Des réductions volumétriques du gyrus cingulé dans le syndrome de délétion 22Q11.2 sont associées à un dysfonctionnement exécutif et des symptômes psychotiques

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" DES REDUCTIONS VOLUMETRIQUES DU GYRUS CINGULE DANS LE SYNDROME DE DELETION 22Q11.2 SONT ASSOCIEES A UN DYSFONCTIONNEMENT EXECUTIF ET DES SYMPTOMES PSYCHOTIQUES "

Thèse

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1. Introduction

1.1 Le syndrome de délétion 22q11.2

1.1.1 Généralités

Le syndrome de délétion 22q11.2, ou syndrome vélo-cardio-facial (VCFS), est associé à des délétions interstitielles du chromosome 22 et a une prévalence estimée approximativement à 1 naissance sur 4000 (Scambler et al., 1992). Shprintzen et al. (1978) ont définit les principales caractéristiques de ce syndrome. Les sujets présentent un langage de tonalité hypernasale (associé à une fente palatine sous-muqueuse), des malformations cardiaques (tétralogie de Fallot, défaut du septum ventriculaire, arc aortique droit), une dysmorphie faciale (rétrognatie, longue face, nez proéminent, aplatissement des pommettes), ainsi que des difficultés d'apprentissages et des perturbations comportementales comme la survenue d'une schizophrénie chez environ un tiers des individus affectés par la délétion 22q11.2 (Murphy et al., 1999). D'autres dénominations ont aussi été utilisées pour décrire ce syndrome, parmi lesquelles on retrouve le syndrome de DiGeorge, le "conotruncal anomaly face syndrome" et le "CATCH 22" (Cardiac anomalies, Abnormal facies, Thymic hypoplasia, Cleft palate and Hypocalcemia) (Hall, 1993; Demczuk and Aurias, 1995; Gong et al., 1996). Plus de 180 autres anomalies associées au syndrome ont également été rapportées à ce jour, mais il existe une grande variabilité du nombre, du type et de la sévérité de celles-ci (Shprintzen, 2000). En effet, le phénotype peut rester discret chez de nombreux individus, qui ne présenteront que peu de symptômes jusqu'à l'adolescence ou l'âge adulte, et vont ainsi échapper à une détection précoce du syndrome. Durant l'enfance, c'est souvent les retards d'apprentissages scolaires ou les troubles du comportement qui vont motiver dans certains cas des examens complémentaires qui vont aboutir au diagnostic. Le seuil de détection se situe probablement en dessous de 20% des sujets affectés (Eliez et al., 2004).

1.1.2 Phénotype cognitif et comportemental

Les déficits cognitifs sont considérés comme une des caractéristiques principales du syndrome de délétion 22q11.2. La majorité des enfants atteints présentent un score de QI plus bas que celui de la population moyenne, se situant entre un niveau intellectuel à la limite inférieur (QI: 70-85) et un retard mental léger (QI: 50-69). De plus, les recherches qui ont été conduites au sein de la population affectée suggèrent fortement la présence d'un profil cognitif spécifique au syndrome (Zinkstok and van Amelsvoort, 2005). En particulier, il existe une différence entre les scores de QI verbal et performance en faveur des compétences verbales (Moss et al., 1999). En effet, les sujets possèdent un niveau clairement inférieur dans le raisonnement abstrait, le raisonnement visuo-spatial et l'arithmétique. Chez les adultes avec la délétion 22q11.2, ces mêmes aptitudes restent diminuées et l'on retrouve par ailleurs aussi des handicaps au niveau de la cognition sociale (Henry et al., 2002). Finalement, il a aussi été démontré que les patients souffrant de la délétion 22q11.2 et également atteints de schizophrénie souffrent de déficits au niveau des fonctions exécutives et de l'attention, aptitudes impliquant normalement le lobe frontal du cerveau (van Amelsvoort et al, 2004). Cela suggère une potentielle atteinte du lobe frontal chez les sujets 22q11.2 schizophrènes. Cependant, il n'est pas encore clair si (1) un mauvais fonctionnement du lobe frontal représente un facteur de risque dans l'apparition et le développement de la schizophrénie ou si (2) c'est la maladie psychotique elle-même qui engendre une atteinte du lobe frontal.

Au niveau comportemental, les enfants qui présentent une délétion 22q11.2 sont particulièrement à risque de présenter des troubles psychopathologiques. En effet, ceux-ci manifestent souvent des problèmes d'attention, de l'impulsivité, une labilité émotionnelle, des comportements désorganisés ainsi que des difficultés socio-relationnelles marquées par de la timidité ou la désinhibition (Eliez et al., 2004). Les diagnostics les plus fréquemment retrouvés dans cette jeune population sont le trouble déficitaire de l'attention (avec ou sans hyperactivité), les troubles de l'humeur et les troubles obsessionnels-compulsifs (Papolos et

al., 1996; Feinstein et al., 2002, Gothelf et al., 2004). De plus, ces patients sont également plus à risque de développer une schizophrénie à début précoce (childhood-onset schizophrenia, COS) par rapport à une population contrôle, établissant le syndrome de délétion 22q11.2 comme une cause génétique identifiée de schizophrénie (Usiskin et al., 1999). Par ailleurs, il est intéressant de noter que 50% des sujets atteints d'une délétion 22q11.2 vont expérimenter des manifestations psychotiques telles délires et/ou hallucinations au moins une fois dans leur vie à partir de l'âge de 9 ans (Baker and Skuse 2005; Debbané et al., 2006a). Finalement, chez les adultes souffrant du syndrome de délétion 22q11.2, une étude de Murphy et al. (1999) a clairement démontré une fréquence élevée de schizophrénie ou trouble schizo-affectif en identifiant grâce à une évaluation psychiatrique structurée chez 50 individus 22q11.2: 24% de sujets avec schizophrénie, 2% de sujets avec des troubles schizo-affectifs et 2% de sujets avec psychoses NOS (not otherwise specified).

1.1.3 La délétion 22q11.2 et la schizophrénie

Récemment, le syndrome de délétion 22q11.2 a été suggéré comme un modèle homogène d'un sous-type génétique de schizophrénie (Basset and Chow, 1999). Celui-ci a ainsi bénéficié ces dernières années d'une attention particulière dans la recherche psychiatrique. Un nombres croissants d'études vise aujourd'hui à analyser et caractériser précisément le développement de la psychose chez les patients atteints de ce syndrome, afin d'élucider progressivement les mécanismes de survenue d'une schizophrénie dans cette population fortement à risque. En effet, la psychose est de plus en plus considérée comme une affection neurodéveloppementale, suivant un continuum (van Os and Tamminga, 2007), qui se manifeste d'une façon évolutive avec des symptômes sub-cliniques prodromiques (manifestations cliniques et cognitives) bien avant la déclaration claire et le diagnostic de la maladie à l'âge adulte (Brewer et al., 2006). Il a par exemple été observé que les manifestions de symptômes psychotiques précoces (dès l'âge de 9 ans, type hallucinations

auditives, chez les enfants avec une délétion 22q11.2) représente un important facteur de risque pour la survenue ultérieure d'une psychose (Poulton et al., 2000; Gothelf et al., 2007a). Ainsi, les études examinant les altérations génétiques, cliniques, cognitives et neuroanatomiques dans le syndrome de délétion 22q11.2 pourraient permettre d'identifier différentes caractéristiques susceptibles de prédisposer les jeunes atteints de la délétion 22q11.2 à développer une maladie psychotique.

1.2 La neuroimagerie dans la délétion 22q11.2

Plusieurs études de neuroimagerie structurelle (qualitative et quantitative) ont été publiées concernant le syndrome de délétion 22q11.2. En effet, les méthodes de neuroimagerie se sont spécialisées et sont devenues au cours de ces dernières années un outil de recherche de plus en plus performant. Elles sont largement utilisées afin de poursuivre et améliorer la compréhension des troubles neuro-psychiatriques associés à la délétion 22q11.2. C'est principalement des études en rapport avec la schizophrénie ou la psychose qui ont été menées dans la population 22q11.2, dans le but de progressivement identifier des anomalies cérébrales structurelles et/ou fonctionnelles spécifiques, qui représenteraient ainsi des marqueurs cérébraux à risque dans le développement de la psychose.

Une revue récente de la littérature résume différentes caractéristiques morphologiques et fonctionnelles observées à ce jour (Zinkstok and van Amelsvoort, 2005). Les principales anomalies qualitatives rapportées chez les individus souffrant de la délétion 22q11.2 sont: une atrophie corticale, une prévalence élevée de défauts de la ligne médiane (agénésie du corps calleux ou septum pellucidum caveux), une atrophie cérébelleuse et la présence d'hyper-intensités dans la matière blanche (Mitnick et al., 1994; Lynch et al., 1995; Vataja and Eomaa, 1998; Chow et al., 1999; van Amelsvoort et al., 2001).

Au niveau quantitatif, de nombreuses études se sont intéressées à la structure cérébrale générale ou à des régions plus spécifiques chez les patients atteints de la délétion 22q11.2. D'une façon générale, on a observé une diminution du tissu cérébral total, de la matière grise

et blanche, des altérations structurelles du cervelet ainsi qu'une augmentation du volume des ventricules et des sulci (Chow et al., 1999; Eliez et al., 2000; Eliez et al., 2001; Kates et al., 2001; Chow et al., 2002; van Amelsvoort et al., 2004). Ces résultats sont partiellement similaires aux anomalies structurelles qu'on retrouve dans la schizophrénie (McCarley et al., 1999; Wright et al., 2000; Shenton et al., 2001; Gur et al., 2007). Par ailleurs, une étude incluant des sujets avec la délétion 22q11.2 *et* atteints de schizophrénie comparés à un groupe contrôle sain montre une forte réduction de matière grise totale plus importante que la diminution de matière blanche (Chow et al., 2002). Au contraire, une autre recherche comparant deux populations 22q11.2 *avec* ou *sans* schizophrénie rapporte une réduction extensive de la matière blanche totale chez les schizophrènes, mais aucune différence au niveau de la matière grise totale (van Amelsvoort et al., 2004).

Concernant les lobes cérébraux, les résultats dépendent du type de population étudiée, étant donnée que l'on retrouve des différences entre les enfants et les adultes souffrant de la délétion 22q11.2. Néanmoins, dans tous les cas, des différences volumétriques du lobe frontal ont été rapportées dans les études comparant des populations 22q11.2 (enfants ou adultes) avec des individus contrôles (Eliez et al., 2000; van Amelsvoort et al., 2001; Kates et al., 2001; Chow et al., 2002). Ces résultats sont cohérents avec des recherches menées chez des patients schizophrènes présentant des déficits de matière grise et blanche dans les régions du lobe frontal (Gur et al., 2000; Shenton et al., 2001). Par ailleurs, ces régions sont impliquées dans le fonctionnement exécutif, la planification, l'attention et les émotions (Kandel et al., 2000), aptitudes la plupart du temps détériorées chez les patients schizophrènes *et* ceux atteints de la délétion 22q11.2 (Keefe et al., 1995; Lewandowski et al., 2007).

Avec ses multiples connexions au lobe frontal, le lobe temporal a lui aussi été extensivement étudié dans la population schizophrène ou à haut-risque de développer une psychose (Wright et al., 2000; Suzuki et al., 2005; Gur et al., 2007). Chez les enfants et adultes portant la délétion 22q11.2, plusieurs articles rapportent des structures temporales (lobe temporal, gyrus temporal supérieur, hippocampe et amygdale) diminuées (Eliez et al., 2001; Kates et

al., 2001; van Amelsvoort et al., 2001; Chow et al., 2002; Debbané et al., 2006b). Des résultats similaires sont fréquemment rapportés dans la population schizophrène (Wright et al., 2000; Shenton et al., 2001). Par ailleurs, la réduction de la matière grise dans les régions temporales est corrélée à des mauvaises performances cognitives dans la schizophrénie (Gur et al., 2000), ainsi qu'à la présence de symptômes psychotiques positifs type hallucinations selon une revue de littérature récente d'Allen et al. (2008).

Plusieurs autres anomalies structurelles ont aussi été identifiées dans la population 22q11.2. Parmi celles-ci, on note une diminution du cortex et de la matière blanche du lobe pariétal (Eliez et al., 2000; Chow et al., 2002; Kates et al., 2001), des altérations morphologiques du thalamus, du gyrus fusiforme et du noyau caudé (Eliez et al., 2002; Bish et al., 2004; Campbell et al., 2006; Deboer et al., 2007; Glaser et al., 2007).

Récemment, une réduction de densité de matière grise dans le gyrus cingulé a aussi été rapporté chez les individus 22q11.2 (Simon et al., 2005), parallèlement à des études mettant spécifiquement en évidence une diminution de la matière grise dans le gyrus cingulé antérieur chez des individus à haut risque de développer une maladie psychotique (Pantelis et al., 2003 ; Yamasue et al., 2004 ; Borgwardt et al., 2007 ; Baiano et al., 2007). Ces derniers résultats sont particulièrement relevants, du fait que le gyrus cingulé est impliqué dans le fonctionnement exécutif et partage de nombreuses connections avec le cortex préfrontal et l'hippocampe (Bush et al., 2000), deux régions significativement altérées dans la schizophrénie.

Finalement, quelques études de neuroimagerie fonctionnelle ont aussi été conduites. Les résultats de celles-ci se sont révélés concordants avec les résultats des études structurelles, observant des anomalies fonctionnelles lors d'épreuves cognitives impliquant des régions structurellement altérées (Eliez ez al., 2000; Eliez et al., 2001; Schmitz et al., 2003; Andersson et al., 2008). Cela démontre que des altérations cérébrales structurelles peuvent entraver les fonctions cognitives associées (Karmiloff-Smith et al.,1998 ; Bush et al., 2000), ce qui résulteraient, suite à des interactions complexes (encore en cours d'élucidation), dans

la manifestation de phénomènes psychopathologiques, tels des hallucinations (Aleman & Laroi, 2008).

1.3 Altération du gyrus cingulé : substrat potentiel d'un dysfonctionnement cognitif et de manifestations psychotiques positives dans la schizophrénie

Des déficits au niveau des fonctions exécutives et de la mémoire de travail sont présents chez la plupart des individus schizophrènes et sont considérés comme des endophénotypes ainsi que des caractéristiques principales de la schizophrénie (Mohammed et al., 1999 ; Bilder et al., 2000 ; Silver et al., 2003 ; Snitz et al., 2005). De plus, des études de neuroimagerie ont directement relié ces handicaps à des altérations structurelles et fonctionelles du gyrus cingulé dans la psychose (Szeszko et al., 2000 ; Carter et al., 2001 ; Morey et al., 2005). Parallèlement, Bush et al. (2000) ont observé que des tests neuropsychologiques évaluant les capacités d'inhibition et la mémoire de travail activent la partie caudale du gyrus cingulé antérieur. Plus précisément, une corrélation positive significative a été retrouvée entre le volume du gyrus cingulé antérieur à droite et la capacité d'exécuter une tâche « go/no-go » (Bush et al., 2000). Ainsi, une diminution du volume du gyrus cingulé pourrait sous-tendre les dysfonctionnements exécutifs rencontrés chez les patients souffrant de schizophrénie.

Par ailleurs, une hypothèse proposée par Frith et al. (1992), appuyée également par plusieurs recherches récentes rapportées dans une revue d'Allen et al. (2008), suggère que le gyrus cingulé antérieur pourrait être impliqué dans la génèse de symptômes psychotiques positifs, tels des hallucinations ou des délires. En effet, Frith et al. pensent que ces symptômes résulteraient d'un contrôle biaisé des événements mentaux internes générés par un individu (pensées, idées, intentions, réflexions), aboutissant à une mauvaise attribution de leur origine, en l'attribuant par exemple à une source externe à soi. Ces déficits du monitorage interne (« self-monitoring ») sont associés à un dysfonctionnement du gyrus cingulé antérieur, comme l'ont observé Allen et al. (2007) dans une étude récente.

Parallèlement, Suzuki et al. (2005) suggèrent que la diminution du contrôle de l'inhibition, normalement régulée par des réseaux incluant le cortex préfrontal et le gyrus cingulé antérieur (Kerns et al., 2004), pourrait également participer à la survenue de symptômes psychotiques positifs, d'autant plus que ce type de manifestations cliniques est relié à une réduction du volume du gyrus cingulé antérieur dans la schizophrénie (Choi et al., 2005 ; Wang et al., 2007). Enfin, une étude de neuroimagerie fonctionnelle a mis en évidence un large réseau d'aires corticales, incluant le gyrus cingulé, durant la survenue d'hallucinations auditives (Shergill et al., 2000).

1.4 Buts de l'étude

Comme nous l'avons mentionné, la population souffrant de la délétion 22q11.2 est considérée comme un groupe à haut risque de développer une maladie psychotique ou une schizophrénie à l'âge adulte (Murphy et al., 1999; Basset and Chow, 1999). Les sujets atteints d'une délétion 22q11.2 et les patients souffrant de schizophrénie présentent des déficits au niveau des fonctions exécutives ainsi que des anomalies neuro-anatomiques semblables (Lewandowski et al., 2007). Ces données, avec celles décrites ci-dessus (section 1.3), suggèrent que certaines altérations cérébrales, au niveau du gyrus cingulé entre autres, pourraient conduire à des dysfonctionnements cognitifs et promouvoir l'apparition de symptômes psychotiques chez les individus atteints d'une délétion 22q11.2.

Les enjeux de notre étude ont donc été d'examiner, à l'aide de la neuroimagerie structurelle, la morphologie du gyrus cingulé dans une population affectée par la délétion 22q11.2. Pour cela, nous avons utilisé une méthode d'analyse sensible et spécifique basée sur une région d'intérêt (ROI-based analysis), dont le protocole se trouve en annexe (page 43). Ensuite, nous avons étudié le rôle potentiel du gyrus cingulé en tant que marqueur cérébral d'un dysfonctionnement exécutif, en essayant de mettre en évidence une altération du gyrus cingulé associée à des déficits au niveau des fonctions exécutives chez les sujets atteints de

la délétion 22q11.2. Des outils neuropsychologiques comme le « digit span » et le « stroop interference test » ont été spécifiquement choisi pour évaluer les fonctions cognitives exécutives chez les participants, en raison de l'importante implication du gyrus cingulé antérieur (division cognitive) dans le contrôle de l'inhibition et la mémoire de travail (Bush et al., 2000). Puis, nous avons comparé le volume du gyrus cingulé entre les patients 22q11.2 présentant des symptômes psychotiques et les patients 22q11.2 *ne* présentant *pas* de symptômes psychotiques. Finalement, dans la discussion de notre étude, nous avons exposé les mécanismes complexes par lesquelles des altérations neuroanatomiques du gyrus cingulé pourraient moduler le fonctionnement exécutif et l'expression de symptômes psychotiques positifs au sein de la population 22q11.2.

2. Travail original

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Title

CINGULATE GYRAL REDUCTIONS ARE RELATED TO LOW EXECUTIVE FUNCTIONING AND PSYCHOTIC SYMPTOMS IN 22q11.2 DELETION SYNDROME

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2.1 Abstract

A similar pattern of deficits in executive function and neuroanatomical abnormalities is shared between 22q11.2 deletion syndrome (22q11DS) and schizophrenia, suggesting that common cerebral alterations may lead to cognitive dysfunction and promote the appearance of psychotic symptoms in 22q11DS individuals. Specifically, there is increasing evidence for involvement of the cingulate gyrus (CG) in executive dysfunction and the expression of positive symptoms in schizophrenia. The aim of our study is to examine CG morphology in a 22q11DS population and its potential role as a cerebral marker of executive dysfunction and the manifestation of psychotic symptoms. Using region of interest (ROI)-based analysis, we compared CG volumes from 58 children and adults affected by 22q11DS with 64 healthy age- and gender-matched controls. After covarying for total cranium grey matter and age, a bilateral reduced CG grey matter volume, driven by a decrease in anterior CG cortex, was observed among 22q11DS patients. Further post-hoc analyses suggest correlations between right CG cortical reductions, low-executive functioning and the occurrence of psychotic symptoms. The CG structural abnormalities observed in 22q11DS are consistent with previous reports in schizophrenic patients and are associated with pre-morbid cognitive impairments. The mechanisms by which these changes may modulate executive functioning and the expression of psychosis are discussed.

2.2 Introduction

Recent studies suggest that individuals at high-risk for psychosis demonstrate structural abnormalities in the cingulate gyrus (CG) (Pantelis et al., 2003), especially reduced grey matter in the anterior cingulate (Borgwardt et al., 2007; Yamasue et al., 2004). As part of the limbic system, the CG is involved in executive function and shares numerous connections with prefrontal cortex and hippocampus (Bush et al., 2000), two other regions significantly altered in schizophrenia (Gur et al., 2007; Suzuki et al., 2005). Moreover, cognitive impairments linked with both of these structures, specifically executive function and working memory, are considered as putative endophenotypes and core features for schizophrenia (Mohammed et al., 1999; Bilder et al., 2000; Silver et al., 2003; Snitz et al., 2005). Increasing interest is given to identifying such potential endophenotypes, which represent important markers for the complex relationships between genes, brain and related cognitive functions.

It is now established that almost a third of individuals affected by 22q11.2 deletion syndrome (22q11DS), a neurogenetic autosomal dominant condition occurring in approximately 1 in 4000 live births (Oskarsdottir et al., 2004), eventually develop schizophrenia (Murphy et al., 1999). Moreover, neuropsychological deficits associated with schizophrenia are already apparent in youngsters with 22q11DS. These deficits include impairments in executive function, sustained attention and verbal skills (Gothelf et al., 2005; Lewandowski et al., 2007). Studies on schizotypal manifestations in 22q11DS show that half of the adolescents with the syndrome experience transient positive psychotic symptoms, such as hallucinations and delusions (Baker and Skuse, 2005; Debbané et al., 2006a). Auditory hallucinations represent the earliest symptomatic manifestation of psychosis in children with 22q11DS, which can be observed as early as the age of 9 (Debbané et al., 2006a), and represent a powerful predictor for subsequent development of psychosis (Poulton et al., 2000; Gothelf et al., 2007a). These observations lend support for the view of psychosis as a continuum (van Os and Tamminga, 2007), according to which cognitive and clinical manifestations of

schizophrenia can be observed, at reduced levels of expression, in individuals prone to psychosis (Brewer et al., 2006).

As previously mentioned, subjects at high-risk for psychosis display brain morphological changes in addition to cognitive changes compared to healthy individuals. Neuroimaging studies of 22q11DS describe how cerebral alterations in the syndrome relate to schizophrenia (Chow et al., 2002; Zinkstok et al., 2005). Individuals with 22q11DS display general structural brain abnormalities, including reduced total brain tissue, grey and white matter volumes (Eliez et al., 2000; Kates et al., 2001), increased ventricular and basal ganglia volumes (Campbell et al., 2006; Eliez et al., 2002), decreased thalamic, hippocampal as well amygdala volumes (Bish et al., 2004; Debbané et al., 2006b; Deboer et al., 2007), and a reduction in cingulate grey matter density (Simon et al., 2005). In schizophrenic 22q11DS subjects compared to non-schizophrenic, further anatomical differences include decreased whole-brain total volume and total white matter and increased total and sulcal cerebrospinal fluid volume (van Amelsvoort et al., 2004). These results provide evidence for a specific pattern of schizophrenic-like cerebral alterations in 22q11DS. Additionally, the executive function deficits in 22q11DS (Lewandowski et al., 2007) have been closely related to structural abnormalities in the anterior CG in schizophrenia (Szeszko et al., 2000; Carter et al., 2001; Morey et al., 2005).

Research has demonstrated that structural cerebral alterations may disrupt related cognitive function (Karmiloff-Smith et al., 1998; Bush et al., 2000), potentially sustaining resulting psychopathological manifestations such as hallucinations (Aleman & Larøi, 2008). Frith et al. (1992) suggests that the anterior CG is key to positive symptom activity, and recent research supports this claim (Allen et al., 2008). In the verbal self-monitoring hypothesis proposed by Frith et al. (1992), positive symptoms involve misattributing the origin of self-generated mental events (thoughts, intentions, internal speech) to a source other than the self. These self-monitoring deficits, shown to involve the anterior CG (Allen et al., 2007), can promote the

expression of hallucinations (Aleman & Larøi, 2008). Accordingly, both structural and functional alterations in the anterior CG are present among psychotic patients with positive symptoms (Shergill et al., 2005; Choi et al., 2005; Wang et al., 2007). Therefore, given that 22q11DS patients are particularly prone to experience positive symptoms like hallucinations from a young age (Baker and Skuse, 2005; Debbané et al., 2006a), a careful analysis of CG structure and associated clinical symptoms seems worthwhile.

The aim of this study is to examine CG structure and its potential relationships with executive dysfunction and positive psychotic symptomatology in a sample of individuals with 22q11DS. To accurately measure CG morphology, we employed a ROI-based analysis method for its high sensitivity and specificity, rather than voxel-based morphometry (VBM)-analysis, which can sometimes produce artifactual results (Eckert et al., 2006). We conducted this research on a large sample of affected children, adults and healthy controls. As suggested by previous VBM results (Simon et al., 2005), we expected CG volumes to be reduced in 22q11DS subjects. Following previous reports on executive dysfunction and CG alterations in schizophrenic patients (Szeszko et al., 2000; Carter et al., 2001; Morey et al., 2005), we explored whether altered CG morphology is associated with the deficits in executive function frequently observed in 22q11DS (Lewandowski et al., 2007). Finally, given evidence for an implication of CG integrity in the expression of positive psychotic symptoms, we expected to find structural differences in CG volume between psychotic and non-psychotic 22q11DS individuals.

2.3 Materials and methods

2.3.1 Subjects

22q11DS group

Fifty-eight patients with 22q11DS aged 6-37 (mean=15.52±8.75) participated in the study. Detailed demographic characteristics are presented in **Table 1**. The sample had a mean full-scale IQ score of 69.03±11.79 as measured by the Wechsler Intelligence Scales for Children or Adults (WISC-III and WAIS-III). The 22q11.2 deletion was confirmed in all patients using PCR direct sequencing. Written informed consent was received from all participating subjects, as well as the parents of subjects younger than 18 years of age, in accordance with protocols approved by the Institutional Review Board of Geneva University School of Medicine. At time of participation, a total of ten patients were taking psychotropic medication, five of which had a diagnosis of schizophrenia.

The presence of positive psychotic symptoms was determined through semi-structured interviews with participants affected by 22q11DS and their parents. The parents of participants younger than 18 years responded to a computerized DICA-P (Reich, 2000), administered by a child and adolescent psychiatrist (S.E.). DICA-P software generated DSM-IV diagnoses as well as a listing by diagnostic criteria of all symptoms reported as present or absent. The DICA-P was supplemented with the K-SADS-PL (Kaufman et al., 1997) for evidence of psychosis and mood cycling. Participants older than 18 years were interviewed separately from their parents by the same psychiatrist (SE) using the SCID-I to generate DSM-IV diagnoses and criteria (First et al., 1993). This procedure was supplemented with the SADS-PL. The “degree of psychosis” scale (**Table 1**) represents a description of patients’ psychotic symptoms and the severity. This scale has been used in a previous publication (Debbané et al., 2007 in press).

22q11DS subgroups

Psychotic (n=24, 11 males and 13 females) and non-psychotic (n=18, 7 males and 11 females) subgroups were created from the 22q11DS group for post-hoc analyses. This division corresponds to a degree of psychosis >0 (psychotic) or =0 (non-psychotic). Only patients older than age 9 were used (n=42), given the age at which psychotic symptoms become relevant in the clinical picture of children with 22q11DS (Debbané et al., 2006a).

These patients also were divided into high- executive functioning (n=20, 12 males and 8 females) and low- executive functioning (n=20, 5 male and 15 female) subgroups. A composite score (WISC III-Digit span subtest + Stroop interference score) was used to assess level of executive function. Only for the executive function analyses, two of the 42 subjects were excluded due to an absence of data. **Table 3** shows detailed group characteristics and Section 2.3 describes the executive function composite score.

Control group

The comparison group consisted of 64 healthy individuals aged 6-39 (mean=15.02±8.09) with a mean IQ of 111.89±13.02. An absence of past or present neurological and psychiatric disorders was established during a medical intake interview and by using scores from standardized screening forms (Medical and Developmental History Form, the CBCL for individuals younger than 18, and the SCL-90 for those older than 18).

2.3.2 Brain Imaging

MRI was performed on a Philips Intera 1.5T scanner; 124 contiguous coronal slices with a thickness of 1.5mm and in-plane resolution of 0.94mm x 0.94mm (TR=35ms, TE=6ms) were acquired. Image optimization was performed in BrainImage 5.2 following standard

procedures whose details have been published elsewhere (Reiss et al., 1998; Schaer et al., 2006).

Manual circumscription of the cingulate gyrus ROI was performed based on a previously published protocol (Woodward et al., 2006) developed by the principal investigator (S.E.). Briefly, we first traced left and right CG on sagittal slices 5 millimeters lateral to the midline. Sagittal landmarks were used to draw CG boundaries on coronal slices. The CG was delimited medially by the interhemispheric cortical surface, and laterally by a line between the deepest extension of the CG sulcus and the deepest extension of CG grey matter adjacent to the corpus callosum (CC), and by the CG sulcus superiorly and the CC or the calcarine fissure inferiorly. A dynamic Talaraich grid (Talaraich and Tournoux, 1988) was then used to define four sub-regions of the CG: ventral anterior (VA; corresponding to A/B/C boxes of Talairach), dorsal anterior (DA; D/E1 boxes), cingulate body (CinB; E2/E3 boxes) and splenium cingulate (SCin; F/G boxes). (**Figure 1**)

For all procedures, two independent raters, blind to the participants' diagnoses (FD, MS), traced the CG volumes of 10 randomly chosen subjects. Intra-class correlation coefficients for total left and right cingulate tissue volumes were 0.94, indicating good inter-rater reliability for measurements.

2.3.3 Statistics

An alpha of 0.05 (two-tailed) was used as the threshold for statistical significance. Specific covariates including total grey or white matter volumes, age, IQ or psychosis degree were used when necessary to exclude any non-significant freestanding results.

Volumetric comparisons between 22q11DS and control groups

First, ANOVA were used to compare total brain tissue, grey and white matter volumes between groups. Second, we used MANCOVA, with total cranium grey or white matter

volumes respectively as covariates, to compare CG grey and white matter volumes bilaterally, and then to compare grey matter volumes from the four CG sub-regions for both hemispheres. Significant regional differences were then retested adding age as a covariate.

Post-hoc volumetric analyses within 22q11DS and control subgroups

For the aforementioned reasons, only patients 9 years of age and older were included in post-hoc analyses.

First, we defined high- and low-executive functioning subgroups by averaging the z-scores converted from standard scores obtained from the Digit Span and Stroop interference tasks. These tests were specifically chosen given that the anterior CG cognitive division is important for executive control (Bush et al., 2000). We then divided our sample of 22q11DS subjects by high (z-score >0) and low (z-score <0) executive-functioning individuals. An ANCOVA using age, IQ and degree of psychosis as covariates, and executive function as a group factor was then performed to compare high and low executive functioning subgroups on left/right CG grey matter volumes.

Second, we employed ANCOVA, with age as a covariate and presence of psychotic symptoms as a group factor to test the effect of psychosis on left and right CG grey matter volumes.

Finally, we repeated the same procedure for posterior CG regions (splenium cingulate sub-region), which were not expected to be related to executive function or psychotic symptoms.

We subsequently tested for any significant relationships between executive functioning and CG grey matter volumes within control subjects older than 9 (48 individuals split in 27 high- and 21 low-executive functioning control subjects).

Relationship between executive function and psychotic symptoms in 22q11DS

ANOVA with psychotic symptoms as group factor and the executive function composite mean z-score as the dependent variable was used to test a potential relationship between level of executive-functioning and presence of psychotic symptoms.

2.4 Results

Volumetric comparisons between 22q11DS subjects and healthy controls

Subjects with 22q11DS showed a significant reduction in total brain tissue and grey and white matter volumes compared to the control group (total brain tissue: p=0.001; total cranium grey matter: p=0.000; total cranium white matter: p=0.001) (**Table 2**). MANCOVA indicated smaller bilateral CG grey matter volumes in 22q11DS compared to controls (Wilks Lambda: p=0.002, Left: p=0.025; Right: p=0.003). Further delineation of the CG sub-regions (**Figure 1**) revealed that dorsal anterior (DA) and cingulate body (CinB) grey matter volumes were also reduced bilaterally (Wilks Lambda: p=0.013; p=0.004 for left DA; p=0.002 for right DA; p=0.027 for left CinB; p=0.004 for right CinB). Neither left nor right CG white matter volumes significantly differed between groups. Adding age as a covariate, we observed the same pattern of reduced CG volumes across 22q11DS subjects.

Post-hoc volumetric analyses within 22q11DS and control subgroups

A significant reduction in right CG grey matter volume was observed in the low-executive functioning 22q11DS subgroup (p=0.02) compared to the high-executive functioning 22q11DS subgroup. When comparing CG grey matter volumes and executive functioning within control subgroups, ANCOVA didn't show any significant differences between groups (p=0.142).

Further, a reduction in right CG grey matter in the 22q11DS psychotic group compared to the 22q11DS non-psychotic group, as well as a trend for reductions in the right DA (p=0.074) and CinB (p=0.054) anterior sub-regions were observed (**Table 3**).

To test whether these results were related to deficits in executive function and psychotic symptoms observed in 22q11DS, we performed the same analyses with the posterior

segment of the CG (splenium cingulate sub-region), and did not observe a significant relationship with executive function or psychotic symptoms.

Relationship between executive function and psychotic symptoms in 22q11DS

ANOVA with psychotic symptoms as a group factor and the executive function mean z-score as a dependent variable indicated a trend ($p=0.064$) toward a relationship between low executive functioning and the presence of psychotic symptoms. Indeed, the general distribution of psychotic symptoms among the low- and high-executive functioning 22q11DS individuals shows that 70% of the low-executive functioning subjects demonstrated psychotic symptoms versus 40% in the high-functioning subgroup (**Table 3**).

2.5 Discussion

To our knowledge, this is the first investigation of the cingulate gyrus structure using ROI-based analyses in 22q11DS individuals. The results demonstrate bilateral reductions in CG cortical volume compared to normal controls, driven by a decrease in anterior CG grey matter volumes, which remain significant after covarying for age and total grey matter volume. Further, post-hoc analyses illustrated a reduction in the right CG grey matter volume in low-executive functioning patients, associating right CG alterations in 22q1DS with executive function deficits. We also observed an overall right CG grey matter reduction in the participants with 22q11DS reporting psychotic symptoms, and post-hoc analyses revealed a trend toward right anterior CG grey matter reduction in relation to the presence of psychotic symptoms. Decreased statistical power in our post-hoc analyses may have prevented the identification of specific CG sub-regional alterations linked to psychosis in 22q11DS. Finally, we observed a trend toward a correlation between low-executive functioning and the presence of psychotic symptoms.

Reductions in anterior cingulate grey matter volumes confirm CG alterations in 22q11DS compared to healthy controls, which were first reported by Simon and colleagues (2005) using voxel-based morphometry analyses. These findings are also compatible with anterior CG structural abnormalities found in individuals at high-risk for psychosis (Borgwardt et al., 2007), as well as in schizophrenia (Baiano et al., 2007). Using support from the literature on psychosis, in this discussion we will focus on the following points: (1) the implication of a relationship between executive function deficits and anterior CG changes; (2) the potential involvement of the anterior CG in the expression of psychotic symptoms in 22q11DS; (3) suggestions for future explorations of brain structure and cognitive functions leading to positive symptom expression.

Cognitive studies have shown that executive function and working memory deficits related to the anterior CG (Szeszko et al., 2000; Carter et al., 2001; Morey et al., 2005) are present in most schizophrenic individuals (Mohammed et al., 1999; Bilder et al., 2000; Silver et al., 2003; Snitz et al., 2005). Neuroimaging studies have directly linked these deficits to the CG. Indeed, executive and working memory tasks normally activate the caudal part of the anterior CG (Bush et al., 2000), and a significant positive correlation between the volume of the right anterior CG and the ability to perform a go/no-go task has been previously reported (Bush et al., 2000). Thus, the relationship between right CG cortical reductions and low-executive functioning in 22q11DS patients may represent an endophenotypic marker signaling neurocognitive deficits associated with schizophrenia. A recent study of children and adolescents with 22q11DS reporting “schizophrenic-like” executive functioning deficits (Lewandowski et al., 2007) further supports this idea.

Pronounced CG structural alterations in individuals with psychosis and 22q11DS may provoke functional disruptions in a cerebral network responsible for the development of positive symptoms such hallucinations. The existing literature on high-risk and schizophrenic samples implicates the CG in the pathology of psychosis (Pantelis et al., 2003; Yamasue et al., 2004; Borgwardt et al., 2007). Suzuki and colleagues (2005) suggest that loss of inhibitory control, typically regulated in networks involving the prefrontal cortex and the anterior CG (Kerns et al., 2004), may be significant to the development of such symptoms, related to an anterior CG grey matter volume reduction in schizophrenia (Choi et al., 2005; Wang et al., 2007). Concordantly, Allen and colleagues (2008) review several reports illustrating anterior CG activity deficits during hallucinatory experiences. For example, the authors suggest that abnormal anterior CG and temporal cortex activation leads, in patients with auditory verbal hallucinations, to the misattribution of inner speech to an external source (Allen et al., 2007). Moreover, abnormal connections between the temporal and anterior CG cortex also contribute to verbal self-monitoring deficits, further sustaining auditory verbal hallucinations (Johns and McGuire, 1999; Mechelli et al., 2007). Finally, consistent with our

structural findings of an altered right CG volume in 22q11DS patients with psychotic symptoms, Shergill et al. (2000) report the involvement of a large network of cortical areas prominently in the right hemisphere, including CG, in auditory hallucinations.

Although our data point to an association between right CG alteration, executive function and psychotic expression, it is difficult at this point to differentiate between the cause and effect of their putative contributions. Considering that CG grey matter reduction is a common finding among 22q11DS people compared to healthy individuals, two developmental hypotheses may be likely: (1) CG cortical alterations may disturb executive functioning in 22q11DS patients, thereby increasing the risk for psychotic symptom expression, or (2) CG cortical alterations may directly support hallucination-proneness thereby affecting executive function deficits in 22q11DS subjects. To date, longitudinal studies in 22q11DS find that cerebral alterations, most notably in dorso-lateral prefrontal cortex, are related to cognitive alterations (verbal IQ decline) that accompany the rise of psychotic symptom expression (Gothelf et al, 2005; Debbané et al, 2006a). However, a direct relationship between developmental brain abnormalities and psychosis expression in 22q11DS has yet to be found (Gothelf et al., 2007b).

One limitation of our study is that we cannot exclude an effect of IQ concerning the structural results between 22q11DS subjects and the control group. Indeed, the groups show significant differences in IQ, which are likely correlated with brain grey matter volume (Reiss et al., 1996). Low IQ is probably a general result of the many specific developmental factors interacting in 22q11DS, like the ones specifically tested in this study. Covarying for IQ is an ongoing debate in research on neurodevelopmental syndromes because it often means covarying out the very effects in question. However, within-group analyses clearly show the association between right CG alteration and psychotic symptoms in 22q11DS, independent of age or IQ. Another limitation is our inability to test for any effects of medication between the 10 individuals with 22q11DS following pharmacological treatment and healthy subjects

because of the large variety of psychotropic drugs (neuroleptic, anti-epileptic, benzodiazepine, methylphenidate) prescribed to the participants. Finally, the assessment of psychotic symptoms will necessitate finer evaluation to better understand their developmental process in 22q11DS. Future studies employing dimensional measures characterizing frequency and intensity of hallucinations and delusions, and the distress and perturbation caused by these symptoms, may help to clarify the complex interactions between brain morphology, cognitive profile and the unfolding of positive symptoms psychosis.

2.6 Tables and figure

Table 1: Demographic and medical data

	22q11DS			*Controls*			ANOVA	
	n	*Mean*	*SD*	*n*	*Mean*	*SD*	F	p
Age	58	15.521	± 8.751	64	15.024	± 8.099	0.106	0.745
IQ	58	69.034	± 11.799	64	111.89	± 13.023	360.102	0.001
Gender 1/2		1.569	± 0.499		1.609	± 0.491	0.202	0.654
male = 1	25			25				
female = 2	33			39				
Degree of psychosis[a]	58	1.21	± 1.67	NA	NA			
Psychotropic medication	10			0				
Schizophrenia	5			0				

Note:

[a]Degree of psychosis: 0 = no symptoms lifetime; 1 = hallucination or delusion (< 3 lifetime); 2 = hallucination or delusion (>3 lifetime); 3 = hallucination or delusion (monthly basis); 4 = hallucination or delusion (weekly basis); 5 = DSM-IV schizophrenia diagnosis

Table 2: Volumetric comparisons between 22q11DS subjects and healthy controls

	22q11DS (n=58)		*Controls (n=64)*		ANCOVA	
	Mean	*SD*	*Mean*	*SD*	F	p
Total Brain Tissue[a]	1124.523	±138.71	1244.722	±102.87	29.915	0.001
Total cranium grey matter[a]	674.682	±85.813	743.839	±74.432	22.714	0.001
Total cranium white matter[a]	449.841	±90.035	500.883	±70.949	12.208	0.001
Cingulate gyrus						
Total left grey matter	11.238	±1.721	12.597	±1.847	5.18	0.025
Total right grey matter	12.167	±2.36	14.274	±2.16	9.31	0.003
Total left white matter	6.94	±1.352	7.288	±1.214	0.004	0.951
Total right white matter	6.677	±1.534	7.247	±1.129	0.864	0.355
Ventral anterior grey matter						
Left	2.484	±0.889	2.862	±1.29	2.059	0.154
Right	3.296	±1.181	3.736	±1.232	1.925	0.168
Dorsal anterior grey matter						
Left	1.904	±0.349	2.314	±0.619	8.46	0.004
Right	2.272	±0.536	2.784	±0.623	10.52	0.002
Cingulate body grey matter						
Left	1.692	±0.321	1.957	±0.346	4.995	0.027
Right	1.739	±0.374	2.091	±0.378	8.721	0.004
Splenium cingulate grey matter						
Left	5.156	±0.942	5.461	±0.945	0.003	0.957
Right	4.859	±1.025	5.661	±1.237	2.816	0.096

Note:

Raw measurements of CG volumes are included in the table. Follow-up ANCOVA show significant differences between groups after covarying for total cranium grey or white matter volume. All volumes are expressed in cm^3.

[a] ANOVA was used to statistically compare volumes.

Table 3 : post-hoc analyses among 22q11DS subjects aged above 9

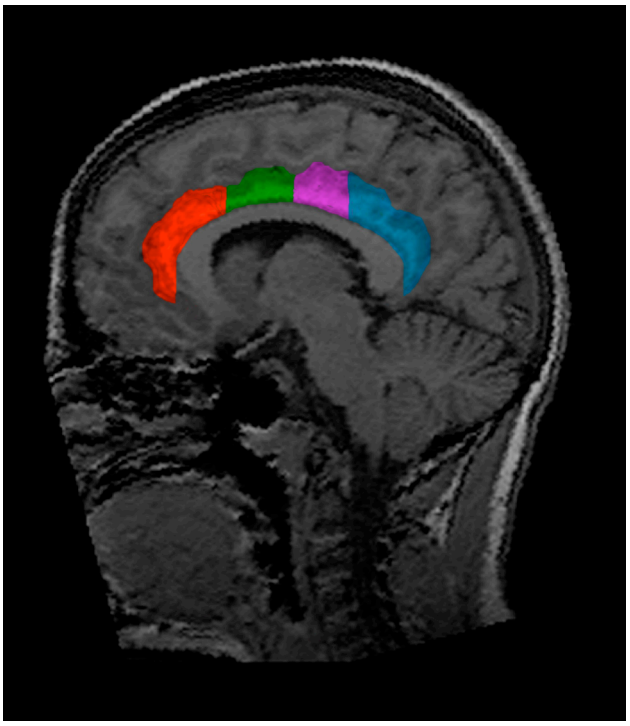
Psychosis versus non-psychosis volumetric comparisons (n = 42)						
	psychotic 22q11DS subgroup (n=24)		*non-psychotic 22q11DS subgroup (n=18)*		ANCOVA	
	Mean	*SD*	*Mean*	*SD*	F	p
Age[a]	20.482	±7.842	16.255	±8.561	2.763	0.104
IQ	64.208	±10.668	72.111	±10.867	5.555	0.066
Cingulate gyrus						
Total left grey matter	10.919	±1.729	11.661	±2.083	0.733	0.397
Total right grey matter	11.438	±2.53	13.485	±2.22	4.479	0.041
Dorsal anterior grey matter						
Left	1.852	±0.325	1.908	±0.345	0.13	0.72
Right	2.111	±0.552	2.519	±0.541	3.371	0.074
Cingulate body grey matter						
Left	1.58	±0.282	1.748	±0.348	1.417	0.241
Right	1.602	±0.292	1.919	±0.483	3.937	0.054
High versus low executive functioning volumetric comparisons (n = 40)						
	high 22q11DS subgroup (n=20)		*low 22q11DS subgroup (n=20)*			
	Mean	*SD*	*Mean*	*SD*	F	p
Age[a]	15.817	±6.91	20.062	±8.229	3.12	0.085
IQ[a]	72.2	±9.299	64.85	±11.065	5.171	0.029
Cingulate gyrus						
Total left grey matter	11.75	±2.008	10.843	±1.735	1.293	0.324
Total right grey matter	13.615	±1.451	11.342	±2.859	6.142	0.020
Dorsal anterior grey matter						
Left	1.915	±0.331	1.838	±0.291	0.279	0.730
Right	2.506	±0.419	2.136	±0.631	2.45	0.105
Cingulate body grey matter						
Left	1.756	±0.314	1.556	±0.255	2.262	0.339
Right	1.918	±0.402	1.593	±0.362	3.576	0.117
Executive functioning-psychotic symptoms relationship						
Psychotic subjects	n = 8		n = 14			
Non-psychotic subjects	n = 12		n = 6			
	Psychotic subjects (n = 22)		*Non-psychotic subjects (n = 18)*			
Executive function composite mean z-score[a]	-0.217	±0.637	0.213	±0.789	3.641	0.064

Note:

Raw measurements of CG volumes are included in the table. Follow-up ANCOVA show significant differences between groups. All volumes are expressed in cm^3.

[a] ANOVA was used to statistically compare groups.

Figure 1:



Note:

Sub-regions of the cingulate gyrus are shown: ventral anterior (red), dorsal anterior (green), cingulate body (pink), splenium cingulate (blue). The ROI excluded sub-genual cingulate gyrus.

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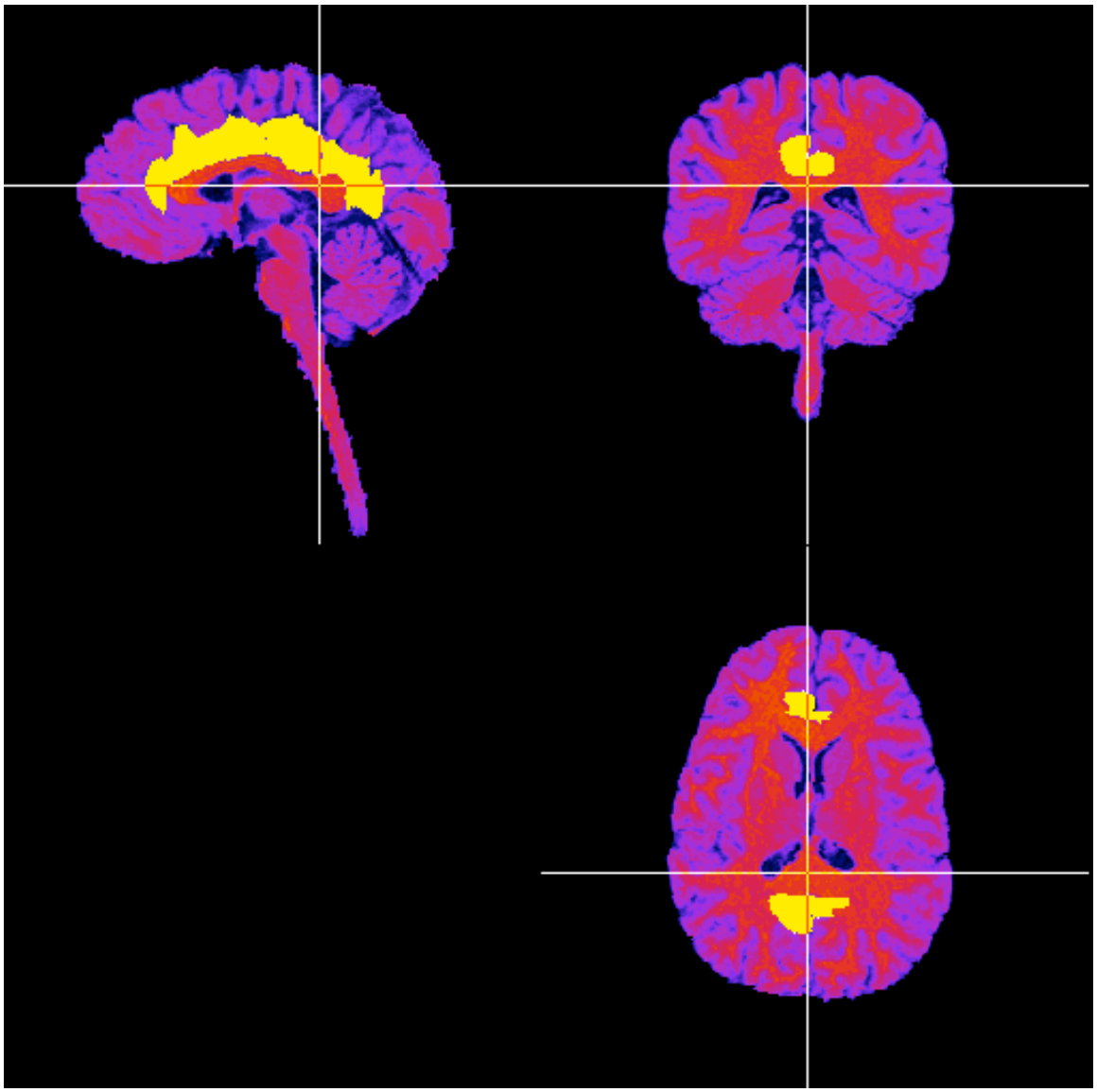
4. Annexe

Delineating Cingulate Gyrus

Last Modified January 16, 2004



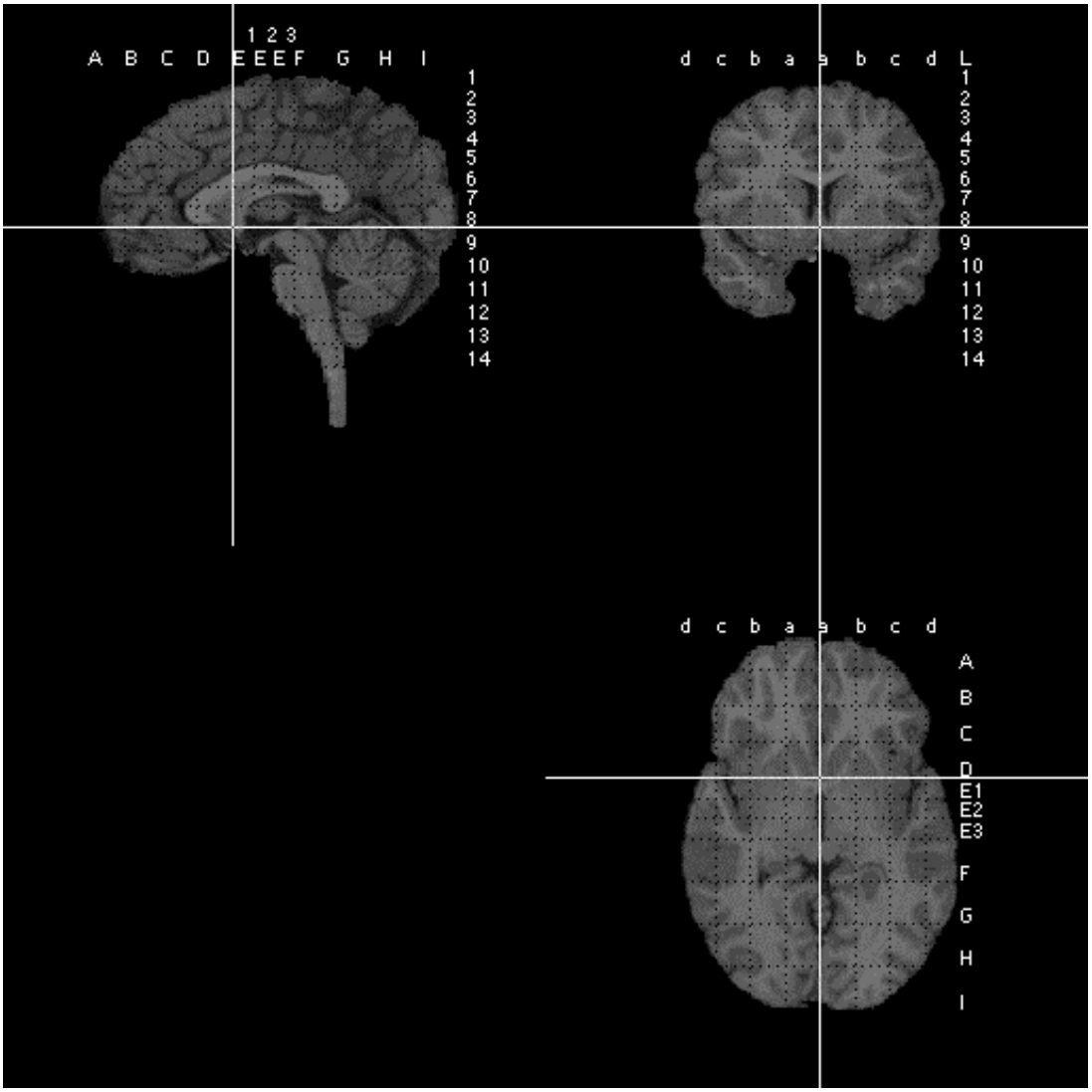
Use When Defininig:

- Cingulate Gyrus & subdivisions

1) Load Macros “CINGULATE MACROS v 1.x” in the latest version of BrainImage.

2) Open resliced stack as a volume and load Talairach.

Figure 1: Talairach



3) Make stack from volume. **Stacks → Make Stack From Volume**.
4) Save stack as subject #_ACPCSTACK.initials

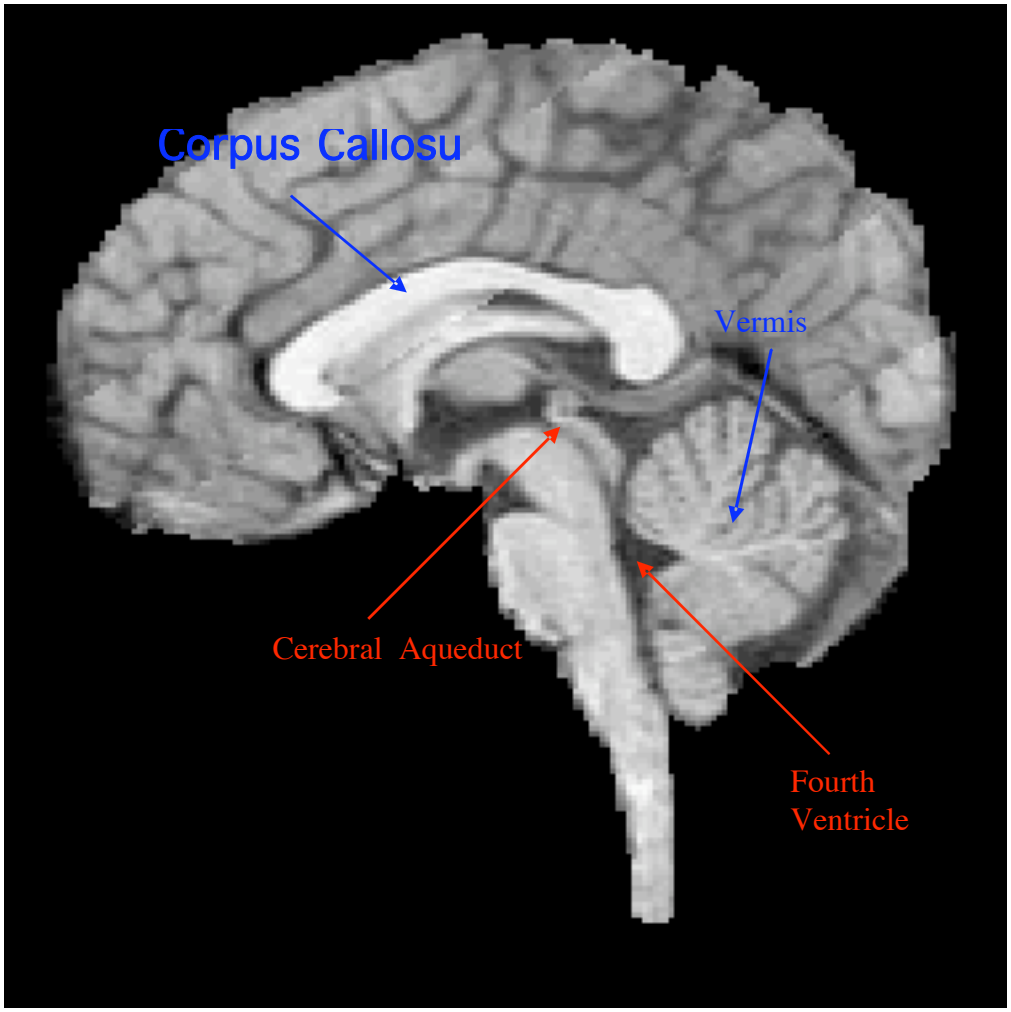
1) Create Sagittal ACPC Stack

1) Open ACPC stack into volume.
2) Under Volume Controls, slide the Bottom Right Panel Rotation **–90** degrees.
3) Make stack from volume.
4) Save as subj#_ACPC.SAG.initials

2) Outline Cingulate on Sagittal ACPC Stack

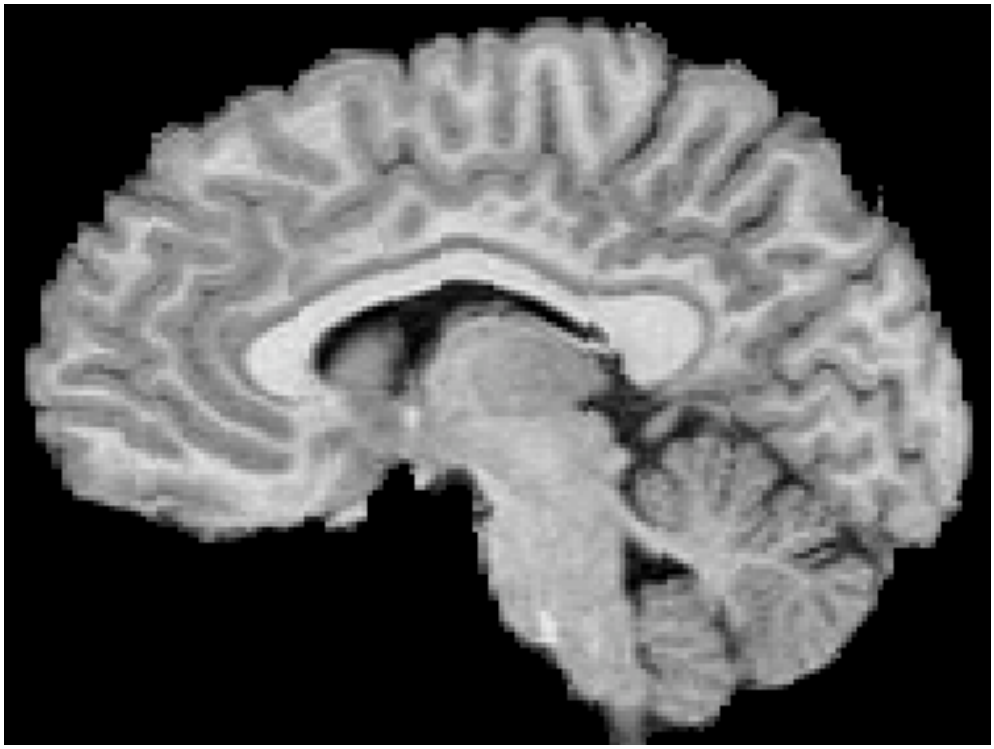
1) Open ACPC.SAG stack.
2) Find the best midsagittal slice. A clearly delineated cerebral aqueduct, corpus callosum, fourth ventricle, and cerebellar vermis mark this slice. Record this slice number.

Figure 2: Example of Perfect Midsagittal Slice



3) Move 5 slices laterally (up in number) from midsagittal slice. See Figure 3.
4) On this slice, outline the superior border of the cingulate. Find the first sulcus that is anterior to the corpus callosum. See Figure 4.
5) Check on either side of the **midsagittal slice** (where cingulate is more visible) to make sure the chosen sulcus is the first one to be anterior to corpus callosum. For an example, see Appendix, Figures 11a and 11b.
6) Follow this sulcus down to its most inferior point bordered by white matter. Now follow this sulcus using white pencil.
7) If the sulcus is not visible, then follow the **middle** of the gray matter border as long as it is continuous.
8) Check on either side of the midsagittal slice to see if outline follows cingulate. See Figures 11b and 11c in Appendix for an example.

Figure 3: Sagittal Slice Located Five Slices from Midsagittal Slice



Defining cingulate Superior Border anterior to Corpus Callosum

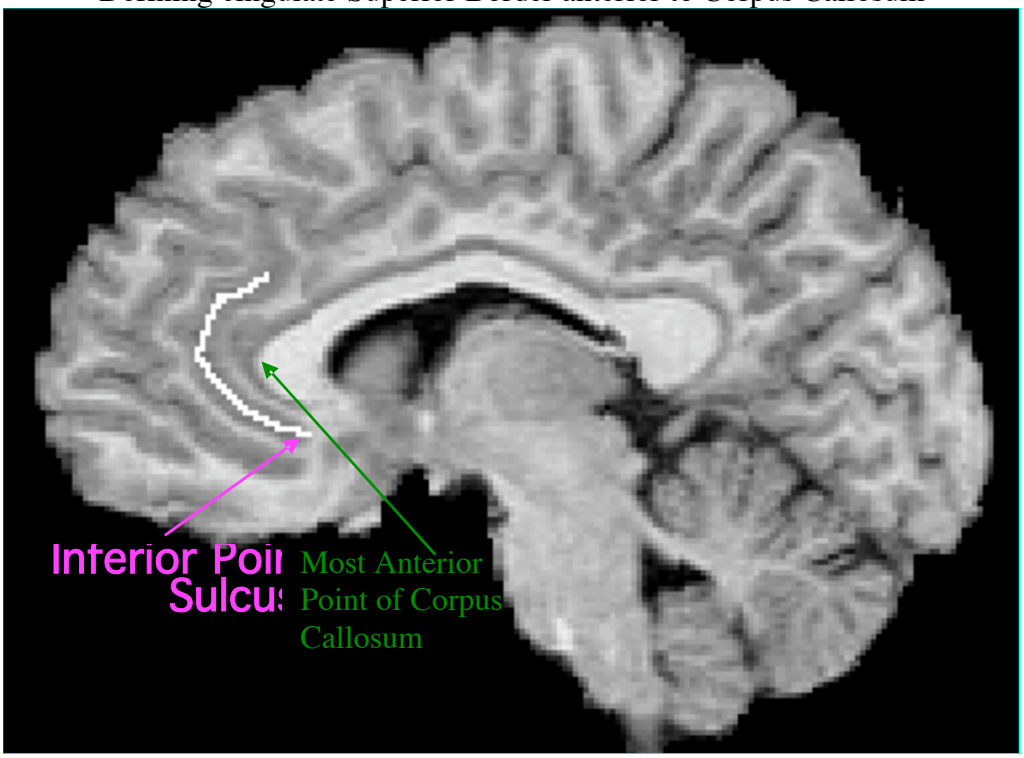
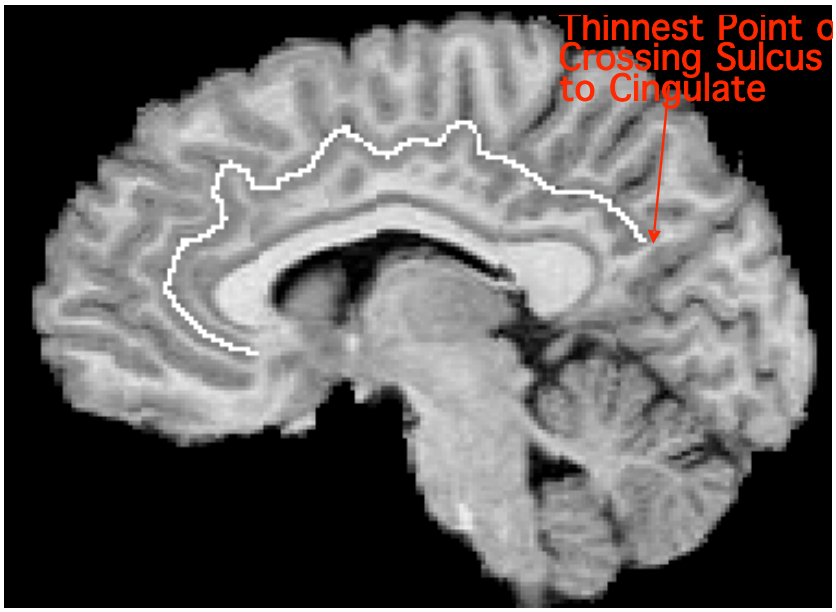


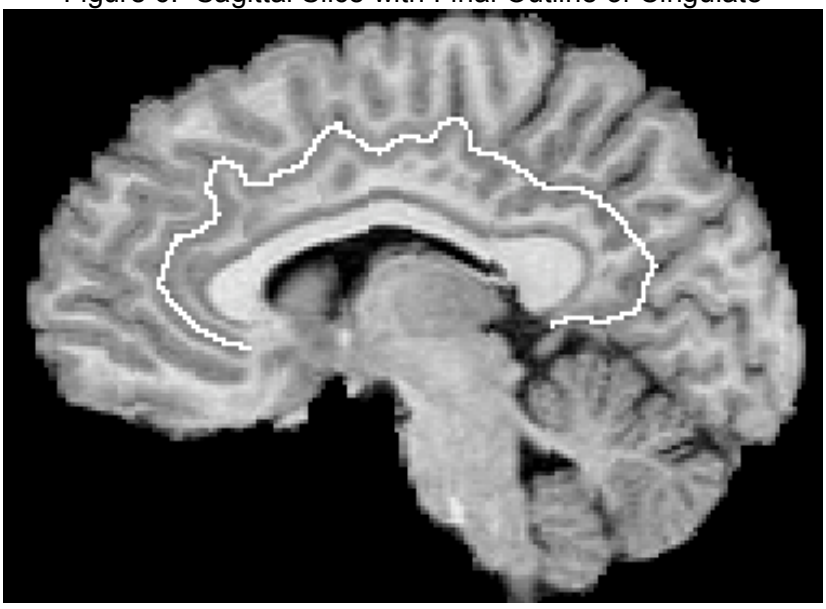
Figure 5: Sagittal Slice with White Branch Crossing Sulcus Superior to Cingulate



6. Branching through the sulcus border usually occurs in the posterior regions of the brain. If the cingulate branches superiorally, cut through these branches by following these rules:

1) Make a cut through the portion of the (white matter) branch that is thinnest. Exclude as much white matter as possible from outline of cingulate.
2) If the branch appears to be equally thin in more than one point, then cut through the branch that is most superior.
3) Check on either side of the midsagittal slice to ensure that outline follows cingulate.

Figure 6: Sagittal Slice with Final Outline of Cingulate



7. In the posterior region, follow the sulcus or gray border that ends closest to the splenium (posterior bulb) of the corpus callosum.

1) If branching occurs, follow the same rules as above.
1) If sulcus does go far enough to reach the occipital lobe, then, before reaching the occipital lobe, go straight down until reaching a sulcus that will reach the splenium.

8. Save this stack as Subj#_ACPC.SAG.CIN

9. Repeat this drawing on the other hemisphere (-5 slices from the midsagittal slice). Resave stack

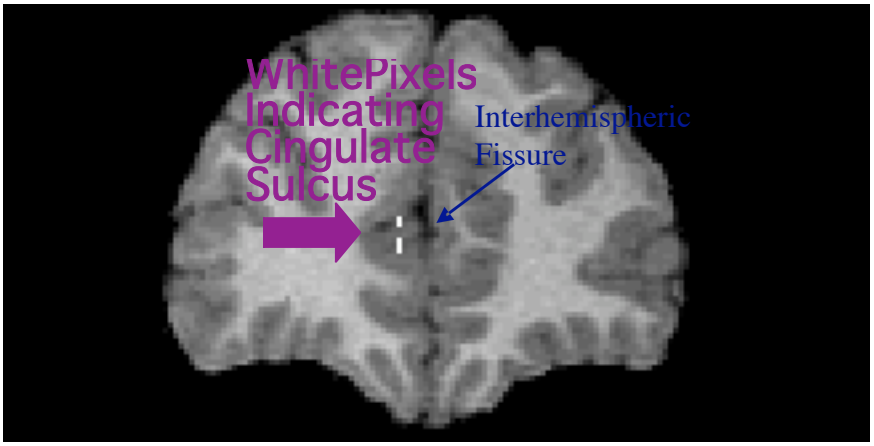
1) Rotate Sagittal Border into ACPC, Coronal View

1) Open ACPC.SAG.CIN as a volume.
2) Under Volume Controls, slide the Bottom Right Panel Rotation **+90** degrees.
3) Make stack from volume.
4) Save stack as Subj#_ACPC.COR.CIN

2) Draw Left Hemisphere Cingulate ROI in Coronal View

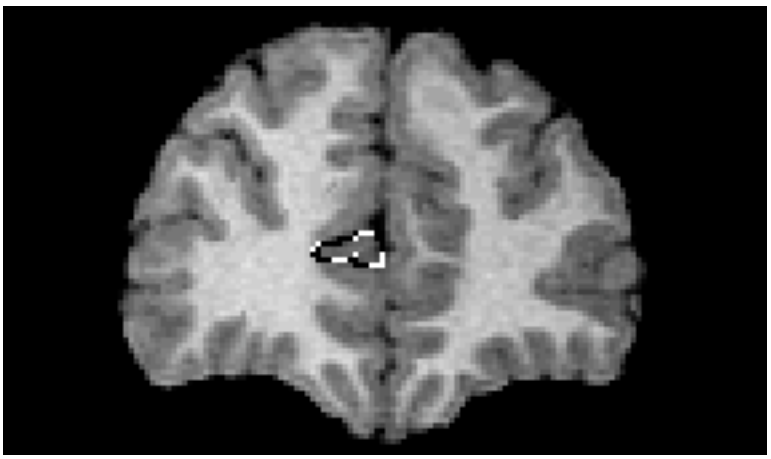
1) Open stack ACPC.COR.CIN
2) On the right hemisphere, move to the anteriormost slice with two white pixels.

Figure 7: Anteriormost Slice on Coronal Stack with Two White Pixels Indicating Superior and Inferior points of Cingulate Sulcus



3) Draw cingulate ROI on this slice.

Figure 8: First Cingulate ROI on Right Hemisphere



4) Continue drawing cingulate ROIs every two slices.

ROI Rules

2) ROI should be drawn to the gray-white border.

2) Begin ROI at the most superior point of the cingulate sulcus, at the gray-white border. The superior point of the cingulate sulcus is marked by the superior white pixel. (If more than one superior, white pixel is available, always utilize the most superior one. This is especially common in the posterior region.)

- Following the cingulate sulcus, draw around the medial region (through the interhemispheric fissure) and into the inferior cingulate sulcus. Draw to the gray-white border and then release. (Allow the superior and inferior points of the ROI to automatically connect.)

- **If the sulcus is not clear, then draw horizontal line through white pixel connecting interhemispheric fissure and gray-white border.**

- ROI goes through **middle** of long, white pixels.

- The inferior white pixel marks the inferior border of the cingulate. However, when the corpus callosum is connected, the Callosal sulcus will be used as the inferior border of the cingulate. Follow callosal sulcus to draw inferior border of cingulate. See Figures 9a and 9b. (Ignore any white pixels below corpus callosum.)

- As soon as the corpus callosum splits posteriorally, use white pixel as guideline for inferior border. However, if white pixel is not available, then follow the Calcarine fissure to lateral gray-white border. (The white pixels take precedence in the determination of the inferior border of the cingulate.) See Figures 10a and 10b.

Figure 9a: Coronal Slice with Corpus Callosum Inferior to Cingulate

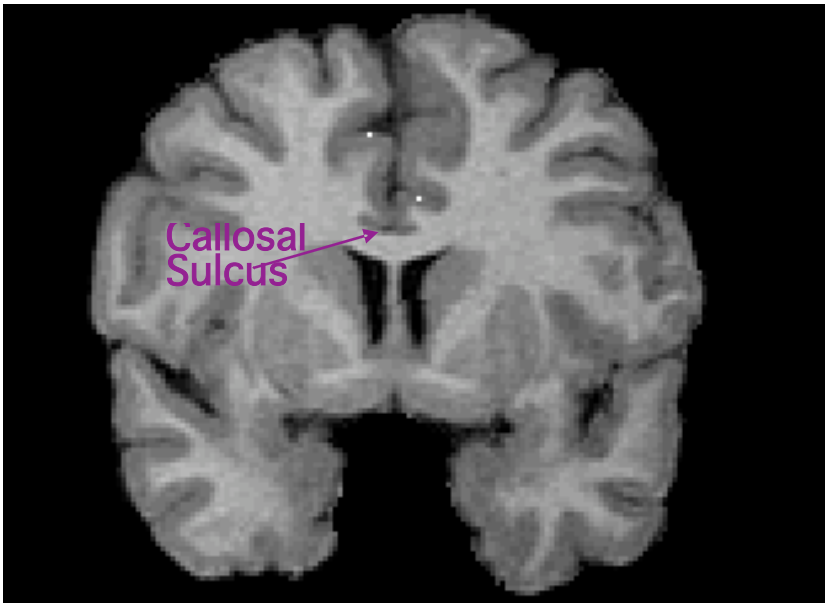


Figure 9b: Coronal Slice with Cingulate ROI Utilizing Corpus Callosum as Inferior Border

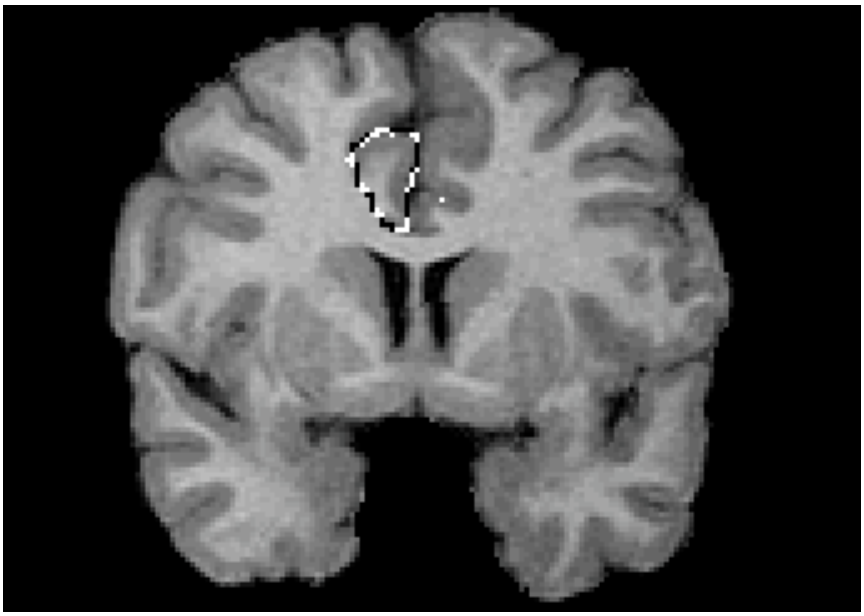


Figure 10a: Coronal Slice Posterior to Corpus Callosum

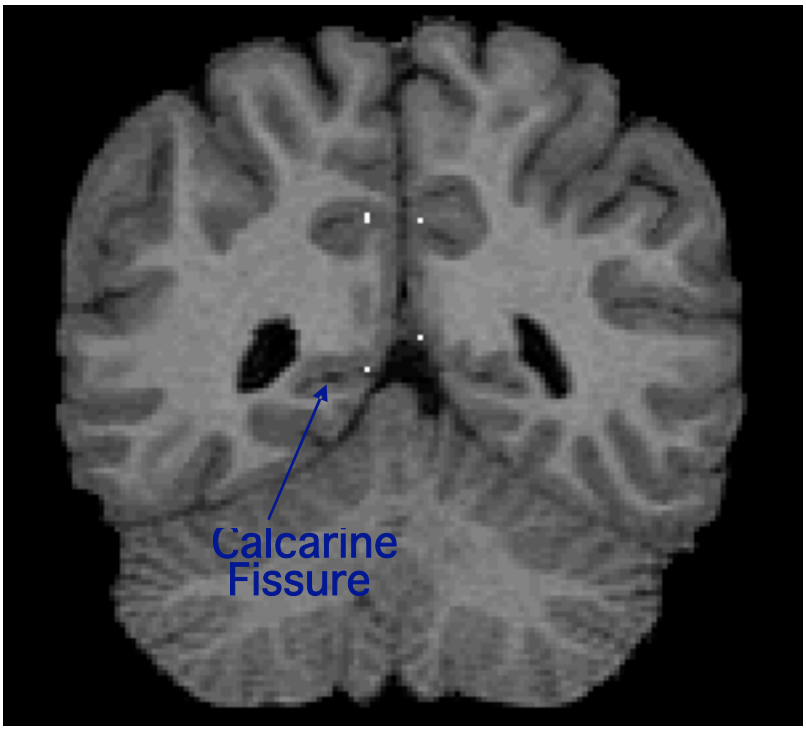
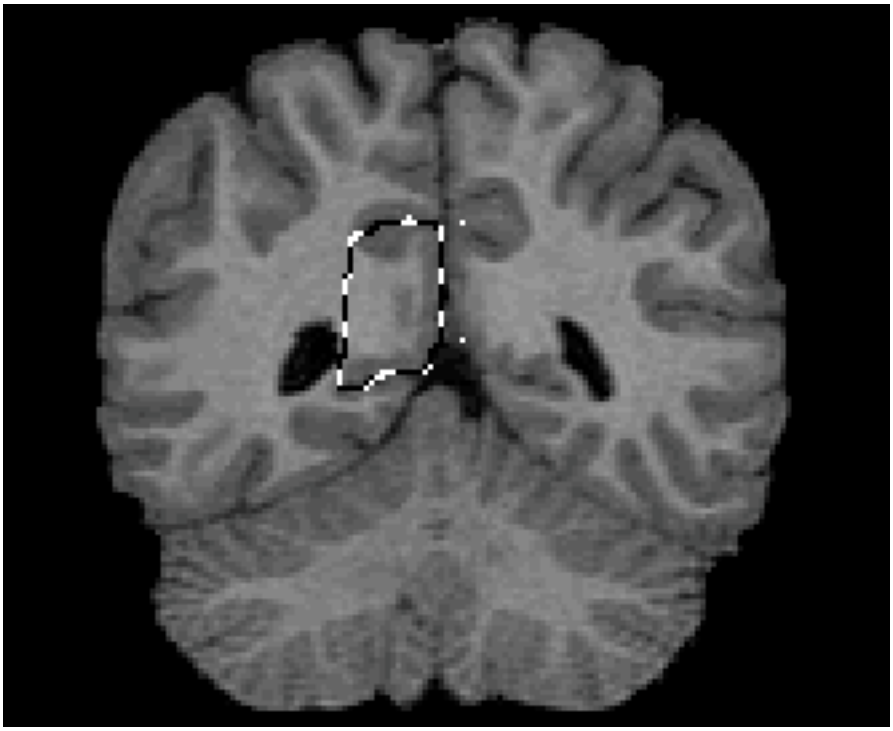


Figure 10b: Cingulate ROI Posterior to Corpus Callosum



5) The last slice of cingulate is the one anterior to the slice with the last white pixel on it. Always draw an ROI on this slice.
6) Save ROI as Subj#_RCIN.initials
7) Interpolate ROIS using Assert ROI warp function.
   Enhance→Experiments→AssertROIs
8) Resave ROIs.
9) Repeat for cingulate on the left hemisphere and save as Subj#_LCIN.initials.

5) Measuring Pixel count (whole cingulate, if subdividing see below):

1. Load RCIN ROIs on the stack ACPC.COR.
2. Fill to black. **Special→Fill ROIs**
3. Reload RCIN ROI onto stack.
4. Draw Stack Histogram. **Stacks →DrawStackHistogram**
5. Measure PDF stack. Alt + Option + cursor on stack histogram. Select “Measure PDF Stack.”
6. Record pixel value.
7. Repeat for LCIN ROIs.

Measuring Cingulate Automatically Using Talairach: (manual protocol described below)

Measuring:

9) Open the ACPCGRAY stack (gray fraction stack rotated into ACPC space).
10) Load the left cingulate ROI. Stack Clear outside to white.
11) Make volume from stack. Load Tal onto ACPC stack and enter dimensions of the **original resliced** image (RS.INV file, *not* the ACPC stack's dimensions!).
12) Press Apple-2 to open the results window. Press apple-3 to clear the window.
13) Under *Analyze*, select option. Make sure the "talairach sum" box is checked and that max measurement is set to 1400.
14) Under the *Talairach* sub-menu of the *analyze* menu, select *Select all*. From the same menu, select *measure (sum)*. The results will appear in the results window. Save these results as LCINGRAYGRAY(MS).rater's initials.
15) Repeat with the RCINGULATE ROI. Saving the results as LCINGRAY(MS).rater.
16) Repeat for left and white cingulate measures for white matter using the ACPCWHITE Stack instead for the gray (producing LCINWHITE and RCINWHITE files.
17) Exit Brainimage.
18) Open up the LCINGREY(MS) sheet in Excel. Highlight the data (furthest left column) and select *copy*.
19) Open the cingulate measurement spreadsheet (a worksheet in the TALC workbook for each subject) and paste the cingulate results into the appropriate column. Use a preexisting sheet if it is there in the PDF folder (make sure that the cingulate spreadsheet version is > v 1.0). If a new TALC sheet needs to be created, the image dimensions (FOV and matrix size) will need to be input on the summary sheet.

Preparing Cingulate For Manual Subdivision (optional):

In order to measure the subregions of the cingulate manually, the tissue emcompassed in the cingulate ROIS needs to be isolated from other brain tissue and rotated into ACPC sagittal space.

1. Load the CIN.COR stack
2. Load the LCIN ROIs
3. Stack Clear Outside (SPECIAL→ STACK CLEAR OUTSIDE)

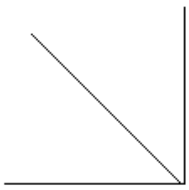
4. Make Volume from Stack (STACKS → MAKE VOLUME FROM STACK)
5. Rotate the Bottom Right Panel -90 degrees.
6. Make a stack from the volume (STACKS → MAKE STACK FROM VOLUME)
   Save the Top Right panel. Name the File **subject number_LCINSTACK.initials**
7. Repeat steps 1-7 for the Right Cingulate ROI.

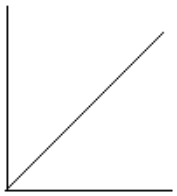
Subdividing Cingulate:

Because the cingulate is thought to perform several neuropsychological functions that are localized to specific regions, it is useful to divide it into several subregions.

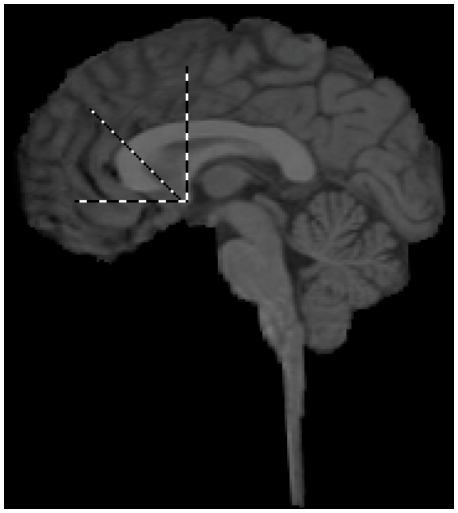
1) Load the sagittal ACPCSTACK and find the best midsagittal slice (should be recorded from previous steps)

2) Open the CINGULATE_SUBDIVIDE STACK There are two template images to be found here: one for delineating the anterior region of the cingulate and one for the posterior region, on separate slices.





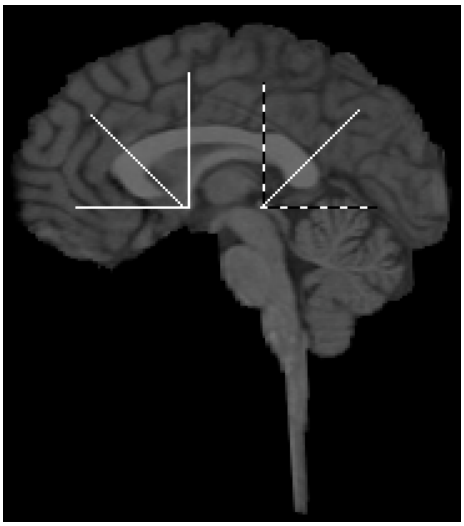
3) Using the wand tool, click on the first images to create an ROI. Select the midline of the sagittal ACPC stack and press apple-4 to transfer the ROI to the stack.



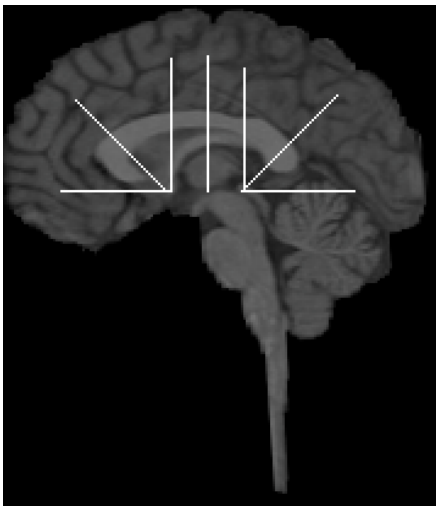
4) Using the arrow tool, maneuver the ROI such that the vertex of the angle lies on the anterior commissure.

5) From the special menu, choose the macro "Fill subdivision to intensity 1." It is important to make sure that you are using this macro and not the "fill" macro with pure white (intensity 0) intensity. If you do not fill to intensity 1, there will problems later when you try to create your ROIs later.

6) Repeat this procedure (steps 3-5) using the second premade ROI for the (posterior cingulate region), except use the posterior commissure (PC) as the vertex of the angle ROI.

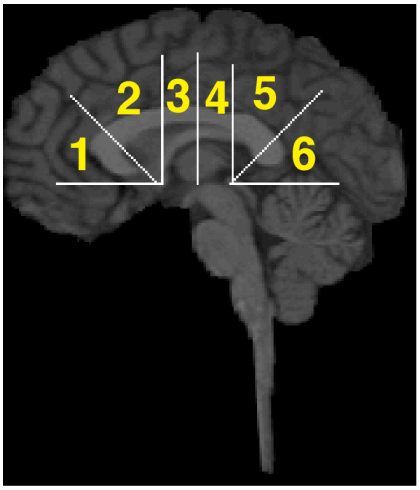


7) Using the line tool and any non intensity 1 color (e.g. pure white or pure black), measure the distance between the vertical lines ant the anterior and posterior ends. Draw a vertical line exactly half the distance between these two lines and fill to intensity 1.



8) From the special menu, select "get ROI from demarcations" This will extract an ROI from all intensity 1 pixels in the stack.

9) Save this ROI as **subject number_CINDEM.initials.**



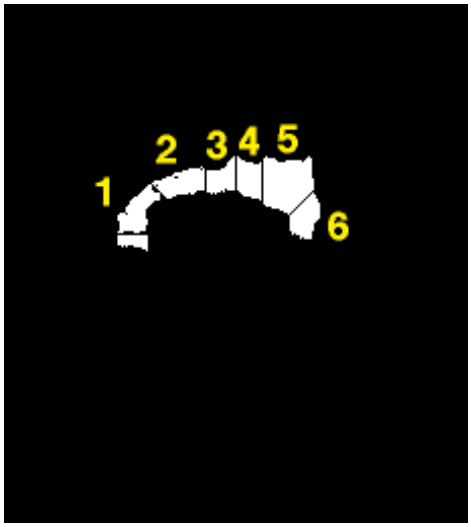
10) Reopen your SAGDIF ROIs and go to the midsagittal slice (where the subdivision ROI is located. Hit "G" and go to slice 1. Press apple-4 to redraw the demarcation son the first slice. From the special menu, select "Draw ROI 1 on every slice". Save these ROIS.

11) Select Black from the LUT. Fill the demarcation ROIS (SPECIAL→FILL ROIs, shortcut F).

12) Set a Dual Threshold. (STACKS→SUAL THRESHOLDS→Otsu)

13) Binarize the stack (SPECIAL→ADJUST THRESHOLD AND BINARIZE)

14) You should have now have a black and white image delineating the boundary of the cingulate and its subdivisions in three dimensions. The next step is to grpw a seed in each subdivision separately and save it as an ROI. The numbers of each segment are shown below:



15) Go to the slice that best shows the division between segments. From the STACKS menu, choose GROW STACK VOL→ SEED VALUE and click on the first segment. An ROI will grow to include exactly the contents of the first segment. Save this ROI as **subject number_LCIN1.initials**.

16) Kill the segment 1 ROI ("K") and repeat this process for segments 2-6.

17) Repeat for the Right cingulate.

18) Measure the pieces using methodology described in the previous section (Page 74). Alternatively, fractions stacks can be used to measure gray and white matter separately.

Appendix

This is an example of a situation in which it is difficult to decide the location of the first sulcus anterior to the corpus callosum. Although there is a clear sulcus anterior to the corpus callosum, gray matter appears in between this sulcus and the corpus callosum, which may define the cingulate better. Therefore, it is necessary to check either side of the midsagittal slice to see whether the sulcus or gray matter provides a better outline of the cingulate. Compare Figure 11a to Figure 11b, which provides a more distinct outline of the cingulate. It is now clear that an ambiguous sulcus exists in the gray matter that should be used as the cingulate border. If the sulcus is not visible, then follow the **middle** of the gray matter border as long as it is continuous. Figure 11c depicts the final outline of the cingulate and should once again be compared to either side of the midsagittal slice for accuracy.

Figure 11a: Sagittal View of Cingulate Five Slices From Midsagittal Slice

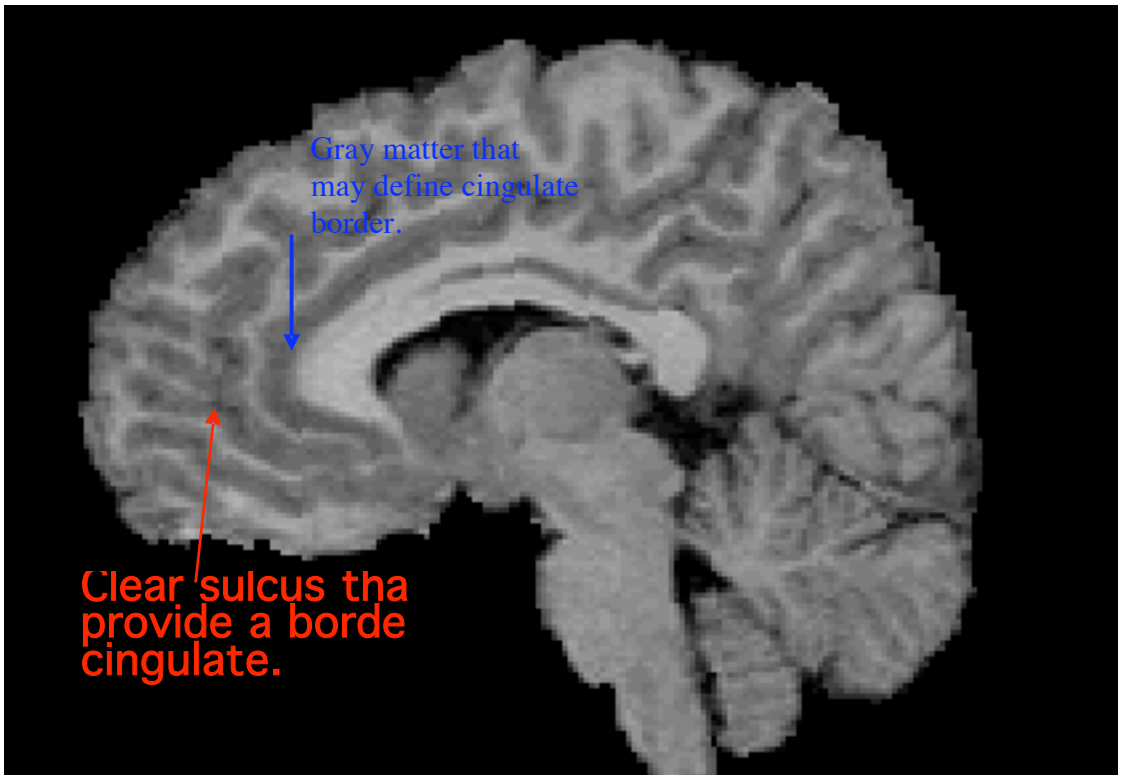


Figure 11b: Sagittal View of Cingulate on Slice Adjacent to Midsagittal Slice

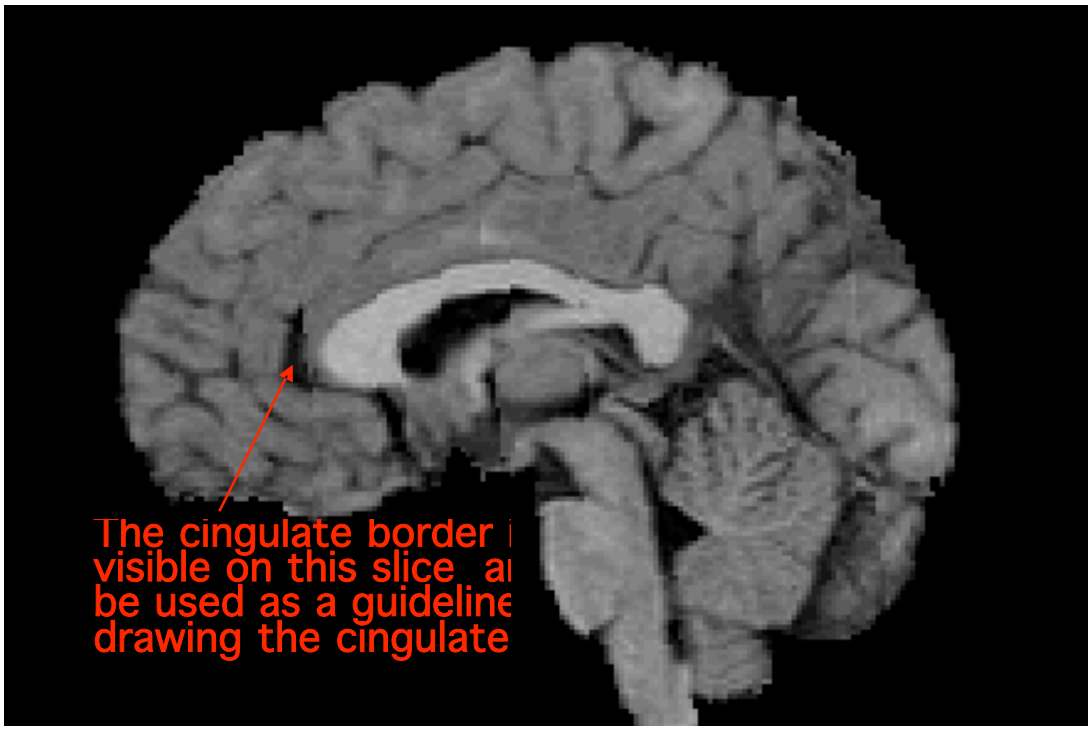


Figure 11c: Sagittal View of Final Outline of Cingulate