (DY).

Together, these findings suggest that BA12 is important for the modulation of fear-response, but not for the learning or retention of fear-safety discriminations. Despite some individual variation and the small sample size, these findings are consistent with previous studies indicating the important role of BA12 in processing negative or aversive cues. Specifically, lesions of BA12 in marmosets produce increased fear response and increased arousal in the presence of threats (Agustín-Pavón et al., 2012; Shiba et al., 2015) and fMRI studies in humans reported increase activity in the lateral OFC when subjects responded to negative conditioned stimuli (Morris & Dolan, 2004; J. O'Doherty et al., 2001). Though damage to BA12 does not consistently impair reversal of stimulus-

outcome associations (Rudebeck, Ripple, Mitz, Averbeck, & Murray, 2017), PET and fMRI neuroimaging in humans identify areas of the lateral OFC that are highly active during the reversal of contingencies (Hampshire et al., 2012) as well as during suppression of previously rewarded associations (Arana et al., 2003; Elliott et al., 2000; Noonan et al., 2011). Thus, BA12 may be critical for not only maintaining negative valence assignment, but for the rapid recalibration of stimulus-outcome associations when contingencies or contexts change. Overall, our findings support the role of BA12 in the modulation of stimulus associations by assessing the value of potentially threatening cues and modulating emotional response, manifesting in deficient processing of aversive cues in animals with BA12 lesions.

The role of BA13 in the expression and modulation of learned fear

Lesions to area BA13 yielded more severe deficits in the expression and modulation of learned fear in the presence of the conditioned safety cue than lesions of BA12. Thus, after BA13 lesions, all three animals displayed poor discrimination of the fear and safe cues they had learned pre-surgery, indicating deficits in fear expression. As a result, their poor discrimination led to a lack of modulation of their fear response in the presence of the safe signal. When presented with a new set of stimuli, the remaining two animals with BA13 lesions that completed the task were able to learn to discriminate between the novel fear and safe cues, but only one of these two animals (BA13-1) showed good retention of the novel discrimination after a delay of 2-4 days and good modulation of fear, the other animal (BA13-2) did not show good retention nor fear modulation. Of these two subjects, BA13-1 had limited BA13 cell loss (2%), whereas BA13-2 had more extensive cell loss in BA13 (20%) as well as inadvertent damage to

BA11; this difference in extent of lesions may explain why BA13-1 showed only a transient impairment compared with the more pronounced impairment seen in BA13-2. Thus, although area BA13 does not affect the acquisition of fear-safe discrimination, the data suggest that this area is critical for the retention of these discriminations and as a result for the modulation of fear response. The effect of increasing age was significantly related to increased fear-potentiated startle to the post-surgical safe cue, driven in part by the most aged subject, BA13-5. However, the subject with the second highest FPS to the post-surgical safe cue (C-10) exhibited intact fear-safe discrimination, suggesting that the effects of increasing age are unrelated to fear-safe discrimination. Despite the small sample size in the present study, the data are consistent with previous nonhuman primate studies indicating that area BA13 plays a critical role in the expression and modulation of learned fear. For example, studies in rhesus macaques show impaired fear expression following lesions to the OFC that include BA13 as revealed by reduced emotional reactivity during social threats (Kalin et al., 2007; Kazama et al., 2014; Machado & Bachevalier, 2008), and reduced fear in the presence of nonsocial threats (Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kalin et al., 2007; Machado & Bachevalier, 2008). Similarly, single neuron electrophysiological recordings in macaques demonstrate that neurons in the OFC, many in BA13, code for the reinforcement value (positive or negative) of a stimulus association (Morrison & Salzman, 2009; Thorpe et al., 1983; Tremblay & Schultz, 1999). In humans, BA13 and the central OFC are implicated in the flexible processing of stimulus-outcome associations across valence. For example, studies using fMRI in humans have shown that activation in BA13 increases during reward processing (O'Doherty, Critchley, Deichmann, & Dolan, 2003; Sescousse, 2010)

and that BA13 represents both positive and negatively valenced outcomes (Gottfried et al., 2002; Rolls, Doherty, Kringelbach, & Francis, 2003). In addition, numerous studies identify BA13 as a critical region for the flexible modulation of behavior in the reinforcer devaluation task, which measures the ability to modify behavioral choice of reinforcing stimuli based on internal states, such as satiety (Izquierdo & Murray, 2004; Kazama et al., 2014; Rudebeck & Murray, 2011). Indeed, humans studies using fMRI have documented changes in BA13 activation from pre- to post-satiety in the reinforcer devaluation task (Gottfried, O'Doherty, & Dolan, 2003), highlighting that this area of the central OFC is important for shifting the representation of an outcome based on the changing reward value of stimuli. Thus, the deficits in the modulation of fear-potentiated startle seen following BA13 lesions may reflect an impairment in retaining stimulus-outcome association value resulting in a failure to modulate behavioral response in shifting or novel contexts. Overall, our findings support the critical role of BA13 in flexible modulation of stimulus associations and highlight the role of BA13 in fear expression.

BA14 and its role in fear conditioning and modulation

Lastly, we demonstrated that lesions to the BA14 area of the OFC produced no effects on the acquisition, retention, or modulation of fear-safe discriminations. Following excitotoxic lesions to BA14, both animals showed fear-safety discriminations and effectively modulated fear response in the presence of the safety signal both during the original post-surgical condition and in the novel post-surgical condition following training with a new stimulus set. This lack of impairment was present even when the lesion extent was significant with 40% of cell loss for both animals. Overall, BA14

lesions did not impair fear learning, retention or modulation in our study. Despite our small sample size, these findings are consistent with reports in humans and macaques indicating that BA14 is not critical for modulating fear response to social or nonsocial objects, perhaps due to a greater role for BA14 in the processing of appetitive, but not aversive, cues. Specifically, compared to control animals, lesions to BA14 in rhesus macaques did not significantly alter behavior in the presence of threatening nonsocial (i.e. snake) or social (i.e. human or macaque stare) cues (Noonan et al., 2010). In addition, fMRI studies in humans using a broad range of appetitive cues (i.e. attractive faces, pleasing music) all show increased activation in the medial OFC to such cues (Gottfried et al., 2002; Hampshire et al., 2012; Li, Chen, & Tsai, 2015; O'Doherty et al., 2001) and support the notion that this region is important for the processing of positively valenced associations. In humans, lesions that include the medial OFC and BA14 impair strategic acquisition of reinforcers (Bechara et al., 2000; Bechara, Damasio, Damasio, & Lee, 1999) and show decreased arousal (measured by pupil diameter) to reward (Manohar & Husain, 2016). In monkeys, BA14 lesions in rhesus macaques yield impaired extinction to reinforcement contingencies (Rudebeck & Murray, 2011) and medial OFC damage in marmosets result in increased arousal to positive cues (Reekie, Braesicke, Man, & Roberts, 2008) but limited impairments to fear cues (Agustín-Pavón et al., 2012). Finally, direct recordings of neurons in the primate OFC also support this notion, as BA14 neurons showed consistently greater activity to appetitive rather than aversive cues (Monosov & Hikosaka, 2012). Together, the findings suggest that BA14 is not critical for the processing of negative or fearful cues.

OFC fields contributions to extinction

Our study revealed no impairment on the extinction of fear associations with lesions to areas BA12, BA13, or BA14 of the OFC and contradict the findings reported in previous studies indicating that the OFC supports extinction of positive reinforcement associations (Butter, 1969; Rudebeck & Murray, 2011). These different outcomes may have occurred because our study measured extinction of fear associations, and not extinction of positive reinforcers. Indeed, previous research has implicated the prelimbic (BA25 in primates) and infralimbic (BA32 in primates) areas of the OFC as well as the amygdala as critical components for fear extinction (Milad et al., 2007; Milad & Quirk, 2002; Phelps et al., 2004) and not OFC areas 12, 13, and 14.

The role of the amygdala in fear-learning and modulation

Importantly, due to the role of the amygdala in fear learning and emotional regulation and its substantial interconnections with subregions of the OFC, the current results should be discussed in relation to the contributions of the amygdala to acquisition, retention, modulation and extinction of learned fear. The amygdala is critical for the association between a fearful unconditioned stimulus and the to-be conditioned stimulus. In rodents as well as human and nonhuman primates, lesions including the lateral amygdala (LA) impair fear association learning (Antoniadis, Winslow, Davis, & Amaral, 2009; Ledoux, Romanski, & Xagoraris, 1990) and in human fMRI studies, amygdala functional activation to the conditioned stimulus correlates with the magnitude of acquisition learning (Phelps et al., 2004). This contrasts with the present results demonstrating that OFC lesions did not disrupt the acquisition of fear-safety discriminations. Thus, the OFC-amygdala network appears not critical for this function, a finding further supported by the minimal connectivity between the lateral amygdala and

the OFC (Barbas, 1993; Carmichael & Price, 1995). However, when amygdala lesions were created after fear-associations were already learned, amygdala lesions had no impact on fear-potentiated startle learning (Antoniadis et al., 2009) as did lesions of OFC BA12 and BA14. Yet, OFC BA13 lesions did impair retention of fear-safe associations after surgery, suggesting that the maintenance of value representations may be a unique functional feature of OFC BA13. This proposal is consistent with several theories of the role of the OFC in fear learning and expression (O'Doherty, 2007; Salzman, Paton, Belova, & Morrison, 2007).

Following learning of stimulus-outcome associations, it appears that the amygdala-OFC network becomes more critical for the modulation of learned fear and safety. In rodents, safety signal learning is associated with amygdala-prefrontal coactivation (Likhtik et al., 2014) and electrophysiological recordings reveal that a population of neurons in the basolateral amygdala complex (BLA) specifically fire in response to the combined fear-safety cue during modulation (Sangha, Chadick, & Janak, 2013). In addition, amygdala lesions combined with electrophysiological recordings in rodents lead to decreased freezing to a conditioned fear-cue as well as increased prefrontal activity during the presentation of the conditioned stimulus (Garcia, Vouimba, Baudry, & Thompson, 1999), demonstrating that the amygdala-PFC connectivity is critical for appropriate behavioral modulation. This suggests that the impaired fear modulation found in animals with lesions to OFC BA12 and BA13 may be due to disconnection of the amygdala-OFC network. This proposal is supported by the substantial anatomical connectivity between the BLA and areas BA12 and BA13 as well as by disconnection studies that have shown that a disconnection of the amygdala and

OFC yield profound deficits in reward modulation by changing context (Baxter et al., 2000). Lastly, studies in humans and nonhumans demonstrate that the amygdala is important for extinction of fear learning (Phelps et al., 2004), especially for the maintenance of extinction over multiple test days. As we found no impairments in extinction learning with lesions to OFC BA12, BA13, or BA14, we can conclude that the amygdala does not require feedback from the above OFC areas to facilitate extinction learning.

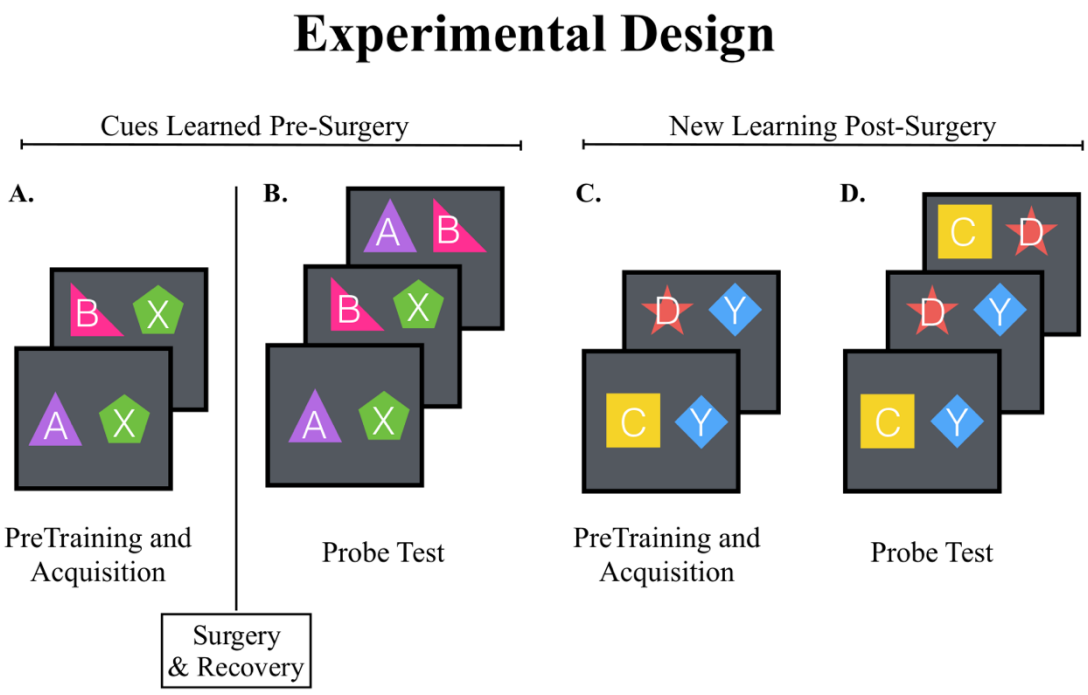

Figure 1. Design of experimental testing for fear-potentiated startle task.
A) Pre-surgical pre-training and acquisition of to-be conditioned stimulus associations (AX+: aversive cue; BX-: safe cue), B) Probe test following surgery to assess retention and modulation of previously learned associations (trials included aversive cue: AX+; safe cue: BX- and combined cue: AB), C) Post-surgical pre-training and acquisitions for novel stimulus associations (aversive cue: CY+, safe cue: DY-), and D) Probe test to assess retention and modulation of stimulus associations learned after surgery (aversive cue: CY+, safe cue: DY-; combined cue: CD).

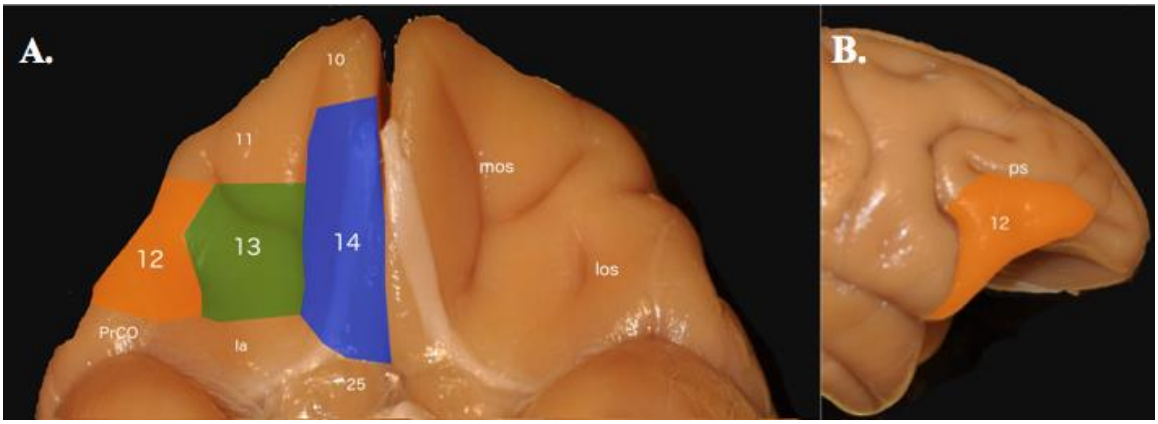

Figure 2. Ventral (A) and lateral (B) view of the macaque frontal cortex.
Depiction of intended lesions to BA12 (orange), BA13 (green), and BA14 (blue). los = lateral orbital sulcus, mos = medial orbital sulcus, PrCo = posterior opercular cortex, Ia = insula, ps = principal sulcus.

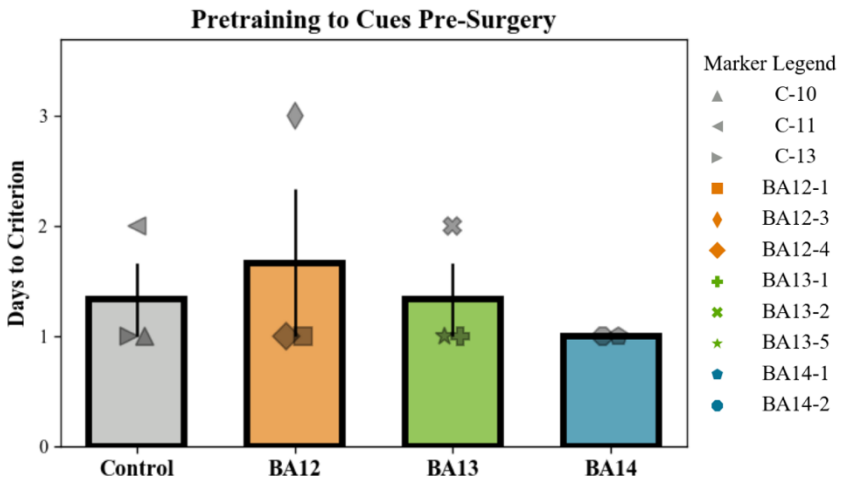

Figure 3. Pre-training phase.
Number of days to reach pre-training criterion for control animals (control, gray bar), animals with OFC BA12 lesions (BA12, orange bar), animals with BA13 lesions (BA13, green bar), and animals with BA14 lesions (BA14, blue bar). The symbols used to represent each subject are listed on the right, and are consistent throughout the FPS testing in the remaining of the text.

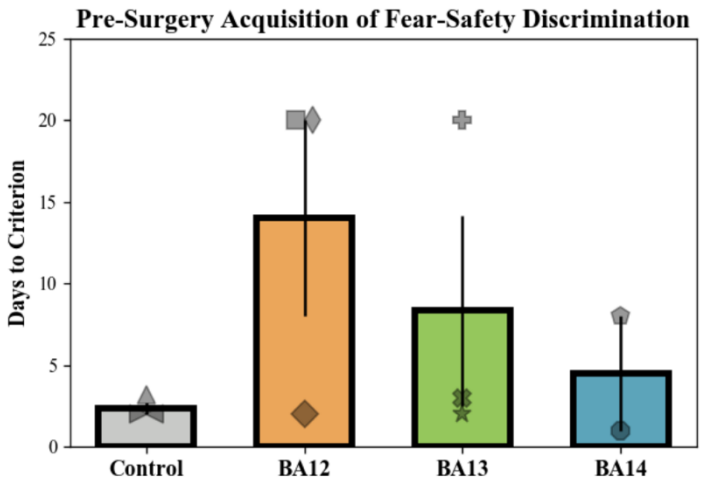

Figure 4. Pre-surgical acquisition.
The number of days to acquire fear-safety discriminations for each group. Conventions as in Figure 3.

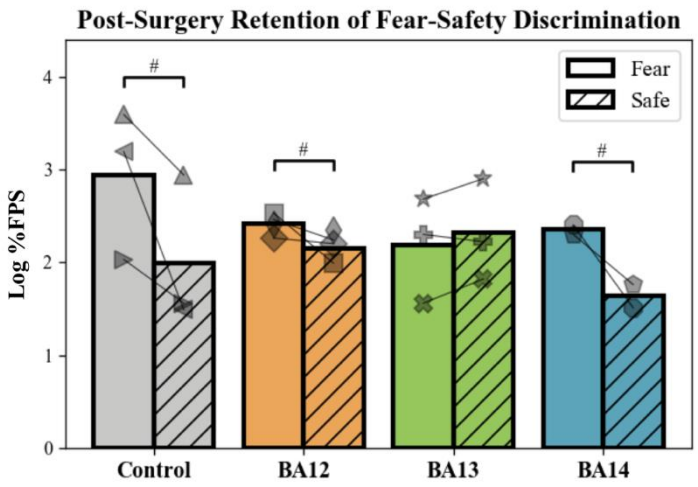

Figure 5. Retention of fear-safe discrimination cues post-surgery.
Log transformed percent fear-potentiated startle to the fear cue (filled bars) and the safe cue (hatched bars) for each group. Conventions as in Figure 3. # indicates $p<0.10$.

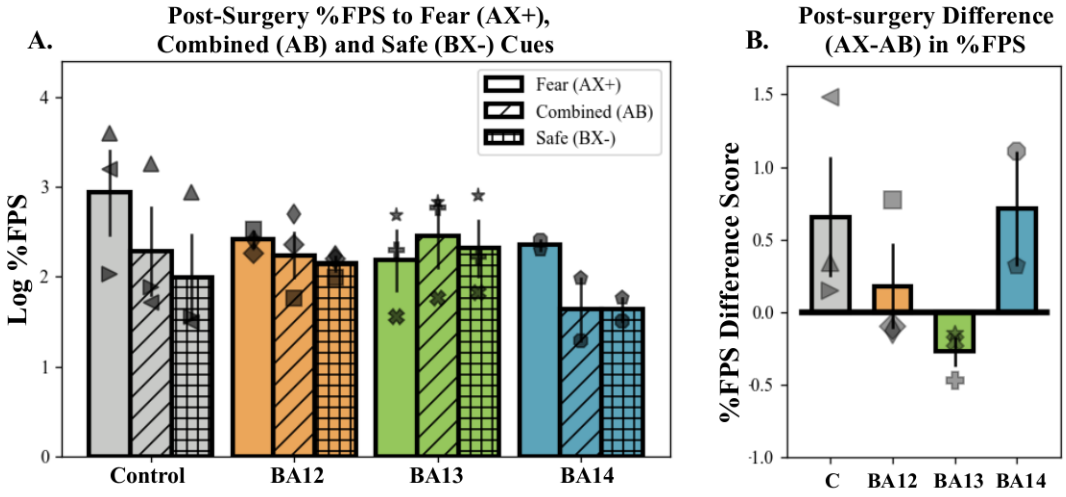

Figure 6. Probe test post-surgery.
A) Log transformed percent fear potentiated startle to the fear cue (filled bars), the combined cue (hatched bars), and the safe cue (crossed bars); B) Difference in %FPS scores between fear cue (AX+) and combined cue (AB). Conventions as in Figure 3.

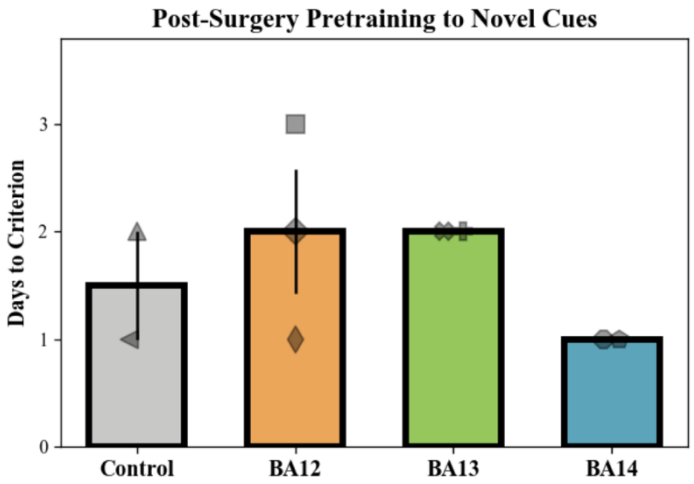

Figure 7. Pre-training of novel cues.
Number of days to reach pre-training criterion for the novel stimulus set (C, D and Y cues) across groups**.** Convention as in Figure 3.

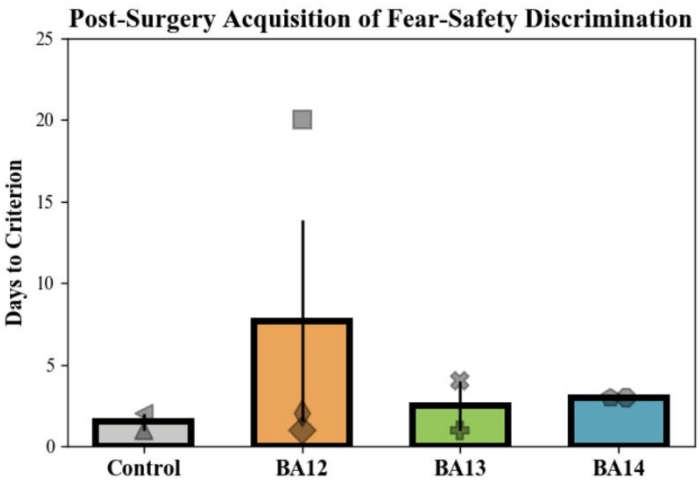

Figure 8. Acquisition of fear-safe discrimination of novel cues.
Number of days to reach criterion for fear-safety discrimination of the novel stimulus set. Conventions as in Figure 3.

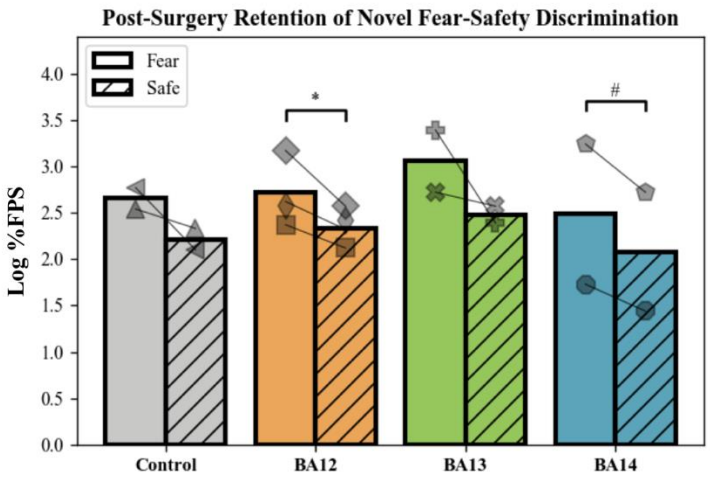

Figure 9. Post-surgery retention of novel Fear-Safety cue discrimination. Log transformed percent fear potentiated startle for the four groups, convention as in Figure 5**.** * indicates $p<0.05$, # indicates $p<0.10$.

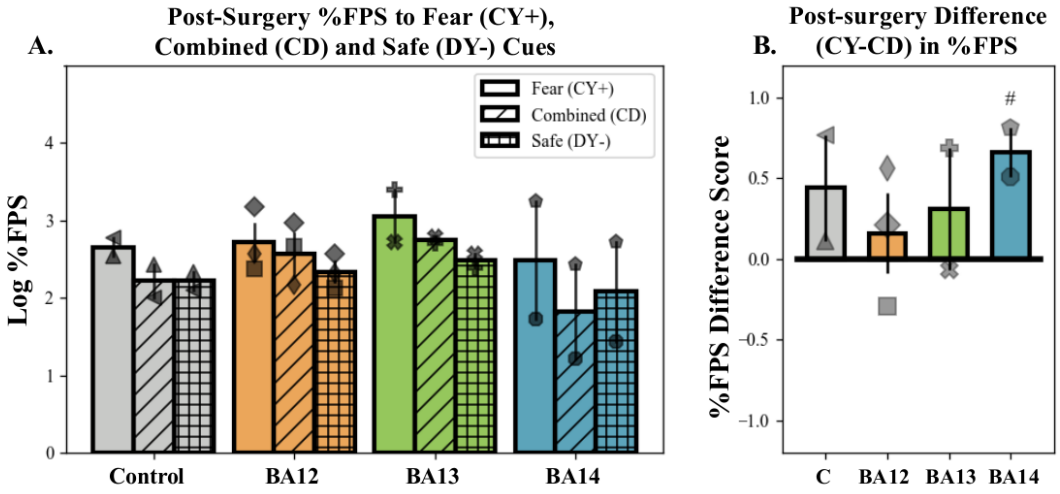

Figure 10. Modulation of fear responses.
A) Log transformed percent fear potentiated startle to the fear cue (filled bars), the combined cue (hatched bars) and safety cue (crossed bars) for the four groups. B) Difference in %FPS scores between fear cue (CY+) and combined cue (CD). Conventions as in Figure 3. # indicates $p<0.10$

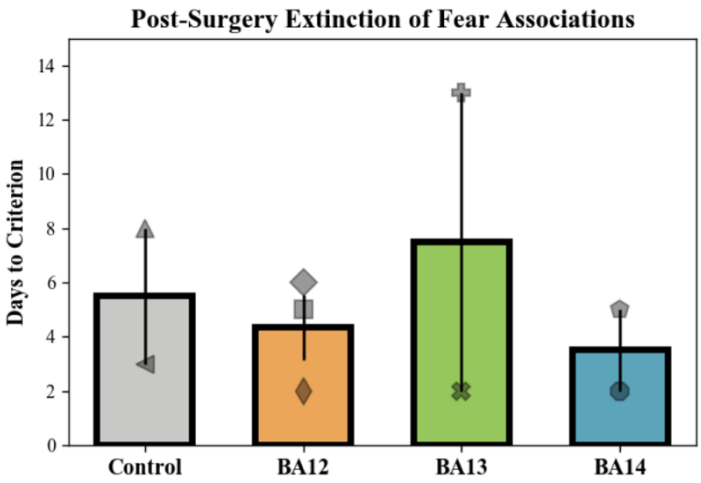

Figure 11. Extinction.
number of days to reach criterion for extinction training. Conventions as in Figure 3.

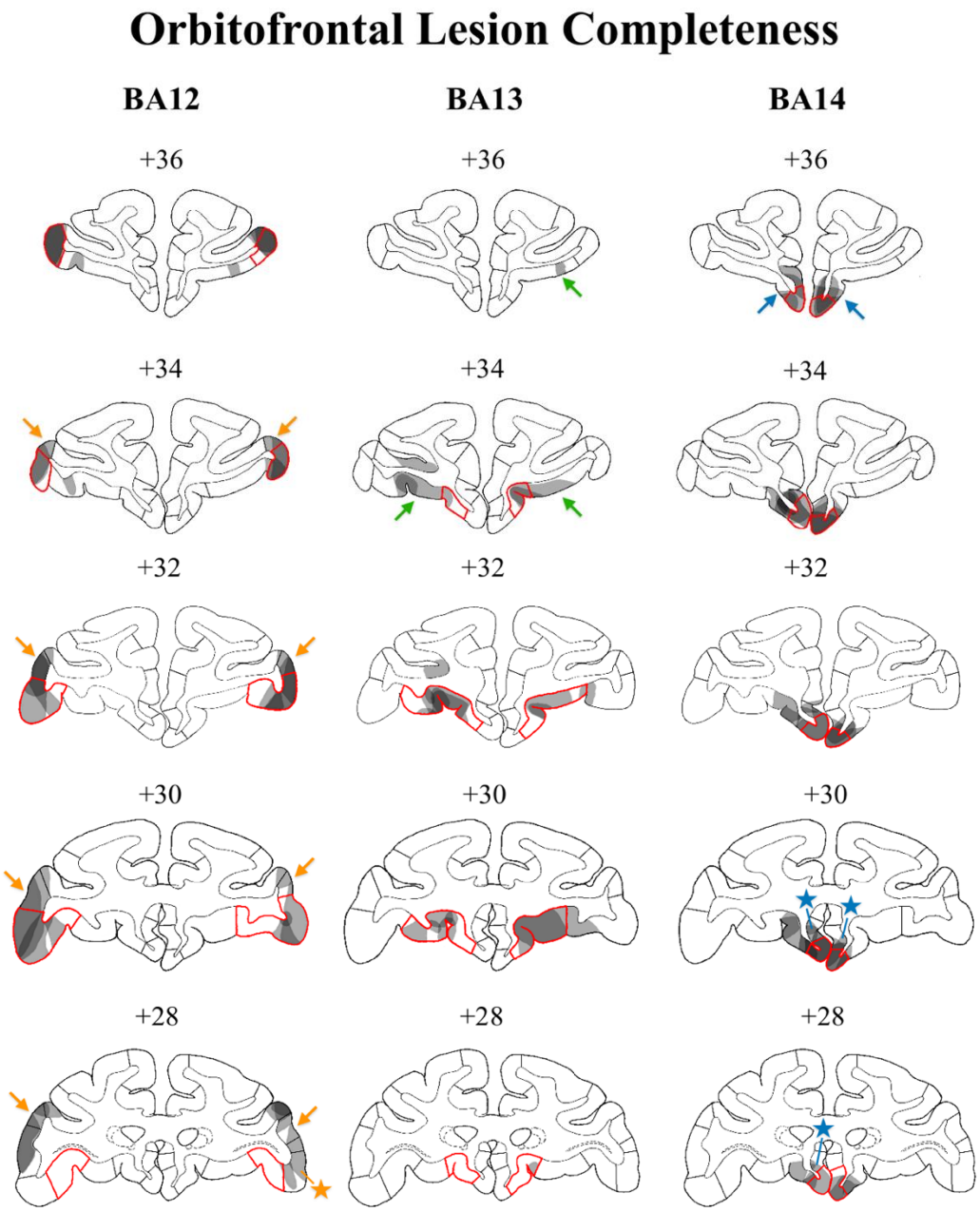

Figure 12. Depiction of lesion extent for each lesion group.
Coronal sections through the extent of area BA12 lesions (left column), area BA13 lesions (middle column), and area BA14 lesions (right column). The red outlines on each section indicate the intended extent of the lesions and numerals above each section refer to the distance in millimeters from the interaural plane. Lesion extent for each subject were layered on each other, such that darker areas indicate area of damage common to all subjects, whereas lighter area indicate damage to only some subjects. Unintended damage included BA45 (orange arrows) and PrCO (orange stars) from Group BA12, BA11 for Group BA13 (green arrows), and BA25 (blue stars) and BA11 (blue arrows) for Group BA14.

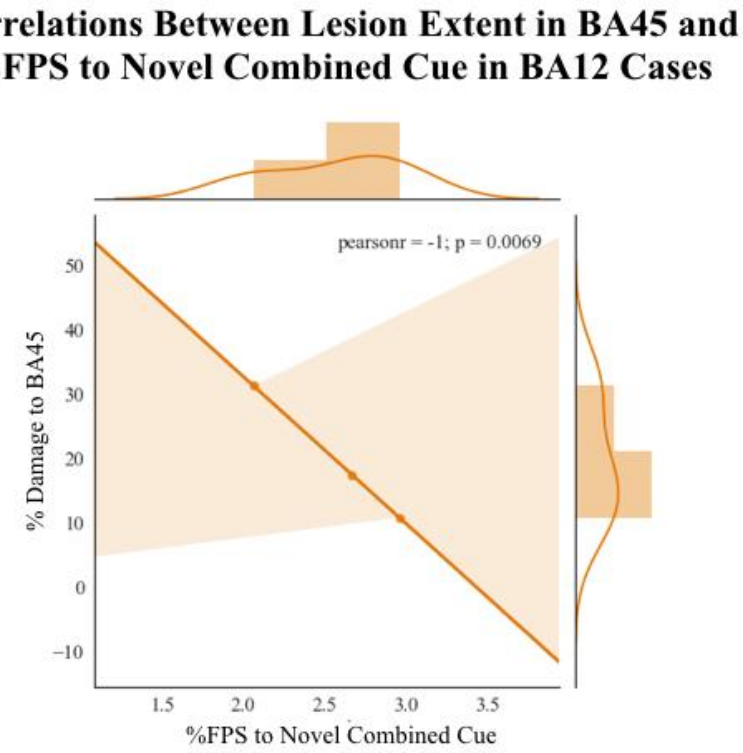

Figure 13. Correlation between lesion extent in BA12 cases and fear-potentiated startle.
In BA12 cases, percent damage to BA45 was negatively correlated with percent FPS to the novel combined cue.

Cases	Area 12				Area 45				Area PrCO			
	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W
BA12-1	20.17	36.99	28.58	7.461	22.04	12.76	17.4	2.813	0	18.5	9.252	0
BA12-3	14.13	17.48	15.8	2.47	15.04	10.14	12.59	1.524	0	2.57	1.285	0
BA12-4	26.82	4.274	15.54	1.146	18.79	2.734	10.76	0.514	3.545	7.196	5.371	0.255
X	**20.37**	**19.58**	**19.98**	**3.692**	**18.62**	**8.544**	**13.58**	**1.617**	**1.182**	**9.424**	**5.303**	**0.085**

Cases	Area 13				Area 11			
	L	R	Avg	W	L	R	Avg	W
BA13-1	2.736	1.945	2.341	0.053	0	0	0	0
BA13-2	18.95	21.58	20.26	4.089	11.78	0.404	6.092	0.048
BA13-5	31.11	22.37	26.74	6.96	22.32	18.72	20.52	4.179
X	**17.6**	**15.3**	**16.45**	**3.701**	**11.37**	**6.376**	**8.871**	**1.409**

Cases	Area 14				Area 11				Area 25			
	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W
BA14-1	45.09	36.85	40.97	16.61	5.545	2.617	4.081	0.145	10.38	6.083	8.231	0.631
BA14-2	48.93	32.83	40.88	16.06	1.857	4.072	2.965	0.076	11.67	14.51	13.09	1.694
X	**47.01**	**34.84**	**40.92**	**16.34**	**3.701**	**3.344**	**3.523**	**0.11**	**11.02**	**10.3**	**10.66**	**1.162**

Table 1. Histological lesion extent across all impacted areas.
Percent damage listed for relevant OFC areas for each case and averaged across each group (below, in bold). Histological damage is given for the left (L) and right (R) hemispheres and both the average (Avg.) and weighted average (W). The weighted average is calculated as (L x R)/100. Ia = insula, PrCo = posterior opercular cortex.

	Retention Set A	Modulation Set A	Learning Set B	Retention Set B	Modulation Set B	Extinction
Group BA12	+ 3/3	↓ 1/3	+ 2/3	+ 3/3	↓ 2/3	+ 3/3
Group BA13	↓ 1/3	↓ 0/3	+ 3/3	↓ 1/2	↓ 1/2	+ 2/2
Group BA14	+ 2/2	+ 2/2	+ 2/2	+ 2/2	+ 2/2	+ 2/2

Table 2. Summary of effects of OFC lesions on fear learning, expression, modulation, and extinction.
Arrows indicate impairments relative to sham-operated controls, plus sign indicates that the behavior remains intact following surgery. Numbers indicate the number of animals per group that successfully demonstrated the behavior.

General Discussion

The two manuscripts that comprise this dissertation extend our understanding of the separable contributions of subregions of the orbitofrontal cortex (OFC) to emotional processing. As a highly interconnected area of the prefrontal cortex (PFC), the OFC has been shown to contribute to complex and multifaceted behaviors, leading many to postulate that the primary function of the OFC is to associate learned behaviors and responses with various outcomes and values. The central goal of this dissertation was to determine how specific subregions of the OFC contribute to this broad function within the specific realm of emotional regulation by measuring the impacts of lesions to OFC areas BA12, BA13, and BA14 separately on attention and arousal to socioemotional stimuli (Study 1) and fear modulation (Study 2). Interestingly, both studies indicated that emotional regulation was impaired by damage to areas BA12 and BA13 but not damage to area BA14. More specifically, Study 1 showed that damage to areas BA12 and BA13 decreased attention to highly salient threatening cues, and increasing arousal to positive cues, yet damage to BA14 spared these functions. Study 2 showed that damage to BA12 and BA13 impaired the expression of fear and safety, resulting in a lack of modulation of fear response in the presence of a safety signal. These changes were again not seen following damage to BA14. Taken together, these studies support the functional dissociation of areas within the orbital network of the OFC (such as BA12 and BA13) and the medial network of the OFC (including BA14) in the modulation of behavioral and autonomic function in response to threatening cues.

The following sections will summarize our conclusions regarding the functions of the three distinct OFC subregions on attention, arousal, and modulation in the presence of

highly salient emotional cues. The next section will contrast the role of the OFC with that of the amygdala and draw hypotheses regarding the mechanisms underlying emotional regulation in the OFC. Lastly, we will discuss the limitations of our study, identify some further directions, and produce general conclusions given these limitations.

The specialized role of the lateral OFC in the processing of negative stimuli

From the time of Phineas Gage, it has been widely assumed that the OFC makes significant contributions to the modulation of emotional behavior. Though Gage experienced extensive trauma to the frontal cortex (Damasio et al., 1994), studies of other natural lesions in humans provide support for the notion that the OFC influences emotionality and social function. In humans, damage to the OFC, including lateral areas such as BA12, results in general social impairments in interpreting and integration socioemotional signals, such as reduced emotional awareness and impaired social interactions (Bramham et al., 2009; Hornak et al., 2003; Saver & Damasio, 1991). Indeed, damage to BA12 is associated with impairments in recognizing facial expressions of emotion (Monte et al., 2013) and decreased gaze to salient face regions, such as the eyes (Wolf et al., 2014). In addition, numerous studies have identified a functional dissociation by emotional valence, with the lateral OFC supporting negative valence and the medial OFC supporting positive valence (O'Doherty et al., 2003). In humans, the lateral OFC, including BA12, shows functional activation to negatively valenced faces (Blair et al., 1999; Gottfried et al., 2002; Vuilleumier et al., 2001) and to negative nonsocial cues (O'Doherty et al., 2003; Morris & Dolan, 2004) and damage including BA12 led to greater impairments in identifying negative emotional expressions (Monte et

al., 2013). Together, the lateral OFC appears to support social function by modulating attention to salient social cues, such as conspecific faces and threats.

One possible mechanism for the impaired social function and perhaps the negative bias present with damage to the lateral OFC may be related to the role of BA12 in inhibiting response to conditioned cues (Blair & Cipolotti, 2000). In both social and nonsocial tasks, it is often important to modulate behavioral response based on changing variables. When stimulus-outcome contingencies have changed an individual must inhibit the previously conditioned response and make the new response. Indeed, in nonhuman primates, damage to BA12 produces broad impairments in response inhibition that are distinct from those found in the medial OFC (Iversen & Mishkin, 1970). In addition, damage to regions of the lateral OFC produce increased fear and arousal in the presence of threats (Agustín-Pavón et al., 2012; Shiba et al., 2015). These findings may be explained by impaired inhibition and modulation of bottom-up arousal from the amygdala resulting in perseverative responding or increased anxiety-like behaviors. In social contexts, the failure to modulate bottom-up arousal to attend to salient cues may result in impaired emotional attention and perception.

In Study 1, damage to BA12 produces broad impairments that suggest inhibitory failure and increased arousal. For example, damage to BA12 yielded specific impairments in attention to the eyes of negative stimuli as well as increased arousal to positive social stimuli, together suggesting heightened anxiety in the presence of social cues manifested in an inability to attend to relevant cues. In rhesus macaques, looking directly at the eyes of a conspecific is a sign of dominance, typically followed by threatening and aggressive behavior. However, like humans, the eyes are also a key

region for social information, and numerous studies have documented high levels of eye attention to social stimuli in macaques, a function that may be mediated by the amygdala (Dal Monte et al., 2015; Hoffman, Gothard, Schmid, & Logothetis, 2007; Mosher, Zimmerman, & Gothard, 2014). The extent of damage to BA12 was specifically correlated with decreased attention to positive social cues and within Group BA12, small amounts of unintended damage to the neighboring region PrCO resulted in a general avoidance of negatively valenced stimuli. Together, these data support a potential negative bias in emotional processing following damage to BA12 resulting from impaired modulation of arousal to highly salient stimuli that may require inhibition of fear response. The findings in Study 1 were limited to the social domain, and damage to BA12 produced no alterations in attention or arousal to nonsocial cues. This is particularly interesting as in Study 2 damage to BA12 specifically impaired the modulation of fear response in the presence of a nonsocial safety signal. Specifically, animals with BA12 lesions treated conditioned nonsocial fear and safe cues identically, again supporting the notion of a negative threat bias and impaired modulation of arousal in fearful contexts. That nonsocial cues evoked impairments in Study 2 but not Study 1 may be due to increased salience due to the predictive value of the nonsocial cues in Study 2. In the AX+/BX- paradigm used in Study 2, the cues were reliable predictors of actual positive or negative events, whereas this was not the case in the eye-tracking task in Study 1. This suggests that again arousal may be a key component in the impaired emotional regulation produced by BA12 lesions, and nonsocial cues in Study 1 were not sufficiently engaging. In Study 2, we found further support for the notion that BA12 is critical in the modulation of emotional behavior. In our experiments, animals with lesions

to BA12 performed similarly to controls on tasks that involved the learning and updating of stimulus associations, but were significantly impaired in modulating behavioral response to a conflicting cue that required behavioral inhibition to recognize that the presence of the safety cue predicted the absence of an aversive event. This impairment in animals with BA12 lesions was associated with the amount of damage to BA45, a prefrontal area important for emotional processing and regulation. Given the consistent involvement of BA12 in modulating response to highly salient aversive cues, and the well-documented role of BA12 in behavioral inhibition found in other studies, we believe that our studies provide additional evidence to support the role of the lateral OFC in emotional regulation through modulation of arousal to salient cues, especially those of negative valence.

The role of the OFC BA13 in emotional behavior and stimulus-outcome associations

Much of our understanding of the functions of the OFC derives from lesion studies in nonhuman animals and almost all lesion studies include damage to OFC area BA13 in whole or in part. The most central region of the OFC, BA13 is highly interconnected both within the OFC and across numerous areas outside the OFC including extensive bidirectional connectivity with the amygdala. Damage to BA13 results in profound deficits in emotionality and social behavior. In nonhuman primates, lesions including BA13 yield decreases in species-typical fear and anxiety behaviors in the presence of social and nonsocial threats (Bachevalier et al., 2011; Izquierdo & Murray, 2004; Izquierdo et al., 2005; Kazama et al., 2014; Machado et al., 2009). Interestingly, one study from our laboratory found that, when placed in social context

interactions, monkeys with lesions to BA13 and BA11 did not change the frequency or intensity of social interactions, and instead, their partners changed their behavior by increasing the frequency of aggressive behaviors they exhibited toward the BA11/13 lesioned animals. This is likely due to impairments in social interactions in the BA11/13 lesioned animals, produced by deficient understanding of socioemotional signals. In humans, damage to OFC areas including BA13 produce aberrant behavioral patterns as well, including impaired recognition of facial expressions of emotion (Hornak et al., 2003, 1996; Rolls, 1999). In addition, electrophysiological studies measuring OFC activity in nonhumans show BA13 neurons fire to facial expressions of emotion (Azzi et al., 2012) and discriminate social categories (Watson & Platt, 2012), further supporting the notion that BA13 is critical for representing flexible emotional cues in social interactions.

One of the most consistent impacts of damage to BA13 is the disruption of the updating of stimulus-outcome associations in reversal learning and reinforcer devaluation. Electrophysiological recordings in nonhumans support the critical role of BA13 in stimulus-outcome associations as BA13 neurons appear to incorporate outcome signals from all modalities (Rolls, Yaxley, & Sienkiewicz, 1990; Rolls, 2007; Thorpe, Rolls, & Maddison, 1983), including abstract reward (Padoa-Schioppa & Assad, 2006), and encode all components of association learning regardless of outcome (Gottfried et al., 2002), including anticipating outcomes and responding to errors and changes in reward contingency (Roesch & Olson, 2005; Schoenbaum et al., 2000; Thorpe et al., 1983). Recent studies from our laboratory and others have utilized restricted, fiber-sparing lesions and demonstrated that BA13 is not critical for reversal learning *per se*

(Bachevalier et al., 2011; Kazama et al., 2014; Machado et al., 2009; Rudebeck & Murray, 2011, 2014), but rather for the flexible adjustment of actions when a change in reward contingencies or context has occurred. Numerous groups have demonstrated that OFC BA13 lesions result in persistent impairments in reinforcer devaluation, which represents a more flexible modulation of behavior based on internal or external states. Indeed, BA13 appears to be critically involved in this function, as studies in healthy humans show that BA13 changes in functional activation between initial reward with a preferred treat and receipt of the same treat following satiety (Gottfried et al., 2002). In addition, extensive electrophysiological data demonstrate that OFC BA13 neurons fire during multiple stages necessary for flexible modulation, include error detection and outcome expectancy (Knutson, Fong, Adams, Varner, & Hommer, 2001). These data suggest a more nuanced role for BA13 in flexibly monitoring and modulating the perceived significance of a cue based on changing contexts. As social interactions are inherently dynamic and flexible, disruption of this function would presumably lead to the socioemotional impairments described above.

Our studies expand on the above literature by highlighting the specific role of area BA13 in modulation, rather than on value assignment or updating associations. Study 1 revealed that BA13 is critical for directing attention to salient social cues, such as the eyes of negative stimuli, potentially through modulating arousal. Damage to BA13 produced increases in pupil dilation to positive stimuli, towards the level of arousal seen for negative cues. These findings are consistent with those in the literature that demonstrate emotional dysregulation and impaired understanding of emotional expressions following damage including BA13. In Study 2, we elaborated on our

understanding of the role of BA13 in arousal modulation by demonstrating that, in addition to producing transient impairments in retention, BA13 is critical for the flexible modulation of fear response in the presence of a safety signal, supporting the notion that flexible modulation of emotional valence is a key process provided by BA13. In addition, histological assessment indicated that though lesions to BA13 were not large, they were sufficient to disrupt normal emotional modulation in our studies, likely due to the disruption of cortical organization. In addition, unintended damage in BA13 included damage to BA11 and BA46, the extent of which was associated with functional impairments related to directing attention to emotional cues and monitoring stimuli for salient cues. Together, these studies highlight the importance of BA13 in modulating attention to highly salient environmental cues, and are quite similar to the effects produced by damage to BA12, supporting the role of the orbital network of the OFC in emotional regulation.

The role of the medial OFC and the ventromedial PFC in regulating arousal

In contrast to areas BA12 and BA13, the medial OFC area BA14 appears to play a critical role in modulating arousal to appetitive and rewarding cues rather than aversive cues. That damage to BA14 causes distinct impairments than damage to BA12 and BA13 is not surprising, given that BA14 differs from the more orbital OFC areas in connectivity to both the amygdala and hypothalamus (Barbas & De Olmos, 1990; Barbas, 1993; Carmichael & Price, 1995), key areas for fear and emotional processing. Specifically, the ventromedial areas of the OFC, including BA14, appear to be critical to modulate appropriate levels of arousal to stimulus-outcome associations, and this arousal appears to help drive advantageous behavioral choices. Individuals with ventromedial PFC (vmPFC)

damage including BA14 show impaired arousal (measured by skin conductance) when they fail to make advantageous selections during a gambling task (Bechara et al., 2000). However, damage to BA14 does not impair the experience of arousal, as individuals with vmPFC damage show increased skin conductance upon receipt of a reward outside the task (Bechara et al., 1999). Rather the impairment is limited to the use of emotional signals to guide behavior, in this case to make a financially rewarding choice. Similarly, during a facial emotion recognition task, patients with damage to the vmPFC reported emotional faces as less arousing and less emotional than controls (Heberlein et al., 2008a) and show reduced pupil dilation (a measure of arousal; Bradley, Miccoli, Escrig, & Lang, 2008) to highly rewarding cues compared to controls (Manohar & Husain, 2016). These data highlight the important role of arousal for cueing behavior, a process that appears to be mediated by the vmPFC. In addition to a role in modulation of arousal, BA14 appears to be specialized for the processing of positive or reinforcing outcomes. Like BA13, BA14 neurons in the monkey OFC are active to all aspects of the reinforcement process, including encoding and anticipation of positive outcomes (Monosov & Hikosaka, 2012; Thorpe et al., 1983). In humans, areas of the vmPFC including BA14 show functional activation to the receipt of reinforcing outcomes (Gottfried et al., 2002; Hampshire et al., 2012; Noonan et al., 2011; O'Doherty et al., 2001) and during the reappraisal of stimuli toward a more positive valence (Winecoff et al., 2013). Though damage including the vmPFC impairs reversals in humans (Fellows & Farah, 2003) and nonhumans (Butter, 1969), these effects are not consistent (Rudebeck & Murray, 2011) and instead, BA14 may contribute to the flexible use of reinforcers to guide behavior, as suggested by decrements in the ability to extinguish a previously rewarded association.

Given these above data, we expected that damage to BA14 in our studies would produce impairments in arousal, specifically for positive cues, such as appetitive social stimuli and familiar food and treat items. Instead, our studies add to the understanding of the functions of BA14 by revealing that the region is not critical for emotional attention, arousal, or modulation of emotional cues. Specifically, damage to BA14 did not yield any deficits in attention or arousal to social or nonsocial cues compared to typical control animals. However, histological analyses did reveal that damage to BA14 was significantly correlated with decreased attention to the bodies of positive and neutral stimuli, though this did not reach significance in our group level analyses. This pattern of results suggests that perhaps BA14 does play only a minor role in directing attention to appetitive cues. One explanation for this outcome may be that our positive cues were not inherently positive to our subjects. The positive social signals used in Study 1 are appeasing facial expressions that convey (to a monkey viewer) a position of high rank and dominance. It is possible that these cues are not as inherently positive as we had assumed, perhaps due to the expression originating from an unknown adult animal. In addition, the positive nonsocial cues used in Study 1 were videos of familiar food treats and toys and, perhaps due to the predictable nature of laboratory life, may not have induced the positive valence that we expected. In Study 2, BA14 lesions again produced no impairment in the modulation of fear response in the presence of safe cues. Finally, we found that damage to BA14 did not produce impairments in the number of trials to extinguish fear associations, suggesting that the extinction deficits caused by BA14 lesions reported in previous studies may be specific to reinforcing associations. Together these results suggest that BA14 alone is not critical for emotional regulation to negative

cues, but we cannot draw strong conclusions regarding the impact of these lesions on processing positive or appetitive cues due to task constraints.

Implications for understanding the role of OFC-amygdala connectivity in emotional regulation

A comparison of the effects of amygdala lesions in socioemotional processing and fear learning is critical given the importance of areas BA12 and BA13 for the regulation of attention and arousal to emotional and fearful cues, the involvement of amygdala nuclei in emotional regulation, and the divergent patterns of amygdala connectivity present across all OFC areas. As discussed in chapter one, the OFC has significant connectivity with the amygdala nuclei: BA12 both sends and receives projections from the amygdala, primarily within the basolateral complex of the amygdala (BLA)(Barbas & De Olmos, 1990; Carmichael & Price, 1995), and BA13 also has reciprocal connectivity with BLA, but also projects to the central and lateral nuclei, and receives projections from the lateral and central amygdala nuclei (Barbas & De Olmos, 1990; Carmichael & Price, 1995). BA14 receives significant projections from the BLA, and fewer from the cortical nuclei, but does not send many projections back to the BLA (Barbas & De Olmos, 1990; Barbas, 1993; Carmichael & Price, 1995). These differences in anatomical projections are informative because the different amygdala nuclei contribute differently to the processing of sensory inputs and have downstream influence on the production or inhibition of arousal to emotional cues. Specifically, the lateral amygdala receives significant inputs from diverse sensory modalities (Phelps & LeDoux, 2005; Schiller & Delgado, 2010), information that is then processed by the BLA to help in the later prediction of outcomes (Garcia et al., 1999; Ghashghaei & Barbas, 2002; Schafe et al.,

2001). In addition to projecting to various cortical regions including the OFC, the BLA projects to the central amygdala, an area with extensive connections to hypothalamic and brain stem areas important for the regulation of autonomic arousal and homeostasis (Phelps & LeDoux, 2005; Schiller & Delgado, 2010). Together, these anatomical differences support the notion that differences in emotional processing across OFC subregions may be mediated by differences in connectivity with amygdala nuclei.

The amygdala has long been regarded a seat of emotional processing, and is particularly relevant to the processing of negative or fear-inducing cues (Adolphs, Baron-Cohen, & Tranel, 2002; Adolphs, Tranel, Damasio, & Damasio, 1994). Within social contexts, amygdala lesions produce decreased attention to the eyes of social stimuli, particularly for those of negative valence, and increased arousal to negatively valenced cues in both humans and nonhumans (Adolphs et al., 2005; Dal Monte et al., 2015). The amygdala also shows functional activation to social cues, especially negative faces (Hoffman et al., 2007). In addition, lesion studies in nonhuman primates show that the decreased eye gaze resulting from amygdala damage is accompanied by increased arousal (i.e. pupil diameter, Dal Monte et al., 2015) and functional activation in the macaque BLA shows specific activation to negative valence and highly salient social cues (Hoffman et al., 2007). When reevaluating threats, humans show decreases in amygdala activation correlated with increased prefrontal activation, including lateral OFC (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003), suggesting that the prefrontal cortex may be responsible for down-regulation of amygdala function based on additional contextual information. That the effects of OFC lesions on attention to socioemotional stimuli mirror those of amygdala lesions, and that the OFC regions with the strongest BLA connectivity

(BA12 and BA13) show the most profound deficits, supports the idea that the OFC-BLA network is a critical substrate for emotional processing and modulation of arousal.

To further understand the role of the interconnections between the amygdala and the OFC, it is important to contrast the role of each region in the process of fear learning, expression, modulation and extinction as measured in Study 2. Damage to the amygdala impairs the development of initial conditioned stimulus (CS) -unconditioned stimulus (US) associations that are critical for the fear conditioning and safety signal learning (Schafe et al., 2001). In addition, lesions of the amygdala impair fear-potentiated startle (Weike et al., 2005) and decreased arousal to the CS (LaBar, LeDoux, Spencer, & Phelps, 1995). This effect is similar to our findings in BA13, in which damage impairs the expression of previously learned fear associations. In addition, the amygdala is critical for the modulation of stimulus-outcome associations, including fear-associations, in changing contexts. In rodents, BLA neurons fire specifically during when modulation of fear response is needed (Sangha et al., 2013) and damage to the amygdala produces decreased fear response and increased PFC activity to conditioned fear cues (Garcia et al., 1999). Given the role of OFC areas BA12 and BA13 in this process, as demonstrated in Study 2, this would suggest that OFC-amygdala connectivity is important for the modulation of fear response, a notion supported by studies showing that inactivation of either the amygdala or the prefrontal cortex results in impaired stimulus-outcome association updating (Baxter et al., 2000) and both structures are co-activated during safety signal processing in rodents (Likhtik et al., 2014) and during emotional reappraisal in humans (Ochsner, Bunge, Gross, & Gabrieli, 2002). Many researchers have proposed that the late-developing OFC is critical for the top-down modulation of amygdala

response (Milad & Quirk, 2002), and that disruption of this relationship leads to impaired emotion processing. Finally, the amygdala is important for fear extinction (Phelps et al., 2004), a process that is not mediated by the OFC. Together, these data highlight a critical role of PFC-amygdala connectivity, especially OFC-BLA connectivity for the evaluation of potential environmental threats and the modulation of behavioral response by down-regulation of the amygdala.

Does damage to the OFC impact fear or anxiety?

It is important to note that we have primarily addressed the effects of OFC lesions on fear processing, yet fear and anxiety are separable emotional experiences that rely on similar structures included in distinct (or independent) neural pathways. Research in humans and rodents has suggested that the fear-potentiated startle paradigm taps into the fear system, yet there is less agreement on translational paradigms that induce anxiety-like behaviors (Davis, Walker, Miles, & Grillon, 2010). Given that our socioemotional eye tracking task included acute stressors, such as a threatening expression from an unknown adult animal, we believe that this task also induced a fear state. However, we cannot exclude the possibility that slight differences across tasks and across areas of damage may be due to differences in the fear and anxiety systems and suggest that further research is needed on this subject.

Limitations and future directions

Multiple limitations in our study design may have impacted outcomes and conclusions, including the number of subjects, the extent of the lesions, and the selection of valenced stimuli. First, though the number of subjects was based on power analyses, some animals failed to meet the minimum baseline criterion at different stages of Study 2

and thus had to be excluded. This led to smaller size groups than expected, and led to some difficulty in interpreting effects due to individual variability. To this end, our data need replication with future studies using larger number of subjects or introducing more stringent within subject manipulations to strengthen the effects. However, despite the small numbers and individual variability across tasks and lesion extent, examining the statistical analyses in the context of effect size as well as significance, compounded by the consistent effects found across studies, leads us to believe that our conclusions are well-founded.

A second issue in our studies is the choice of stimulus set for Study 1. To avoid the effects of training and conditioning across subjects, we chose to select stimuli that had preexisting value to rhesus macaques, namely environmentally or evolutionarily significant positive and negative cues. However, given the predictable nature of laboratory life, it is possible that some of the valenced stimuli did not produce the desired effect due to lack of predictive validity. However, the social stimuli used in our study have been validated in numerous publications and reliably produced different behavioral or neuronal response across valence categories (Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007; Hoffman et al., 2007; Mosher, Zimmerman, & Gothard, 2011; Mosher et al., 2014). Even so, it would be wise to construct a validated stimulus set for use in nonhuman primates, similar to the International Affective Picture System (IAPS) used with humans (Lang, Bradley, & Cuthbert, 1997), to both improve the validity of experimental research as well as improve reproducibility and replication across groups.

In addition, though our study has helped to shed light on the contributions of individual subregions of the OFC to emotional regulation, we are left to hypothesize

about the functional mechanisms that produce these impairments. Future studies may utilize advanced techniques to deeply probe the relationship between OFC subregions and emotional centers such as the amygdala and hypothalamus. For example, recent advances in the use of temporary inactivation of cortical and subcortical structures through the use of designer receptors exclusively activated by designer drugs (DREADDS) would allow for inactivation paired with excitotoxic lesions or electrophysiological recordings and may help shed light on the functions networks critical for emotional regulation.

General conclusions and the contributions of the orbital and medial networks of the OFC

Based on the findings presented in this dissertation, our data support the distinction of two functional OFC networks and elaborate on the current understanding of the contributions of each network. The orbital network, including BA12 and BA13, has been suggested to play a role in multimodal integration and associations based on the networks vast inputs from sensory areas. The data from our studies support this notion and expand it to include an important role in modulating arousal based on stimulus associations. BA12 and BA13 appear to have complimentary functions in the modulation of attention to emotional cues, particularly for those of negative valence. Though the importance of BA13 is well established, this study also demonstrates the critical contributions of BA12. The medial network of the OFC, including BA14 as well as areas BA25 and BA10, has been proposed to regulate emotional arousal through the networks interconnections with the hypothalamus and brainstem. Our data do not support this notion but also do not contradict it. As discussed above, our studies clearly demonstrate

that BA14 does not play a role in modulation to fearful or threatening cues, but the effects of BA14 damage on arousal and modulation to positive cues were not specifically measured. Further studies should seek to probe these questions, as well as to determine whether BA12 and BA13 differ in the processing of positive or appetitive cues.

The effects of damage to areas in the orbital network of the OFC are likely explained by the OFC-AMY disconnection. Given the importance of the OFC in down-regulating fear expression by the amygdala, the removal of this inhibitory influence likely results in the impaired fear discrimination seen in Study 2. This is also likely the mechanism for the decreased gaze and heightened arousal found in Study 1, as the lack of OFC inhibition may increase the potential threat of gaze from an unknown adult macaque and promote avoidance. In contrast, as damage to BA14 does not impact the behaviors measured in our study, but amygdala damage does, this suggests that cross-talk between the medial OFC network and the amygdala is not critical for modulation of attention and arousal to fearful stimuli.

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