

12 months. There were no differences in symptoms between IGHD and MPHD, or between GHD and non-GHD. These findings corresponded temporally with a decline in IGF-I. IGF-I concentrations did not differentiate the MPHD and IGHD groups. Depressive symptoms, assessed by the Profile of Mood States (POMS), increased in both IGHD and MPHD groups by 6 months of GH discontinuation and thereafter increased further for the IGHD, but decreased within the MPHD group. The opposite pattern was observed for the POMS Tension scale, which increased across the 12 months for the MPHD group, but declined for those with IGHD. Lower IGF-I concentrations were associated with more negative mood states and somatic complaints for the combined group, whereas higher IGF-I was associated with greater 'vigor'.

Nine of 14 patients (64%; 4 males, 5 females; 2 with IGHD and 7 with MPHD) from the GH discontinuation study who remained GHD when retested as adults subsequently participated in the GH treatment study. This sample was augmented with an additional 11 patients (6 males and 5 females; 3 IGHD and 8 MPHD) who were GHD both as children and adults, had not been treated with GH in the past year, and had not participated in the GH discontinuation study. Upon reintroduction of GH to only those patients meeting adult criteria for GHD, IGF-I levels increased between 0 and 6 months in both IGHD and MPHD, but without further change by 12 months. Accompanying this increase, scores on the insecure and depression scales (of the SCL-90) decreased across the entire 12 months for both IGHD and MPHD groups, whereas anxiety (assessed by the State-Trait Anxiety Scale) decreased significantly only from baseline to 6 months. QoL scores showed a significant improvement from 0 to 6 months of GH treatment. IGF-I levels were negatively correlated with negative mood states, but positively correlated with vigor, QoL, and short-term memory. The investigators concluded that GH-modulation of IGF-I concentrations is responsible both for deteriorating mood states during GH discontinuation and improved psychological status during the return to treatment.

Stouthart PJ, et al. Quality of Life of Growth Hormone (GH) Deficient Young Adults During Discontinuation and Restart of GH Therapy. *Psychoneuroendocrinology* 2003;28:612-626.

**Editor's Comment:** As recognition has grown that the actions of GH extend beyond linear growth, the practice of treating GHD in adulthood has become more widely accepted. Unlike most studies assessing the benefits of adult GH replacement, these outcome variables were psychological rather than metabolic. In this study, both the IGHD (73% of whom retested GH-sufficient by adult criteria) and MPHD subgroups exhibited similar deterioration in emotional state upon discontinuation of GH with improvement after reinstating GH therapy. The investigators related these psychological changes to lower and subsequently improved IGF-I concentrations.

Several methodological features of this study should be taken into account before factoring them into clinical management algorithms. For instance, the investigators provide no indication of how representative study participants were of those in this clinic in meeting diagnostic and age criteria. Were those who agreed to participate more emotionally symptomatic? Research suggests considerable variability among patients in responsiveness to the QoL benefits of adult GH replacement.<sup>1,2</sup> The potential contribution of a placebo effect to mental health indices also needs to be considered. A meta-analysis suggests that placebo effects are stronger in small trials with continuous subjective outcomes.<sup>3</sup> The investigators may be attributing some psychological benefits to GH that are potentially due to response bias or placebo effect. Nonetheless this study is of great interest and provides important information.

David E. Sandberg, PhD

## References

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## Non-Hormonal Genetic Influence on Brain Development

Current dogma holds that differences in brain development and behavior between males and females depend primarily on gonadal steroid hormones, especially testosterone and its metabolites that induce the masculine pattern and inhibit the female pattern of brain development. However, there is also evidence that genetic factors may act directly on the developing brain contributing to these differences. Until recently, this alternative view has been difficult to document, but Dewing et al provide new and convincing evidence for non-hormonal genetic effects.

Their work was done in a mouse embryo 10.5 days

after conception. This is just before the first sign of sexual differentiation of the genital ridges occurs, thus the influence of gonadal hormones could be excluded. Their strategy was to harvest whole heads from the embryos, isolate RNA into separate pools for males and females and then analyze for differential gene expression in the male and female brains. For screening analysis, they used gene (microarray) chip (Affymetrix) technology which allowed the relative expression of nearly 10,000 characterized mouse genes and over 3,000 less well defined expressed sequences (Expressed Sequence Tags – ESTs) to be determined. The normalized gene

chip results reported as fold change or difference between male and female brain RNA revealed 36 genes or ESTs with enhanced expression in females and 18 genes or ESTs with enhanced expression in males. These genes exhibited a significant fold difference of greater than 1.1 and 7 genes or ESTs for each sex displayed a fold difference of 2.0 or more. The gene showing highest differential expression in females was *Xist*, which was 18.5 fold higher in females, while genes showing the highest differential expression in males included DEAD box peptide (*Dby*) and eukaryotic translation initiation factor 2,Y (*Eif2s3Y*) with fold differences of 10.0 and 8.8, respectively. *Xist* maps to the X chromosome, while the latter two genes reside on the Y chromosome.

Real-time quantitative analysis (RT-PCR) of littermate-matched male and female embryonic brain RNA confirmed and validated the results of the gene chip screening for a small number of genes based on their potential roles in brain development. The authors concluded that developmental differences in male and female brains in mice are due in part to the differential expression of genes before gonadal secretion starts.

Dewing P, et al. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Mol Brain Res* 2003;118:82-90.

**First Editor's Comment:** *This is an important paper that documents the differential expression of genes in the male and female brain prior to any influence from gonadal hormones. If confirmed, it will have a substantial impact on understanding how genetic factors influence brain development. The design of the study allows for the identification of non-hormonal factors that act before the gonads are formed. However, there is no reason to think that genes act through mechanisms that do not involve gonadal hormones after gonadal hormone secretion begins, although other investigational approaches will be needed to demonstrate this. Dewing and colleagues provide no insight into the nature of the non-hormonal mechanisms through which genes may act before the appearance of gonadal hormones, although they could presumably be multiple and diverse.*

*One should note that the most dramatic differences were found for genes whose expression is expected to be limited to one sex or the other. For example, one would expect genes located on the Y chromosome to be expressed only in the male brain and *Xist* mRNA, which is expressed only by the inactive X chromosome in XX females, to be detected only in the female brain. That they were detected at all, seemingly reflects how the assays distinguish negative results from background signals. When these results are excluded the differences were diminished. Microarray gene chip and related approaches for studying gene expression are relatively new and evolving rapidly as is bioinformatics, the discipline that deals with analysis of the vast amounts of data this technology generates. Its novelty combined*

*with the complexity of its data has led to a certain amount of caution in the biomedical field with regard to the biological significance of microarray results. Initially, a 2-fold difference in expression was considered an informal threshold for biological significance. Many of the results in this study fall below this level and therefore would not be considered significant by this criteria even though they are statistically significant. However, as the analytical methods advance, the threshold is being progressively lowered such that a cut-off, such as the 1.1-fold difference used in this paper, is becoming acceptable. It is still probably wise, however, to view small differences in gene expression with caution until they are confirmed by others and placed in a biological context.*

William A. Horton, MD

**Second Editor's Comment:** *The findings of this study are important and exciting, and will likely contribute to a transformation of the dominant conceptual model regarding sexual differentiation of somatic phenotype, brain, and behavior. There is a risk that the findings may be misinterpreted in a manner potentially harmful to the clinical decision-making process in cases involving intersexuality. The findings force us to rethink the classic view of brain sexual differentiation and behavior which posits that the role of genes in the development of sex differences is restricted to the process of sex determination, i.e., the development of a bipotential and undifferentiated gonad into either an ovary or a testis. Evidence of a direct role of genes (not mediated by sex hormones) may lead clinicians to question the flexibility in decision-making they may currently exercise when sex assignment is in question. But should they?*

*The basic finding of the study is that over 50 candidate genes are differentially expressed in the brains of male and female mice, ostensibly prior to gonadal production of sex hormones. Although a remarkable observation, these findings are not necessarily relevant for one psychological outcome variable of great importance in intersex cases, that is the stability of gender identity across the lifespan. (Gender identity refers to the individual's self identification as either girl/woman or boy/man.) Readers of media reports of this article will likely draw different conclusions. The headline of one well-publicized report of this study states "Sexual Identity Hard-Wired by Genetics."<sup>1</sup> Quotes within the article imply that gender identity springs directly from our genome. If so, then how do we account for the consistent finding in the literature that 46,XY individuals with complete androgen insensitivity syndrome develop an unambiguous gender identity as girls, and later women?<sup>2</sup>*

*The conflict between research findings and their interpretation is likely more apparent than real and is promoted by an oversimplification of the process of psychosexual differentiation in humans. An individual's*

gender identity need not be congruent with their gender-role (which refers to behaviors that differ in frequency or level between males and females in this culture and time such as toy play or maternal interest), and sexual orientation (the pattern of sexual arousal). At the present time, the clinical research literature suggests that gender identity generally conforms with the gender of rearing, even when gender assignment is discordant with genetic sex. The picture is quite different, however, with respect to the variables of gender-role behavior and sexual orientation. It is clear that many new findings will stem

from the line of research described in this report. However, it would be unfortunate if these data were to be interpreted as suggesting that gender assignment must conform with genotype to foster a stable gender identity.

David E. Sandberg, PhD

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## IGF, Learning & Memory

Lupien et al tested the following hypotheses: (1) IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory; (2) IGF-I can support cognitive function across the blood-brain barrier; (3) IGF can preserve brain function in diabetes independently of hyperglycemia; and (4) brain IGF contributes to hippocampal-based cognitive functions.

The first three hypotheses were tested by comparing normal rats versus streptozocin (STZ) diabetic rats. Four weeks after STZ, minipumps were implanted to deliver continuous infusions of 20 µg/day IGF-I or vehicle (10 mM acetic acid, pH 6.0) for 7.5 weeks. (For reference, daily IGF-I production by the adult rat liver is about 31 µg/day.) The hidden platform or "place" test was performed to assess spatial learning and memory; the "probe" test to examine memory; and the "cued" test to detect sensorimotor deficits. Following these tests, the mean blood glucose levels were 125.0±11 mg/dl in the non-diabetic rats versus 515±73 in the STZ + vehicle and 495±99 in the STZ + IGF rats. Body weights of both STZ groups were about half that of the non-diabetic rats.

All 3 groups decreased their latency times to escape the hidden platform, but there was a 3-day lag before latencies began to decline in the STZ + vehicle group. STZ+IGF performed similarly to the non-diabetic rats, and both groups decreased their latencies by shortening their search paths. The STZ + vehicle group decreased their latencies by increasing their swim velocity; their paths did not shorten. The average latency was more prolonged in the STZ + vehicle, than in the STZ + IGF rats. The STZ + vehicle rats also swam the furthest distance; STZ + IGF were again like the non-diabetics. Swim velocities were not significantly different, thus motor or proprioceptive disturbances were not the cause of the poorer performance of the STZ + vehicle rats. IGF infusion improved learning/memory performance without ameliorating the hyperglycemia or the catabolism of the STZ rats. Total brain weight and hippocampal weight were significantly reduced in the STZ rats, and these were not attenuated by IGF infusion. The second experimental design tested IGF's contribution to normal learning/memory by passive avoidance of electric shocks after two-weeks of continuous infusion into the lateral ventricle of either 40% anti-IGF-II

antiserum or 40% preimmune serum. Whereas the latencies of the preimmune serum rats increased, those of the IGF-II antiserum rats were significantly diminished. The authors concluded that IGF treatment can prevent brain disturbances that contribute to impaired spatial learning/memory in experimental diabetes in rats.

Lupien SB, et al. Systematic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. *J Neurosci Res* 2003;74:512-523.

**Editor's Comment:** *The authors integrated their results into a review of prior studies of the effects of diabetes and IGF on neurologic function. Experimentation in rats allowed controlled manipulations that cannot be made in humans, like the examination of brain tissues and the continuous intraventricular infusion of IGF antiserum. These data add to the evidence supporting IGF benefits for neurologic function. Aleman and colleagues demonstrate significant associations between circulating IGF-I concentrations and performance on perceptual-motor performance and mental processing speed in healthy men aged 65-76 years.<sup>1</sup> Although it is tempting to attribute the better performance to the higher IGF-I levels, associations are NEVER sufficient to prove causation and require corroborative evidence.*

*While the associations between high circulating IGF-I concentrations and increased cancer risk have garnered a lot of attention, the neurologic effects of IGF should be considered, particularly pertaining to diabetes-induced learning/memory impairments and increased risk of dementia. Gasparini and Xu recently reviewed IGF-I and insulin as it related to the pathophysiology of Alzheimer's disease.<sup>2</sup> It appears that there may also be risks to having low IGF-I levels; IGF-I does more than promote somatic growth.*

Adda Grimberg, MD

## References

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