The Neurobiology of Executive Function Under Stress and Optimization of Performance

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Abstract. Much basic and clinical research to date has investigated predictors of stress resilience and vulnerability, indicating, for example, that broad impact neurobiological factors, such as neuropeptide Y (NPY) and neuroactive steroids, are mechanistically related to short term stress resilience, as well as longterm patterns of stress-related medical and neuropsychiatric comorbidities. The problem is that we lack good methods for identifying predictors of stress resilience or vulnerability at an individual level, so that human performance and therapeutic interventions can be targeted precisely to underlying points of malfunction for maximum effectiveness. We thus propose modified experimental designs that capitalize on our growing capacities to query and analyze multimodal data across the translational levels of human biology and behavior. We propose that use of these methods in studies of individuals participating in intense military training or returning from deployment could enable better prediction of performance, and development of more effective personalized interventions aimed at optimizing and maintaining stress resilience over time.

Keywords: Resilience · PTSD · Translational neuroscience · Neuropeptide Y · Allopregnanolone · Neuroactive steroids · Predictive algorithms · Functional data analysis · Non-linear modeling · Machine learning

1 Introduction

Hallmarks of resilience in the face of threat to survival, which is typically accompanied by intense psychological and physiological demand, include the capacity to: (1) respond physiologically and behaviorally as needed in the moment, (2) moderate arousal, so as to think rapidly and flexibly, (3) recover rapidly, and (4) adaptively upregulate systems under demand so that the challenge can be met more easily the next time. Then, should environmental threat recede over time, the downregulation of formerly adaptive stress reactions promotes readjustment. The failure of these capacities may present immediate risk, as well as the potential for longterm psychological, interpersonal, vocational and medical disabilities. As a result, there is great interest in enhancing the stress resilience of military personnel who confront life threat as a routine part of their duties.

From a research perspective, four major developments from studies of stress resilience and posttraumatic stress disorder (PTSD) conducted over the past 15 years

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promise to advance this goal. First, a plethora of relatively small scale studies, appropriately constrained in breadth and depth by data analytic attention to risk for Type I error, have demonstrated that *multiple individually variable* biological components of the complex stress response *interact* to influence both: (a) *short-term* physiological, psychological, cognitive and behavioral reactions *during acute stress*, as well as (b) *longterm adaptive and maladaptive responses to stress*. The latter include posttraumatic stress disorder (PTSD) and a number of PTSD-comorbid conditions, such as depression, chronic pain, substance abuse disorders, traumatic brain injury, autoimmune disorders, cardiovascular disorders and metabolic syndrome [1–3]. Secondly, there is now much evidence to suggest that many biopredictors of acute stress resilience *reciprocally* predict PTSD risk [1, 4]. Third, advances in epigenetics have both dispelled the notion that genotype is "determinant" and revealed means by which dysregulated stress systems may be modified or even restored to normal function—using techniques likely to evolve quickly over the coming years. Fourth, new and rapidly evolving bioinformatics tools now allow multimodal, multi-level data analysis to help: (a) elucidate the multiple interacting *translational mechanisms* that contribute to higher order *behavioral phenotypes*, and (b) develop algorithms for prediction of stress resilience or PTSD risk on an individual basis. Combined, these capacities promise to facilitate development of individualized interventions to enhance human performance or therapeutically restore normal function.

These new opportunities in the field of stress research also have generated new prospective study designs aimed at identifying individual level predictors of stress resilience or vulnerability amenable to modification. Such studies *collect a dense translational array of known as well as prospective resilience relevant biomarkers from individual participants* exposed to field relevant stress conditions or more convenient proxy conditions validated against field relevant conditions. While more costly and labor intensive in the short term, this approach seems most likely to accelerate our understanding of "how stress resilience works"—a prerequisite to effective individual targeting of performance enhancers and therapeutics.

In contrast, previous neurobiological predictors of military performance and selection, or of PTSD risk and severity, have been studied alone or, at most, a few at a time in different populations. For example, in the seminal series of studies by Morgan et al. [5–8], each hormone predictor [e.g., peak stress plasma levels of neuropeptide Y (NPY) or ratios of salivary dehydroepiandrosterone sulfate (DHEAS) to plasma cortisol, or resting plasma DHEA(S) levels measured across intense training] accounted for about ∼16–30 % of the variance in military performance and ∼16–40 % of the variance in dissociation at peak stress. Thus, it is clear that other factors must be considered in addition to these in order to achieve adequate predictive power. In addition, these hormone predictors were not correlated within individuals when more than one predictor was measured. This suggests that diverse physiological systems mediate stress resilience in different individuals. This observation is consistent with a study [9] showing that PTSD symptoms were uniquely exacerbated by norardrenergic (NA) system activation in about one-third of Viet Nam veterans tested, by serotonergic system activation in another third, and by activation of either system in yet another third.

These earlier military stress resilience studies demonstrate another principle to consider in the design of future studies. Dissociation, as measured by the Clinician Administered Dissociative States Scale [10], was a stronger predictor of military performance than hormone levels or ratios that predicted dissociation. This is to be expected. As indicated in Fig. 1, the contribution of a particular predictive factor to the variance in a behavioral phenotype (e.g., selection for Special Forces, PTSD risk) increases as the translational level of organization increases (i.e., from genes to gene products to physiological processes to higher order neuropsychological function to observable behavior). This is generally due to the fact that higher order phenomena emerge from combinations of lower order components that vary and interact.

2 Broad Impact Biological Contributors to Stress Resilience

Some stress-sensitive neurobiological factors, however, have particularly large effects on stress-related outcomes due to their *broad impact* in the brain and peripheral physiological systems. Pathological variations in such broad-impact factors, in turn, have *manifold* pathological effects—which may account for the high rates of comorbidity among what might otherwise appear to be discrete clinical phenomena resulting from stress. High rates of association among apparently discrete stress-related disorders thus may signal the presence of shared underlying pathophysiologic processes amenable to common therapeutic approaches. The precise point of dysfunction within such broad impact systems may, however, vary from one individual to another. The following subsections therefore discuss the subcomponents of two broad-impact stress-sensitive systems previously found to influence stress resilience and/or stress vulnerability. Knowledge of the translational subcomponents of each system, as well as of extra-system factors that may impact each system, will be critical to developing maximally effective, individually targeted interventions to enhance stress resilience.

2.1 Neuropeptide Y

As previously reviewed [2, 4], NPY is co-localized with a variety of neurotransmitters in the brain and periphery. During the resting state, extra-neuronal NPY reduces the release of neurotransmitters with which NPY is co-localized intra-neuronally by activating pre-synaptic NPY-Y_2 autoreceptors. Once released in response to intense neuronal stimulation, NPY facilitates the post-synaptic effects of its co-localized neurotransmitter. NPY thus functions generally as a neurobiological capacitor or high-pressure valve, effectively conserving bioenergy for use under high demand situations. NPY also promotes adaptive anabolic processes when environmental demands exceed physiological capacities. For example, NPY stimulates growth of cardiac collateral vessels in response to ischemia [11] by inducing platelet-derived growth factor (PDGF) and exerting mitogenic effects on vascular smooth muscle and endothelial cells [12]. As previously reviewed [1], NPY promotes post-stress feeding and lipogenesis, which is advantageous in the aftermath of stress that drains energy reserves. However, NPY acting at NPY-Y_2 receptors, promotes metabolic syndrome when stress is coupled

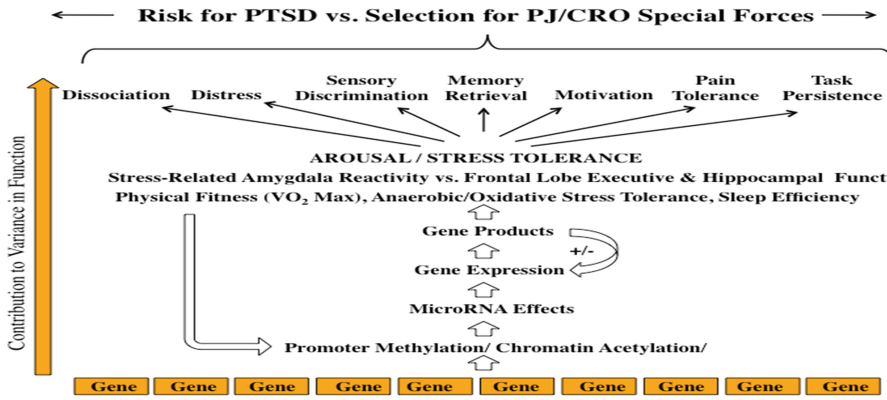

Fig. 1. Interactive translational processes that determine and define stress resilience

with excess sugar and fat calories [13]. NPY also has anti-inflammatory and anti-nociceptive properties [3], preventing opiate tolerance and withdrawal, and the progression of acute pain to chronic pain. It also promotes sleep [14] and likely enhances function of the recently discovered "glymphatic system", which clears damaging byproducts of oxidative stress from brain during sleep [15]. In addition, NPY stimulates neurogenesis [16] and enhances nucleus accumbens mediated reward [17]. Thus it is not surprising that dysregulation of the NPY system has been related to risk for PTSD, chronic pain, neurodegeneration, cardiovascular disease, metabolic syndrome, and immune system dysfunction—clinical phenomena that frequently co-occur among persons exposed to chronic severe or traumatic stress.

The importance of investigating sub-components of the NPY system that contribute to resilience or PTSD risk is particularly well exemplified by the sympathetic NPY-NA subsystem. As reviewed and illustrated in Pitman et al. [4], mild to moderate stress increases arousal by activating the release of norepinephrine (NE), which at moderate levels acts at high-affinity NA α_2 receptors in the frontal lobe to enhance frontal lobe executive capacities and frontal lobe inhibition of the amygdala. Intense stress, however, may induce hyperarousal by releasing NE in greater amounts, resulting in activation of low-affinity NA α_1 receptors in the frontal lobe and degradation of frontal lobe function [18]. This results in a lifting of the frontal lobe brake on amygdala-mediated reactions, including defensive behaviors, cardiovascular system responses, hypothalamic-pituitary-adrenal (HPA) axis activation, and brain monoamine release [19].

During resting and low stress conditions, NPY restrains NE release from sympathetic neurons by activating pre-synaptic NPY-Y_2 receptors that interact with NA-α_2 autoreceptors [20] —thus moderating arousal and facilitating calm. However, during intense stress, NPY is released from sympathetic neurons along with NE to facilitate post-synaptic NA effects. In the meantime, dipeptidyl peptidase 4 (DPP-4) cleaves the first two N-terminal amino acids from NPY to produce $NPY_{3–36}$, a selective NPY-Y_2 receptor agonist with low NPY-Y_1 receptor affinity [21]. Activated NPY-Y_2 receptors likely then interact with NA-α_2 receptors to reduce the firing rate of sympathetic neurons and release of NE—restoring calm and frontal lobe function.

Consistent with this schema, NPY measured at peak stress during military survival training was highest among Special Forces, and correlated inversely with dissociative symptoms and positively with military performance reliant on *use of previously learned tactics*, metrics likely reflecting frontal lobe capacity [5]. In contrast, Vietnam veterans with severe chronic PTSD have been shown to have low cerebrospinal fluid (CSF) levels of NPY [22], as well as low resting plasma NPY levels and blunted NPY responses to stress along with abnormally high and prolonged plasma NE responses [23]. In the latter study, low resting plasma NPY levels were inversely correlated with PTSD symptoms. In another study [24], high resting plasma NPY levels were correlated with greater improvement in PTSD symptoms over time.

It is therefore noteworthy that severe stress reduces baseline plasma NPY levels [25, 26], an *individually variable effect* that differed between unselected and selected Special Forces over intense selection training (CA Morgan, personal communication). Of possible relevance to these findings, NPY gene expression and synthesis are upregulated by glucocorticoids [13] and testosterone, but downregulated by estrogen [21], a basis for possible general gender differences in the mediation of military relevant stress resilience and PTSD risk. Both NE and NPY release during stress are also influenced by genotype [27–29], including possible alterations in the Kozak sequences of two NPY gene start codons that determine whether NPY is made available for release or targeted to mitochondria [30]. In mitochondria, NPY diminishes oxidative metabolism, which may be immediately protective during stress, but promote longterm stress intolerance, "burn-out", depression, fatigue, or weight gain. The NPY gene also has a large CpG island in its promoter, suggesting the potential for methylation dependent epigenetic downregulation, a potentially reversible process that may contribute to stress-induced downregulation of NPY synthesis.

We therefore believe that full characterization of NPY-NA system subcomponent function could enable more effective prediction of military performance. For example, possession of a gain-in-function NPY gene polymorphism might predict short-term stress resilience [5], but in the longer term, metabolic syndrome [13] —although perhaps not if compensated by possession of a loss-of-function NPY-Y_2 receptor polymorphism. A gain-in-function NPY gene polymorphism might not be predictive of resilience in a model relying just on genotype data, however. Combining genotype and gene methylation data may be more effective, as a normal NPY gene with a methylated promoter conferring loss-of-function can be equated with loss-of-function NPY gene polymorphisms to predict risk.

Knowledge of precise points of NPY-NA system malfunction may also enable more precise treatment targeting. For example, selective NA α_1 receptor antagonists (e.g., prazosin) have been shown to enhance sleep and moderate daytime reactivity to stressors in PTSD [31]. These medications might be prescribed as a temporary measure to individuals in whom the NPY-NA system has been temporarily re-programmed by exposure to extreme stress, but they may need to be prescribed chronically to those with gene polymorphisms associated with excessive NE release even at baseline (e.g., the NA α_{2C}Del322-325 gene polymorphism [27] or loss-of-function NPY gene polymorphisms [29]). Intranasal NPY could potentially be used to facilitate engagement of the frontal lobe during cognitive reprocessing therapies to promote fear extinction or extinction recall. Deacetylase inhibitors perhaps could be prescribed briefly to reduce

NPY promoter methylation and thereby enhance NPY gene expression. Exercise or use of ketogenic diets may have similar benefits. Moderate progressive exercise has been used in already trained male rowers to further increase the capacity for NPY release during intense exercise challenge [32]. We hypothesize that global stimulation of free fatty acid oxidation to acetyl CoA by exercise or use of a ketogenic diet may enhance chromatin acetylation, and thus facilitate access of regulatory elements (e.g., testosterone or cortisol) to DNA response elements in the NPY gene promoter during stress exposure—with consequent upregulation of NPY synthesis.

2.2 GABAergic Neuroactive Steroids

As reviewed [2, 4], the neuroactive progesterone metabolites, allopregnanolone and its equipotent stereoisomer, pregnanolone (collectively termed ALLO), potently and positively modulate GABA action at brain $GABA_A$ receptors by increasing Cl- influx 7–10 fold [33]. ALLO thus function generally as a resistor in stress sensitive brain circuits connecting somatosensory cortex, thalamus, prefrontal cortex and the amygdala [34]. However, ALLO is also critical for production of myelin, which speeds neurotransmission in select circuits, suggesting a broader role for ALLO in coordinating neuronal networks. Of note, ALLO has highest affinity for extrasynaptic benzodiazepine-resistant $GABA_A$ receptors that *reduce gain* in neuronal firing rates during periods of increased activation, as occurs during stress [35]. This is an important characteristic, because synaptic benzodiazepine-sensitive $GABA_A$ receptors and GABA synthesis are downregulated in the amygdala in response to fear conditioning [36], which in the extreme, models exposure to traumatic stress. Thus restraint of amygdala reactivity after trauma exposure may rely on extrasynaptic $GABA_A$ receptor activation by ALLO synthesized locally or released from the adrenal gland.

Like NPY, ALLO has broad impact in the brain and periphery, exerting potent anxiolytic, anti-conflict, anticonvulsant, anti-nociceptive, anesthetic, sedative, anti-inflammatory, pro-myelination, neurogenerative and neuroprotective effects. ALLO levels peak about an hour after stress exposure [37], to provide delayed negative feedback and return stress responses back to baseline [38]. Thus it is noteworthy that CSF levels of ALLO were found to be low (39 % of normal) in women with PTSD and to correlate inversely with PTSD reexperiencing and negative mood symptoms [39]. Similarly, male OEF/OIF veterans showed a reduction in the plasma ratio of allopregnanolone to progesterone, in association with increased depression and PTSD symptoms [40]. Pilot work showing a correlation between maximum load exercise-induced plasma ALLO levels and pain tolerance is also consistent with the anti-nociceptive properties of ALLO (E. Scioli-Salter and A. Rasmusson, personal communication). In addition, ALLO synthesis deficits have been induced by binge alcohol consumption in a rodent model [41]. Together, these studies support the idea that PTSD and comorbid conditions such as depression, chronic pain, and alcohol abuse may share underlying pathophysiological processes—that may respond to common therapeutics.

Two enzymes are involved in the synthesis of ALLO from progesterone, 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD). A rodent model of PTSD

[42] and a recent genotype study in humans [43] suggest that diminished function of 5α-reductase contributes to PTSD symptomatology in males. In contrast, Rasmusson et al. [39] suggest that a block in ALLO synthesis at 3α-HSD promotes PTSD in women. Cortisol and testosterone upregulate expression of the 3α-HSD gene, suggesting that low levels of these steroids might also contribute to deficient ALLO production and PTSD risk. Alcohol-induced reductions in nicotinamide adenine dinucleotide phosphate (NADPH), a protective antioxidant and 3α-HSD enzyme cofactor may also contribute. Such causes for ALLO synthesis deficits may apply to both males and females. The enzymes 5α-reductase and 3α-HSD also synthesize GABAergic neuroactive steroids from testosterone, androstenedione and cortisol. Thus, measurement of the full complement of GABAergic neuroactive steroids may be needed to accurately assess stress resilience across gender and at the individual level in order to improve predictive models and best target potential therapeutics.

Based on these studies, it seems reasonable to consider ALLO "replacement" by synthetic derivatives (e.g., ganaxolone) in the prevention or treatment of PTSD and its comorbidities. Recent work suggests that ganaxolone blocks fear memory reconsolidation during extinction training and promotes extinction retention [44]. Of note, the beneficial effects of ganaxolone were not observed in animals without ALLO deficiency. Alternatively, administration of selective brain steroidogenic precursors such as pregnenolone [45] or other stimulants of GABAergic neuroactive steroid production [46] may be helpful. The presence of a CpG island in the 5α-reductase gene promoter also suggests the potential for epigenetic regulation of ALLO synthesis [47].

3 Modeling and Analysis of Contributors to Stress Resilience

Development of prediction models is a multi-step process that begins with investigation of individual measurements and builds to a multimodal characterization of the data. Traditional analytic methods employ standard statistical techniques, such as linear modeling, to examine specific factors in isolation or in limited combinations. While these approaches have yielded valuable insights about groups of people as described above, they have yet to reveal a richer understanding of the mechanisms operating at the level of the individual. Recent advances in functional data analysis, non-linear methods, and machine learning offer the potential to develop new models that consider a broader set of factors to predict stress resilience or stress vulnerability for the individual. A brief discussion of these methods is presented here.

3.1 Functional Data Analysis

An individual's performance can vary over time with repeated exposures to stress or life threat followed by periods of recovery. While traditional statistical methods can analyze a point in time, understanding trajectories is necessary to characterize short-term responses, recovery, and long-term adaptations. Functional data analysis is a new approach to handling these challenges by representing values across time as mathematical functions [48]. Measurements collected at various times, representing

various stages of stress exposure and recovery, can be expressed as a simple function of time, such as a polynomial. The parameters that define the function relationship then become the variables employed in the statistical analysis. The terms of the functional representation provide a powerful method for characterizing resilience over time. The constant and linear terms, for example, represent the level and general trend in performance over time. Quadratic and higher-order terms indicate the temporal dynamics of the process within such overall trends and thus can capture changes in performance more finely. Functional data analysis methods have proven useful in other biomedical applications, such as modeling acute limb ischemia and reperfusion injury [49]. For modeling resilience, functional data analysis provides a natural framework for analyzing temporal changes in individual performance.

3.2 Non-linear Modeling

Multi-modal data acquired through studies of stress resilience and vulnerability yield rich data sets, providing new opportunities for discovery and analysis. However, the size and complexity of the data pose challenges. Variables of interest can include psychophysiological indices, levels of various neurotransmitters, and performance scores for various cognitive and physical tests. Taken at face value the number of variables can appear daunting, but the underlying physiology will constrain the relationships among the measurements, causing the data to be in some lower dimensional arrangement within the high dimensional space implied by the full set of measurements. Standard methods for data reduction, such as principal component analysis (PCA) or factor analysis, seek to identify the implicit dimensions, which capture the major variance in the data. These methods work well when a simple affine procedure will transform the data into an appropriate low-dimensional space. However, because of complex and possible mechanistic mediational relationships among the various measurements, in many cases the very high-dimensional data actually exist on a much lower dimensional manifold. A new class of methods, known as manifold learning [50], has emerged to handle these problems. These methods for non-linear dimensionality reduction seek to discover the underlying structure of the data. A number of techniques have been proposed for this type of analysis – principal curves, Gaussian process latent variable models, and kernel PCA, to name a few. The goal of these techniques is to learn the low-dimensional manifold and thereby develop a compact representation of the data that is more tractable. Once this new representation is known, data analysis and prediction modeling can operate off of the lower-dimensional representation to achieve robust prediction modeling.

3.3 Machine Learning

Machine learning encompasses a class of methods for discovering relationships by letting the data speak, producing a mapping from a set of measurements to a set of class labels. For analyzing resilience, the class labels might represent different trajectories that were identified during the functional data analysis. The common approach is to use a set of “labeled data” (i.e., a set where the class labels and the various measurements

are known) as the training data for learning the mapping between the measurements and the classes. This learning stage produces a classifier that is then evaluated on new data to assess its accuracy. For predicting trajectories of human performance capacity, a major challenge is the multi-model nature of resilience relevant data. To develop a robust classifier requires finding a common framework for merging the diverse set of measurements. For similar research problems, where data arise from a diverse set of measurements and observations, multiple kernel learning (MKL) has proven successful. This novel machine learning technique mathematically "homogenizes" different types of data through an optimized estimation of the contributions from each data type. MKL has proven effective in modeling heterogeneous data for a number of applications [51, 52]. Use of this approach will allow for comparisons of different variable combinations to identify the most predictive set of variables, while ensuring the model is stable and does not over fit to the data.

4 Summary

Much research to date has investigated predictors of stress resilience and vulnerability, and indicates that broad impact neurobiological factors such as NPY and neuroactive steroids may be mechanistically related to short-term military performance and long-term stress-related medical and neuropsychiatric outcomes. The problem is that we lack good methods for identifying predictors of stress resilience and vulnerability at the individual level. We thus propose modified experimental designs that capitalize on our growing capacities to query and analyze multimodal data across the translational levels of human biology and behavior. This approach allows consideration of critical interactions among multiple stress reactive systems that vary across individuals along the translational spectrum. It also allows discovery of novel factors that may contribute to resilience. In addition, use of new data analytic methods may help define underlying mechanisms shared among stress-related outcomes. We propose that use of these methods will enhance our ability to both predict human performance under high stress conditions, as well as diagnose dysfunction of resilience-related systems, at the individual level—thus, enabling personalization of performance interventions and therapeutics to increase, maintain and restore stress resilience over time.

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