Neurobiology of PTSD

by John J. Medina, PhD

This is the last installment in a 3-part series discussing the behavioral, cellular, and molecular characteristics of posttraumatic stress disorder (PTSD). In Part 1, I described some basic clinical observations of PTSD and the challenges these observations pose to researchers attempting to understand underlying biological substrates.[1] Part 2 examined progress on addressing these challenges at the level of the tissue and cell.[2] In Part 3, I will discuss efforts to understand PTSD at the level of DNA, including potential genetic underpinnings and heritable risk factors.

As with the previous installments, we will quickly see that much research remains to be done before the overarching biological basis of PTSD is fully characterized. There may even be some epigenetic influences to consider. To begin examining these genetic issues, I will discuss research on variable responses to stress in outbred laboratory animals and then move on to genetic and environmental influences in humans.

Hints of genes

One of the most difficult challenges facing researchers interested in the biological explanation of PTSD lies in the extraordinary variability of stress responses. Can the disorder be explained genetically? Environmentally? It is in many ways the age-old question of nature versus nurture, without the convenient rejoinder that it is "both."

The strongest hint that PTSD variability may be explained as a preexisting condition rose from the twin studies discussed in Part 2.[2] Researchers found an increased prevalence of PTSD in monozygotic twins who were discordant when exposed to intensely stressful events (combat). The sibling who was exposed to the trauma had an alteration in hippocampal volume and morphology; the other twin did as well, even though that twin had not experienced the trauma (and showed no signs of PTSD). These studies attempted to explain the striking individual differences by posing the question: Does preexisting brain morphology serve as a risk factor of PTSD? The not-so-subtle subcontext was: Might this morphology be genetic?

The answer to these questions must be a resounding "no," or at least "not yet proved." (As shall be seen at the end of this column, the very nature of hippocampal volume has been called into question.) The ideas behind biological susceptibility, however, have been gaining traction among scientists investigating animal models of PTSD. One particularly well-designed experiment that was recently published involved a group of researchers who examined reactions to fear in groups of outbred laboratory rodents (looking at conditioned fear reactivities). Two days after being conditioned, the researchers noticed clear individual differences in reactions to stressful situations. Taken as a population, the effect actually had a normal distribution.

Part 3

The novel approach to this work came from what the researchers did next (**Figure**). Many scientists conducting this type of study look at the distribution of reactions in the populations that are being observed and then focus on the average responses of the group (the middle hump). But what about the animals at either end of the population curve? Those at one end showed weak reactivity and actually appeared to be stress-resistant. Those at the other end showed strong reactivity and appeared to be stress-sensitive. If you are interested in PTSD, you might very well be interested not in the average response population but in the highly reactive response population.

That is exactly the population on which these researchers focused next, testing additional reactions in an increasingly detailed manner. Two additional phenotypes were discovered in this overly reactive population. One group of animals appeared to recover much more quickly in fear extinction experiments; another population did not. Could this second group represent a clinically useful animal model for PTSD? Because the genetic histories of these animals are well-characterized, it is possible to start answering genetic questions related to PTSD. That is where the current state of the research lies.

Of course, it is not only animal populations that have been examined. Researchers have focused for years on heritability issues regarding stress responses in humans. To understand one of these experiments, I must review something called "variable number terminal repeat (VNTR) analysis."

VNTR analysis

Certain regions of the mammalian genome consist of short, repeated, noncoding sequences called VNTRs. There are 4 useful things to know about VNTRs. First, everybody has them. Second, they are liberally sprinkled throughout the chromosomes of most creatures, from fruit flies to humans. Third, there are wide variations between individuals in VNTR length and sequence order. Fourth, because VNTRs populate chromosomes, they can be inherited.

Put these 4 facts together and you get a terrific way of identifying individuals—you can even determine something about the genes they are capable of inheriting. Every person has individual VNTR patterns and every person passes these patterns on to the next generation. All persons can be individually identified (and even assigned relative genetic relationships) simply by taking blood samples from family members and looking at discrete VNTR patterns.

This can be very useful if you are interested in understanding the genetic basis of certain pathologies. VNTRs can act like homing beacons identifying snippets of DNA potentially responsible for specific diseases. If you can associate a particular pattern of VNTR inheritance with a particular disease, you can begin examining local genes for structural abnormalities within the vicinity of the inherited VNTR (or even genes that possess the VNTR). If you find a gene that is defective, it may be a candidate gene for the disorder. This is the first important step in whittling down the number of candidate genes from "all" to "those that reside close to the VNTR."

Although this can take a lot of work, researchers have tried to do such analyses in PTSD. The results have been decidedly mixed. In one case, a VNTR variant was found near a dopamine transporter gene. This variant was found in 93 patients who had experienced PTSD but was *not* found in 95 patients who had undergone severe trauma and did not present with PTSD. Another group looked at the association between mutations in the glucocorticoid receptor and PTSD. They were able to show an association between variants in this gene and the severity of symptoms of PTSD (patients possessing one variant displayed more severe symptoms than patients with another). Other groups have shown associations between mutations in part of the regulatory circuits for a serotonin transporter gene.

How can we organize all this important work? The bottom line is that we cannot. It is very difficult to do these investigations in a responsible manner and even harder to replicate certain findings. Indeed, PTSD may be represented by mutations that must be carried by many genes. There may even be epigenetic reasons for the disorder that have nothing to do with heritable mutations per se. Epigenetic means related to functional changes in the genome that can be regulated by external environmental events that do *not* involve alterations in the genetic code. It is to epigenetic investigations that I now turn.

Epigenetic studies

One epigenetic mechanism is called "methylation," a molecular process that affects the activity of a large percentage of genes throughout the animal kingdom. Methylation literally means to add a methyl group (CH_3) to certain nucleotides in the double helix, which usually results in the methylated gene becoming inactive.

Methylation may be involved in the development of stress regulation in early life. One particularly interesting programming alteration involves the methylation of genes regulating certain stress responses found in the hypothalamic-pituitary-adrenal (HPA) axis. This methylation occurs

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because of early life events, one of the most obvious being maternal care. In rat pups, permanent alterations in hippocampal glucocorticoid receptor expression and subsequent HPA function have been demonstrated to occur as a direct result of environmental influence. Some researchers believe this linkage may explain why events that happen early in life can predict both the development of PTSD and the development of the HPA axis. Such clear mechanisms are currently a focus of intensive research.

Other epigenetic explanations are not so clear-cut and may be epigenetic only in the loosest sense of the term. A number of recent longitudinal studies have looked at independent variables spanning the entire course of the illness. A 10-year follow-up study in Holocaust survivors clearly demonstrated decline in stress hormone levels for those patients in whom PTSD developed or in whom PTSD was chronic. No such decline was shown for those survivors who did not present with PTSD. Indeed, they showed a relative *increase* in hormone levels.

Hippocampal volume has also been studied (using the same types of experiments as the twin studies described previously). Hippocampal volume can be quite variable. It can change as a result of environmental exposure, duration of certain illnesses, and even the age of the subject. As noted, shrunken hippocampal volume has been shown in PTSD patients, but most of these are younger cohorts. When one examines PTSD in older cohorts, the differences in volume evaporate, and no linkage with disease is observed. What does that mean? Simply that the association is not a simple one. Age-related changes—as well as a host of other environmental factors—must be taken into account when one is attempting to understand the genetic basis of complex symptoms like those of PTSD.

The future

That last sentence summarizes these 3 articles. It doesn't really matter at what level you choose to look—behavioral, cellular, or genetic—you run into the same types of success and the same types of turbulence. From the behavioral side, there is still a need to create discrete diagnostic categories that are robust enough to make sense to a test tube. Tying such behavioral protocols to neuroanatomical interactions will go a long way toward creating distinct disease subtypes and ferreting out the cellular basis for the surprising individuality of PTSD. Once such phenotypes are established, the genes functioning within the characterized neural circuits can be examined. Microarray analysis—a powerful way to look at global gene expression profiles in tissues—can identify relevant molecular processes. Only then will we have enough tools on hand to find the genes responsible for the disorder. Studying such genes can lead to creating effective treatments, including designing medications that are not only specific to the disorder but also specific to an individual's experience with the disorder.

I started this series by discussing some of the combat veterans I met while growing up in a military family. Many of them were real heroes to me. They still are. Given the present political climate and military situation, we are poised to add to their ranks in ways not seen since Vietnam. We owe it not only to them but also to future cohorts to fund more research now in order to create tailor-made treatments as soon as possible. It is not a simple task, but it may be one of the best things we can do with our research dollars.

Dr Medina is a developmental molecular biologist and private consultant, with research interests in the genetics of psychiatric disorders.

References

1. Medina JJ. Neurobiology of PTSD: part 1. *Psychiatr Times.* 2008;25(1):29-34.
2. Medina JJ. Neurobiology of PTSD: part 2. *Psychiatr Times.* 2008;25(2):18-20.

Figure

A genetic approach to PTSD

The experience of PTSD can vary from one individual to the next. Attempts to create animal models mimicking this variability are an important step in understanding potential underlying genetic mechanisms. Shown below are the results of one attempt (see text for a more detailed explanation).