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Brain connectivity in autism: the significance of null findings.

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Autism Spectrum Disorder (ASD) has become a case study for illustrating the difficulty in tracing a psychiatric disorder to its underlying causes -- psychological, genetic, or neurological. Aside from theories derived from the very criteria used to diagnose ASD, one would be hard pressed to find a psychological theory that is not hotly disputed. Genetic investigations have revealed that very large sample sizes are required to tease out the very small effects of very many genes, or large effects of genes present in only very few cases. Meanwhile, those doing work at the neurological level have held out hope that their theories might, finally, serve to provide a compact and reliable explanation of ASD that could best mediate between the genes and the phenotype. A new study by Lefebvre and colleagues (1) in this issue of *Biological Psychiatry* is beginning to cast some doubt that a simple anatomic story will emerge.

Arguably the leading neurological theory of ASD is about brain connectivity (2). This theory's popularity is unsurprising, since ASD is a neurodevelopmental disorder affecting most of the brain, and since the emergent properties of brain function are based on neural connectivity. Yet ever since its inception, the connectivity hypothesis has been vague, morphing from a theory about underconnectivity in ASD, to one about distal *under*connectivity paired with local *over*connectivity, to one about just atypical connectivity in either direction (or both).

The new work by Lefebvre *et al.* (1) now suggests that at least one specific indication of atypical connectivity may be a false positive altogether. Their study looked at the largest white matter tract in the human brain, the corpus callosum, whose 200 million axons enable rapid communication between the two cerebral hemispheres (i.e., inter-hemispheric communication). The corpus callosum arose only once in phylogeny, with the evolution of placental mammals: indeed, marsupials like opossums and kangaroos have no corpus callosum. The most parsimonious explanation for callosal evolution is that it arose to facilitate long-distance integration within large brains. As cognitive and clinical neuroscience have shifted away from a focus on individual brain areas studied in isolation and toward greater appreciation of how these brain areas operate within and across brain *networks*, there has been a proliferation of studies targeting the corpus callosum in the search for neural bases of psychiatric disorders.

Corpus callosum abnormalities are found in a wide range of developmental disorders caused by both genetic and environmental factors: for instance, callosal enlargement has been reported in Neurofibromatosis 1 and in 22q11.2 Deletion Syndrome; callosal reduction has been reported in Williams Syndrome, low-birth weight, fetal alcohol syndrome, attention deficit hyperactivity disorder -- and autism spectrum disorders (ASD). Furthermore, recent work confirms that congenital abnormalities of the corpus callosum can produce ASD symptomatology largely indistinguishable from idiopathic ASD (3). So there is little question that developing in the absence of a corpus callosum can result in an atypical mind and behavior. People with ASD, of course, do not generally lack a corpus callosum altogether. But how strong is the evidence that it is even abnormal at all?

In the current investigation of corpus callosum size in idiopathic ASD, Lefebvre and colleagues capitalized on a data-sharing initiative, known as the ABIDE consortium (4), to overcome the power limitations from small sample sizes evident in prior studies. Using a subset of this large sample (N=694) gave the study sufficient power to identify even a weak effect, yet they found no evidence of diminished callosal size in ASD. This finding is largely corroborated by another large recent study showing only a weak effect in a specific portion of the corpus callosum (5). If there is in fact reduced connectivity in ASD, it is not manifest in reduced callosum size.

In providing compelling negative findings, Lefebvre *et al.* (1) help us to focus our search on the possibilities for positive findings. First off, it may be that the corpus callosum in particular, or gross white matter volume in general, are simply too coarse a measure, and that the structural signature of atypical connectivity in ASD will reside in the microstructure of axons. This could be addressed by focusing on the histological study of post-mortem brains or on different kinds of imaging methods, such as diffusion imaging **(see Figure)**. However, it is possible that measures based on structural connectivity may simply be too insensitive, and we should be looking more closely at the phenotype: functional connectivity. Indeed studies of functional connectivity in ASD are now more common than those of structural connectivity, but no clear picture has yet emerged here either. Recent work suggests that functional connectivity is atypical in ASD, but in ways that are both idiosyncratic and heterogeneous across individuals (6), a pattern that has also recently emerged in neural activation studies using complex stimuli (7) and that may represent a general principle of the condition. This heterogeneity would make it more difficult to find group differences on a single structural measure, and highlights the need for more nuanced analysis of individual variations in order to find the most salient connections between brain and behavior. Finally, the negative findings in the present study apply only to individuals aged 7.5 to 40 years, leaving open the possibility that structural abnormalities of the corpus callosum may be present and detectable earlier in development (8) but become less apparent later in life.

The study by Lefebvre *et al.* (1) is valuable also in raising a number of specific methodological considerations. First, studies of callosal size must consider variations in brain volume in the most effective manner. While there is a robust relation between the size of the corpus callosum and brain size across and within mammal species (including humans), the correlation is non-linear and as a result, larger brains actually have proportionally smaller corpora callosa. Based on simulations, the authors conclude that due to this non-linearity, brain volume is more accurately controlled through covariation rather than normalization.

The second consideration relates to presumptions about potential confounds. Carefully matching ASD and control groups on IQ is typical to ensure that observed group differences cannot simply be explained by differences in IQ. Yet, Lefebvre *et al.* (1) found a weaker correlation between verbal IQ and brain volume in the ASD group, and therefore matching groups on IQ may actually introduce unintended artifactual differences in callosal size. In short: controlling for potentially confounding covariates can sometimes create spurious group differences.

There is perhaps an even more important message from the study by Lefevbre *et al.* (1). Despite their failure to find abnormal callosal size in such a large sample with ASD, the authors also conducted a meta-analysis of prior studies -- of which more than half reported significant reductions in corpus callosum size. All of those prior studies were vastly underpowered in sample size, and from the pattern of publications it is also apparent that there was a strong bias to publish those whose findings happened to achieve the magical "$p<0.05$" threshold. The problems inherent in this kind of reporting are by now well known, but unfortunately not yet eliminated. P-values are among the least reliable metrics we can report, so much so that some journals recommend dispensing with them altogether (9). If a question is important and the research is done carefully, it should be irrelevant whether the finding is deemed "statistically significant" or not. So-called negative findings can be as informative as positive ones, and help to narrow our search for causal explanations of psychiatric illness. The study by Lefebvre *et al.* (1) provides a patent demonstration of the value of this approach.

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Figure Caption:

Figure. Examples of two types of MRI-based structural images of the corpus callosum. **(A)** T1 weighted MRI can be used to quantify cross-sectional area, volume, and morphology of the corpus callosum. **(B)** Diffusion MRI can provide more detailed information about microstructural properties [e.g., white matter fiber orientation (indicated here by different colors) and organization]. Images courtesy of Mike Tyszka (Caltech).

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