A. SPECIFIC AIMS

Faces are a rich source of information, offering a window to identity, ethnicity, age, gender, and changing mental and emotional states. Recognizing this facial information is critical for social interaction and represents a fundamental component of complex social behavior and is atypical in developmental disorders such as autism and Williams Syndrome. The processing involved in decoding facial information is thought to depend on a network of core perceptual face processing regions in the brain [e.g.,1, 2] that undergo prolonged maturation well into adolescence [3-5]. However, little is known about the anatomical pattern of connectivity of the core perceptual face processing network or about the typical developmental timecourse of these connections.

I propose to combine fMRI and DTI tractography to characterize the pattern of anatomical connections that define the network for face processing in the typically-developing brain. By charting the typical developmental timecourse of white matter connections, this research is a critical prerequisite for understanding how the structural organization of the face processing network is disturbed in developmental disorders of social information processing such as autism and Williams Syndrome [6-8].

Aim 1: Characterize the pattern of connections between functionally-defined core perceptual face processing regions in adults

We hypothesize that the shared functional property of face-selectivity across core face processing regions is the result of their shared white matter connectivity “fingerprint”, which we predict is distinct from the pattern of connectivity between non-face selective regions.

In healthy adults, I will combine fMRI and DTI probabilistic tractography to identify white matter pathways likely to connect core perceptual face processing regions in adults including face-selective regions in the fusiform gyrus (FFA), occipital cortex (OFA), and superior temporal sulcus (pSTS). This study will be the first to provide estimates of the likelihood and strength of connectivity between core perceptual face-selective regions in human adults. In the same subjects, I will also collect behavioral measurements of face recognition (identity, expression). This will allow me to relate quantitative measures of structural properties of likely white matter connections between face processing regions with the degree of gray matter face-selectivity at the neurophysiological level, and face recognition performance at the behavioral level.

Aim 2: Assess the development of white matter connectivity for core perceptual face processing regions from age 7 through adulthood

We hypothesize that increases in the extent of face-selective gray matter in the typically developing brain are the result of changes in the pattern of white matter connections to these regions.

Previous work in the lab has found that the functionally-defined volume of face-selective regions in the FFA and OFA, but not pSTS, increases between ages 7 and 16 [3, 4], correlating with improvements in face recognition memory. In Aim 2a, I will determine whether increases in the extent of face-selective gray matter in the typically developing brain are correlated with changes in white matter structure immediately adjacent to these regions. In Aim 2b, as in Aim 1, I will determine how developmental changes in gray matter function are related to changes in the connectivity of the face processing network. This work will be the first to establish the developmental timecourse for white matter connections related to face processing in typical development.

B. BACKGROUND AND SIGNIFICANCE

Face processing in adults depends on a network of face-selective cortical regions

Over a decade of neuroimaging results have consistently identified a suite of anatomically distinct, bilateral face-selective regions in ventral and lateral cortex including regions in the mid-fusiform gyrus (FFA), posterior fusiform gyrus (pFus), lateral occipital cortex (OFA) and posterior superior temporal sulcus (pSTS) (Figure 1, [1, 9-11]). Studies of acquired prosopagnosia following lesions to the FFA and OFA demonstrate the causal necessity of at least two of these regions for successful face identification [12, 13]. These results and others have motivated several models of human neural face processing [1, 2, 14]. A key prediction shared by all of these models is that the different face-selective regions form a coherent network with functionally interdependent components. Although these models predict the existence of white matter connections between
core perceptual occipitotemporal regions as well as extensions into socioemotional processing centers like the amygdala, there is no empirical evidence for these connections thus far. Invasive anatomical tracer measurements are not possible in humans in vivo, yet in vivo functional localization of gray matter regions is critical for identifying the white matter connections of interest. DTI tractography therefore presents an ideal solution by enabling in vivo estimation of the likelihood and strength of white matter connections between functionally-defined cortical regions [15]. By combining fMRI and DTI tractography to identify white matter pathways likely to connect core perceptual face processing regions in adults, this research will place new and important constraints on theories of neural face processing as well as establish for the first time a portrait of adult connectivity that can then be examined across development (Aim 1).

The pattern of white matter connections to a neural region is a major determinant of functionality—this pattern is unknown for core perceptual face-selective regions

The functionality of a neural region is heavily constrained by its connections, which define what information may influence the operations of the region and what influence can be exerted on other operations downstream [16, 17]. While the pattern of intrinsic connections within a region no doubt plays an important role in determining functionality, disrupting extrinsic white matter connectivity disrupts function even when intrinsic connectivity is left intact [18-20]. Moreover, the pattern of long-range white matter connectivity to a particular region is a macrostructural “fingerprint” that can be used to segregate gray matter regions into functionally and hierarchically distinct processing components [21, 22]. These connectivity profiles can be estimated using DTI fiber tractography and have been validated by fMRI studies in visual and motor systems [23-25], as well as by ex vivo tracer studies [26]. While no studies have yet estimated the pattern of white matter connectivity between face-selective regions in either humans or non-human animals, recent experimental work in the macaque has shown that electrical stimulation within the boundaries of one face-selective region results in a spatially patchy pattern of activation. This pattern of activation is largely confined to other face-selective patches, which the authors interpret to be linked to one another [27]. Estimates of white matter connectivity between anatomically distinct face-selective regions in the human is therefore an important area of future research as it would serve as an independent source of evidence for defining the components of the face processing network, as well as for understanding how functional properties change across development.

We can use DTI probabilistic tractography to estimate the most likely white matter pathways connecting two regions and measure their structural properties

Diffusion tensor imaging (DTI) capitalizes on the fact that a freely diffusing water molecule will move at a constant rate in any direction. In structured tissue like the white matter, however, diffusion is impeded in a direction-selective way that enables principled inferences about the underlying tissue structure, such as fiber pathway trajectory, volume and length, as well as tissue diffusivity and anisotropy [28, 29]. The relationships between neighboring voxels can also be used to estimate the trajectories of the underlying fiber tracts. The benefit of using a DTI fiber tractography algorithm over a whole brain voxel-based search is that we can be more confident that we are finding and analyzing white matter voxels likely to contain pathways of interest, and additionally, conduct tests with greater statistical power [25, 28-30]. Two classes of tractography algorithms exist, deterministic and probabilistic. While deterministic algorithms such as STT are commonly and successfully used to identify portions of large fasciculi [24, 25, 28-33], these algorithms estimate white matter pathways based only on local information and therefore suffer from low sensitivity including false negatives in regions of low anisotropy and false positives due to pathway conflation. Probabilistic algorithms overcome these limitations in sensitivity by expanding the pathway search space and estimating the relative likelihood of candidate pathways based on several factors including fit to the observed diffusion data, anatomical priors, and discovery frequency [34-37]. Probabilistic algorithms have been able to identify white matter tracts of known

Figure 1. Ventral (left) and lateral (top) right hemisphere face-selective regions on an example subject with depicted contrast
existence that deterministic algorithms have been unable to discover, including the Meyer’s loop portion of the optic radiation [37] and medial portion of the superior longitudinal fasciculus [34].

The timecourse of white matter development varies by region and extends through childhood

Previous research in typically developing populations has found region-specific maturational changes in neural tissue volume from early childhood through adulthood despite comparable total brain volume during this period [32, 33, 38]. In the largest cross-sectional studies of brain development conducted thus far, white matter volume has been found to increase linearly from age 4-22 [39] with considerable regional variation in the timing and pattern of developmental change of tissue diffusivity, reflecting changes in axonal diameter, packing density and/or myelination [33, 40]. Of particular relevance to this proposal, structural properties of the inferior longitudinal fasciculus (ILF), a major white matter tract connecting the occipital and temporal lobe, change between age 5-30; including a 10-25% increase in fractional anisotropy and 8-19% decrease in mean diffusivity [33]. It is therefore likely that understanding the trajectory of typical development in the specific white matter connections important for face processing may help to select appropriate time windows for intervention.

Recognition memory for novel faces develops slowly in parallel with increases in the size of some, but not all core face-selective regions

Despite a perceptual bias operating from birth that ensures the visual system’s preferential orientation towards faces from the earliest moments of life [2] and the presence of behavioral markers of adult-like holistic/configural processing of faces from early infancy through early childhood [c.f.,41], it is not until adolescence that recognition memory for novel faces reaches adult-like levels [3-5]. This long trajectory of behavioral maturation in face recognition memory parallels the long trajectory of changes in the extent of face-selective cortex in regions of the brain like the right hemisphere FFA (Figure 2) and OFA, but not others such as the pSTS [3, 4]. Development changes in the functional organization of visual cortex during childhood and adolescence appear to be specific to face processing regions and not object or place processing regions [42]. However, neither the mechanisms underlying the developmental increase in functionally-defined regional “size,” nor the explanation for anatomical heterogeneity in these developmental changes (right but not left hemisphere; FFA and OFA, but not pSTS) is known. These changes may result from a variety of factors, including changes in the pattern of inter-regional white matter connectivity [16].

Understanding the typical development of the face processing network is a critical prerequisite for testing hypotheses about the neural basis of developmental disorders of social communication

Although the pattern of anatomical connections between core perceptual face processing regions is unknown, converging evidence supports the hypothesis that the typical progression of development in the neural face processing network is disturbed in developmental disorders such as autism and Williams Syndrome [6, 7]. Evidence from fMRI studies in humans find atypical functionality in FFA and STS activation in developmental disorders such as autism and Williams Syndrome [43-48]. Comorbidity of autism with congenital right occipital lobe seizures [49], as well as reports of white matter structural differences between autistic and typically-developing brains [50, 51] and between adults with typical and impaired skill in face recognition [52] are consistent with, but do not directly test, the proposal that disturbances to the typical pattern of anatomical connectivity between these regions could account for these results. Measuring the specific pattern of connectivity between core perceptual face processing regions and how this pattern changes during typical development is a logical next step (Aim 2). More than that, this work is a critical prerequisite for testing hypotheses about the neuroanatomical basis and progression of developmental disorders of social communication such as autism and Williams syndrome.

C. PRELIMINARY DATA
Probabilistic tractography shows that the FFA is heterogeneously connected to lateral occipital cortex. White matter pathways connecting the FFA with object- and face-selective regions were more frequently identified than those connecting the FFA with place- and motion-selective regions.

C1 Design & rationale: As a first attempt to characterize the pattern of connectivity for the FFA, I identified the most likely white matter pathways between the FFA (seed ROI) and a large lateral anatomical ROI (target ROI). Paths were estimated using conTrack, a previously validated probabilistic tractography algorithm developed at Stanford [36, 37]. The broad goal was to determine whether the most likely white matter pathways between the FFA and lateral cortex would be uniformly distributed or spatially heterogeneous. The specific goal was to determine whether the most likely pathways connecting the FFA to lateral cortex coincided with locations showing particular kinds of stimulus selectivity. I therefore plotted the pathway endpoints in the target ROI with the locations of functionally-defined ROIs showing face-, place-, object-, or motion-selective regions in the same ROI. Finally, I compared pathways generated from the observed diffusion data with control pathways that were generated by replacing the observed data with a control data set (no directional information, spherical tensors) and thus determined solely by the tractography algorithm’s priors. The aim of this comparison was to assess the degree to which pathways found in the observed diffusion data were dependent on the data or the algorithm’s priors. The conTrack algorithm estimates likelihood based not only on a pathway’s fit to the data, but also the fit to anatomical priors. 

C2 Methods: Adults ages 18-40 (n=6) were recruited from Stanford University and community to participate in both an fMRI and DTI scan as described in D.1. 

Seed ROI definition (FFA): The seed ROI used in the tractography analysis was defined as the largest contiguous cluster of face-selectivity in the right hemisphere mid-fusiform gyrus, the RH FFA. It can be seen in figure 3A2 and 3B2 as the purple outline on the most inferior visible portion of the temporal lobe. 

Target ROI definition (lateral occipital): The target ROI used in the tractography analysis was defined using anatomically-based landmarks. This included all cortex within a disk centered on the occipital pole, that extended 3/4-total length of the right hemisphere temporal lobe, excluding retinotopic cortex posteriorly. The ROI was bounded by the intraparietal sulcus dorsally and occipitotemporal sulcus ventrally. It can be seen in the right column of Figure 3 as the larger black outline. The target ROI contained face-selective (purple), object-selective (blue), place-selective (green), and motion-selective (smaller black) regions (Figure 3).

![Figure 3. All panels show the same example subject’s right hemisphere. Top row (A1,A2): Most likely white matter pathways given the observed DTI data on a representative subject; Bottom row (B1,B2): Control: most likely white matter pathways based on anatomical priors alone after replacing the observed DTI data with spherical tensors; Left column (A1,B1) shows fiber pathways in blue on a brain section. Right column (A2,B2) shows endpoints of fiber pathways in orange relative to visual regions of known stimulus-specificity on the inflated cortical surface. Colored outlines show ROIs, black: anatomical target ROI; purple: face-selective; blue: object-selective; green: place-selective; black (ITS): motion-selective. Seed ROI: FFA.](image)

C3 Tractography results with observed DTI data (Figure 3, top row): We used conTrack to estimate the most likely white matter connections between the FFA (seed ROI) and lateral occipital anatomical region (target ROI). Parameters: 200,000 samples, 1mm step size, log2 likelihood > -1.568 (details, C5). Visual inspection suggests the pattern of connectivity between these regions is spatially heterogeneous (Figure 3, A2). A follow-up test marginally confirms this: 4 x 1 repeated-measures ANOVA with stimulus-selectivity type and number of endpoints per voxel in the target ROI as the dependent measure, F(3,15)=2.76, p=0.0783. The data more strongly supports the existence of white matter connections between the FFA and face-selective regions.
of face recognition, social skill, and general intelligence.

D1.1 Behavioral methods

All participants undergo a battery of five behavioral tests to assess various aspects of face recognition, social skill, and general intelligence.
Recall memory test: This test assesses participants’ ability to encode and retrieve novel images [3, 4]. The number of study images and the similarity of the test lures were selected to equate adults’ performance (d-prime) across six image categories: child male faces, adult male faces, indoor scenes, outdoor scenes, cars, and abstract sculptures.

Benton facial recognition test: This test assesses perceptual aspects of face identification in which a target face is presented simultaneously with several choice faces varying in viewpoint, illumination, etc. Participants choose which face from an array is the same identity as a target face.

NEPSY-II affect recognition test: This is a standardized neuropsychological test of facial expression/emotion recognition. Participants choose which face from an array matches the expression of a target face across changes in identity, gender, and ethnicity.

Autism spectrum questionnaire (AQ): This is a standardized questionnaire assessing the degree to which a typically developing individual has traits associated with the autism spectrum [53] and has been previously used by the trainee to predict individual differences in face-related gaze behavior [54].

Wechsler abbreviated scale of intelligence (WASI): This is an age-normed neuropsychological test that assesses general verbal and nonverbal IQ. It is included to allow us to match general cognitive factors not specific to face processing.

D1.2 Neuroimaging methods Experiments will be conducted on a 3T GE scanner at the Lucas Imaging Center at Stanford University. We use software developed in Dr. Grill-Spector’s lab and Dr. Wandell’s lab for the analysis of anatomical, functional and diffusion MR data: http://vistalab.stanford.edu/Software.

Whole brain anatomy: We acquire 4 high resolution whole brain anatomy SPGR scans using an 8-channel head coil. Scans are averaged, aligned, and resampled to 1mm voxels. Anatomy data is segmented into gray and white matter, and a cortical surface of each hemisphere is generated for each subject.

fMRI data acquisition and analysis: fMRI scans use a surface coil covering the occipital and posterior temporal and parietal lobes. We acquire 32 slices with TR=2s, and voxel size = 3x3x3mm. Data is corrected for motion, high-pass filtered, corrected for spatial inhomogeneities, and aligned to that individual subject’s whole brain anatomy. Subjects participate in block-design localizer scans including: 2 scans with blocks of face, object, place, and to identify face-, object-, and place-selective regions; an MT localizer scan; and 4 retinotopy scans with flickering checkerboard stimuli including 2 eccentricity and 2 polar angle scans.

ROI selection and definition: In each individual, we define several face-, place- and object-selective ROIs as well as MT+ and retinotopic regions. Face-selective ROIs (faces > objects & scenes, p<10^{-3}) include a region in the fusiform gyrus (FFA), a face-selective region in the lateral occipital cortex (LO-faces) and a face-selective region in the ascending limb of the posterior superior temporal sulcus (pSTS). Place-selective regions (scenes> faces & objects, p<10^{-3}) include a region overlapping the collateral sulcus and parahippocampal gyrus (PPA), a region in the retrosplenial cortex (RSC-places) and a region between the transverse occipital sulcus and intraparietal sulcus overlapping the middle occipital gyrus (MOG-places). We also define the object-selective regions (objects > scrambled objects, p<10^{-3}) including a lateral region (LO-objects) and a region overlapping the posterior fusiform gyrus and occipital temporal sulcus (pFus/OTS). MT+ is defined as a region in the posterior inferotemporal sulcus that responds more to low contrast moving gratings than stationary gratings (p<10^{-5}). Retinotopic regions V1, V2, V3, V3a, V4, V7 and LO1 are defined from retinotopic scans [55]. In follow-up analyses, we will define these ROIs at several different thresholds to determine the extent to which any results we find are threshold-dependent [3-5].

DTI data acquisition and analysis: DTI analysis is conducted in individual subjects, to relate fiber tracts and their development to functionally defined ROIs. Each subject participates in diffusion-weighted scans using an 8-channel head coil on a 3T GE scanner at the Lucas Center at Stanford University. We acquire 60 slices, 2mm isotropic voxels, using an EPI spin-echo measurement, TR=6.7s and TE=73ms. We acquire 4 repetitions of each of 30 diffusion directions (b=900s/mm^2), as well as 10 non-diffusion-weighted (b=0) measurements per slice resulting in 7800 images per subject.

Tensor model: We summarize the set of measurements for each voxel in the brain as a diffusion tensor. Because each standard resolution voxel (2x2x2mm) contains anywhere from 40,000-4,000,000 axons in each voxel and the tensor models only the dominant component where mixtures of distinct fascicles are included in a single voxel, tensors reflect bulk tissue microstructure [29].

Preprocessing: Data preprocessing corrects for eddy currents and motion [56], aligns the DTI data to that individual’s anatomy, and computes a standard diffusion tensor estimation using a least-squares fit [28].
Fractional anisotropy (FA): quantifies the degree of anisotropy. FA is a commonly used indicator of structural orientation coherence in each voxel. Higher FA values could indicate a decrease in axon diameter in the dominant orientation and/or a decrease in the presence of non-dominant fiber orientations. Several recent studies reveal regional asynchrony in FA across development, with the corpus callosum and the inferior longitudinal fasciculus, increasing in FA (and presumably developing) faster and earlier than other tracts that appear to develop quite slowly, such as the fronto-temporal connections [32, 33, 40].

Mean diffusivity (MD): is a rotationally invariant measure of the magnitude of diffusion. Recent studies indicate that MD decreases with age for many white matter structures. MD in major fiber bundles decreases during development, and seems to follow a different temporal trajectory than FA changes in the same tracts [33].

Volume: Because white matter pathways that result from tractography analyses have no thickness, the volume of a white matter connection is estimated as the number of voxels containing one or more fiber pathways.

Length: will be estimated by multiplying the number of nodes in a pathway and the step size of the algorithm.

Tractography algorithms: Previous research has motivated the development of various tractography algorithms in order to study white matter pathways whose identification is known to be algorithm-dependent. As part of Aims 1-2, we will therefore use two previously validated probabilistic tractography algorithms, conTrack [36, 37] and FSL-FDT [34, 35], in order to understand the extent to which estimates of connection likelihood and strength are algorithm-dependent.

**D1.3 Methodological issues**

**Age differences in fMRI and DTI data acquisition quality:** Because MRI data acquisition quality depends on subject compliance, we have four strategies to ensure that fMRI and DTI data quality is matched across age.

1. Prior to participating in fMRI or DTI sessions, we will train subjects to control motion, maintain fixation and perform tasks while lying in an MRI simulator. We have found these methods to lead to highly successful fMRI sessions in scanning young children.
2. To control for any between-age-group differences in the patterns of eye movements (potentially accounting for differences in the BOLD response), during each fMRI session we use an infrared eye-tracking system (ViewPoint EyeTracker, Arrington Research). Scans including eye movements > 2° will be excluded and follow-up analyses will compare eye movements across ages and stimulus conditions to confirm that there are no between subject, between image category eye movement confounds.
3. As previously described [3-5], we will match subjects across age for the potential BOLD-related confounds of motion, pre-stimulus activation fluctuation, and general linear model (GLM) residual error.
4. Because signal-to-noise (SNR) impacts DTI analyses, we will ensure comparable SNR across all data sets regardless of age using the strategies above. If this is not sufficient, we will match SNR by discarding data from the adult data sets or by increasing the data acquisitions in children.

**Selecting seed and target ROIs for tractography experiments:** Individual differences in the size of seed and target ROIs, as well as their distance from one another may impact measures of white matter properties, such as length and volume. This is particularly important because data from our lab shows age-dependent changes in the size of face-selective ROIs. Depending on the specific outcomes of the proposed analyses, I will conduct controls to understand whether the age-related results I find depend on any of the above-mentioned factors. These controls will include defining seed and target ROIs of a fixed size and distance across ages, comparing diffusion properties of fiber tracts known to change across development with those thought to remain constant across the ages I plan to measure (e.g., fornix major/minor). This will determine whether any observed age-related differences are specific to particular white matter connection hypothesized to undergo developmental change.

**Interpreting the results of DTI fiber tractography:** We will conduct controls to estimate the degree to which pathway likelihood estimates depend on specific algorithm parameters (e.g., anatomical priors, see section C1.4) and how much they depend on the observed diffusion data. While probabilistic tractography algorithms can identify pathways of non-zero probability for any pair of ROIs, they can nonetheless support inferences about which pathways are significantly more likely to exist than others, given the observed diffusion data.

**D1.4 Tractography data analysis**

**Tractography method 1:** Estimate the most likely connections between a functionally-defined seed ROI (e.g., FFA) and a large anatomically-defined target ROI (e.g., lateral occipital cortex) containing many regions of heterogeneous (face-, place-, object-, and motion-selective) functional selectivity (Figure 5).
Rationale: This analysis will determine whether the most likely white matter connections between a functionally defined ROI and an anatomical ROI are uniformly distributed or are spatially heterogeneous. By choosing seed ROIs of varying anatomical location and functional selectivity but keeping the target ROI constant, we will learn whether the pattern of connection endpoints in the target ROI is best explained by the anatomical properties of the target ROI alone regardless of the precise spatial location and extent of functional activation of the seed ROI for that individual. Finally, we can determine whether the pattern of connection endpoints coincides with regions of particular functional selectivity within the target ROI.

Analysis plan: The dependent measure is the location and number of connection endpoints in the target ROI as the seed ROI is varied according to factors of interest (e.g., type of functional selectivity, anatomical location). I will compute a two-way repeated-measures ANOVA with seed ROI location as one factor, and target ROI endpoint location classified by factor of interest (e.g., type of functional selectivity, anatomical location) as the second factor. Greater numbers of connection endpoints in a particular region of the target ROI indicate greater likelihood of connectivity to that region.

Potential outcomes and interpretations: If neither seed ROI location nor target ROI endpoint location explain significant variance in the location and number of connection endpoints, the data would support the conclusion that the pattern of connectivity between the seed and target ROIs is spatially homogeneous. All other outcomes would indicate that the connectivity pattern is spatially heterogeneous, in which case hypotheses predicting specific patterns of connectivity could be tested.

Tractography method 2: As another approach to assessing connectivity that does not require threshold-setting, I will track a matched number of pathways between a seed ROI and different target ROIs that are matched in size and distance to the seed ROI. (Figure 6, purple circle dotted line), or anatomically-adjacent ROIs of matched surface area (Figure 6, gray circles dotted line). I will measure structural properties of these pathways to estimate connectivity strength.

Rationale: This analysis will determine whether the structural properties of a likely connection between a pair of ROIs is specific to their precise location, or instead reflects general connectivity strength between two anatomical regions that coincide, but are not restricted to, the locations of functionally-defined ROIs. It will also serve to test the hypothesis that structural properties of estimated connections between pairs of ROIs whose connectivity is predicted will systematically differ from those of matched pairs of ROIs in anatomically adjacent locations whose connectivity is not predicted (e.g. between a pair of face-selective ROIs versus a face-selective ROI and non-face-selective cortex).

Analysis plan: The dependent measures are quantities known to relate to the structural strength of a white matter connection. These include FA, MD, and volume (see section D1.2 for more details). I will compute a one-way repeated measures ANOVA with target ROI location as the main factor. Higher FA, lower MD, and/or greater volume may indicate greater structural connectivity strength.

Possible outcomes and interpretations: If target ROI type does not explain significant variance in FA, MD, or volume of the connection between a pair of ROIs, the data would support the conclusion that the connectivity strength between the ROI pair of interest is not specific to that pair, but instead reflects the connectivity strength of the local anatomical neighborhood. If target ROI type is a significant explanatory factor, interpretation depends on the direction of the effect, where higher FA and volume may indicate a stronger connection.

D2. Experimental Design

Specific Aim 1: Estimate the likelihood and strength of white matter connections between functionally-defined core perceptual face processing regions in adults.

Rationale: The pattern of white matter connections to a neural region is a major determinant of functionality, however, this pattern is unknown for core perceptual face-selective regions. This work will test the hypothesis that the shared functional property of face-selectivity across core perceptual face processing regions is the result of their shared white matter connectivity “fingerprint”. Specifically, we predict white matter pathways connecting face-selective regions with other face-selective regions to be more likely and of greater strength.
than pathways connecting face-selective regions with non-face-selective regions of equivalent size and distance.

Methods: Subjects will include twelve adults (>21yo) recruited from Stanford and the surrounding community. Behavioral and neuroimaging methods are as described in Section D1.

Analysis plan for Tractography Method 1: For each ROI pair, I will use Tractography Method 1 to assess the spatial specificity of a likely connection between the pair when designating one element in the pair as the seed ROI and a large anatomical region including the second element in the pair as the target ROI. We will seed the tractography algorithm on each of the three core face processing regions (FFA, OFA and pSTS). When seeding with the FFA we will define a larger anatomical ROI on the lateral surface as the target (see Figure 3) When seeding with either the OFA or pSTS, we will use a large ventral anatomical ROI spanning the fusiform and parahippocampal gyri as the target. The dependent measure is the distribution of connection endpoints in the target ROI given the seed ROI. Greater numbers of connection endpoints in a particular region of the target ROI containing the second ROI pair element indicates greater likelihood of connectivity between the ROI pair elements versus adjacent anatomical regions of dissimilar stimulus-selectivity, given the diffusion data.

Analysis plan for Tractography Method 2: For each ROI pair, I will use Tractography Method 2 to assess the connectivity strength of a connection between the two ROI pair elements relative to the connectivity strength of one element in the pair as the seed ROI and an anatomically-adjacent ROI of matched surface area as the target ROI. The dependent measures are quantities known to relate to the structural strength of a white matter connection including FA, MD, and volume. Additionally, I will compute correlations between the estimated connection strength between face-selective ROI pairs with fMRI indices of face-selectivity [3, 4] and with behavioral measures of face recognition performance. This will allow me to relate quantitative measures of white matter structural properties with the degree of gray matter face-selectivity at the neurophysiological level, and face recognition performance at the behavioral level.

Potential outcomes and interpretations: Motivated by models of human neural face processing [1, 2, 14] and microstimulation experiments in monkeys [27], the predicted outcome is that probabilistic tractography will estimate white matter pathways connecting face-selective regions with other face-selective regions to be more likely and of greater strength than pathways connecting face-selective regions with non-face-selective regions of equivalent size and distance. Alternatively, the shared functional property of face-selectivity across core perceptual face processing regions is the result of their shared white matter connectivity with some other functional region (e.g. connections between face- and object-selective region) or that connections closely track anatomical structures, rather than functional regions. Potential pitfalls include outcomes where probabilistic tractography estimates spatially homogeneous connectivity between all regions in the brain and/or idiosyncratic estimates of connectivity patterns that do not generalize across individuals. However, preliminary data suggests neither of these outcomes are likely (see Section C). Regardless of outcome, this study will be the first to provide estimates of the likelihood and strength of connectivity between core perceptual face-selective regions in human adults.

Specific Aim 2: Assess the development of white matter connectivity for core perceptual face processing regions from age 7 through adulthood

Aim 2a: Determine whether increases in the extent of face-selective gray matter in the typically developing brain are the result of changes in white matter structure immediately adjacent to these regions

Rationale: Postnatal cortical development is widely believed to consist of a process of ongoing synaptic remodeling until the pattern of synaptic connectivity becomes adult-like [17, 57]. Increases in the extent of face-selectivity in the FFA and OFA, but not pSTS, from age 7 through adulthood [3, 4] may therefore reflect increasing specificity in the pattern of white matter connections, revealing additional face-selective cortex in the anatomical penumbral region that does not show strong face-selectivity earlier in development. Consistent with this hypothesis is the finding that the anatomical penumbra surrounding the face-selective region in young children shows high levels of activity for both faces and objects [3]. Either pruning of connections to object-selective regions, or strengthening of connections to face-selective regions (e.g., through myelination) would result in increased face-selectivity in this penumbral region [58]. Both would result in increased structural coherence (increased FA), in the white matter adjacent to the region of face-selective gray matter.
Methods: Subjects will include twenty-four children ages 7-18, recruited from the local community. Behavioral and neuroimaging methods, including controls for non-face-specific, age-dependent confounds are as described in Section D1.

Analysis plan: For each of the three core perceptual face-selective regions, FFA, OFA, and pSTS, we will define two types of white matter ROIs: (1) a white matter ROI adjacent to the gray matter shown to have adult-like face-selectivity, which will be defined in all subjects, (2) a white matter ROI adjacent to gray matter in an anatomical penumbra surrounding the face-selective ROI that is expected to develop adult-like face-selectivity, which will be defined in children only. FA and MD will be computed for all ROIs and correlated with age. Potential outcomes and interpretations: If age-related increases in the extent of face-selective cortex are the result of increasing specificity in the pattern of white matter connections, we should find age-related increases in FA in white matter adjacent to the right hemisphere FFA and OFA, but not right hemisphere pSTS or left hemisphere face-selective regions because only those regions show age-related changes in spatial extent. Age-related increases in FA may be specific to the white matter adjacent to the latent face-selective penumbra only, the observed face-selective region only, or both; and may be accompanied by decreases in MD. Alternatively, there may be no significant age-related changes in FA, suggesting that increases in the extent of face-selective gray matter during typical development are either too small to be measurable with standard resolution voxel-wise DTI analysis, or that changes in intra-regional gray matter connectivity, not inter-regional white matter connectivity, are responsible. Regardless of outcome, this work will be the first to measure white matter structure adjacent to regions of face-selectivity in the typically developing population.

Aim 2b: Building on Specific Aim 1, assess the extent to which estimates of the likelihood and strength of white matter connections between functionally-defined core perceptual face processing regions changes across development

Rationale: We will repeat the analyses in Aim 1 in children age 7-18. We will determine the most likely pathways between the core face-processing network in children and adolescents and identify which white matter connections change with development and how these changes relate to improved proficiency in face processing. These white matter connections will be the focus of Specific Aim 2.

Methods: Identical to Aim 1, only the white matter connections of interest will be identified in children age 7-18.

Analysis plan: The experimental approach, using tractography methods 1 and 2, is identical to that described in Aim 1, with the exception that individuals will include children age from 7-18 as well as adults 21+. In addition to the factors of interest described in Aim 1, each test will now include the factor of age. In addition, analyses will be modified to controlling for non-face-specific, age-dependent functional and anatomical confounds, such as size of seed and target ROI.

Potential outcomes and interpretations: This work will be the first to determine the timecourse of changes in the likelihood and strength of white matter connections of the face processing network. We will examine whether these changes are correlated with known behavioral and neurophysiological changes during typical development. We predict that structural changes in the likelihood and strength of white matter connections likely to be involved in face processing will reflect pruning of connections to object-selective regions, or strengthening of connections to face-selective regions (e.g., through myelination). Therefore, we expect age-related increases in FA of these connections, and will examine correlations with behavioral face recognition performance, as well as size and selectivity of the FFA, OFA, and pSTS. One potential outcome would be that neither Specific Aim 2a nor 2b will find age-related changes in white matter structure. This would indicate that an adult-like pattern of connectivity can be found in the typically developing brain as young as age 7, and that the timecourse of development in anatomical connections of the face processing network may be more subtle in magnitude or earlier developing in timing than was expected given the neurophysiological changes during this time period [3-5, 42].

Projected timeline
Present – Dec 2009: Data collection
Present – Feb 2010: Aim 1, data analysis and manuscript preparation
Feb 2010 – Sep 2010: Aim 2b, data analysis
Sep 2010 – Feb 2011: Aim 2a, data analysis
Feb 2011 – Sep 2011: Manuscript preparation, Aim 2a and 2b
E. HUMAN SUBJECTS

Protections for Human Subjects

**Subjects:** A total of 36 participants will be recruited for this study: 12 adult participants (>21 years) and 24 children age 7-18.

**Inclusion criteria:** We will include males and females.

**Exclusion criteria:** Metal implants in the body, dental work involving metal, psychosis or pervasive developmental disorder, treatment with psychometric medication, overt neurological injury or disease, seizure disorder, Tourette’s syndrome, obsessive compulsive disorder, conduct disorder, oppositional defiant disorder, anxiety disorder, depression and cardiac disease.

**Sources of Research Material:** The bulk of the research material will consist of MRI image files and behavioral performance.

**Human subjects involvement:** In the fMRI experiments, subjects will lie on a table within the magnet for less than 45 minutes while MRI scans are acquired. They will view a standard computer monitor (via a mirror). Images of the brain will be made in one or more of the following conditions:

1. Subjects passively perceive stimulus information (e.g., visually presented patterns, visually or auditorially presented words).
2. Subjects perceive stimuli and are required to make a response (e.g., a button press) to the stimuli perceived.
3. Subjects move specific regions of their body (e.g., tapping fingers).
4. Subjects perform cognitive activity, e.g., making a decision about a visual stimulus.

**Recruitment and Consent Procedures:** Children will be recruited through advertisements in local newspapers, parenting magazines, and in the parents-Teacher Association newsletter of the Palo Alto School District and from local private schools. Adults will be recruited from advertisements in student newspapers of Stanford University and local community colleges.

Each child’s family will be mailed a packet including information material. These will explain our aim to “study brain mechanisms for remembrance of pictures”, describe the procedures of the proposed project and emphasize the voluntary nature of participation, and include consent forms. This will be followed up by a telephone call from one of the Ph.D. level investigators, for the purposes of answering questions and clarifying information included in the packet. Families and children will be invited to view the facility housing the MRI scanner and scanning in progress. Written consent will be obtained from parents and children for child participants, and from adult participants.

1. **Risks to the Subject:**

The potential risks from this study to the subject will be minimal. There are no known significant risks with the MRI procedure. Due to the rapid rate of change of magnetic field gradients that can be attained with this imaging system the possibility exists for peripheral nerve stimulation which would result in twitching. Dizziness and nausea may occur if the head is moved within the bore of the magnet. There is a risk of heating from the radiofrequency coils, the cables of the coils, the cables from response boxes and other accessories, and the cables from physiological monitoring devices. The most serious potential risks are related to the possibility of ferromagnetic objects in the vicinity of the high-field magnet in the scanner. These objects could conceivably become projectiles due to the powerful magnetic field.

2. **Adequacy of Protection against Risks:**

**Recruitment and informed consent:** The procedures will be in compliance with the safety guidelines for MRI research. Subjects will be informed of the risks and benefits of the study, and must sign an Informed Consent Form prior to participating in any study. Subjects will have the right to withdraw at any time without prejudice or
affect on their medical care. The human subjects protocols will be reviewed annually by the University's IRB. There will be no costs to the subjects for participation in this study. Any data that may be published in scientific journals will not reveal the identity of the subjects. Families of the volunteered children will receive a copy of all the consent forms including the information on potential risks at least a week prior to the scheduled experiments.

**Protection against risk:** To insure subject safety every researcher in the lab must complete a safety training course, every year. These safety courses are offered by the staff at the Lucas Center for Magnetic Resonance Spectroscopy and Imaging on the Stanford University campus. Subjects will be carefully screened to make sure they do not have any metal before being taken into the room with the MRI scanner. Subjects will wear earplugs to protect their hearing while in the MRI scanner. Volunteers will be excluded for the usual contraindications to MRI exams, including pacemakers, surgical aneurysm clips, and known metal fragments embedded in the body, including eyes. Women of childbearing age will not be included in this study if they might be pregnant. Subjects who are unable to attend to or respond to stimuli as required by the experimental protocol will also be excluded. Subjects with suspected cerebrovascular or pulmonary disease or a history of such will be excluded. Patients with a history of migraine, arterial hypertension, coronary heart disease, asthma, anemia, or epilepsy will also be excluded.

Subject data will remain confidential. All collected data will be coded and stored without the person’s name.

### 3. Potential Benefits of the Proposed Research to the Subjects and Others

Given the paucity of the risks in the present study, the potential benefits to society are salient. There are no direct benefits to the individual participants. No clinical trials are proposed.

### 4. Importance of the Knowledge to be Gained

Understanding the normal perceptual, cognitive and neural mechanisms of face and object perception is a prerequisite to deeper understanding of a variety of developmental disorders such as autism, Williams Syndrome, and a number of learning disabilities. Thus, the proposed experiments address significant gaps in our knowledge in these areas. Importantly, the proposed use of fMRI and DTI will facilitate future research involving adult and pediatric imaging.

**Inclusion of Women and Minorities:**

We plan to recruit females as half of our participants in every age group. This research subjects will include a maximum of 24 child subjects ages 7-18, and 12 adult subjects age 21 and older, equally divided between males and females, living in the San Francisco Bay Area. Efforts will be made to represent the ethnic diversity of the Bay area in the sample, 55% Caucasian, 20% African-American, 15% Latina, 1-2% Native American, and 8-9% Asian/Pacific Islander.

**Inclusion of Children:** Given our goal to study the development of face and object recognition between ages 7 to 18, we will recruit 24 participants in this age range.

**Data and Safety Monitoring Plan:** No clinical trials are proposed. Subject data will remain confidential. All collected data will be coded and stored without the person’s name.

### F. BIBLIOGRAPHY AND REFERENCES

4. Golarai, G., et al., *Evidence for development of face-selective and place-selective cortex during*


34. Behrens, T.E., et al., Probabilistic diffusion tractography with multiple fibre orientations: What can we

G. Respective Contributions

This proposal was written by the applicant. Background information on face processing development, empirical hypotheses and design of fMRI, DTI, and behavioral experiments, were developed and written by the applicant.
in consultation with the sponsor, Dr. Kalanit Grill-Spector and the co-sponsor, Dr. Robert Dougherty. Preliminary data was collected and analyzed by the applicant with the supervision of both sponsors.

H. Selection of Sponsors and Institution

Kalanit Grill-Spector was selected as the primary sponsor due to her international reputation as an expert in fMRI approaches to face processing and high-level vision. Her work with Golijeh Golarai was the first to report age-related changes in the extent of face-selective cortex. This work was a key motivator of the proposed research, and one of the main reasons I joined the lab to conduct my dissertation research.

Robert Dougherty was selected as co-sponsor of the proposal due to his central role in pioneering DTI fiber tractography software, validating deterministic and probabilistic tractography algorithms, developing novel methods for modeling and interpreting diffusion MR measurements, and relating white matter development to high-level vision (reading). He has extensive experience developing open-source software for the analysis of functional and diffusion MR research and his technical expertise in all aspects of a DTI research study, from acquisition to preprocessing to analysis design to interpretation, is indispensable to the proposed research.

Stanford University is an ideal institution in which to conduct this pre-doctoral training due to its wealth of resources. In addition to my listed advisors, I will benefit greatly from consultation with other faculty such as dissertation committee member Brian Wandell, an internationally recognized expert in functional and diffusion MR research in visual neuroscience; James McClelland, a pioneer in learning models of cognitive development; and Anthony Wagner, a leading cognitive neuroscientist. Additionally, Stanford University is home to one of the most well-equipped and supported neuroimaging facilities in the nation, the Richard M. Lucas Center. Stanford is also home to the academic community provided through a vibrant series of interdisciplinary seminars and colloquia that provide opportunities for conceptual discussion, methodological training, and exposure to clinical concepts. Together, these experiences will help me learn to relate my research to mental health issues and mental disorders that are the mission of the NIMH.

I. Responsible Conduct of Research

Stanford University provides a considerable amount of training in the responsible conduct of research. I have participated in and completed the following official training components: (1) Stanford Psychology Department Human Subjects Orientation – every fall, the university’s human subjects research manager gives a detailed overview of the guidelines set forth by the Stanford IRB to enforce university and federal policy for the protection of human subjects. (2) Stanford Human Subjects Tutorial – prior to any interaction with human subjects or human subjects data collected, all students, faculty, and staff must complete an extensive online tutorial. This tutorial covers all topics related to the responsible conduct of research including conflict of interest, adverse report handling, data handling, human subjects policies, the responsibilities of researchers, and the responsibilities of the institute as a whole.

In addition, I have demonstrated my commitment to the responsible conduct of research by organizing a departmental workshop on “Professional Ethics and Scientific Integrity,” in which students, faculty, and university ombudspeople gathered to discuss how to agree on and implement individual, research group, and institutional level standards of professional conduct. The goal was to encourage scientific integrity in the face of the many pressures researchers face in academia and to efficiently identify and rectify errors and misconduct—and better yet, discourage them from happening in the first place. The participants maintain an informal email list to discuss ongoing issues of professional standards and scientific best practices.