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The complexity of simplicity: Role of sex, development, and environment in modulation of the stress response

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Abstract

Anecdotally, we all know stress is “complicated”, but most stress research is undertaken using incredibly simplified models that may not allow us to fully understand underlying interactive mechanisms that are present in the “real world”. This attempt at simplification, while sometimes necessary, may explain some of the difficulties in translating basic science findings to the clinical and epidemiological data on stress and stress-related disorders. In a symposium at the 2015 International Society for Psychoneuroendocrinology meeting in Edinburgh, UK, a series of speakers explored “*The many pathways to plasticity in the stress system*”, specifically focusing on variables that are in many cases eliminated from studies of stress in order to provide increased experimental control. Specifically, four speakers tackled the complex contributions of *Sex*, *Development*, and *Environment* in stress research, providing evidence from published and unpublished work from their own laboratories demonstrating that in our race for simplicity in experimentation, the stories we tell become all the more complex.

Keywords

Sex differences; puberty; metabolism; immune; circadian; plasticity

Anecdotally, we all know stress is “complicated”. While it is an oversimplification to state that there is good stress (eustress) and bad stress (distress), this type of simplification can be helpful as a way to structure experiments and characterize observed responses. However, simplification, and more specifically oversimplification, can also be an enemy to scientific progress. In fact, the difficulty in translating many basic science findings to the clinic (or wider “real world”) may occur because in our attempts to control for all “confounds” we have missed important mechanisms. As such, the adage that the whole is greater than the sum of its parts may be particularly relevant in our explorations of stress and its effects on human health.

The stress response involves myriad neurobehavioral and physiological systems that allow an organism to adapt to environmental demands, hopefully ensuring survival. Given that the stress response and stress physiology is inherently complicated, it is not surprising that researchers who study stress strive to reduce some of this complexity, attempting to control

(as any good experimentalist tries) all of the variables except for the one being measured. However, in some cases, trying to simplify or reduce a response as complex and integrative as stress can result in a fundamental misinterpretation of the experimental outcomes, possibly serving as a confound that defeats the purpose of the simplified experimental preparation in the first place. In a symposium at the 2015 International Society for Psychoneuroendocrinology meeting in Edinburgh, UK, a series of speakers explored “*The many pathways to plasticity in the stress system*”, specifically focusing on variables that are in many cases eliminated from studies of stress in order to provide increased experimental control. Specifically, four speakers tackled the complex contributions of *Sex*, *Development*, and *Environment* in stress research, providing evidence from published and unpublished work from their own laboratories demonstrating that in our race for simplicity in experimentation, the stories we tell become all the more complex.

It is obvious that development can fundamentally alter the function of neural and physiological circuits. By controlling for age, investigators attempt to mitigate these significant effects. However, by reducing variability due to developmental processes, we potentially miss important outcomes that could inform our understanding of the basic function of these circuits. Moreover, without careful examination of the timing of these processes it is highly unlikely that laboratories will rationally pick the optimal age to do their experiments, and instead remain locked in the “this is the way our lab has always done it” mentality. In his presentation, Dr. Russell Romeo discussed his work, and the work of others, in exploring the impact of age on physiological and behavioral stress responses.

Dr. Romeo presented data from his lab that demonstrate age can affect the stress response. Specifically, prepubertal Sprague Dawley rats (30d old) show a protracted hormonal response to a 30min restraint stressor as measured by both corticosterone (CORT) and adrenocorticotrophic hormone (ACTH). Moreover, females show a higher peak response than males, but still show the same prolonged response compared to adults (1). In addition to differential responses to acute stress, the Romeo lab also has shown differential response to repeated (sub-chronic) stressors. In response to a homotypic (7d repeated 30min restraint), prepubertal males show a much higher response to this same restraint stress at the 30min time point on day 8 than do adults, while the protracted response observed using acute stress is no longer observed. Conversely, when exposed to a heterotypic stress (7d repeated cold exposure), a further exposure to 30min of restraint on day 8 does not result in a higher hormonal stress response at the 30min time point in prepubertal rats, but does recapitulate the acute stress findings showing a protracted response compared to adults (2). These findings illustrate quite clearly that age, sex, and experience can result in differential responses to stress. At the level of the paraventricular nucleus (PVN), a key node in the hypothalamic pituitary adrenal (HPA) axis, expression of the immediate early gene *cfos* seems to mirror the physiological findings quite closely, with prepubertal rats showing enhanced *cfos* protein levels following stress. More specific dissection of the PVN by specifically focusing on the corticotropin releasing hormone (CRH) positive cells of the PVN indicate that this differential response is clearest in this cell group, thus suggesting that some of these differences are generated by altered HPA drive. However, to more fully explore changes in the circuitry, and current work in the Romeo lab is focused on understanding how developmental changes in both excitatory drive to the PVN (e.g. from the

anterior bed nucleus of the stria terminalis (aBST) and nucleus of the solitary tract (NTS), or inhibitory tone to the PVN (e.g. peri-PVN, posterior BST, and medial preoptic area (MPO)) contribute to these obvious hormonal changes. In addition to changes in the HPA, Dr. Romeo also presented new data demonstrating developmental and sex differences in another stress-related neuroendocrine circuit, the hypothalamic-neurohypophyseal tract (HNT). While the HPA is routed through the anterior pituitary, the HNT involves direct release of the hormone oxytocin (OXT) into the bloodstream via the posterior pituitary. Unpublished work from the Romeo lab shows that prepubertal males have nearly undetectable levels of plasma OXT, which shows no increase following restraint stress, while adult males show a robust and time-dependent increase in plasma OXT. Prepubertal females, on the other hand show similarly low basal OXT levels, but show a robust increase in plasma OXT in response to restraint. Adult females show very high basal OXT, but a modest drop in OXT following restraint, that returns back up to normal levels after about 60min. Together, the data presented by Dr. Romeo illustrate the incredibly complex interactions between development, sex, and experience. These interactions can be used as tools to specifically probe the mechanisms by which development, sex, and stress exposures can differentially shape responses in both juveniles and adults. Given we know that many stress-related disorders seem to surface during the juvenile-adult transition, and that men and women differentially respond to stressors, such tools are vital.

While development and sex certainly interact to modulate stress responses, additional factors can be overlaid to further “complicate” our understanding of an individual’s response to stress. The work of Dr. Gretchen Neigh clearly demonstrates that sex differences are not simply a single variable influence on stress (i.e. males don’t just respond more or less than females). Instead, sex can differentially modulate the effects of tertiary variables on stress responsivity. One of the most significant of these variables is diet. In humans, changes in diet can be considered a “self imposed” modulating factor, and understanding how sex, diet, and stress interact to affect behavioral and physiological responses is thus essential. In addition, it is important to note that the mobilization of energy stores (specifically glucose) is a primary role of CORT. Thus the links between stress and energy balance are bi-directional.

Dr. Neigh framed her presentation with the overall theme that “opportunity costs”. That is, decisions are made by weighing the potential benefit against potential gain, and that any opportunity has associated costs. Thus, many of the choices we make have costs that are sometimes hidden or unclear at the time we make the choice. The choice of calorie dense foods, and foods high in sugars may be a good choice when one is calorie deprived. However, in states of abundant food availability, that choice can have significant consequences. Dr. Neigh brought the focus of her talk to fructose, a sugar that has seen a rapid increase in consumption in the past several decades, and closely tracks the rise in obesity around the globe. Fructose has a very different pattern of absorption and metabolism when compared to glucose, which facilitates its storage as fat. In addition, fructose does not provide the stimulation of insulin and leptin signaling that normally follows a meal, thus impairing satiety signals and likely leading to excess consumption. The significance of these effects for neurobehavioral and physiological function has also been demonstrated, with male rats exposed to high levels of fructose showing reduced hippocampal neurogenesis and

increased circulating tumor necrosis factor alpha (TNF α) (3). A major public health concern is that adolescents consume the most fructose of any age group. Thus, in the theme of this symposium, Dr. Neigh's lab explored how development altered the effects of high fructose on stress responses, anxiety, and depressive-like behaviors. Her findings indicated that while subchronic peri-adolescent stress in male rats did not lead to major effects on affective behaviors in adulthood, peri-adolescent exposure to a diet rich in fructose caused significant increases in both anxiety and depressive-like behaviors in adulthood (4). Remarkably, exposure to high-fructose diet for the same amount of time in adulthood did not lead to significant alterations in these same behaviors, clearly indicating that developmental processes and metabolic exposures interact to shape affective behaviors in adulthood. These behavioral effects were also mirrored in changes in CORT, with significant increases in stress-induced plasma CORT in adulthood when rats were exposed to high-fructose diet during the peri-adolescent period (4). At the molecular level, Dr. Neigh showed very convincing data that high-fructose diet during adolescence could remodel the hypothalamic transcriptome, causing changes in several gene pathways involved in myriad disorders, from depression to asthma, and calcium signaling to neurodegeneration. Therefore, it is clear that exposure to "choice" stressors such as high-fructose diets, can have significantly different effects in the adolescent period compared to adults. It is becoming clearer that early life experiences can have significant effects on adult function. Using this to our advantage as a tool, we gain increased understanding of the mechanisms by which the timing of environmental exposures, both voluntary and involuntary, can have long-term effects on stress reactivity. Understanding how these effects manifest at the physiological, neural, and molecular levels in animal models can provide us with valuable data to inform our understanding of the complex effects of early life exposures, nutrition, and stress in humans.

Since the stress response involves many different biological and behavioral systems, it is no surprise that there are many variables that can impinge upon, and shape, stress outputs. This is especially true when using more "complex" psychosocial stressors such as social defeat, or in the case of the work of Dr. Stefan Reber, chronic subordinate colony (CSC) housing stress. Psychosocial stressors are clearly ecologically relevant to the human condition, and development of models that can replicate many of the long term effects of psychosocial stress in humans with fidelity is thus an important goal. The CSC model that has been spearheaded by the group at Regensburg University, including Dr. Stefan Reber. CSC has been an incredibly useful tool to help unravel many of the physiological and behavioral sequelae of chronic psychosocial stress (5), and has many similarities to multifaceted human stress disorders such as post traumatic stress disorder (5,6). When Dr. Reber established his own research group at Ulm University, located about 200km west of Regensburg, one of the first tasks the new group set upon was to re-establish the CSC model in Ulm, using the identical parameters as were used in Regensburg. This initially simple task turned into a tour de force of trouble shooting and experimentation showing how many variables we take for granted can distinctly affect both physiology and behavior. More importantly, these studies now provide excellent empirical data to further investigate the mechanisms of psychosocial stress effects on health.

One of the clearest effects of the CSC model is hypocorticism, largely caused by a reduced morning peak and increased night time levels, decreased glucocorticoid resistance, increased

anxiety-like behavior, and increased inflammatory state of the colon. When the first experiments using CSC were undertaken in the new location at Ulm these changes were not detected, and in fact colon inflammation was actually reduced. What had changed? The first variable that researchers in Dr. Reber's group found was different was that the light cycle in the vivarium in Ulm was on a 14h light, 10h dark "summer-like" photoperiod, while back in Regensburg the light cycle was set to an equal 12h light, 12h dark (more on the importance of such changes in the section below). Upon making this discovery, and returning the mice to a 12:12 light-dark cycle, the model was still not working as it previously had. What else was different? Continuing the detective story, the Reber group determined that in Ulm vivarium water was acidified (low pH) while water in Regensburg was simple tap water of a much higher pH. Changing both the light cycle back to 12:12, and the water to normal tap water, remarkably, the CSC mice showed the expected hypocorticism and decreased GC resistance. However, there was still no anxiety-like phenotype, and now there was no difference in GI inflammation. Further sleuthing was needed. After much additional research, Dr. Reber's group discovered that rather than a "macro" variable like light or water content, a "micro" variable was at work: *Helicobacter*. It was determined that resident mice in Regensburg were positive for enteric *Helicobacter* species, while in Ulm they were not. Given that *Helicobacter* is known to promote colitis in a variety of mouse lines with compromised immune function (7), it was safe to hypothesize that perhaps this was another main culprit, at least for the change in the colon. Upon repopulating resident mice in Ulm with *Helicobacter typhlonius*, in a 12:12 light-dark cycle, using non-acidified water, and exposing them to CSC, the hypocorticism and somatic effects (i.e. increased colon inflammation) were finally observed. Remarkably, the combination of these three different variables also restored the effects on emotional behavior, with CSC mice showing increased anxiety-like behavior. This frustrating problem, coupled with a heroic set of careful investigative work and experimentation, uncovered the remarkably complex way both external (e.g. light, water) and internal (e.g. microbiota) interacted to change the trajectory of stress-induced changes in physiology and behavior.

Much as is in humor, it is becoming an accepted truism in many areas of biological research that "timing is everything". This should come as no surprise to neuroscientists and neuroendocrinologists. From understanding the temporal nature of firing patterns of individual neurons to appreciating how seasonality can affect gonadal and metabolic hormones, time seems to be a universal factor in nearly any biological system. Thus, it is surprising how time is sometimes ignored as an important tool to understand the function of neural circuits, behavior, and physiological outputs.

The work presented from Dr. Karatsoreos' lab at Washington State University was grounded in the overall concept of "allostasis". Allostasis, or the concept of stability through active intervention, provides a framework by which an organism employs physiological and/or behavioral responses to cope with stressors in the environment. Mediators of allostasis allow physiological systems to operate outside of their normal parameters, thus providing the capacity to respond to short term excesses in physiological demands. These mediators (e.g. glucocorticoids, inflammatory cytokines, etc.) interact in a non-linear fashion, and when engaged for too long, or when not terminated efficiently, can result in "wear and tear" on the very systems that they were originally deployed to protect. This allostatic "load" can

eventually lead to “allostatic *overload*” when combined with additional environmental demands over a long period of time. Dr. Karatsoreos’ hypothesis that circadian clocks ensure temporal organization of myriad physiological systems, and that disruption of this organization can serve as an allostatic load. He shared both published and unpublished work that demonstrated disruption of the circadian clock when combined with additional environmental stressors, can lead to negative outcomes and potentially allostatic overload. Using a model characterized in his laboratory in which mice are exposed to shortened 20h days for a prolonged period of time, Dr. Karatsoreos showed that circadian disruption leads to multi-system dysregulation, using neurobehavioral, metabolic, and immune function as model systems. Neurobehavioral effects included decreases in cognitive flexibility coupled with reduced complexity of pyramidal neurons in the medial prefrontal cortex (8). Further, long-term (8–10wk) housing in 20h days resulted in metabolic dysregulation, including weight gain, and elevated levels of insulin, leptin, and plasma triglycerides (8). Intriguingly, short-term 20h housing (4–5wks) did not lead to significant changes in weight, but did significantly raise plasma triglyceride levels. However, combining this short-term circadian disruption with a high fat diet (60% fat) “metabolic stress” resulted in much accelerated weight gain, and metabolic hormone changes. Given the important role of cytokines in allostatic responses, exploring how this system was impacted by circadian disruption was also undertaken. Short-term circadian disruption did not result in any observed changes in basal plasma cytokine responses (9), but when circadian disrupted mice were challenged with an immune stressor (lipopolysaccharide; LPS), the circadian disrupted mice showed a significantly disrupted plasma cytokine response, with some cytokines showing exaggerated activity, while others showed blunted activity following challenge (9). Importantly, all of these effects seem to be independent of sleep deprivation, as the 20h day model used in the Karatsoreos lab resulted in no demonstrable sleep loss, while showing decreased sleep quality, and disorganized sleep patterns (9). Together, this work clearly illustrates the important role of the circadian system in regulating both basal function and stress-induced changes in several different physiological systems, strengthening the notion that the circadian clock may well be an important modulator of stress resilience.

Together, the four talks presented in *The many pathways to plasticity in the stress system: Sex, Development, and Environment* symposium highlighted the fact that stress is not simple. Sex can fundamentally alter the way cells, circuits, and organisms respond to stressors. Developmental stages significantly impact how stress and stress history modulate plasticity of the many central and peripheral systems respond to stress, and what the long-term consequences of stress exposure are. Additionally, environmental factors, from the chemical composition of the drinking water to the presence of different pathogens in an animal colony can significantly affect outcomes of even the most well-characterized experimental models. Finally, circadian time has enormous impacts on both normal and abnormal function, impacting nearly all physiological and biochemical processes, thereby modulating the response to environmental challenges. Disrupting the circadian system may then lead to altered resilience or vulnerability to stress, further complicating the picture. The goal of this symposium, and this overview of the presented talks, was to help bring the vast complexity and interconnectedness of the stress response system into specific relief. While it may be necessary in some cases to try to eliminate the potential “confounds” of sex,

developmental stage, and time of day from experiments, it is also quite likely that ignoring the way these systems impact stress responses may very well prevent us from fully understanding the nuances of stress responsivity that are likely responsible for the many differential responses observed in humans, and the difficulty of translating from bench to bedside. Rather than eliminating such variables, we should instead find clever and creative ways to employ them as tools to better understand the cellular, systemic, and organismal responses to stress. Sometimes, in attempting to simplify a system to facilitate experimentation, we forget that the complexity we are trying to remove may be an integral facet of the very thing we are trying to understand.

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