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Sex-related differences in amygdala functional connectivity during resting conditions

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Recent neuroimaging studies have established a sex-related hemispheric lateralization of amygdala involvement in memory for emotionally arousing material. Here, we examine the possibility that sex-related differences in amygdala involvement in memory for emotional material develop from differential patterns of amygdala functional connectivity evident in the resting brain. Seed voxel partial least square analyses of regional cerebral blood flow data revealed significant sex-related differences in amygdala functional connectivity during resting conditions. The right amygdala was associated with greater functional connectivity in men than in women. In contrast, the left amygdala was associated with greater functional connectivity in women than in men. Furthermore, the regions displaying stronger functional connectivity with the right amygdala in males (sensorimotor cortex, striatum, pulvinar) differed from those displaying stronger functional connectivity with the left amygdala in females (subgenual cortex, hypothalamus). These differences in functional connectivity at rest may link to sex-related differences in medical and psychiatric disorders.

The amygdala is a heterogeneous structure involved in a range of neuroendocrine, autonomic, emotional, and cognitive functions (Aggleton, 1992; Gray, 1993; Aggleton, 2000). Converging evidence from both human and animal studies indicates that one major function of the amygdala is the modulation of memory processes involving other brain regions during emotional arousal (Cahill, 2000; McGaugh, 2000). Human imaging studies have demonstrated a significant relationship between amygdala activity at encoding and long-term memory for emotionally arousing material but not with memory for relatively nonemotionally arousing material (Cahill et al., 1996; Hamann et al., 1999; Canli et al., 2000, 2002; Cahill et al., 2001a). Recent neuroimaging studies have established a sex-related hemispheric lateralization of the role of the amygdala in the modulation of memory for emotionally arousing material. Cahill et al. (2001b) demonstrated a sex-related lateralization of amygdala involvement in emotionally influenced memory by directly comparing amygdala effects in men and women during the viewing of emotional film clips. Sex-related differences in the relationship between the amygdala and subsequent recall of the emotional films arose in the absence of a sex-related difference in arousal ratings or in free recall performance for the two sets of film clips. In men, enhanced activity of the right amygdala, but not the left, was related to enhanced recall for emotional film clips. In women, enhanced activity of the left amygdala, but not the right, was related to enhanced recall for emotional film clips. This basic finding was later confirmed in an event-related fMRI investigation of the neural bases of the evaluation and encoding of emotional stimuli (Canli et al., 2002). Recognition accuracy for maximally arousing pictures was correlated with greater left hemisphere amygdala activation in women but with greater right hemisphere amygdala activation in men. Recognition accuracy for less arousing pictures was not correlated with amygdala activity in either hemisphere for both men and women.

Although the consistency in the results of Cahill et al. (2001b) and Canli et al. (2002) provides substantial support for the existence of a sex-related lateralization of amygdala involvement in emotionally influenced memory, the most compelling single demonstration of this lateralization has been provided by Cahill et al. (2004). Using event-related fMRI investigation of sex-related differences in amygdala function in emotionally influenced memory, the same hemispheric lateralization was found. In a between-groups, random effect analysis comparing women and men, the left amygdala demonstrated a significantly stronger relationship between its activity at encoding and long-term memory for arousing pictures in women relative to men. No such difference occurred in the right amygdala. Conversely, the right amygdala demonstrated a significantly stronger relationship

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between its activity at encoding and long-term memory for arousing pictures in men relative to women. No such difference occurred in the left amygdala. Furthermore, an analysis of variance (ANOVA) of the parameter estimates for the height of the BOLD response in the amygdala demonstrated a significant sex-by-hemisphere interaction. The existence of a significant sex-by-hemisphere interaction provides the strongest evidence that the sex-related lateralization is not an artifact of thresholding.

To better understand the scope of sex-related hemispheric differences in amygdala function, we examined potential sex-related differences during resting conditions. Although cognitive processes during resting states are unregulated, measurement of rCBF during rest in healthy subjects demonstrates reasonable reliability and is considered to provide a relatively stable estimate of brain activity in the absence of stimulation (Zald et al., 2002; Coles et al., 2005). Regional brain activity during the resting state has been associated with trait-related measures and performance during task conditions (Ragland et al., 2000; Sugiura et al., 2000; Zald et al., 2002). Neuroimaging investigations of the resting state highlight the view that the brain is not a system that simply responds to changing contingencies but has an intrinsic organization of its own that interacts with incoming information (Xiong et al., 1999).

A number of investigations have shown sex-related differences in resting regional activity levels and laterality indexes (Mathew et al., 1986; Baxter et al., 1987; Miura et al., 1990; Azari et al., 1992; Andreason et al., 1994; Gur et al., 1995; George et al., 1996; Murphy et al., 1996; Volkow et al., 1997; Ragland et al., 2000; Kawachi et al., 2002). The majority of these investigations did not include a region of interest exclusive to the amygdala. Of those investigations that included the amygdala as a region of interest or that used whole brain analyses (Gur et al., 1995; George et al., 1996; Murphy et al., 1996; Ragland et al., 2000; Kawachi et al., 2002), only one demonstrated sex-related differences concerning the amygdala. Gur et al. (1995) found significantly higher relative glucose metabolism in the amygdala (right and left averaged) in men than in women during an eyes open resting condition but did not find significant sex-related differences in the laterality of amygdala metabolism.

Despite the fact that the majority of studies mentioned above failed to show a sex-related difference in amygdala activity per se during resting conditions, these studies do not directly address whether there are sex-related differences in its functional connectivity. Such differences in connectivity could exist, even in the absence of differences resting glucose or blood flow differences. Horwitz (1990) provides an initial demonstration of the ability for differential functional connectivity to exist in the absence of differential activation using computer simulations. Consistent with such a hypothesis, Azari et al. (1992) investigated sex-related differences in intra- and inter-hemispheric correlations among sixty-five regions of interest (none exclusive to the amygdala) during a resting condition. Although there were no more than a chance number of sex-related differences in metabolic rates, there were numerous sex-related differences in functional connectivity. Women had more positive left hemisphere correlations that were significantly greater than those of men, while men had more positive right hemisphere correlations that were significantly greater than those of women.

The purpose of the current study was to investigate the possibility that sex-related differences in amygdala involvement in memory for emotional material develop from differential patterns of amygdala functional connectivity already evident during periods of relative rest. It may be, for example, that even in a resting state, activity in the right hemisphere amygdala is, in general, more strongly coupled to that of the rest of the brain in men than it is in women, and conversely, that activity in the left hemisphere amygdala is more strongly coupled to that of the rest of the brain in women than in men. Such a finding would suggest that the sex-related hemispheric laterality of amygdala function in relation to memory does not occur exclusively as a result of emotional stimulation. While sex-related differences regarding amygdala function have been primarily noted in emotional memory paradigms, amygdala functional connectivity patterns could differ in a more general fashion. Sex-related differences in resting amygdala functional connectivity would suggest a general effect of sex on emotional and cognitive processing rather than a specific effect of sex on the neurobiology of emotional memory.

The functional connectivity of the right and left amygdala was investigated using "seed voxel" partial least square analyses (seed-PLS) of regional cerebral blood flow data (rCBF). Seed-PLS is a multivariate analytical technique used to examine the relationship between activity of a target region ("seed") and activity across the whole brain as a function of experimental condition (McIntosh et al., 1996; McIntosh and Lobaugh, 2004). Seed-PLS was applied to 100 voxels from each amygdala region. In each analysis, the dominant latent variable was examined for the presence of significant sex-related differences in amygdala functional connectivity.

Results

Fig. 1 shows the location of the amygdala seed voxels in which the dominant latent variable expressed significant sex-related differences in amygdala functional connectivity during resting conditions. The direction of the difference is expressed by color: red indicates a stronger amygdala–brain relationship in women than in men; blue indicates a stronger amygdala–brain relationship in men than in women. Interestingly, seed voxels associated with significantly greater functional connectivity in men than in women were found exclusively in the right hemisphere amygdala (17 voxels). In contrast, seed voxels

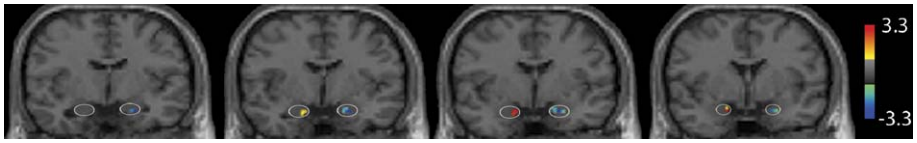

Fig. 1. Location of amygdala seed voxels demonstrating significant sex-related differences in amygdala functional connectivity is depicted. Red voxels are associated with greater functional connectivity in women than in men. Blue voxels are associated with greater functional connectivity in men than in women. Coronal slices start at −6.75 mm from the anterior commissure and increase in increments of 2.25 mm to 0 mm. Circle represents approximate location of voxels considered as potential seeds. A fifth coronal slice at −9 mm from the anterior commissure also contained potential seeds; however, none were associated with sex-related differences and the slice was not included in the figure.

associated with significantly greater functional connectivity in women than in men were found exclusively in the left hemisphere amygdala (8 voxels). Furthermore, the location of the seed voxel maximally associated with sex-related differences in functional connectivity within the left hemisphere amygdala ([−14, −2, −24]) was more medially located than was the maximum within the right hemisphere amygdala ([20, −4, −22]). The results of the seed-PLS analyses for these two voxels are further elaborated below.

It should be noted that the relatively small number of voxels in Fig. 1 expressed significant sex-related differences in functional connectivity as the *dominant* pattern. It is possible that other amygdala voxels displayed significant sex-related differences in amygdala functional connectivity with specific regions as assessed by pairwise correlations, but this was not the dominant pattern when considering the whole brain. The purpose of selecting voxels expressing sex-related differences in amygdala functional connectivity as the dominant pattern was to select amygdala voxels with the most widespread sex-related differences in functional connectivity.

Right amygdala functional connectivity

The first (dominant) latent variable (LV) (accounting for 65% of the cross-block correlation, $P < .002$) revealed brain areas that showed stronger functional connectivity with the right amygdala in men than in women (shown in Fig. 2a). Unlike Fig. 1, color indicates the direction of the *correlation*: red indicates a greater positive correlation; blue indicates a greater negative correlation, a convention used in Figs. 2 and 3. Bootstrapping analysis revealed that reliable regions were almost exclusively associated with a stronger positive relationship with the right amygdala in men than in women (Table 1). Pulvinar, sensorimotor cortices, and striatal areas were strongly represented. The second (minor) LV (accounting for 35% of the cross-block correlation, $P < .216$) revealed brain areas associated with greater functional connectivity with the right amygdala in women than in men (shown in Fig. 2b). The relatively few reliable regions mainly displayed a stronger negative relationship with right amygdala activity in women than in men (Table 2).

Left amygdala functional connectivity

The first (dominant) LV (accounting for 76% of the cross-block correlation, $P < .004$) revealed brain areas that showed stronger functional connectivity with the left amygdala in women than in men (shown in Fig. 3a). Bootstrap analysis indicated that reliable regions were almost exclusively associated with a stronger positive relationship with the left amygdala in women than in men (Table 3). Subgenual cortex and hypothalamus were strongly represented. The second (minor) LV (accounting for 24% of the cross-block correlation, $P < .614$) revealed brain areas displaying greater functional connectivity with the left amygdala in men than in women (shown in Fig. 3b). The very few reliable regions mainly displayed a stronger negative relationship with left amygdala activity in men than in women (Table 4).

Discussion

Although no sex-related differences in absolute activity levels in the amygdala were detected (consistent with earlier results (George et al., 1996; Murphy et al., 1996; Ragland et al., 2000; Kawachi et al., 2002)), the results of the seed-PLS analyses revealed significant sex-related differences in amygdala functional connectivity during an eyes closed, "resting" condition. The right hemisphere amygdala possessed a much more widespread distribution of functional connectivity at rest in men than it did in women. In striking contrast, the left hemisphere amygdala possessed a much more widespread distribution of functional connectivity at rest in women than it did in men.

These results reveal a pattern of hemispheric lateralization ("women left/men right") similar to that of previous investigations of amygdala function in emotionally influenced memory (Cahill et al., 2001b, 2004; Canli et al., 2002). Thus, these results are consistent with the hypothesis that sex-related differences in amygdala involvement in memory for emotional material arise from differential patterns existing in the resting brain, in the absence of explicit emotional stimulation. The present results indicate that dramatic sex-related differences in the functional connectivity of the human amygdala exist even at rest.

The current study focused on sex-related differences in the functional connectivity of the right and left amygdala. It is not clear to what extent sex-related differences in functional connectivity of the right and left hemisphere are specific to the amygdala and to what extent the findings may be more general. Other brain regions may display a similar sex-related lateralized pattern of functional connectivity.

Internally vs. externally oriented networks

In general, the regions displaying stronger functional connectivity with the right amygdala in males at rest (sensorimotor cortex, striatum, pulvinar) are involved in attending to and acting onto the external environment. The pulvinar has limbic, sensory, and motor connections and has been associated with visuospatial and selective attention functions (Romanski et al., 1997; Gutierrez et al., 2000). The caudate and putamen have motor, cognitive, and emotional functions. Damage to the putamen, caudate nucleus, and pulvinar has been associated with spatial neglect in humans (Karnath et al., 2002). Spatial neglect is characterized by a failure to explore, react, or respond to items in the side of space contralateral to the lesion. The underlying function is asymmetrically represented: right-sided lesions result in more complete spatial neglect (Mesulam, 1999).

In contrast, the regions displaying stronger functional connectivity with the left amygdala in females (subgenual cortex, hypothalamus) have functions more oriented towards attending to and controlling aspects of the internal milieu. The hypothalamus has well-known neuroendocrine functions (Morgane and Panksepp, 1981). The subgenual prefrontal cortex (PFC) is involved in visceromotor functions. The subgenual PFC has extensive connections with the amygdala, hypothalamus, and other structures in the autonomic system such as the periaqueductal gray and brainstem autonomic nuclei (Carmichael and Price, 1995). Stimulation of this region produces changes in heart rate, blood pressure, gastric motility, and respiration (Davey et al., 1949; Hurley-Gius and Neafsey, 1986). From these differences, we may speculate that during resting conditions, there is a distinct difference in the prevailing functional networks expressed in men and women, such that for men, this network is more outwardly oriented, while for women, it is more inwardly oriented.

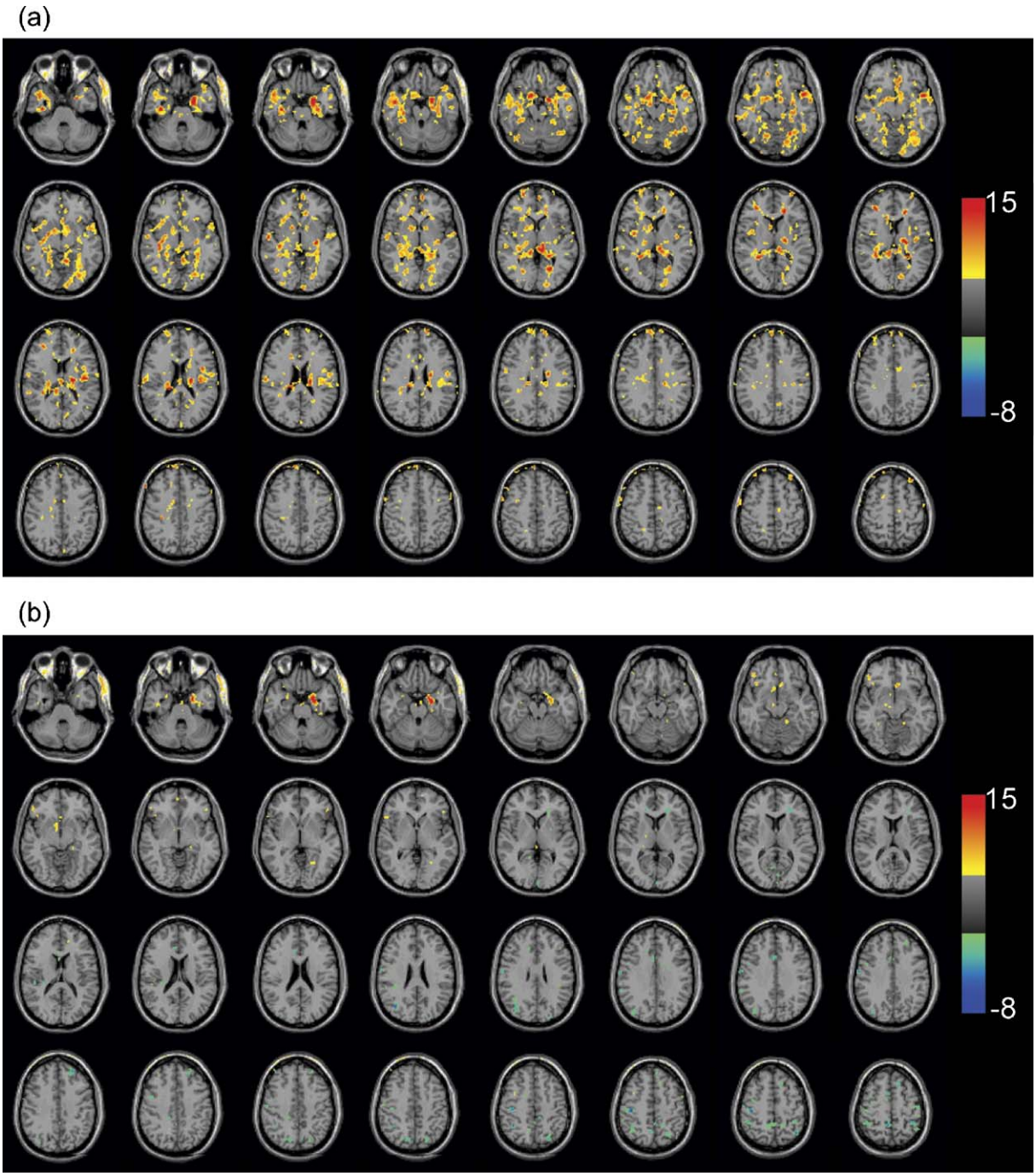

Fig. 2. Sex-related differences in right amygdala functional connectivity. (a) Regions displaying stronger functional connectivity with the right amygdala in men than in women. Red areas display a greater positive correlation with right amygdala activity in men than in women. Blue areas display a greater negative correlation with right amygdala activity in men than in women. (b) Regions displaying stronger functional connectivity with the right amygdala in women than in men. Red areas display a greater positive correlation with right amygdala activity in women than in men. Blue areas display a greater negative correlation with right amygdala activity in women than in men. Horizontal slices start at −29 mm from the anterior commissure and increase in increments of 2.25 mm to 41 mm.

Potential explanations for the existence of sex-related differences in resting amygdala functional connectivity

As mentioned in the Introduction, cognitive processes during the resting state are unregulated. It is possible that sex-related tendencies to engage in different cognitive processes during rest exist and impact the functional connectivity of the amygdala. However, we are not aware of any report suggesting that men and women engage in different cognitive processes during resting state paradigms.

Alternatively, sex-related differences in resting amygdala functional connectivity may be related to fundamental neuroanatomical differences. Sexual dimorphism of the amygdala nuclei has been well established in animals (Nishizuka and Arai, 1981;

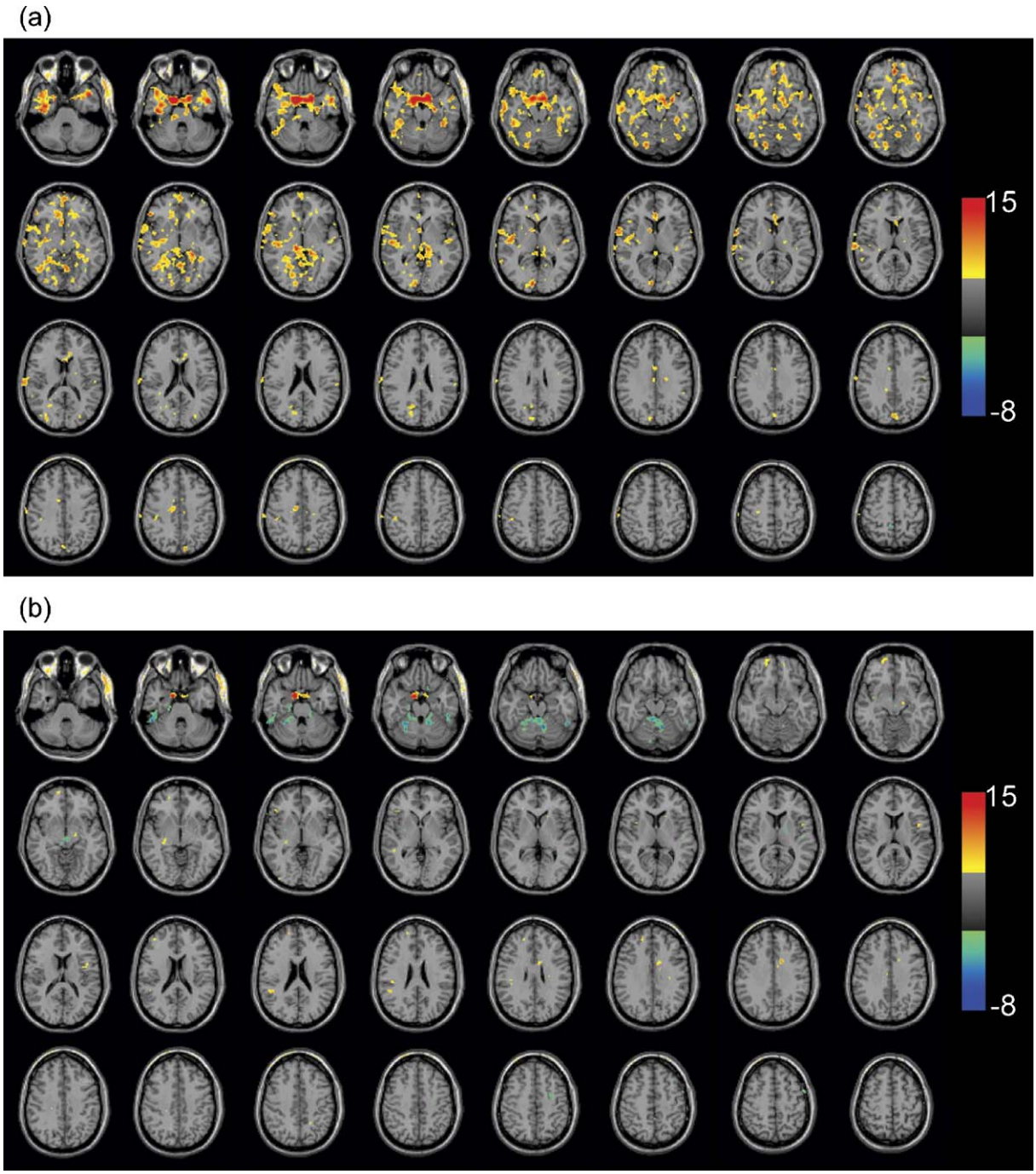

Fig. 3. Sex-related differences in left amygdala functional connectivity. (a) Regions displaying stronger functional connectivity with the left amygdala in women than in men. Red areas display a greater positive correlation with left amygdala activity in women than in men. Blue areas display a greater negative correlation with left amygdala activity in women than in men. (b) Regions displaying stronger functional connectivity with the left amygdala in men than in women. Red areas display a greater positive correlation with left amygdala activity in men than in women. Blue areas display a greater negative correlation with left amygdala activity in men than in women. Horizontal slices start at −29 mm from the anterior commissure and increase in increments of 2.25 mm to 41 mm.

Kalimullina, 1989; Hines et al., 1992; Stefanova and Ovtscharoff, 2000). Sexual dimorphisms of amygdala nuclei have been demonstrated in terms of volume, neuronal density, and density of specific neuronal populations (Stefanova and Ovtscharoff, 2000). Human studies have demonstrated larger amygdala relative to total cerebral size in men compared with women (Goldstein et al., 2001). Also, sex-related differences in opioid receptor binding within the human amygdala exist prior to menopause (Zubieta et al., 1999). Such relatively stable neuroanatomical differences may impact the way the amygdala interacts with other brain regions, even in the absence of explicit emotional stimulation. Sexual dimorphism of other regions may also impact amygdala functional connectivity by changing the neural context in which the amygdala operates. The idea of a neural context underscores the importance of considering the status of anatomically related elements in elucidating the

Table 1
Regions associated with stronger right amygdala functional connectivity in men than in women

	Coordinates	Ratio	Correlation	
			Female	Male
Temporal cortex				
BA 21	61, −5, −16	7.69	.34	.57
	−41, 2, −29	6.28	.01	.57
BA 38	50, 7, −14	9.65	.03	.74
BA 20/37	50, −54, −18	6.48	.26	.55
Prefrontal cortex				
BA 25	−11, 5, −20	8.66	.48	.71
BA 24	11, 32, −7	6.73	.09	.61
BA 32	7, 34, −14	6.70	.32	.56
BA 9	16, 65, 16	6.29	−.02	.50
	−34, 63, 20	4.86	−.02	.50
BA 46	−29, 41, 5	8.36	.09	.64
BA 10	7, 63, −5	7.17	.30	.54
	−20, 65, −2	7.31	.23	.62
Occipital cortex				
BA 17/18	11, −92, −11	5.74	.07	.64
Insular cortex	41, −11, 7	8.71	−.16	.78
Thalamus				
Pulvinar	11, −29, −2	9.78	.11	.64
	−7, −32, 5	6.50	−.10	.70
Hippo/PHG				
	29, −25, −16	6.42	.34	.60
	−29, −43, −2	5.83	−.16	.55
	27, −38, −18	6.82	.29	.54
	−34, −18, −27	9.27	.45	.68
	34, −38, −9	6.08	.37	.51
	−9, −36, −2	7.93	.12	.64
Striatum				
Caudate head	9, 11, −14	6.02	.31	.54
	−11, 7, −7	6.70	.08	.60
Caudate body	20, −7, 1	9.05	.33	.64
	−14, −20, 16	5.52	−.08	.55
Caudate tail	−16, −32, 9	9.96	.02	.70
Putamen	−30, −16, −9	7.59	.36	.61

Note. Tables 1–4 do not represent an exhaustive list of all reliable clusters but are meant to reflect the more prominent elements of Figs. 2 and 3.

contribution of a particular region to a function (McIntosh, 2000; McIntosh et al., 2001).

Sex-related differences in the presence/absence or relative density of projections may also contribute to sex-related differences in the functional networks of the amygdala during rest. Unfortunately, many anatomical tracing studies in animals do not even report the sex of the subjects. A possible sex-related difference in the anatomical connectivity of the subgenual cortex in primates has been noted (Freedman et al., 2000). Freedman et al. (2000) failed to demonstrate a projection from the subgenual cortex to the ventromedial hypothalamus in male monkeys while a robust projection had been previously demonstrated in a female monkey (Ongur et al., 1998). Diffusion tensor imaging (DTI) allows in vivo explorations of the anatomical connectivity of the human brain and can address issues of sex-related differences in anatomical connectivity in humans. DTI has already begun to enhance our knowledge of sex-related as well as hemispheric differences in anatomical connectivity in humans (Szeszko et al., 2003; Westerhausen et al., 2003; Buchel et al., 2004; Zhang et al., 2004).

Table 2
Regions associated with stronger right amygdala functional connectivity in women than in men

	Coordinates	Ratio	Correlation	
			Female	Male
Prefrontal cortex				
BA 47	−45, 34, −11	5.08	.53	.02
BA 9	25, 50, 25	−5.43	−.53	.03
BA 24	−5, 20, 20	−5.95	−.47	.15
BA 8	14, 25, 43	−5.11	−.54	.09
Occipital cortex				
BA 18/19	−36, −70, 14	−6.06	−.49	.25
Parietal cortex				
BA 7	27, −70, 34	−4.95	−.51	.11
	−5, −43, 41	−5.81	−.58	.12
BA 40	−36, −47, 34	−6.73	−.60	.21
BA 3/4	38, −2, 45	−7.35	−.67	.10
	38, −18, 36	−7.99	−.66	.32

It has been suggested that female-specific antinociceptive and anxiolytic systems have evolved around sex-specific stressors

Table 3
Regions associated with stronger left amygdala functional connectivity in women than in men

	Coordinates	Ratio	Correlation	
			Female	Male
Temporal cortex				
BA 21	47, −2, −27	10.92	.79	.23
	−36, −23, −27	7.64	.67	.09
BA 38	27, −2, −18	7.45	.71	.18
	−41, 11, −27	6.53	.63	.22
BA 20/37	36, −43, −23	6.20	.68	.09
	−43, −41, −20	6.55	.70	.13
BA 22	−50, −16, −5	7.04	.72	.18
Prefrontal cortex				
BA 25	11, 7, −23	7.90	.66	.20
	−9, 2, −23	14.36	.71	.53
BA 24	2, 34, 0	6.40	.60	.26
	−9, −9, 29	5.38	.54	.07
BA 32	−2, 47, −14	8.42	.65	.33
BA 11	−2, 47, −20	5.10	.57	.35
BA 10	−5, 68, −7	4.56	.55	.40
BA 47	−50, 34, −9	6.95	.56	.26
Occipital cortex				
BA 17/18	−7, −88, −2	8.17	.72	.25
BA 18/19	11, −81, 27	6.10	.57	.37
Hypothalamus	2, 0, −20	6.78	.64	.32
	−2, 0, −20	5.60	.64	.34
Thalamus				
V. Anterior Nucleus	−9, 7, −2	5.67	.70	.11
Hippo/PHG				
	16, −34, −7	7.71	.62	.20
	−29, −50, −11	5.77	.69	.10
	23, −18, −23	7.32	.69	.28
	−32, −29, −16	7.56	.79	−.09

Table 4
Regions associated with stronger left amygdala functional connectivity in men than in women

	Coordinates	Ratio	Correlation	
			Female	Male
Temporal cortex				
BA 20/37	50, −36, −23	−6.24	.25	−.48
	−47, −45, −27	−5.95	.59	−.46
Prefrontal cortex				
BA 10	−11, 61, −14	4.38	.04	.58
BA 24	16, 9, 20	5.95	−.01	.62
Hippo/PHG	−18, −20, −25	4.72	.30	.49
Cerebellum	11, −56, −20	−6.60	.46	−.53
	−25, −61, −23	−5.92	.34	−.49

(Berkley, 1997). Women experience a number of stressors that men never directly experience such as menstruation, pregnancy, and childbirth. All of these stressors are visceral in nature and, likewise, are stressors for which a fight or flight response is not particularly useful. Although these stressors are specific to reproduction, it may be that a stress system designed to deal with these vitally important stressors would generalize to other types of stressors. It seems reasonable to speculate that the resources needed to support such a system would influence how the individual handles stress in general. Numerous studies show sex-related differences in sympathetic and parasympathetic activity in response to a wide variety of stressors (reviewed in Dart et al., 2002). In general, there is a preponderance of sympathetic mediated responses in males and a preponderance of parasympathetic mediated responses in females. Indeed, it has been argued that the "fight or flight" response more appropriately characterizes the prototypical stress response in males, while the prototypical stress response in females is better characterized as "tend and befriend" (Taylor et al., 2000). Although the possibility that men and women are engaged in different cognitive processes during rest cannot be ruled out, there is considerable evidence to suggest that there are fundamental sex-related differences in the biology of the stress system that are likely to influence amygdala functional connectivity in the absence of explicit stimulation.

Finally, although the data were collected before exposure to any emotional experimental stimuli, there are emotional components to the experiment that could be not controlled (such as catheterization, unease with radioactive material and medical scanners, as well as the simple act of being a subject of study) which may be contributing factors to the observed sex-related differences in amygdala functional connectivity. But even in this event, the results would reflect a basic sex-related difference in response to these stressors.

Potential links to sex-related differences in psychiatric and medical disorders

Sex-related differences in resting amygdala functional networks may relate to sex-related differences in the prevalence and treatment of some psychiatric and medical disorders. Although the subjects in the current study had no history of psychological disorders, there was a striking sex-related difference such that women had greater functional connectivity than did men between the left amygdala and subgenual PFC. The subgenual PFC has been implicated in disorders such as depression and irritable bowel syndrome (Drevets et al., 1998; Hirayasu et al., 1999; Botteron et al., 2002; Naliboff et al., 2003). It is notable in this regard that women show greater activation of the left amygdala and subgenual PFC than men during exposure to a visceral stressor in patients with irritable bowel syndrome (Naliboff et al., 2003). Furthermore, resting regional cerebral blood flow in the subgenual cingulate has previously been associated with trait-wise differences in negative affect in healthy controls, with higher levels of resting rCBF associated with greater negative affect (Zald et al., 2002). The sex-related differences in functional connectivity between the amygdala and subgenual cortex may thus have important effects on the regulation of negative affect that extend beyond psychiatric illnesses.

The current results may also relate to sex-related differences in the effects of brain lesions in nonemotional cognitive tasks. Recently, a sex-related difference in the severity of symptoms following ventromedial prefrontal cortical lesions has been demonstrated (Denburg et al., 2004). Interestingly, the results show the same lateralization pattern (female left/male right) as seen in our study. In women, left-sided lesions were associated with profound disturbances in social conduct, emotional processing, decision making, and personality, while right-sided lesions were associated with normal performance. Conversely, in men, right-sided lesions were associated with profound disturbances, while left-sided lesions were associated with normal performance. This apparent sex-related lateralization in the role of the prefrontal cortex in decision-making tasks has also been demonstrated in healthy controls (Bolla et al., 2004). During performance of a decision-making task (Iowa Gambling Task), men showed greater activation in the right lateral orbitofrontal cortex, while women showed greater activation in the left dorsolateral prefrontal cortex.

Concluding remarks

The previously reported sex-related hemispheric laterality of amygdala function in relation to memory for emotional material parallels the differential patterns of amygdala functional connectivity found in the resting brain. The findings strengthen the argument for consideration of influences of both sex and hemisphere in neurobiological investigations. These results have importance beyond understanding sex-differences in emotionally influenced memory and may improve our understanding of sex-related differences in psychiatric and medical disorders.

Methods

Overview

Data in this study were derived from multiple PET studies conducted by Drs. Pardo, Zald and colleagues at the Cognitive Neuroimaging Unit of the Minneapolis Veterans Affairs Medical Center. The studies from which the data were pooled employed various cognitive and sensory stimulation paradigms. There were no substantial differences in the number of male and female subjects participating in individual studies. Also, the selection criteria for participation were similar across the studies.

Subjects

Seventy-two healthy, right-handed subjects (36 male, 36 female) were selected from a pool of 100 subjects (43 female, 57

male) who had participated in various imaging experiments as described above. Subjects were selected on the basis of handedness (all were right-handed) and the brain coverage of their scans. Male and female subjects had comparable brain coverage that included the amygdala and as much of the rest of the brain as possible. Subjects were screened for psychiatric and neurological problems, and only subjects with no history of any major neurological or medical disorders, psychiatric illness, substance abuse, or major head trauma were included All subjects gave informed consent in accordance with the Minneapolis Veterans Affairs Medical Center's Institutional Review Board.

PET scan procedures

During the baseline resting scan, subjects were instructed to relax with their eyes closed. No further instruction was given. The baseline scan was always separated from any cognitive or sensory task scan by at least 8 min. Regional cerebral blood flow (rCBF) was assayed using an ECAT 953B camera (Siemens, Knoxville, TN) with septa retracted; a slow-bolus injection of $H_2^{15}O$ (.25 mCi/kg) infused at a constant rate over 30 s, and a 90-s scan acquisition. Images were reconstructed with a three-dimensional reconstruction algorithm with a Hanning filter (.5 cycle-per-pixel) (Kinahan and Rogers, 1989). Automated software (Minoshima et al., 1994) performed normalization for global activity (scaled to a mean of 1000 counts), detection of the intercommisural plane, intra-subject scan alignment, resampling to a pixel size of 2.25 mm^3 and nonlinear warping to Talairach space (Talairach and Tournoux, 1988). Eight of the subjects (4 male, 4 female) received two or more resting scans that were averaged together before analysis.

Statistical analysis

Seed-PLS was performed in three steps using Matlab (Mathworks, Sherborn, MA). (1) The correlation between activity of amygdala seed voxel and activity values for all other brain voxels was computed across subjects within each group, producing one correlation map for the men and one for the women. (2) Singular value decomposition of the combined correlation maps was performed, resulting in pairs of mutually orthogonal latent variables (LVs), or patterns of activity across the whole brain, that were related to amygdala activity. These LVs completely reproduce the cross-correlation matrix computed in the first step. Each latent variable contains a singular image identifying brain regions whose activity covaries, as a whole, with amygdala activity. With this particular dataset, two LVs were produced. The first LV accounts for more of the cross-block correlation and is considered the "dominant" LV while the second LV is considered the "minor" LV. Each voxel in the singular image has a weight (salience) that is proportional to the covariance of its activity with amygdala activity. (3) The singular image was multiplied by the raw images (dot product) for each subject to produce individual brain scores. These brain scores indicate the extent to which an individual subject's scan reflects the pattern represented in the singular image.

Separate PLS analyses were performed for the right and left amygdala. Unlike previous investigations of amygdala functional connectivity using PLS (Keightley et al., 2003; Kilpatrick and Cahill, 2003), there was no obviously appropriate seed voxel to select. Also, consistent with published work mentioned above, no significant sex-related differences in amygdala activity were found in a standard subtraction analysis (data not shown). Therefore, seed-PLS was applied to 100 potential seeds from each amygdala region. In each analysis, the correlation between brain scores and amygdala seed activity was calculated for men and for women. The significance of the difference (women minus men) in correlation was determined using Fisher's z transformation (David, 1949). A significant difference in these correlations indicates that the singular image identifies brain regions whose functional connectivity with the amygdala varies as a function of sex. The latent variables for the right and left seed voxels displaying maximal sex-related differences in the dominant LV were then selected to be submitted to further statistical analysis. The statistical significance of each LV was assessed by a permutation test (1000 permutations).

The reliability of the voxels identified in each singular image was assessed by a bootstrap estimation of the standard error (500 iterations). Bootstrap samples were generated using sampling with replacement and re-running PLS. A voxel salience whose value remains stable regardless of the sample chosen is considered reliable (Sampson et al., 1989). The bootstrap has advantages over more traditional null significance tests in that it provides evidence of the stability of the findings, rather than simply demonstrating whether an effect exists (McIntosh and Lobaugh, 2004). Voxels with saliences greater than 3.3 times their standard error (corresponding to $P < .001$ on a two-tailed normal distribution) were considered reliable. Since the primary metric reflects stability, rather than a test of a null hypothesis, no correction for multiple comparisons is necessary for data exceeding the threshold (McIntosh and Lobaugh, 2004).

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References

Aggleton, J., 1992. The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. Wiley-Liss, New York, NY.

Aggleton, J. (Ed.), 2002. The Amygdala: A Functional Analysis. Oxford Univ. Press, Oxford.

Andreason, P., Zametkin, A., Guo, A., Baldwin, P., Cohen, R., 1994. Gender-related differences in regional cerebral glucose metabolism in normal volunteers. Psychiatry Res. 51, 175–183.

Azari, N., Rapoport, S., Grady, C.L., DeCarli, C., Haxby, J.V., Schapiro, M.B., Horwitz, B., 1992. Gender differences in correlations of cerebral glucose metabolic rates in young normal adults. Brain Res. 574, 198–208.

Baxter, L., Mazziotta, J., Phelps, M., Selin, C., Guze, B., Fairbanks, L., 1987. Cerebral glucose metabolic rates in normal human females versus normal males. Psychiatry Res. 21, 237–245.

Berkley, K.J., 1997. Sex differences in pain. Behav. Brain Sci. 20, 371–380. (discussion 435–513).

Bolla, K., Eldreth, D., Matochik, J., Cadet, J., 2004. Sex-related differences in a gambling task and its neurological correlates. Cereb. Cortex 14, 1226–1232.

Botteron, K.N., Raichle, M.E., Drevets, W.C., Heath, A.C., Todd, R.D., 2002. Volumetric reduction in left subgenual prefrontal cortex in early onset depression. Biol. Psychiatry 51, 342–344.

Buchel, C., Raedler, T., Sommer, M., Sach, M., Weiller, C., Koch, M.A., 2004. White matter asymmetry in the human brain: a diffusion tensor MRI study. Cereb. Cortex 14, 945–951.

Cahill, L., 2000. Emotional modulation of long-term memory storage in humans: adrenergic activation and the amygdala. In: Aggleton, J. (Ed.), The Amygdala: A Functional Analysis. Oxford Univ. Press, Oxford, pp. 425–444.

Cahill, L., Haier, R.J., Fallon, J., Alkire, M.T., Tang, C., Keator, D., Wu, J., McGaugh, J.L., 1996. Amygdala activity at encoding correlated with long-term, free recall of emotional information. Proc. Natl. Acad. Sci. U. S. A. 93, 8016–8021.

Cahill, L., Haier, R.J., White, N., Fallon, J., Kilpatrick, L., Lawrence, C., Potkin, S., Alkire, M.T., 2001a. Sex-related difference in amygdala activity during emotionally influenced memory storage. Neurobiol. Learn. Mem. 75, 1–9.

Cahill, L., Haier, R.J., White, N.S., Fallon, J., Kilpatrick, L., Lawrence, C., Potkin, S.G., Alkire, M.T., 2001b. Sex-related difference in amygdala activity during emotionally influenced memory storage. Neurobiol. Learn. Mem. 75, 1–9.

Cahill, L., Uncapher, M., Kilpatrick, L., Alkire, M., Turner, J., 2004. Sex-related hemispheric lateralization of amygdala function in emotionally influenced memory: an fMRI investigation. Learn. Mem. 11, 261–266.

Canli, T., Zhao, Z., Brewer, J., Gabrieli, J.D., Cahill, L., 2000. Event-related activation in the human amygdala associates with later memory for individual emotional experience. J. Neurosci. 20, RC99.

Canli, T., Desmond, J.E., Zhao, Z., Gabrieli, J.D., 2002. Sex differences in the neural basis of emotional memories. Proc. Natl. Acad. Sci. U. S. A. 99, 10789–10794.

Carmichael, S.T., Price, J.L., 1995. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J. Comp. Neurol. 363, 615–641.

Coles, J., Fryer, T., Bradley, P., Nortie, J., Smilelewski, P., Rice, K., Clark, J., Pickard, J., Menon, D., 2005. Intersubject variability and reproducibility of (15)O PET studies. J. Cereb. Blood Flow Metab. 29, 1–10. (electronic publication June).

Dart, A.M., Du, X.J., Kingwell, B.A., 2002. Gender, sex hormones and autonomic nervous control of the cardiovascular system. Cardiovasc. Res. 53, 678–687.

Davey, L.M., Kaada, B.R., Fulton, J.F., 1949. Effects on gastric secretion of frontal lobe stimulation. Res. Publ.-Assoc. Res. Nerv. Ment. Dis. 29, 617–627.

David, F., 1949. The moments of the z and F distributions. Biometrika 36, 394–403.

Denburg, N., Tarnel, D., Bechara, A., 2004. Gender Modulates Functional Asymmetry of the Ventromedial Prefrontal Cortex. Society for Neuroscience, San Diego.

Drevets, W.C., Ongur, D., Price, J.L., 1998. Reduced glucose metabolism in the subgenual prefrontal cortex in unipolar depression. Mol. Psychiatry 3, 190–191.

Freedman, L.J., Insel, T.R., Smith, Y., 2000. Subcortical projections of area 25 (subgenual cortex) of the macaque monkey. J. Comp. Neurol. 421, 172–188.

George, M., Ketter, T., Parekh, P., Herscovitch, P., Post, R., 1996. Gender differences in regional cerebral blood flow during transient self-induced sadness or happiness. Biol. Psychiatry 40, 859–871.

Goldstein, J.M., Seidman, L.J., Horton, N.J., Makris, N., Kennedy, D.N., Caviness Jr., V.S., Faraone, S.V., Tsuang, M.T., 2001. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb. Cortex 11, 490–497.

Gray, T.S., 1993. Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Ann. N. Y. Acad. Sci. 697, 53–60.

Gur, R.C., Mozley, L.H., Mozley, D., Resnick, S.M., Karp, J.S., Alavi, A., Arnold, S.E., Gur, R.E., 1995. Sex differences in regional cerebral glucose metabolism during a resting state. Science 267, 528–531.

Gutierrez, C., Cola, M.G., Seltzer, B., Cusick, C., 2000. Neurochemical and connectional organization of the dorsal pulvinar complex in monkeys. J. Comp. Neurol. 419, 61–86.

Hamann, S.B., Ely, T.D., Grafton, S.T., Kilts, C.D., 1999. Amygdala activity related to enhanced memory for pleasant and aversive stimuli. Nat. Neurosci. 2, 289–293.

Hines, M., Allen, L.S., Gorski, R.A., 1992. Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. Brain Res. 579, 321–326.

Hirayasu, Y., Shenton, M.E., Salisbury, D.F., Kwon, J.S., Wible, C.G., Fischer, I.A., Yurgelun-Todd, D., Zarate, C., Kikinis, R., Jolesz, F.A., McCarley, R.W., 1999. Subgenual cingulate cortex volume in first-episode psychosis. Am. J. Psychiatry 156, 1091–1093.

Horwitz, B., 1990. Simulating functional interactions in the brain: a model for examining correlations between regional cerebral metabolic rates. Int. J. Biomed. Comput. 26, 149–170.

Hurley-Gius, K.M., Neafsey, E.J., 1986. The medial frontal cortex and gastric motility: microstimulation results and their possible significance for the overall pattern of organization of rat frontal and parietal cortex. Brain Res. 365, 241–248.

Kalimullina, L.B., 1989. Zones of sexual dimorphism in the cortico-medial group of nuclei of the amygdaloid complex. Neurosci. Behav. Physiol. 19, 79–83.

Karnath, H.O., Himmelbach, M., Rorden, C., 2002. The subcortical anatomy of human spatial neglect: putamen, caudate nucleus and pulvinar. Brain 125, 350–360.

Kawachi, T., Ishii, K., Sakamoto, S., Matsui, M., Mori, T., Sasaki, M., 2002. Gender differences in cerebral glucose metabolism: a PET study. J. Neurosci. 199, 79–83.

Keightley, M.L., Winocur, G., Graham, S.J., Mayberg, H.S., Hevenor, S.J., Grady, C.L., 2003. An fMRI study investigating cognitive modulation of brain regions associated with emotional processing of visual stimuli. Neuropsychologia 41, 585–596.

Kilpatrick, L., Cahill, L., 2003. Amygdala modulation of parahippocampal and frontal regions during emotionally influenced memory storage. NeuroImage, 20.

Kinahan, P., Rogers, J., 1989. Analytic 3D image reconstruction using all detected events. IEEE Trans. Med. Imaging 36, 964–986.

Mathew, R., Wilson, W., Tant, S., 1986. Determinants of resting regional cerebral blood flow in normal subjects. Biol. Psychiatry 21, 907–914.

McGaugh, J.L., 2000. Memory—A century of consolidation. Science 287, 248–251.

McIntosh, A.R., 2000. Towards a network theory of cognition. Neural Netw. 13, 861–870.

McIntosh, A., Lobaugh, N., 2004. Partial least squares analysis of neuroimaging data: applications and advances. NeuroImage 23, S250–S263.

McIntosh, A.R., Bookstein, F.L., Haxby, J.V., Grady, C.L., 1996. Spatial pattern analysis of functional brain images using partial least squares. NeuroImage 3, 143–157.

McIntosh, A.R., Fitzpatrick, S.M., Friston, K.J., 2001. On the marriage of cognition and neuroscience. NeuroImage 14, 1231–1237.

Mesulam, M.M., 1999. Spatial attention and neglect: parietal, frontal and cingulate contributions to the mental representation and attentional targeting of salient extrapersonal events. Philos. Trans. R. Soc. Lond., B Biol. Sci. 354, 1325–1346.

Minoshima, S., Koeppe, R., Frey, K., Kuhl, D., 1994. Anatomic standardization: linear scaling and nonlinear warping of functional brain images. J. Nucl. Med. 35, 1528–1537.

Miura, S., Schapiro, M.B., Grady, C.L., Kumar, A., Salerno, J.A., Kozachuk, W., Wagner, E.K., Rapoport, S., Horwitz, B., 1990. Effect of gender on glucose utilization rates in healthy humans: a positron emission tomography study. J. Neurosci. Res. 27, 500–504.

Morgane, P., Panksepp, J., 1981. Handbook of the Hypothalamus. M. Dekker, New York.

Murphy, D.G., DeCarli, C., McIntosh, A.R., Daly, E., Mentis, M.J., Pietrini, P., Szczepanik, J., Schapiro, M.B., Grady, C.L., Horwitz, B., Rapoport, S.I., 1996. Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch. Gen. Psychiatry 53, 585–594.

Naliboff, B.D., Berman, S., Chang, L., Derbyshire, S.W., Suyenobu, B., Vogt, B.A., Mandelkern, M., Mayer, E.A., 2003. Sex-related differences in IBS patients: central processing of visceral stimuli. Gastroenterology 124, 1738–1747.

Nishizuka, M., Arai, Y., 1981. Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. Brain Res. 212, 31–38.

Ongur, D., An, X., Price, J.L., 1998. Prefrontal cortical projections to the hypothalamus in macaque monkeys. J. Comp. Neurol. 401, 480–505.

Ragland, J., Coleman, A., Gur, R., Glahn, D., Gur, R., 2000. Sex differences in brain–behavior relationships between verbal episodic memory and resting regional cerebral blood flow. Neuropsychologia 38, 451–461.

Romanski, L.M., Giguere, M., Bates, J.F., Goldman-Rakic, P.S., 1997. Topographic organization of medial pulvinar connections with the prefrontal cortex in the rhesus monkey. J. Comp. Neurol. 379, 313–332.

Sampson, P.D., Streissguth, A.P., Barr, H.M., Bookstein, F.L., 1989. Neurobehavioral effects of prenatal alcohol: Part II. Partial least squares analysis. Neurotoxicol. Teratol. 11, 477–491.

Stefanova, N., Ovtscharoff, W., 2000. Sexual dimorphism of the bed nucleus of the stria terminalis and the amygdala. Adv. Anat. Embryol. Cell Biol. 158 (III-X), 1–78.

Sugiura, M., Kawashima, R., Nakagawa, M., Okada, K., Sato, T., Goto, R., Sato, K., Ono, S., Schormann, T., Zilles, K., Fukuda, H., 2000. Correlation between human personality and neural activity in cerebral cortex. NeuroImage 11, 541–546.

Szeszko, P.R., Vogel, J., Ashtari, M., Malhotra, A.K., Bates, J., Kane, J.M., Bilder, R.M., Frevert, T., Lim, K., 2003. Sex differences in frontal lobe white matter microstructure: a DTI study. NeuroReport 14, 2469–2473.

Talairach, J., Tournoux, P., 1988. A Co-Planar Stereotaxic Atlas of the Human Brain: An Approach to Medical Cerebral Imaging. Thieme, New York.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A., Updegraff, J.A., 2000. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychol. Rev. 107, 411–429.

Volkow, N., Wang, G., Fowler, J., Hitzemann, R., Pappas, N., Pascani, K., Wong, C., 1997. Gender differences in cerebeller metabolism: test–retest reproducibility. Am. J. Psychiatry 54, 119–121.

Westerhausen, R., Walter, C., Kreuder, F., Wittling, R.A., Schweiger, E., Wittling, W., 2003. The influence of handedness and gender on the microstructure of the human corpus callosum: a diffusion-tensor magnetic resonance imaging study. Neurosci. Lett. 351, 99–102.

Xiong, J., Parsons, L., Gao, J., Fox, P., 1999. Interregional connectivity to primary motor cortex revealed using MRI resting state images. Hum. Brain Mapp. 8, 151–156.

Zald, D.H., Mattson, D.L., Pardo, J.V., 2002. Brain activity in ventromedial prefrontal cortex correlates with individual differences in negative affect. Proc. Natl. Acad. Sci. U. S. A. 99, 2450–2454.

Zhang, S., Bastin, M.E., Laidlaw, D.H., Sinha, S., Armitage, P.A., Deisboeck, T.S., 2004. Visualization and analysis of white matter structural asymmetry in diffusion tensor MRI data. Magn. Reson. Med. 51, 140–147.

Zubieta, J.K., Dannals, R.F., Frost, J.J., 1999. Gender and age influences on human brain mu-opioid receptor binding measured by PET. Am. J. Psychiatry 156, 842–848.