neurons, but only for stimuli placed within the inactivated region of space [12]. This highlights LIP's role in analyzing stimuli and/or directing attention toward stimuli within their RFs. An interesting follow-up to the Katz study will be to silence LIP using the same stimulus configuration under which MT was tested; namely, by placing the motion stimulus, not one of the saccade targets, in the RF (Figure 1A).

By using reversible inactivation to reveal a dissociation between decision-correlated neuronal responses and their causal impact on behavior, the Katz study presents an important challenge to understanding the mechanisms of perceptual decisions. Deploying emerging new approaches for large-scale monitoring and precise manipulation of neuronal activity across brain networks that span the sensory-motor continuum offers new opportunities to meeting the challenge. The coming years offer a particularly fruitful period in uncovering neural circuit mechanisms of decision-making.

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Forum

Intergenerational Neuroimaging of Human Brain Circuitry

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Neuroscientists are increasingly using advanced neuroimaging methods to elucidate the intergenerational transmission of human brain circuitry. This new line of work promises to shed light on the ontogeny of complex behavioral traits, including psychiatric disorders, and possible mechanisms of transmission. Here we highlight recent intergenerational neuroimaging studies and provide recommendations for future work.

Extensive work identifying risk genes indicates that complex behaviors (e.g., depression, anxiety) in humans are in part heritable [1]. Evidence that parental behavior and experiences (e.g., trauma exposure) can lead to **epigenetic** changes in offspring nevertheless indicates that **intergenerational transmission** of traits and behaviors includes both genetic and non-genetic (epigenetic, environmental) influences [2,3]. Genetic and epigenetic effects, however, occur at the molecular level and

Glossary

Cross-fostering: a study design wherein offspring are removed from their biological parents at various stages of development and raised by surrogates. This design has the potential to disentangle genetic from prenatal and postnatal environmental effects [3,12].
Endophenotype: a stable phenotype that is heritable, co-segregates with the illness of interest, is not state dependent, is present at a higher rate within affected families, can be reliably measured, and is specific to the illness of interest [4].
Epigenetic: regarding changes in the microstructure or expression of genes (e.g., DNA methylation, histone modification) without altering the DNA sequence. While parental experience and environmental effects (prenatal and postnatal) can lead to epigenetic changes in offspring, whether acquired epigenetic changes can propagate through the germline and cause behavioral change in subsequent generations in humans remains controversial [3].
Genetic correlation: the proportion of the variance in two traits that is due to genetic causes.
Heritability: the amount of variation in a phenotypic trait that is attributable to genetics and therefore not specific to intergenerational (i.e., parent to offspring) effects, which may include non-genetic effects.
Intergenerational transmission: he transfer of traits from parents to offspring, including genetic and non-genetic influences. For example, the impact of prenatal effects (e.g., parent nutrition, *in utero* environment) as well as postnatal rearing effects and other environmental factors could lead to epigenetic or behavioral changes in the offspring, which are thereby intergenerationally transmitted.
Kinship matrix: a matrix representing the probability that a random gene is identical by descent in pairs of related individuals (e.g., identical twins have approximately 100% probability, parent–offspring have approximately 50% probability).
Mega-analysis: because meta-analyses are limited in detecting effects since summary statistics are computed from each cohort separately, this technique for combining post-processed data from independent studies into a single analysis is more powerful and allows more complex analyses.
Meta-analysis: a statistical technique for combining results from independent studies without requiring raw data. The weights of effect sizes are based on the precision of the effect-size estimates per study. Generally, the precision of the effect size is directly related to the study's sample size; thus, sample-size-weighted estimates are often used in meta-analyses [7].
Parent-of-origin effects: when the phenotypic effect of an allele depends on whether it is inherited from the mother or father; typically characterized through epigenetic mechanisms of genomic imprinting. Parent-of-origin effects are implicated in complex trait variation.

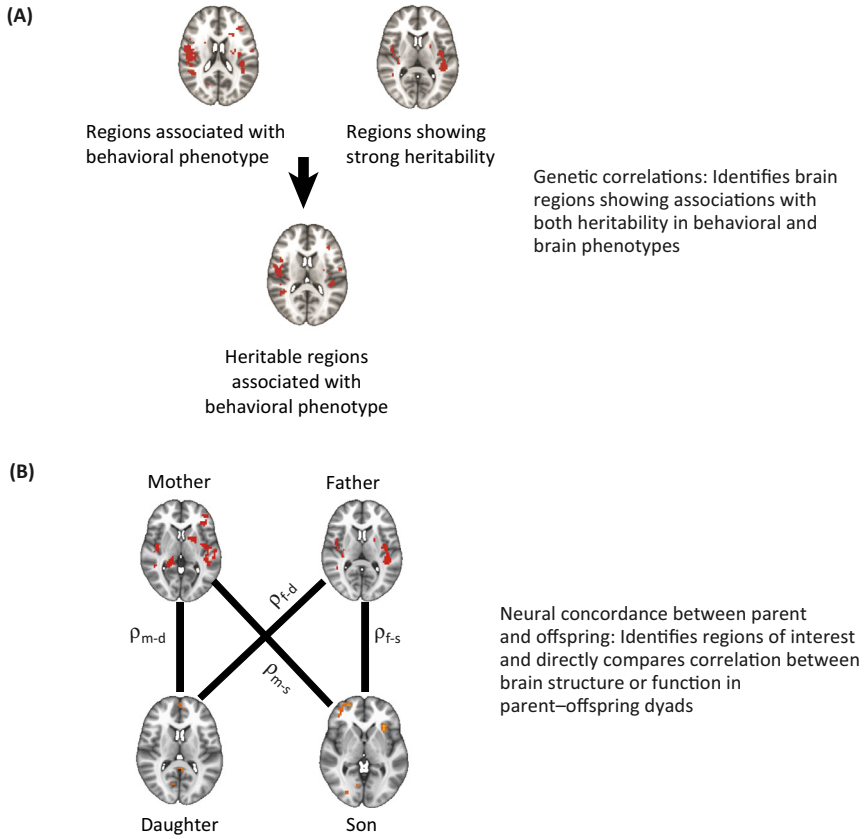
are distal from complex behavioral phenotypes [4]. Intermediate phenotypes or **endophenotypes** at the level of brain circuitry lie in the lacuna between DNA sequences and clinical symptoms and presumably have a simpler molecular basis than disease states, thereby allowing researchers to focus on delineating the neurobiological architecture specific to the illness [4]. Thus, understanding the intergenerational transmission of brain circuitry by examining similarity or concordance of endophenotypes in parent–offspring dyads may shed light on the inheritance mechanisms involved in complex behavioral traits, the pathophysiology of brain-based diseases, potential biomarkers of treatment success (e.g., increased myelination in corticolimbic tracts), and modifiable targets (e.g., prenatal nutrition) for interventions.

Here we highlight recent neuroimaging studies that advance our understanding of the intergenerational transmission of human brain circuitry, with a focus on endophenotypes for psychiatric disorders. We discuss the strengths and limitations of each approach and offer recommendations for future research.

Consortia pooling genomic and neuroimaging data from multiple sites have been important in generating normative data across diverse populations and identifying potential endophenotypes of psychiatric disease [5]. The ENIGMA Consortium, for example, has applied standardized preprocessing protocols to diffusion imaging data from five large twin/sibling studies and one extended pedigree study [6]. Researchers then computed **heritability** estimates of fractional anisotropy (FA), a quantitative index of white matter properties useful for understanding tract organization, using **meta-analysis** and **mega-analysis** approaches. In both approaches, the variance of the brain phenotype of interest, FA, was modeled by the sum of the variance due to additive genetic factors and the variance due to environmental effects (shared and individual). The additive genetic effects were estimated from correlations among family members, structured by a **kinship matrix**, and heritability was computed as the ratio of additive genetic variance to total phenotypic variance. Researchers found significant heritability effects in whole-brain and tract-specific FA across all cohorts (although cohort-specific effects were also found), with the highest heritability in the corpus callosum and the lowest heritability in the fornix. Importantly, these studies identified whole-brain and tract-specific FA as potential endophenotypes for future imaging genetics studies investigating psychiatric disorders. These studies, however, relied heavily on twin/sibling data, which do not provide parental information and therefore cannot directly assess intergenerational effects. Furthermore, different correlation structures depending on the family design (e.g., including grandparents or cousins) will yield different heritability estimates that may have an impact on meta-analytic approaches, which assume that larger cohorts yield more precise heritability estimates [7]. Assuming equal sample sizes, twin designs provide more precise estimates of heritability, but a sufficiently large extended pedigree design has the advantage of better estimating the covariance structure in a kinship matrix and providing heritability estimates that are less likely to be inflated by the effects of shared environment [7].

Some researchers have begun to estimate shared heritability of brain and behavior phenotypes using extended pedigree designs. For instance, in a multiplex–multigenerational study of people with schizophrenia, Roalf *et al.* used a standard measure to compute heritability and modeled each individual's regional brain volume (or shape) as a function of additive genetic effects estimated from correlations among family members, individual-specific residual environmental factors, and covariates (age, sex, site); the authors found significant heritability effects in limbic volume and shape, suggesting these to be potential endophenotypes for schizophrenia [8]. Similarly, Fox *et al.* measured FDG-PET and behavioral responses during a well-standardized task of threat processing in a large familial sample of preadolescent rhesus monkeys [9]. The authors computed the heritability of brain metabolism, the heritability of a behavioral anxiety phenotype, and the bivariate heritability of both phenotypes, then conducted voxelwise bivariate **genetic correlations** and found strong associations between metabolism in a prefrontal–limbic–midbrain circuit and anxious behavior. Therefore, using neuroimaging data to conduct genetic correlations is a powerful way to identify brain regions that share genetic factors with behavioral traits (Figure 1A). Extended pedigree designs, however, are more susceptible to uncontrolled age-related influences (which we discuss further below when discussing general limitations and future directions) and are more logistically difficult to recruit (the sample studied by Fox *et al.*, while representative of rhesus monkey families who interbreed, is not typical of human families). Nevertheless, we expect that future intergenerational neuroimaging studies in humans utilizing extended pedigree designs will be poised to identify robust endophenotypes.

Although we anticipate that large studies with extended pedigree designs will aid in identifying robust intergenerationally transmitted endophenotypes, other researchers have directly measured the concordance of an endophenotype of interest between parent–offspring dyads using smaller cohorts that are more logistically feasible. Foland-Ross *et al.* compared cortical thickness measurements in two groups of mothers (depressed, non-depressed) and their non-depressed daughters (categorized accordingly as high or low risk) [10]. Cortical thickness in regions of interest (ROIs) comprising fusiform cortex that showed significant differences between depressed and non-depressed mothers were computed for each daughter; hierarchical linear



(C)

	Homologous Surrogate	Donor Egg	Heterologous Surrogate
Genetic	X		X
Prenatal	X	X	
Postnatal	X	X	X

IVF as a natural cross-fostering design: Permits assessments of genetic, pre-natal, and post-natal effects on parent-offspring brain phenotypes

Trends in Neurosciences

Figure 1. Schematic of Intergenerational Imaging Designs and Methods. Images are illustrative only. (A) As in Fox *et al.* [9], the heritability of behavioral and brain phenotypes can be used to compute genetic correlations in neuroimaging data to identify regions where there is shared genetic influence common to the two phenotypes. (B) As in Yamagata *et al.* [12], neural concordance among all parent–offspring dyads as measured by correlations can be compared and sex-specific tests can be performed. (C) Future directions: natural cross-fostering using *in vitro* fertilization (IVF) permits the assessment of genetic, prenatal, and postnatal influences between parent and offspring by comparing parents who have offspring through homologous surrogacy (mother is egg donor and birth mother), donor egg pregnancy (mother is not egg donor but is birth mother), or heterologous surrogacy (mother is egg donor but not birth mother).

regression was then performed with the mother's cortical thickness and risk status as predictors of regional cortical thickness. The authors found that cortical thinning in depressed but not non-depressed mothers significantly predicted cortical thinning of the same regions of fusiform cortex in their daughters. While these results suggest that cortical thinning is a matrilineal endophenotype of depression risk, no other dyads (e.g., father–daughter) were assessed.

In the first study to test sex-specific intergenerational effects of human brain structure, Yamagata *et al.* examined gray matter volumes (GMVs) in a voxelwise manner in biologically related parent–offspring dyads: mother–daughter, mother–son, father–daughter, and father–son [11]. Voxelwise statistical maps within the corticolimbic ROI comparing the GMVs of mother–daughter dyads with other dyads showed stronger positive correlations between mother–daughter dyads in the amygdala, hippocampus, and prefrontal cortex, suggesting female-specific transmission of this circuitry and consistent with other work strongly implicating corticolimbic circuits in mood and anxiety disorders [1]. While this approach differs from a sex-specific kinship matrix in that the proportion of shared genetic information among individuals is not modeled, this approach by Yamagata *et al.* represents an important next step in this area of research by assessing sex-specific transmission patterns (and possibly **parent-of-origin effects**) and is ideal for investigators with specific hypotheses regarding the mechanisms of intergenerational transmission of brain circuitry (e.g., matrilineal versus patrilineal transmission; Figure 1B).

Nevertheless, the relative contributions of genetic, epigenetic, and environmental factors to the intergenerational transmission of brain circuitry are unclear. Moreover, the developmental stage (prenatal, postnatal) at which these different influences are instantiated is unknown. In animal work, **cross-fostering** designs are used to disentangle inherited factors from prenatal and postnatal influences [12]. Although human studies cannot randomly assign offspring to prenatal conditions, the rapidly increasing number of children born via *in vitro* fertilization (IVF) (1% of ~4 million newborns in 2010 in the USA) with surrogate parents now makes it possible to conduct natural cross-fostering studies in humans. For example, Rice *et al.* examined the records of 779 first-grade children born through IVF by either a related or an unrelated mother [13]. While the authors found that smoking during pregnancy predicted offspring birth weight as well as offspring antisocial behavior in both genetically related and unrelated pregnancies, only related dyads showed a

significant association between maternal smoking and offspring antisocial behavior. These results exclude prenatal factors as a mechanism between maternal smoking behavior and offspring antisocial behavior and demonstrated the feasibility of human cross-fostering studies in disentangling the origins of complex behavioral traits during early life. Using similar methods, Gaysina *et al.* assessed children from biological, adopted, and IVF families and found significant associations between maternal smoking during pregnancy and conduct problems in children reared by genetically related and unrelated mothers [14], suggesting that, unlike for antisocial behavior, maternal smoking is a prenatal risk factor for conduct disorder. While this design is not without confounds (e.g., age differences between donor and recipient parents, potential medical issues in recipients, possibility of IVF inducing epigenetic effects in offspring), natural cross-fostering neuroimaging studies using IVF designs will allow for the first time the dissociation of prenatal influence from other intergenerational mechanisms of brain circuitry.

Future studies are needed to address current limitations and gaps in this emerging field. Specifically, neurodevelopmental factors must be considered; for instance, the structural and functional characteristics of most brain regions differ between children and adolescents and older adults [15]. While the studies reviewed here often included age as a covariate, age was typically modeled as a linear effect (although see the studies by ENIGMA [6] and Fox *et al.* [9]) despite evidence that nonlinear trajectories exist depending on the brain structure (e.g., the trajectory for the hippocampus differs from that for the prefrontal cortex) and characteristic (e.g., the trajectory for cortical thickness differs from that of surface area) [15]. Future studies comparing parent–offspring dyads or that include individuals spanning a wide age range will need to account for developmental effects specific to the trajectory of the endophenotype of interest, perhaps by computing individual deviance from normative data. Finally, no studies to date have examined the concordance of brain phenotypes between all genetic combinations (related and unrelated) of parent–offspring dyads or prenatal versus postnatal effects. Future cross-fostering IVF neuroimaging studies will be able to compare different types of IVF families such as homologous surrogacy, donor egg pregnancy, and heterologous surrogacy to dissociate genetic, prenatal, and postnatal environmental influences on parental and offspring endophenotypes (Figure 1C). While it is likely that mega-analyses across multiple sites will ultimately be needed to robustly detect intergenerational patterns of neural circuitry, such methods are more amenable to task-independent data (e.g., structure, resting state) that are less heterogeneous in experimental design and pre- and post-processing methods. No human studies to date have examined intergenerational neural patterns using task-based neuroimaging. Circuits derived from well-validated tasks have the advantage of directly measuring brain function associated with a behavioral or psychological construct of interest rather than assuming function based on reverse inference. Genetic correlations computed from bivariate estimates of heritability from task-based brain and behavioral phenotypes are therefore capable of identifying robust endophenotypes underlying key disease-related constructs [9]. To promote mega-analyses, future studies may consider adopting standardized tasks, such as those recommended by NIMH RDoC.

In summary, intergenerational neuroimaging in humans has significant implications for basic, developmental, and clinical neurosciences. We have highlighted recent approaches including genetic correlations from large multiplex cohorts, direct estimates of concordance in parent–offspring dyads, and the exciting possibility of natural cross-fostering designs using IVF. We anticipate that these approaches will initially be used by individual research groups and should therefore adopt standardized neuroimaging tasks and preprocessing protocols with the aim that consortia conducting mega-analyses of pooled data will identify the most reproducible and robust intergenerational patterns of human brain circuitry.

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