This lecture will give an overview of our progress in using noninvasive brain imaging to study the developing brains (and minds) of children as they are growing up.
I will touch on these 4 topics – by “neural architecture” I mean the biology and morphology of brain structures and tissues, e.g., the cerebral cortex and subcortical gray matter structures, as well as the connecting fiber tracts between different brain structures that make up the white matter of the brain.
Imaging Studies of Postnatal Brain Development

The neural architecture undergoes continuous remodeling, not just during the preschool years, but throughout childhood and adolescence.

The images in this slide are just different kinds of MRI – all 4 of the same section of a child’s brain – illustrating that different kinds of MR imaging can be used to image different tissue characteristics.
What We Thought About Development of Brain Structure Before MRI

- Neuronal populations and their connections (the basic architecture of the brain) are established during the prenatal period.
- Soon after birth oligodendrocytes proliferate, differentiate, and wrap the connecting axons with insulating, fatty, sheaths of myelin.
- This more or less completes the development of the hardware, and little additional growth of the brain occurs after age 5 or 6.

The picture here shows a cell, called an oligodendrocyte, and how it wraps “myelin sheaths” around the axons of neurons. This fatty myelin sheath dramatically improves the conduction of electrical signals down the axon. These signals are one way that neurons in one part of the brain send information to other neurons elsewhere.
These images of a 5 year old are cross-sections through the same brain taken in 3 different planes of section, and with 3 different MRI methods. They show the basic structure of the brain: where the gray and white matter structures are, what the folding cortical surface looks like, etc. They are a bit like faces, everyone's looks a bit different – but it would be hard to know whether this one was a child or an adult just by looking at it.
The slide before this one showed that in kids – especially after about the age of 3 – it is very hard to tell how old they are just by looking at the MRI – especially since everyone’s brain looks different. So the research in this area was helped tremendously by the development of more sensitive, and quantitative, techniques for measuring subtle features of the brain’s structure, tissue characteristics, and architecture. This slide illustrates three approaches that have been used, among many that are now available. The three vertically arranged slices top left show a method that requires an expert anatomist to carefully draw around the anatomical boundaries of brain structures visualized in the images. The four panels top right illustrate a method that uses some input (what we call supervision) from a trained anatomist but uses computer algorithms to separate the gray matter, white matter, and fluid areas of the brain, and to facilitate the measurement of the sizes of different brain structures. The bottom two images were produced using no “supervision” but they use a very intelligent algorithm, based on analysis of many images that were segmented by supervisors. This “intelligence” can now be used to segment any brain automatically into the principal structures, in this case the major parcels of the cortex. This method was developed and is used in our colleague Anders Dale’s laboratory.
These are the predictions we made about what MRI would show us about normal brain structure (across the lifespan) when MRI first became available. We were mainly looking at brain scans of normal people of different ages in those days to try to figure out when the atrophy of old age sets in, and how that relates to the presence of dementia.

**Predictions Based on Conventional Views of Brain Morphology**

- Adult brain structure in school-aged children.
- Stable brain morphological characteristics across childhood, adolescence, and adult years.
- Atrophy of some brain structures in old age.
So at this early stage in MRI research we set out to measure brain structures from the MRIs of living people; for example, the total volume of the gray matter in the cerebral hemispheres (the dark gray regions in the picture here of the cerebrum). The curve shown here (with no data points) expresses graphically what we were expecting – stable values in children and adults, but a dip in the values as the atrophy of older age occurred.

The second view here (the curve with blue circles showing the actual observations) is what our research actually showed! Not only was there never a time in the lifespan when the gray matter volume was stable, the most dramatic age-related changes were in the school-aged children we studied – between 6 and 20.
Now, a couple of decades after we did this early research, we can use advanced morphometric techniques to map the changes in the architecture of the cortex – here we summarize the results using some data from a very large study of typically developing children recruited in a multi-site study (called PING) conducted at 10 different universities around the country.

The top row shows how the surface area of the cortex expands (yellow and red) and then begins to contract later (blue), and how these changes show somewhat different time courses in different parts of the cortex.

The middle row shows that the change in the apparent thickness of the cortex shows a different pattern – getting thinner throughout the entire period – though again, at different rates in different parts of the cortex.

The volume (bottom row) is just the surface area multiplied by the thickness.

Clearly, the architecture of the cortex is changing dynamically throughout development, but little is known about exactly what biological factors underlie these measurable changes on MR images.
Just a movie of the changes.
This is just a slide to show that it is not just the cerebral cortex that changes with age. There are also changes across the same age range in subcortical gray matter structures like the hippocampus and the thalamus (again, data are from the large multi-site study called PING).
At the same time that these gray matter changes are occurring, the volume of the white matter is increasing so that the proportion of the total brain that is white matter is steadily increasing. This is presumably because the oligodendrocytes are continuing to myelinate the axons that connect the neurons to other neurons throughout the brain.
Myelination of axons occurs after birth, when virtually all of the brain's neurons are in place and their axons are already connecting them to each other. However, myelination and many other forms of maturation of the neural circuitry will continue for many years. The myelination of fiber tracts is so dramatic in the first year of life that it can easily be seen in the changing contrast on MR images. The top row shows the gradual spreading out of the BRIGHT areas (myelinated white matter) on one type of MRI, and the bottom row shows the gradual spreading out of DARK areas on another type of MR images. From left to right the images show brains from infants ranging from 3 months to one year of age. After this the changes continue but are more subtle visually.
The imaging results I have shown so far were from conventional structural MRI. However, another kind of MR imaging, called diffusion weighted imaging, provides much more information about the brain fiber tracts, and works by measuring the degree and direction of the diffusion of water molecules within the brain tissue.

Diffusion imaging provides new information about brain connectivity
Maturation-dependent microstructure length scale in the corpus callosum of fixed rat brains by magnetic resonance diffusion-diffraction (Weng et al., 2007)

Increase in size and myelination in corpus callosum axons of rats 84 (left) and 21 (right) days old.

This large image shows cross sections of the corpus callosum in rats. The corpus callosum is the large fiber structure formed by the axons connecting the right and left hemispheres of the brain (bright structure in midline of the human brain shown top right). The two right panes of the large image are from a 21 day old rat, and are at 2 levels of magnification. The two panes on the left are from an 84 day old rat and at the same magnification. The axons are the circular structures and are viewed from the cut ends – like looking at the end of a bunch of spaghetti. The black outer rings of the axons are myelin. What is clear is that the axons of the 84 day old rat are larger in diameter and more consistently and thickly myelinated. There is also less space between the axons in the 84 day old rat. Although we know much less about the development of human than rat fiber tracts, we assume changes a bit like this happen in the postnatal human brain. These changes produce substantial alterations in the diffusivity of water molecules in and around the axons.
This slide shows more data from PING – the left image shows the position of a major fiber tract in the human brain – called the superior longitudinal fasciculus (SLF). The plot shows the slow protracted reduction in ADC – a measure of diffusivity – in this fiber tract. This is probably mostly because there is so much less space (water) around the axons as they grow and mature – thus less diffusivity overall.
This is another measure – called fractional anisotropy, or FA – from the same tract, in the same children from PING. As is often observed, this measure of the “directionality” of the diffusivity increases as the fiber tracts mature – because diffusivity is greater along the long axis of the maturing tracts – that is, it is less random in direction.
This just shows that many different fiber tracts in the brain exhibit similar changes in FA (directionality of diffusion) with age, but each seems to have its own unique trajectory.
So far, we have seen that MRI allows us to monitor lots of changes in the biology and structure of the brain over the childhood and adolescent age range. But we still don’t know how much of what we are seeing is due to the ongoing myelination of the different kinds of connections in the brain (e.g., between thalamus and cortex, between other subcortical structures and cortex, or between cortical neurons and other (distant or nearby) cortical neurons.
Although there are robust age-related changes in many structural measures from the brain during development, there is also lots of variability in these measures even among children of the same age. We are asking several questions about this variability.
Many investigators are asking whether the brain variability is related to behavioral variability, but at this point I’d like to highlight a few of the recent results from our research linking behavioral variability in children to multimodal imaging results.
One study from the PING consortium looked at the children’s performance on a “flanker” task, in which they had to press a right hand button if the center fish on a display like these was facing right, and a left hand button if it was facing left. We are all faster pressing the correct button when all of the fish are facing the same way than when the “flanking” fish are facing in the opposite direction from the center fish. But children show a larger difference (interference effect) than adults. We observed that children who showed less interference from the incongruent flankers had expanded surface areas in the (blue and green) region, called the anterior cingulate cortex, which has been linked to “conflict resolution” in adults. So there is an association between the pattern of cortical area expansion (shape of the cortex) and individual differences in children’s behavioral performance.
In another study I work on with Danish colleagues in Copenhagen, we have observed several associations between scores on cognitive tests and diffusion imaging measurements from the brain fiber tracts.
One of the tests we give to the kids measures the time it takes children to “cancel” a primed motor response (button press) when they hear an auditory signal indicating they should STOP. We know from other studies, mostly in adults, that particular brain structures seem to play an important role in this kind of response inhibition. So we looked at the diffusion measures in the tracts that connect those areas.
These figures just show that children with higher FA (directionality of diffusion) in those tracts had better response inhibition.
Another task required the kids to play a computer game and try to find the positions (designated by light blue) where tokens (dark blue) were hiding. When they found the dark blue tokens they could drag them over to the side bar. But the real challenge was to remember not to look in (click on) locations where a token had already been found – because those never had new tokens. Kids get much better with age at remembering the locations they should not return to.
This slide shows that the better they are at remembering (that is, the fewer errors they make), the higher the FA (directionality of diffusivity) in the left superior longitudinal fasciculus, a tract implicated in these kinds of functions in other studies using other methods.
In summary, the Danish studies show that variability in different cognitive functions is reflected in diffusivity differences in different parts of the fiber structure.

Differences in tracts to right inferior frontal lobe (yellow) related to response inhibition, from parietal to frontal lobes (blue) related to spatial processing, ascending sensorimotor fibers (aqua) related to reaction time.
It is not only cognitive differences that show an association with the brain’s architecture, in PING we also observed an association between anxious temperament and areal expansion of the ventromedial prefrontal cortex (area shown in pink), and the relationship suggested that perhaps children with anxious temperament exhibited a later expansion of this region.
Behavioral Variability is Related to Neural Architecture in Children

- Cognitive and emotional differences correlate with structural characteristics in different cortical regions and fiber tracts.
- The associations remain after controlling for age and global parameters.
- It appears that profiles of behavioral attributes are reflected in neural architectures.

This summary is pretty much self-explanatory. By global parameters, I mean the mean or total values for the brain as a whole, such as total surface area of cortex or mean FA averaged across all brain fiber tracts. The fact that the associations remain after controlling for age means that the associations are present even among children of the same age.
So important questions remain about how these associations arise. Could genetic factors produce different patterns of brain structure that then influence the functional characteristics? Or could the experience and learning of the child change the way the neural architectures develop? Or both?

How should we interpret these associations?

- To what extent might they reflect early patterning in the nervous system due to genetic variability?
- To what extent might they reflect cumulative neuroplastic effects on the neural architecture associated with experience and learning?
Since we are controlling for chronological age in these studies, we are basically looking for variability among children of the same age. If children’s brain architectures develop at different rates, then at any given age there will be variability in these brain parameters from one child to the next, even if the parameters all reach about the same asymptote later on… So maybe some of these associations are due to differences in the developmental trajectories of individual children. Of course, they could also be due to differences like those illustrated in the lower image – differences that remain the same across development, even though the parameter may be developing rapidly. Such differences could be due to genetic factors, for example.
We know from much research that brain structure is influenced by genetic variation. Identical twins, who are genetically identical, have brains that, like their faces, look much more alike than those of fraternal twins (who are no more similar genetically than other siblings). But other research tells us that the links between genetic variation and brain architectures is likely to be very complex – MANY, MANY genetic variants are likely to influence brain characteristics.
Arealization of the Mammalian Cortex

- Has been linked to gradients of gene expression (transcription factors) in the neocortical proliferative zone during the embryonic period.
- Transgenic (loss and gain of function) models have shown that alterations of these gradients lead to different relative sizes of the primary sensorimotor regions in the later developing cortex.
- Common (normal) genetic variants may contribute to individual differences in cortical arealization.

Arealization here means the determination of some cortical areas as visual cortex, others as auditory, somatosensory, or motor cortex. We know from basic research in developmental neurobiology that our brains, like the rest of our bodies, are patterned through complex molecular and cellular interactions guided by the action of genes but also involving interactions with the environment.
MRI data from 400 middle-aged male veterans from the Vietnam War Era, who were monozygotic or dizygotic twins, were obtained. Conventional twin methodology was applied to compute the genetic correlation of a measure of cortical surface expansion at each cortical vertex location (2500 vertices after smoothing) with the measure at every other vertex. This matrix of genetic correlations was then further analyzed using a data-driven, fuzzy clustering method to identify sets of vertices with relatively higher genetic overlap. This figure visualizes the pattern obtained for the 4 cluster solution on an inflated cortical surface for comparison with the pattern of cortical arealization, now well established in mouse brain to be under control of gradients of gene expression of specific transcription factors in the cortical primordium. This was a complex study, but the main point is that the human pattern suggests that common genetic variation (that is small, relatively frequent, differences in our genomes) probably gives rise to some individual differences among us in the shape of our cortex.
Further analyses in this study showed that the genetic factors that influence relative sizes of each of the color-coded regions shown above are distinct from the genetic factors that influence relative sizes of the others.
Are the associations related to activity-dependent neuroplastic changes in the tissue?

But is there evidence that brain structural differences could be driven by differences in our experiences and brain activity?
Two famous “juggling” studies in adults suggest that learning a taxing new skill can change the biology of both the cortex and the underlying white matter tracts. And other studies have provided further evidence for these activity-related changes in structural brain measures.
One study of particular interest to me showed that 6 months of remedial reading instruction in 8 to 10 year old children with reading difficulties was associated with increased FA in tracts underlying the medial superior frontal cortex – a site very similar to the region where poor readers initially showed lower FA than good readers.
Conclusions

- There is evidence that biological development of brain tissues continues throughout childhood and adolescence.
- The biological changes can be linked to individual differences in behavior in developing children.
- There is much left to learn about the meaning of these associations – i.e., about how genetic variation, experience, and other environmental factors interact to influence the developing mental characteristics (and individuality) of a child.

These conclusions are pretty self-explanatory.
Our research is conducted in the Center for Human Development – come and visit us on the 5th floor of AP&M.
Study Probe 1

• A functional consequence of myelination is:
  – A) To glue fiber tracts together
  – B) To improve conduction of electrical signals by axons
  – C) To reduce the water molecules in the space around the axons
Study Probe 2

- The surface area of the cortex:
  - A) Declines continuously across the age range from 3 to 20 but at different rates in different parts of the cortex.
  - B) Expands until middle childhood but at different rates in different parts of the cortex then contracts, again at different rates in different regions.
  - C) Expands continuously across the age range from 3 to 20 but at different rates in different parts of the cortex.
Study Probe 3

- Which is true of the development of brain fiber tracts:
  - A) Diffusivity declines during development, probably in association with reduced water in the space between axons.
  - B) Diffusivity increases in fiber tracts during development in a tract specific manner.
  - C) Directionality of diffusivity in fiber tracts becomes more random as development proceeds.
Study Probe 4

- Which is true of correlations between behavioral individual differences and individual differences in brain structural measures:
  - A) Better performance on multiple behavioral measures is associated with global increase in FA (directionality of diffusivity)
  - B) Associations are observed between cognitive measures and surface area differences, but not fiber tract parameters.
  - C) Individual differences in specific behavioral characteristics are generally associated with brain structural measures from the neural systems previously implicated in those functions.
Study Probe 5

- Associations between brain structural differences and behavioral differences:
  - A) Imply that behavioral differences are due to differences in the underlying neural substrate for behavior.
  - B) Mean that differences in the way the brain was patterned in the embryo give rise to differences in behavioral development.
  - C) Leave open the possibility that differences in genetic patterning, differences in experiences and environmental exposure, and differences in the pace of brain development may play a role in these associations, as may other factors.
Study Probe 6

- The evidence regarding genetic influences on brain morphology suggests:
  - A) That brain structure is not highly heritable and most differences are due to effects of experience on the brain’s development.
  - B) That genetic variation has a strong influence on brain structure and many genetic variants are likely to contribute to this influence.
  - C) That a few genes give rise to the morphology of the brain during embryonic life.
Study Probe 7

- Studies of neuroplastic effects on brain structure:
  - A) Suggest that intensive training on motor and cognitive tasks can alter biological measures in gray matter and fiber tracts.
  - B) Suggest that because genetic factors control brain structure, experience has little neuroplastic effect.
  - C) Suggest that differences in brain morphology are mostly due to neuroplastic effects of life experiences.
Key to Study Probes

- 1,B
- 2,B
- 3,A
- 4,C
- 5,C
- 6,B
- 7,A