THE NEUROANATOMY AND NEUROENDOCRINOLOGY OF FRAGILE X SYNDROME

David Hessl, 1,2 Susan M. Rivera, 1,3 and Allan L. Reiss 4*

1 M.I.N.D. Institute, University of California, Davis, Sacramento, California
2 Department of Psychiatry and Behavioral Sciences, University of California, Davis, Sacramento, California
3 Department of Psychology, University of California, Davis, Davis, California
4 Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California

Fragile X syndrome (FXS), caused by a single gene mutation on the X chromosome, offers a unique opportunity for investigation of gene–brain–behavior relationships. Recent advances in molecular genetics, human brain imaging, and behavioral studies have started to unravel the complex pathways leading to the cognitive, psychiatric, and physical features that are unique to this syndrome. In this article, we summarize studies focused on the neuroanatomy and neuroendocrinology of FXS. A review of structural imaging studies of individuals with the full mutation shows that several brain regions are enlarged, including the hippocampus, amygdala, caudate nucleus, and thalamus, even after controlling for overall brain volume. These regions mediate several cognitive and behavioral functions known to be aberrant in FXS such as memory and learning, information and sensory processing, and social and emotional behavior. Two regions, the cerebellar vermis, important for a variety of cognitive tasks and regulation of motor behavior, and the superior temporal gyrus, involved in processing complex auditory stimuli, are reported to be reduced in size relative to controls. Functional imaging, typically limited to females, has emphasized that individuals with FXS do not adequately recruit brain regions that are normally utilized by unaffected individuals to carry out various cognitive tasks, such as arithmetic processing or visual memory tasks. Finally, we review a number of neuroendocrine studies implicating hypothalamic dysfunction in FXS, including abnormal activation of the hypothalamic–pituitary–adrenal (HPA) axis. These studies may help to explain the abnormal stress responses, sleep abnormalities, and physical growth patterns commonly seen in affected individuals. In the future, innovative longitudinal studies to investigate development of neurobiologic and behavioral features over time, and ultimately empirical testing of pharmacological, behavioral, and even molecular genetic interventions using MRI are likely to yield significant positive changes in the lives of persons with FXS, as well as increase our understanding of the development of psychiatric and learning problems in the general population.

Key Words: fragile X syndrome; FMR1 protein; MRI; fMRI; endocrine; cortisol

NEUROPATHOLOGICAL STUDIES

It has long been suggested that the decrease or absence of FMR1 protein production associated with fragile X syndrome (FXS) may lead to brain abnormalities in affected individuals [Devys et al., 1993; Hinton et al., 1991; Rudelli et al., 1985; Tamanini et al., 1997; Wisniewski et al., 1991]. Postmortem microscopic examinations of brain tissue from patients with fragile X have revealed that although the number of neurons falls within the normal range, abnormalities in neuronal dendritic spines exist. These abnormalities are characterized by unusually long and thin, tortuous spines, with very few of the stubby, mushroom-shaped spines found in unaffected controls, and a higher density of spines along dendrites, suggesting a possible failure of synapse elimination [Hinton et al., 1991; Irwin et al., 2000a].

Brain Structure

Structural magnetic resonance imaging (MRI) studies in FXS have further explored the effects of the FMR1 full mutation on neuroanatomy. The first brain abnormality reported in an MRI study was that of hypoplasia of the cerebellar vermis—the connecting tissue between the right and left hemispheres of the cerebellum [Reiss et al., 1988]. Vermis hypoplasia, particularly in posterior lobules VI and VII, has been confirmed in several subsequent studies [Mostofsky et al., 1998; Reiss et al., 1991a; Reiss et al., 1991b]. The vermis, which is anatomically connected to limbic structures, including the hippocampus and amygdala, has been implicated in the execution and regulation of motor behavior [Rosenthal et al., 1988], visual saccades [Hayakawa et al., 2002], auditory processing [Huang and Burkard 1986], and some aspects of language [Moretti et al., 2002, Schmitt et al., 2001]. Abnormalities of the cerebellar vermis may therefore be linked to some of the behavioral anomalies associated with FXS, including hyperactivity and repetitive movements, tactile defensiveness, attention deficits, and language dysfunction.
Structural MRI studies also have shown that the fourth ventricle is enlarged in individuals with FXS [Mostofsky et al., 1998; Reiss et al., 1991a; Reiss et al., 1991b; Reiss et al., 1988]. Increased lateral ventricular volume also has been noted in males with FXS [Reiss et al., 1995] and it was larger in both affected males and affected females in a study of children and adolescents with FXS [Eliez et al., 2001].

Abnormalities of the temporal lobe also have been noted in FXS. Reiss and colleagues [Reiss et al., 1994] studied a sample of 15 young males and females with FXS and 26 intelligence quotient (IQ) matched controls and reported a volumetric decrease with age of the superior temporal gyrus, an area important for processing complex auditory stimuli, including speech. In contrast to this age-related decrease, this study reported an age-related increase in both left and right hippocampal volume, a medial temporal lobe structure important for learning, memory, and processing visuospatial information—a cognitive area known to be particularly problematic in persons with FXS [Freund and Reiss 1991; Mazzocco et al., 1993]. Similarly, Kates and colleagues [Kates et al., 1997] reported increased hippocampal volumes in children with FXS as compared to age- and gender-matched controls. Jakälä and colleagues [Jakälä et al., 1997] found no significant changes in normalized hippocampal volumes between full mutation and premutation groups (n = 20 in each group) but did see atypical hippocampal morphology in MRI.

Another medial temporal lobe structure, the amygdala, was first hypothesized to be affected in FXS after a study of monozygotic twin girls with the full mutation who were discordant for mental retardation [Mazzocco et al., 1995; Reiss et al., 1995]. Both twins had similar CGG expansions, activation ratios, and neonatal course without brain trauma. Twin A, however, had a full-scale IQ of 105, whereas twin B had a full-scale IQ of 47. Structural MRI analyses showed that the amygdala in twin B was 35% larger than that in twin A. In addition, whereas their overall brain size was similar, twin A also had enlarged lateral and fourth ventricles, enlarged caudate and thalamus, and a smaller posterior cerebellar vermis than twin B [Reiss et al., 1995]. Another source of information about amygdala dysfunction in FXS has come from FMR1 knockout mouse studies. Paradee and colleagues [Paradee et al., 1999] demonstrated an abnormal conditioned fear response (less freezing behavior during contextual and cue-induced fear responses) in the knockout mouse compared to controls. The amygdala, a complex structure consisting of approximately 10 distinct nuclei, is thought to mediate both conscious and unconscious emotion processing.

Reiss and colleagues [1995] also reported larger caudate nuclei in young male and female patients with FXS, as compared to controls, and replicated this finding in a subsequent study [Eliez et al., 2001]. The caudate nucleus (which, along with the putamen, forms the structure known as the basal ganglia) has many cortical connections, the most numerous being those to the frontal lobes. The function of the caudate nucleus is to regulate, organize, and filter information. Thus, the many connections between the caudate and the frontal lobes play a large role in determining behavior.

Some of the frontal–subcortical circuits that involve the caudate include those important for shifting attention, motor planning and executive functions, all of which constitute deficits in FXS.

Thus, the many connections between the caudate and the frontal lobes play a large role in determining behavior.

Correlations among MRI, Cognitive/Behavioral Profiles, and Molecular Variables

If it is the case that the aforementioned differences in neuroanatomy between FXS and comparison subjects reflect the effect of absence of the FMR1 protein on brain function and development, an association among brain-based measurements, protein expression, and cognitive/behavioral profiles should be evident. A number of studies have now examined correlations between cognitive/behavioral testing and MRI. Findings. These studies provide a more direct insight into brain–behavior relationships in FXS. Whereas some early studies found no correlations between temporal lobe and posterior fossa structure measures and cognitive/behavioral measures [Reiss et al., 1994, Reiss et al., 1991a, 1991b], more recent studies have found this association. Mazzocco and colleagues [1997], in a study of 30 girls with FXS and age- and IQ-matched controls, found that the size of the posterior cerebellar vermis was negatively correlated with measures of stereotypic/restricted behavior, communication dysfunction, and autistic items on a parental interview, where higher scores represent more dysfunction. By contrast, measures of anxiety did not correlate with IQ or with the size of the posterior cerebellar vermis, suggesting that anxiety, which is part of the behavioral phenotype of girls with FXS, may have a different neuropathological mechanism that is unrelated to morphology of the posterior cerebellar vermis.

Similarly, Mostofsky and colleagues [1998] studied 32 males and 37 females with FXS, along with age-matched typically developing controls as...
well as controls with developmental disabilities (for the males). In females with FXS, hierarchical/stepwise regression was used to determine if size of the posterior vermis predicts cognitive performance. After statistically removing the effect of mean parental IQ (which is a strong predictor of a child’s cognitive ability), they found that posterior vermis size predicted 10%–23% of the variance in performance on full-scale, performance and verbal IQ, block design (a test of visuospatial ability), the Rey –Osterreith Complex Figure Test (a test of visuospatial perception/construction and memory), and the categories achieved on the Wisconsin Card Sorting Test (a test of executive function, including strategic planning and set shifting). No correlation was observed between size of the posterior vermis and age, suggesting that hypoplasia, not atrophy, is likely the primary cause of the small size of the vermis in FXS. These results confirm not only that the posterior cerebellar vermis is significantly affected by the FMR1 mutation but also that the vermis is important for a variety of cognitive tasks, including executive function abilities and visuospatial abilities.

Correlations also have been observed between the size of both the caudate and lateral ventricular volume and IQ. Specifically, Reiss and colleagues [1995] found that both brain structures were significantly and inversely correlated with IQ in subjects with the full mutation, whereas for control subjects, larger caudate predicted higher IQs. The authors suggested that the mechanism for increases in caudate size could be different for controls than for individuals with FXS.

Caudate volume also has been examined with respect to FMR1 protein expression. Reiss and colleagues [1995] used activation ratio (estimated proportion of cells that are expressing the FMR1 protein over the total number of cells) as an indirect measure of variable FMR1 protein expression. Multiple-regression analysis showed that a higher activation ratio (FMR1 expression) predicted a lower (more normal) caudate volume in FXS subjects with the full mutation.

Links between neuroanatomical abnormalities and HPA axis function in FXS have been hypothesized by several authors [Wisbeck et al., 2000; Hessel et al., 2002; Miayashiro et al., 2003; Sun et al., 2001], Table 1). Whereas HPA axis function and FXS are covered extensively below, in the discussion of the neuroendocrinology of FXS, it is relevant to note here that a direct neurotransmitter role for corticotropin-releasing hormone, which activates the HPA axis, has been identified in the pathogenesis of stress-related behaviors [Dunn and Berridge, 1990]. Several brain regions contain binding sites for this hormone, including the hippocampus and the amygdala—regions that have been shown, either directly or indirectly, to be anomalous in FXS [Abrams et al., 1995]. Despite many endocrine and case studies implicating hypothalamic dysfunction (described below), to date there have been no published studies that directly examine the structure or function of this brain region in individuals with FXS.

### Functional Magnetic Resonance Imaging (fMRI)

Functional MRI provides a picture of the brain’s dynamic activity rather than its static structure. Thus, it permits the in vivo study of the neural substrates implicated in the pathogenesis of FXS.

Although there are no published fMRI studies of FXS males, there have been a handful of published studies using female participants with the full mutation. The technique has been shown to be useful in helping to elucidate the neuropathology associated with the deficits in cognitive function that characterize the syndrome. Tamm and colleagues [2002] showed that females with FXS had a significantly different pattern of activation than comparison subjects on a cognitive interference task—the Counting Stroop. The task included interference trials (during which, for example, the word three might be presented on the screen two times) and neutral, control trials (during which the word fish was presented one, two, three, or four times on the screen). For both types of trials, subjects were instructed to press the button (1, 2, 3, or 4) that corresponded to the number of words on the screen, regardless of the word. Whereas comparison subjects showed significant activation in the inferior/middle frontal gyrus and inferior/superior parietal lobe, females with FXS showed more extensive activation in the anterior region of the prefrontal cortex and failed to show expected activation in the inferior/superior parietal lobe.

Another study [Kwon et al., 2001] examined the neural substrates of visuospatial working memory in female subjects with FXS using standard one-back and two-back tasks. During these tasks, subjects saw a circle presented in one of nine distinct visuospatial locations in a $3 \times 3$ matrix. In the one-back task, the subject was instructed to respond if the stimulus was in the same location as in the previous trial. In the two-back task, the subject was instructed to respond if the stimulus was in the same location as it was two trials back. They found that subjects with FXS performed significantly worse on the more difficult, two-back task than did age-matched control subjects. Whereas comparison subjects showed a significant increase in the inferior frontal gyrus, middle frontal gyrus, superior parietal lobule, and supramarginal gyrus on the two-back as compared to the one-back task, subjects with FXS showed no change in activation between the two. Furthermore, molecular measures correlated with brain activation on this task. That is, in subjects with FXS syndrome, significant correlations were found, during the two-back task, between FMRP expression and activation in the right inferior and bilateral middle

### Table 1. Neuroanatomic Abnormalities in Fragile X Patients

<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>Abnormality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellar vermis</td>
<td>Decreased</td>
<td>Mostofsky et al. [1998], Reiss et al.</td>
</tr>
<tr>
<td>Fourth ventricle</td>
<td>Enlarged</td>
<td>[1988, 1991a,b]</td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>Enlarged</td>
<td>Mostofsky et al. [1998], Reiss et al.</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>Decreased</td>
<td>[1998]</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Enlarged</td>
<td>Reiss et al. [1994]</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Enlarged</td>
<td>Kates et al. [1997], Reiss et al. [1994]</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>Enlarged</td>
<td>Mazzocco et al. [1995]</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Enlarged (in females)</td>
<td>Eliez et al. [2001], Reiss et al. [1995]</td>
</tr>
</tbody>
</table>
frONTAL gyri and the bilateral supramarginal gyri. A similar finding was obtained in an fMRI study of arithmetic processing in females with the FXS full mutation [Rivera et al., 2002]. In this study, subjects with FXS exhibited less overall activation than did unaffected subjects during both two-operand (e.g., $2 + 1 = 3$) and three-operand (e.g., $3 + 2 - 1 = 5$) trials. Moreover, unlike the unaffected group, subjects with FXS showed no increased extent of activation in association with greater task difficulty (see Fig. 1). Between-group comparisons confirmed that, in response to increasing arithmetic complexity, unaffected subjects recruit a functional network known to be involved in arithmetic processing, but subjects with FXS do not. With respect to molecular measures, this investigation showed that with increasing levels of FMRP expression, subjects with FXS were found to activate more during the three-operand trials in areas that are involved in arithmetic processing in typically developing subjects. This result, along with the results of Kwon and colleagues [2001], provides direct evidence that decreased FMRP expression underlies deficits in cognitive performance in persons with FXS.

**Neuroanatomical Findings in Male Premutation Carriers**

Thus far our discussion has focused on individuals with the *FMR1* full mutation. It is important to note here that a subgroup of older males with the *FMR1* premutation (those with 55 to 200 CGG repeats, often referred to as premutation “carriers”) develop a progressive neurological disorder, typically beginning after age 50, characterized by cerebellar ataxia and/or intention tremor and cognitive dysfunction [Hagerman et al., 2001; Brunberg et al., 2002; Jacquemont et al., 2003]. Structural brain MRI of these patients has revealed several abnormalities, including increased white matter signal intensity in the middle cerebellar peduncles, cerebellar cortical atrophy, and ventricular enlargement. The affected patients are reported to have *FMR1* messenger RNA (mRNA) levels 2 to 10 times higher than normal, despite mildly reduced levels of FMRP. It has been hypothesized that the elevated mRNA leads to the neuropathology, including the finding of eosinophilic intranuclear inclusions [Greco et al., 2002] and consequent clinical features. The prevalence of this newly identified syndrome is not known; however, a recent report [Macpherson et al., 2003] suggests that a significant proportion of adult males with late-onset cerebellar ataxia carry the *FMR1* premutation. (See Hagerman and Hagerman in this issue).

**Neuroendocrinology**

A number of studies documenting neuroendocrine dysfunction in FXS suggest that the hypothalamus may be especially affected by the decrease or absence of FMRP. Early work focused on the endocrine system to explain the physical features of the disorder such as macroorchidism (enlarged testicles). In some investigations, measures of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid hormone were reported to be normal [Bovens et al., 1978; Brondum Nielsen et al., 1983; Cantu et al., 1978], however, elevated LH and FSH were documented in others [McDermott et al., 1983; Ruvalcaba et al., 1977; Turner et al., 1975]. In an evaluation of hypothalamic–pituitary–thyroid (HPT) function in 12 males with FXS, Bregman et al. [1990] reported normal levels of thyroid-stimulating hormone (TSH) but a blunted TSH response to thyrotropin-releasing hormone (TRH).

Despite a high rate of physical growth in the preadolescent period, individuals with FXS show less pubertal growth compared to normal relatives [Loesch et al., 1995]. As suggested by the investigators, this growth pattern may be an indication of premature activation of the hypothalamic–pituitary–gonadal (HPG) axis. Interestingly, growth hormone abnormalities also have been described in three separate case reports of precocious puberty in girls with FXS, each with advanced bone age and a mature response to gonadotropin-releasing hormone [Butler and Najjar 1988; Kowalczyk et al., 1996; Moore et al., 1990]. Despite the clear growth abnormalities seen in this population, to date there have been no group endocrine studies of growth hormone in FXS.

Premature ovarian failure (POF), or menopause before age 40, is found in 16%–24% of women with the fragile X premutation [Schwartz et al., 1994; Allingham-Hawkins et al., 1999; Parving et al., 1984; Vianna-Morgante et al., 1996; Cronister, 1991; Hundscheid, 2003]. In contrast, the incidence of POF in the general population of women under 40 is estimated to be 1% [Coulam et al., 1986]. The hypothesis that POF is due to hypothalamic overstimulation has been supported by several studies documenting elevated FSH in these women [Braith et al., 1999; Murray et al., 1999]. However, whether POF in the premutation is because of a hormonal abnor-

**Fig. 1.** Brain areas that show significantly greater activation during two- and three-operand arithmetic equations for unaffected subjects and subjects with FXS. All activations reported [Rivera et al., 2002] were significant after height ($z > 2.33$, $p < 0.01$) and extent ($p < 0.01$) thresholding.
mality, a more fundamental ovarian problem, or both is not clear.

Case studies have also supported the potential importance of hypothalamic dysfunction in FXS. For example, Fryns and colleagues [1987] described a sub-phenotype of FXS characterized by extreme obesity, short stature, stubby hands and feet, and hyperpigmentation similar to the features of Prader–Willi syndrome, another genetic condition associated with hypothalamic dysfunction. Interestingly, this author also described a male patient with an acquired hypothalamic lesion, macroorchidism, and facial features of FXS who was normal with regard to the FMR1 mutation [Fryns et al., 1986].

Gould and colleagues [Gould et al., 2000] found disturbed sleep patterns and elevated day and night melatonin levels in young males with FXS in comparison to controls. Melatonin, an indoleamine derived from serotonin and regulated by the hypothalamus, is directly involved in regulating circadian rhythm and has soporific properties. It is interesting to note that these authors hypothesized that increased melatonin could be caused by malfunctioning melatonin receptors, where melatonin is overproduced to compensate for diminished receptor activation, perhaps as a consequence of the FMRP deficit. In fact, this reasoning is reminiscent of some of our own research focused on the stress hormone cortisol, also regulated by the hypothalamus. We review this work below.

Given the numerous endocrine findings, neuroanatomical abnormalities in limbic areas, and the now well-described behavioral features of social anxiety and avoidance, we have focused our neuroendocrine research on the HPA axis, the primary biological stress response system in humans. The HPA axis is among the most intensively studied and best-described components of the neuroendocrine system. Regulation of the HPA axis is complex and involves feedback mechanisms occurring at the level of the hypothalamus, pituitary, hippocampus, amygdala, and frontal cortex. The HPA axis reacts to stress by causing the hypothalamus to secrete corticotropin-releasing hormone (CRH), which stimulates the pituitary to secrete ACTH, which then stimulates the adrenal to secrete cortisol. Cortisol is found in plasma but also is reliably measured in saliva. Secretion of ACTH by the pituitary is episodic through the 24-hour cycle, and, similarly to other hormones, basal cortisol secretion by the adrenal gland shows prominent circadian variation characterized by peak levels in the morning followed by a steady decline to the nadir at night. The HPA response to stress is adaptive in that it prepares the individual for dealing with the source of the stress; however, chronic elevations or disruptions in the typical diurnal rhythm of cortisol can lead to medical problems associated with immune suppression [McEwen et al., 1997] and adverse effects on the brain that interfere with learning and memory [Sapolsky 2000]. Recent evidence has clearly implicated several limbic regions, in particular the amygdala and the hippocampus, as being intimately involved in the regulation of the HPA system [Herman and Cullinan 1997].

A pilot study of salivary cortisol in eight male and seven female children with the fragile X full mutation was conducted [Wisbeck et al., 2000]. In comparison to normative cortisol data taken from a large group of typically developing children, children with FXS had higher levels of cortisol on 2 routine days. On an experimental day during which subjects were engaged in a socially challenging task, males with FXS had cortisol elevations following the task, and they continued to have higher levels at bedtime. In an effort to replicate and extend these results, we conducted a comprehensive, in-home study of 120 families throughout the United States and Canada, including assessment of the HPA axis via measures of salivary cortisol [Hessl et al., 2002]. The families each had at least one child with the FXS full mutation (proband) and one unaffected biological sibling (sibling comparison). Two experimenters spent a full day with each family in the home, completing neuropsychological testing and behavioral assessment of each child and both parents, interviewing parents about the children and their educational and therapeutic services, observing the physical and social qualities of the home environment, and engaging the children in a structured, socially challenging series of tasks (the interview included insistence on direct eye contact, silent and oral reading, and singing). A blood sample was obtained from each child for FXS DNA testing and to measure expression of the FMR1 protein in lymphocytes. To measure cortisol, four samples of saliva were collected on each of 2ss weekend days (estimate of basal diurnal rhythm of cortisol) and six samples were collected during the home visit (to measure cortisol response to social and cognitive challenge). Cortisol concentration was determined by radioimmunoassay.

The results of the study documented that males with FXS had elevated basal cortisol during the day and before bedtime, and they had a greater cortisol response to the diverse challenges of the home visit (meeting the examiners, un-
boys and siblings. However, in both boys with the full mutation, the girls infected biological siblings (Fig. 2). Despite undergoing neuropsychological testing, and the “family” effect was present after accounting for several independent variables in the multiple regression analyses showed that, after removing other factors, including IQ, FMR1 gene protein level, and the effectiveness of educational and therapeutic services, cortisol accounted for a significant proportion of the variance (8% and 14% in boys and girls, respectively) in total behavior problems such that increased levels were associated with greater severity of problems. Cortisol levels were standardized by z-score transformation and then averaged to derive the composite score. Residual scores represent behavior scores after the effects of the independent variables in the multiple regression are removed.

One of the most telling results of the study was a highly significant familial pattern in cortisol response. That is, although the children with FXS tended to have higher cortisol levels and were more cortisol-reactive than their unaffected brothers and sisters, the “family” effect indicated that the matched siblings had very similar cortisol profiles. This finding is quite consistent with studies of inheritance and genetics of HPA function, but it may also reflect social, emotional, and physical environmental factors that are shared in each proband–sibling pair. Thus, as previous studies of cognition, behavior, and physical features have emphasized, accounting for background familial and genetic variance is especially important in future studies of individuals with FXS. Prospective, longitudinal studies of individuals affected by FXS will be critical in unraveling the complex interaction among factors related to genetic influences (fragile X mutation characteristics), brain function, learning and behavior, the environment, and HPA function. Two such studies have recently commenced at Stanford, one involving follow-up of the families described above and the other focusing on young children with FXS throughout the preschool age range.

Because the HPA axis is the primary stress response system, it may mediate causal connections between molecular alterations and stress-related behavior in FXS. Glucocorticoid hormones regulate neuron birth, death, and dendritic arborization, which may explain brain morphological alterations found in several neuropsychiatric disorders associated with stress [Sapolsky, 2000]. Studies of both animals and humans show that abnormal corticosteroid levels can affect hippocampal morphology and volume [Bremner et al., 1995; McEwen et al., 1992]. Glucocorticoid receptors are found in many regions of the brain involved in the regulation of emotion, attention, and memory, including the hippocampus and amygdala [McEwen et al., 1986]. These brain regions are known to mediate emotional appraisal of social stimuli, memory, and learning, all of which are significantly impacted by FXS. Furthermore, as cited above, alterations in the structure or function of several brain regions, including the hippocampus, amygdala, thalamus, and frontal cortex, have been implicated in the pathogenesis of the FXS neurobehavioral phenotype. Thus, although decreased FMRP contributes to neuronal dysmorphology [Weiler and Greenough, 1999] and diminished synaptic transmission [Irwin et al., 2000b], we have hypothesized that there may be secondary HPA axis effects in brain regions that have an abundance of glucocorticoid receptors, including mesial temporal and frontal regions. We are currently testing this hypothesis with studies examining the relationship between cortisol and brain structure and function in individuals with FXS.

The hypothesis that the HPA axis mediates causal connections between molecular alterations and stress-related behavior in FXS has recently gained empirical support by studies examining the molecular targets of FMRP in the brains of FMR1 knockout mice and in human lymphocytes. For example, Miyashiro and colleagues [Miyashiro et al., 2003] recently showed that FMRP directly interacts with a number of other gene mRNAs, including the mRNA of the glucocorticoid receptor. In addition, these authors showed that decreased concentration of glucocorticoid receptors are found in the hippocampal dendrites of FMR1 knockout as compared to wild-type mice. These studies suggest that the FMRP deficit alters GR mRNA and perhaps alters the balance of GR receptors and subsequent HPA activity. Additional support for HPA axis disruption in FXS comes from Sun and colleagues [2001], who discovered abnormal expression of a glucocorticoid-modulating protein, Annexin-1, in blood lymphocytes in a group of adult males with FXS in comparison to those with other developmental disabilities and typical controls.

If the HPA axis plays a causal or mediating role in development of the fragile X behavioral phenotype, pharmacological or environmental interventions designed to normalize HPA function might help to reduce stress-related behavior and improve mood and emotion regulation in affected individuals. For example, the glucocorticoid receptor agonist mifepristone, also known as RU486, rapidly reduces psychosis and depression in adults with psychotic major depression [Belanoff et al., 2002], a group known to...
have significant HPA abnormality. In addition, alterations in the home and educational environment, such as increased structure and predictability and reduced sensory stimuli and social demands, may normalize HPA responses to stress and reduce anxiety in affected individuals.

Neuroanatomy, Neuroendocrinology, and Genetics in Context

Fragile X syndrome offers a unique opportunity to study complex relations among genetic, neurobiological, and environmental systems leading to cognitive and emotional dysfunction. Since the discovery of the FMR1 gene a little over a decade ago, there have been important breakthroughs in understanding the molecular genetics and neurobiology of FXS, many of which are reviewed in this volume. But we know that the life experience of each person with FXS is determined by more than his or her FMR1 gene, neuroanatomy, or other biological factors. To exemplify this point, we have shown that, after accounting for FMR1 protein expression and other background factors, the quality of the home environment (i.e., parent responsivity, availability of learning materials, cultural, recreational, or artistic enrichment, family companionship, and the quality of the physical environment) is independently associated with cognitive ability [Dyer-Friedman et al., 2002], adaptive skills [Glaser et al., 2003], and autistic behavior [Hessl et al., 2001] in children with FXS. Thus, the degree to which individuals with FXS are susceptible to both neurobiological and environmental factors is critical in charting the course of effective treatment studies.

REFERENCES


MRDD RESEARCH REVIEWS • NEUROANATOMY AND NEUROENDOCRINOLOGY • HESSL ET AL.

23


Sapolsky RM. 2000. Glucocorticoids and hippocampal atrophy in neuropathic disorders. Arch Gen Psychiat 57:925–935.


