LONG-TERM BEHAVIORAL AND COGNITIVE CHANGES FOLLOWING STRESS IN ADOLESCENCE

A Dissertation in Neuroscience by Lauren Chaby

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ABSTRACT

This dissertation focuses on understanding the potential for exposure to stress during adolescent development to shape adult phenotype. Chapter 1 summarizes and reviews current hypotheses addressing the effects of developmental stress, including models formulated by primarily human or biomedical researchers (i.e. the differential susceptibility and biological sensitivity to context theories) and hypotheses proposed by researchers spanning the fields of ecology, biology, and neurophysiology (i.e. the mismatch hypothesis, and allostasis and reactive scope models). Subsequent chapters (2-6) empirically assess the long-term effects of exposure to chronic unpredictable stress during adolescence (e.g. isolation, crowding, damp bedding) on adult phenotype in laboratory rats (*Rattus norvegicus*). These adolescent-stressed rats were compared to unstressed, control animals that were maintained in standard, predictable conditions throughout development. Chapter 2 investigates the lasting effects of exposure to chronic unpredictable stress in adolescence on adult decision making, coping response, cognitive bias, and exploratory behavior in rats. The results showed that exposure to adolescent-stress can have long-term impacts on behavior and cognition by shaping the interpretation of ambiguous stimuli, behavioral response to adverse events, and how animals make decisions. It was also found that adolescent-stress can induce short-term changes in the way animals interact with novel stimuli and explore an environment. Chapter 3 examines the effects of adolescent-stress on learning (both associative and reversal) and memory (both reference and working) starting 110 days after completion of the adolescent-stress treatment. Adolescent-stressed rats exhibited enhanced reversal learning, an indicator of behavioral flexibility, but showed no change in associative learning and reference memory abilities compared to rats reared without stress. Chapter 4 assesses the lasting effects of adolescent-stress on anxiety in a novelty suppressed feeding test and found elevated anxiety levels 6.5 months after exposure to stress ceased, which is after the median lifespan of wild Norway rats. In Chapter 5, I test the mismatch hypothesis, described in Chapter 1, by quantifying
the effect of adolescent-stress on foraging behavior and performance in adulthood, under both low and high-threat conditions. The results suggest that adolescent-stress exposure enabled rats to forage more effectively under novel threat in adulthood and that phenotypic changes resulting from stressful experiences during adolescence may enhance function in future high-threat conditions, supporting the mismatch hypothesis. Chapter 6 investigates whether adolescent-stress alters the allocation of time between foraging and vigilance behaviors in low and high-threat conditions in adulthood. I found no evidence of a tradeoff between foraging and vigilance, but under low-threat conditions adolescent-stressed rats spent more time foraging and being vigilant than unstressed rats, suggesting that adolescent-stress may enhance anticipation of threat in adulthood. Finally, Chapter 7 integrates the prior chapters by contextualizing the findings of this thesis in modern theories of developmental stress and introducing the arousal-shift hypothesis to explain the lasting effects of developmental stress on cognition. Some chapters were submitted to British journals and so use the European spelling of words such as ‘behaviour’ and ‘colour’. Together the results described in this thesis suggest that stress in adolescence can cause lasting changes in phenotype that persist into adulthood and serve some functional role that is dependent upon the environmental conditions an animal experiences later in life.
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DEDICATION

*Plant trees under whose shade you will never sit – Jennifer Granholm*

To the next, next generation of scientists. May they having funding, time and space to support their ideas, and the opportunity to ask questions of which we would never dream.
Chapter 1

How can we understand the lasting effects of early stress?

Analysis of current models of early stress

1) Exposure to stress in early life can cause lasting changes in adult phenotype

The potential for exposure to stress in early development to induce persistent changes in phenotype has been of interest to the scientific community for at least six decades (Bowlby, 1953; Thompson, 1957; McEwen & Wingfield, 2004). Exposure to adverse environmental conditions in early life can modulate fitness and human health outcomes by causing lasting changes in behavior, cognition, and physiology (Chaby et al., 2013; Caruso et al., 2014; Mathews et al., 2008; Toledo-Rodriguez & Sandi, 2011; Torregrossa et al., 2012). Lasting changes in phenotype following early exposure to stress (Box 1) have been well studied in mammals and documented in numerous non-mammalian taxa including fish (Pomacentrus amboinensis, McCormick, 1997; Gasterosteus aculeatus, Giesing et al., 2011), birds (Sturnus vulgaris, Coturnix japonica, reviewed in Henriksen et al., 2011), lizards (Lacerta vivipara, De Fraipont et al., 2001; Pseudomoia pagenstecheri, Shine & Downes, 1999), and invertebrates (Daphnia cucullata, Agrawal et al., 1999). For example, L. vivipara exposed to exogenous corticosterone during prenatal development are smaller at birth, have a reduced growth rate and, as juveniles, show reduced body condition and altered activity and basking behaviors in comparison with placebo-exposed offspring (modulated by sex and maternal condition; Belliure et al., 2004; Meylan & Clobert, 2004). In this system, the effects of prenatal-corticosterone exposure lead to increased philopatry and attraction to maternal odor (also mediated by maternal condition; de Fraipont et al., 2000; Meylan et al., 2004), and ultimately cause decreased mortality, which is the most important factor in determining fitness for L. vivipara (Lorenzon et al., 2001; Meylan & Clobert,
Many hypotheses have been proposed to explain the teleological role of lasting effects of early stress; the purpose of this review is to discuss these hypotheses in order to (i) further dialogues between those approaching early stress from biomedical and evolutionary/ecological perspectives, (ii) outline strengths and limitations of current hypotheses given the species and context-specific effects of early stress exposure, and (iii) address recent evidence suggesting that stress exposure early in development can have beneficial effects later in life.

Box 1: Why is stress difficult to define?

Defining stress is both a modern and historical challenge, in part because the term is used to refer to three distinct phenomena: (i) stimuli in the environment that are aversive or challenging, (ii) behavioral and somatic emergency responses, and (iii) the potentially pathological consequences of over-stimulation of the emergency response (Romero et al., 2009). Further complications arise because these processes can be species-specific, which makes it challenging to devise a single definition that can apply to all systems. For example, most organisms would collapse under 5,000 meters of water (at nearly 500 times more pressure than above water), however some deep sea organisms tolerate these conditions, including the common fangtooth fish (*Anoplogaster cornuta*), but may not survive if brought to shallow waters (Wharton, 2002). Though remarkably different, both mesophiles and extremophiles (e.g. deep sea organisms) are likely to experience stress in conditions outside of their preferred environments. However, the effects of exposure to conditions outside preferred ranges can also be species-specific; extreme environmental changes can be devastating for some species but a necessary part of the life cycle of others, even if they are ultimately fatal (e.g. fire-stimulated flowering or seed release, Bruce & Bickford, 1950; Enright & Hill, 1995; Lamont & Downes, 2011). Aspects of the stress response can also vary independently across species; for example, even though the physiological stress response is highly conserved across taxa and conditions (some aspects are common even between yeast and
mammals), behavioral responses to stress can vary considerably, even in closely related species 
(Welch, 1992; Yoshida et al., 1998; Eilam et al., 1999). Behavioral responses to stress vary from 
“active” responses, such as tail flagging in squirrels and inflation in pufferfish, to “passive” 
responses, such as freezing in rats and octopi (Hennessy et al., 1981; Brainerd, 2005; Hanlon, 2007). These inter-species differences, along with issues of self-report and potential observer 
biases across species, complicate definition and measurement of stress across species and 
contexts.

2) **Does early stress make individuals vulnerable to later stress, or prepare them for it?**

Extensive literature describes adverse effects of exposure to stress in early life, yet a growing 
number of studies are suggesting positive, potentially fitness enhancing effects of early stress on 
behavior, morphology, physiology, and reproductive outcomes (Table 1). The concept that stress 
can have seemingly beneficial effects is not new; Southam & Ehrlich (1943) called it “hormesis” 
and Hans Selye (1956) called it “eustress”, though these terms are no longer frequently used. 
Selye, often referred to as the father of stress, advised that “we must not suppress stress in all 
forms, but diminish distress and facilitate eustress…”, emphasizing the potential positive effects 
of stress exposure (Selye, 1976).

Currently, opinions of whether early stress can have beneficial effects later in life and/or prepare 
an individual for subsequent stress are mixed, and the available evidence supports both beneficial 
and detrimental consequences of early stress. For example, exposure to early stress has been 
robustly shown to increase anxiety in a myriad of taxa (Vyas et al., 2004; Sufka et al., 2006; Egan 
et al., 2009; Gatt et al., 2009), but humans with higher levels of anxiety are (i) more attentive to 
threats (Macleod et al., 1986; Macleod & Mathews, 1988), and (ii) faster at identifying 
threatening stimuli (Byrne & Eysenck, 1995). Highly anxious rats and humans also have a larger
amplitude startle response (Kaviani et al., 2004; reviewed in Davis, 1990), increased activity of the autonomic nervous system during anticipation of threat (Melzig et al., 2008), and elevated resting levels of norepinephrine, all of which serve as vital components of the initial stages of the stress response (Mathew et al., 1981; Sevy et al., 1989). Thus, an increase in anxiety caused by early life stress might have beneficial effects by facilitating threat avoidance in a high-threat environment, however, in a low-threat environment, an increase in anxiety might prohibit an animal from pursuing available resources. Though some effects of early stress appear to be dependent upon environmental conditions later in life (Sheriff & Love, 2013), some responses to stress may be so extreme as to not convey an advantage in any environment (Yehuda & LeDoux, 2007). Early stress can increase susceptibility in adulthood to hippocampal atrophy, cognitive impairment, and numerous pathologies in humans including posttraumatic stress disorder (PTSD). PTSD can impair functioning and detract from health and wellbeing through chronic symptoms including flashbacks, dissociation, nightmares and general sleep disruption, memory problems, emotional numbness, and self-destructive behavior (Bremner et al., 1997; Libezon et al., 2014).

The idea that stress exposure may prepare an individual for subsequent stress crosses disciplines; in 1986, Murry et al. reported that exposure to small episodes of cardiac hypoxia stress can buffer the effects of a subsequent large hypoxic episode and reduce cardiac damage and cell death in dogs. This phenomenon is now called ischemic preconditioning and is due in part to action of opiates and cardiac mitochondria (Gross, 2003; Korzick et al., 2007). Extensive research has replicated this effect in additional species, including humans, and found similar phenomena in other organs (Ytrehus et al., 1994; Kharbanda et al., 2002; Hausenloy & Yellon, 2008). Interestingly, similar preconditioning effects have been documented for heat stress (Marber et al., 1993; Joyeux et al., 2002) and oxidative stress (Candelario-Jalil et al., 2001; Niagara et al., 2007).
3) How can we explain the effects of developmental stress?

Several distinct hypotheses, developed from both clinical and animal research, seek to explain why early life experiences influence later development. Below we introduce and discuss seven different hypotheses that address this phenomenon; though this is not a complete list of current and historic hypotheses related to developmental stress, we believe that it represents the current state of developmental stress research. It should be noted that some of the hypotheses discussed here, particularly the allostasis and reactive scope models, were not proposed as models of developmental stress, but rather as more general models of how animals deal with stress. Here, these hypotheses are discussed in a developmental framework and compared to hypotheses that more explicitly attempt to model ontogenetic change.

1) Differential susceptibility theory (DST)

The differential susceptibility theory (DST) proposed by Belsky (1997) addresses the question “what makes some children vulnerable and other resilient to effects of their rearing conditions on later development?”. The DST suggests that some individuals are highly context sensitive (“conditional strategists”) while others are resistant to environmental/social influences (“alternative strategists”). According to the DST, parents should produce both of these strategists in order to “hedge their bets” given that parents cannot predict future conditions. In this model, conditional strategists thrive in favorable conditions and better exploit resource-rich environments compared with alternative strategists. However, conditional strategists exposed to adverse conditions (including poor parenting) will be more negatively affected than alternative strategists, who are more insensitive to both positive and negative aspects of the environment. The DST suggests that conditional strategists will adjust their phenotype in adverse conditions using strategies that once acted to maximize reproductive output, but now, in modern society, are categorized as mental illnesses or undesirable behaviors (e.g. gang membership, sexual
promiscuity, limited parental investment,\textit{sensu} Ellis et al., 2011). The DST model incorporates many disciplines (psychology, evolutionary biology, etc.); though the integration of disparate fields can be wrought with complications, the DST has much to offer in considering variation in responsivity to stress exhibited throughout development.

The DST posits that the production of offspring of both strategies evolved because it is advantageous both for parents and for offspring. The DST suggests that because siblings “share 50% of the same genetic alleles”, there are inclusive fitness benefits to having siblings of the opposite strategy: “…if one child benefited from parental influence (e.g. good vs. poor parental care), so would the other, less susceptible sibling, albeit indirectly via shared genes” (Ellis et al., 2011). If parental influences increase an individual’s ultimate reproductive rate and fitness, its siblings may benefit though shared genes, however, siblings can share from 0 to 100% of their genes, rather than a constant 50% (ranging from 0% for adopted or parasitic siblings to 100% for twins). The 50% estimate is likely derived from an assumption of monogamy, but historically humans have been largely non-monogamous, and are frequently categorized as strategic pluralists (Gangestad & Simpson, 2000). Across taxa, the average relatedness of siblings varies with mating and life history strategy (cuckoldry prevalence, group structure, communal vs solitary offspring rearing, etc.). Further, evidence from numerous passerine birds shows that the degree of relatedness in siblings can affect interactions between siblings in early life (Briskie et al., 1994). Extended monogamy is an uncommon strategy even amongst mammals and life history strategies vary widely across taxa. This suggests that the degree to which offspring may accrue indirect fitness benefits from opposite strategy siblings will be species (and context) dependent, thus complicating the application of the DST to non-human species.

Applying the DST in an evolutionary or inter-species context is also limited by the dearth of studies connecting responsivity to parental care and fitness. To demonstrate that the DST
strategies can be shaped by evolution it is necessary to show that the strategies provide a functional advantage, result in differential reproductive success, and are heritable (Lewontin, 1970; Godfrey-Smith, 2007). Given current rates of environmental instability, it is not directly obvious how phenotypic responses to early life conditions improve function or affect fitness, particularly in species with longer lifespans (Chin et al., 2009; Douhard et al., 2014). Before adaptive arguments can be made it may be necessary to determine whether dichotomous stress reactivity strategies are present in early life in systems where fitness measurements are possible. In humans, however, variation in stress responsivity in early life may have great explanatory value for the relative vulnerability and resilience of even closely related children to rearing influences.

II) Biological sensitivity to context theory (BSCT)

The biological sensitivity to context theory (BSCT) addresses the same question as the DST and suggests that some children are highly sensitive (or reactive) to their environment, causing them to be more strongly influenced by either extremely poor or favorable conditions compared with less sensitive children (similarly to the DST conditional and alternative strategies, respectively, Boyce et al., 1995; Boyce & Ellis, 2005). A key difference between the BSCT and the DST is that the BSCT states that exposure to extreme conditions can increase responsivity to an environment (i.e. make an individual more reactive/conditional) while the DST suggests that offspring are born as either conditional or alternative strategies. In light of this, Belsky (2005) suggested that a combination of influences both before birth and during rearing shape responsivity to environmental conditions. Supporting this, we now have extensive evidence from multiple taxa that both gestational and early life environment can affect reactivity of the hypothalamic-pituitary-adrenal (HPA) axis, which plays a key role in regulating the stress response (Weaver et al., 2004; Hayward & Wingfield, 2004; Seckl, 2004; Sheriff et al., 2010). However, this relationship is not straightforward; the assertion of the BSCT that exposure to stimulation
increases stress responsivity is met with two challenges: (1) there are many studies showing that stimulation in early life can decrease stress reactivity (reviewed in Meaney et al., 1991a), (2) the effect of stimulation on stress responsivity is dependent upon developmental stage (Lupien et al., 2009). The first challenge is exemplified by studies showing that neonatal rats separated from their mothers and “handled” by humans (triggering an increase in corticosterone production and a drop in body temperature) can show lower emotionality and decreased HPA reactivity after weaning (Meaney et al., 1988, 1991b; Núñez et al., 1995, 1996; Liu et al. 1997; Kalinichev et al., 2002). Contrastingly, prenatal stress typically increases HPA reactivity, but depending upon the severity of the stress and the age at exposure these effects can be negated or even reversed (reviewed in Glover et al., 2010). The second challenge, that the effects of stress exposure on stress responsivity are specific to developmental stage, can be illustrated by the following example: neonatal rats separated from their dam at postnatal days 3–4 show enhanced stress responsivity (adrenocorticotrophic hormone (ACTH) response) at postnatal day 20, while pups separated at postnatal days 11–12 exhibit a reduced ACTH response to stress at postnatal day 20 (van Oers et al., 1998). Generally, the effects of stress vary with developmental stage and windows of plasticity associated with the maturation of biological systems, including the central nervous system (Lupien et al., 2009). For example, adults with PTSD have consistently shown smaller hippocampal volumes (Gurvits et al., 1996; McEwen, 1999), but the same is not true for children and adolescents with PTSD who show a general reduction in cerebral volume but no changes in hippocampus size (De Bellis et al., 1999).

The BSCT suggests that increased stress reactivity enhances fitness in high-stress environments by increasing vigilance for threats, which is both supported and challenged by current empirical evidence. Supporting evidence shows that exposure to early life stress can increase functioning of the stress response system in the absence of stimuli (i.e. increased basal corticosterone and circulating levels of norepinephrine, Takahashi & Kalin, 1991). Changes in the stress response in
the absence of stimuli can indicate that fear has shifted from being a ‘state’ to a ‘trait’, thereby affecting cognition, behavior, and physiology in the absence of threat (Obradovic et al., 2010). However, contrasting evidence shows that although hyperarousal can enhance vigilance, it can also precipitate internalizing coping strategies, such as dissociation and tonic immobility.

Internalizing coping strategies are well documented in humans and across taxonomic groups, particularly in response to stress in early life (Erhard et al., 1999; Heidt et al., 2005). In humans, dissociation is the disruption of consciousness, identity, or perception (American Psychiatric Association, 2000). Individuals are more likely to dissociate if they are forced to be immobile, experience physical pain, or feel powerless - dissociation is especially common in children, likely due in part to the relative size and power differences between children and their abusers (Macfie et al., 2001). Dissociation is posited to allow children to bond with abusive caregivers and mentally escape trauma when physical escape is not possible (Freyd, 1996). Data on the prevalence of dissociation in response to childhood trauma are scarce, but Kirby et al. (1993) showed that 85% of adult female psychiatric patients that were abused before age 14 exhibit high levels of dissociation and Zlotnick et al. (1994) found dissociation to be the primary coping method for females exposed to multiple sexual abusers as children.

The relationship between dissociation and attention is complex and dependent upon developmental stage, but in children dissociation is negatively correlated with attention (in relation to cognitive inhibition) and dissociation may actually bias attention away from threats (Kaplow et al., 2008; Becker-Blease et al., 2004; Corbetta et al., 1991; Freyd et al., 1998; DePrince & Freyd, 1999). Further, children who dissociate when exposed to stress can actually lower their physiological hyperarousal over time (Perry 1994, 1995). Dissociation can become a long-term coping strategy; one of every four adults exposed to abuse in childhood report experiencing three or more symptoms of dissociation that occur “often” or “always or almost always”; symptoms are likely more frequent closer to when the abuse occurs (Mulder et al., 1998;
Carrion & Steiner, 2000). Given the prevalence of dissociation in response to early life stress, the relationship between stress reactivity and vigilance may be dependent upon life stage, at least in humans. In the context of the BSCT, the hypothesis that high stress reactivity can increase fitness by increasing vigilance may be best evaluated in adults that are capable of dispelling threats autonomously, rather than in earlier life stages when individuals are more dependent (Macfie et al., 2001).

Aside from considerations of the relationship between stress exposure, stress responsivity, and vigilance, Boyce & Ellis (2005) identified key premises of the BSCT and possible limitations derived from these premises. One premise of the BSCT they identified is that chronic stress is only associated with morbidities and pathologies. They recognize, however, that in some conditions stress exposure or high stress reactivity can have normative effects or improve health outcomes. The question of how and when stress can have beneficial effects is addressed by the hypotheses discussed below. Though the BSCT may have limitations, it has helped transform the way we think about stress responsivity across development, and has much to offer in advancing our understanding of stress.

**III) Thrifty phenotype**

The thrifty phenotype hypothesis was proposed by Hales and Barker (1992) to advance earlier lines of thought by Neel (1962) and address the question “why do maternal effects cause context-dependent health outcomes?”. The thrifty phenotype hypothesis suggests that maternal cues during gestation signal information about resources availability in the environment that cause immediate but permanent changes in offspring. They suggest that these changes function to prepare offspring for an environment consistent with their gestational environment – if the adult environment is consistent with the gestational environment, the changes can be beneficial, but if the adult environment is not consistent with the gestational environment, then the changes can be
detrimental (Hales & Barker, 1992, 2001). For example, communities in the west Netherlands, including pregnant females, were exposed to famine conditions in the winter of 1944-1945 as a consequence of an Axis embargo on food transport in WWII. This tragedy has become a natural case study for the effects of temporary food restriction on human development. Hundreds of studies have tracked the survivors, we now know that gestational famine exposure is correlated with low birth weight and adverse health outcomes, including increased risk of coronary disease, glucose intolerance, and obesity (reviewed in Roseboom et al., 2006; Ravelli et al., 1976; Veenendaal et al., 2013). These findings support Hales and Barker’s (1992) assertion that a mismatch between early environment (food restriction/insulin deficiency during gestation) and adult environment (food-rich environment) can cause adverse human health outcomes. The thrifty phenotype hypothesis has also been successfully applied to other mammalian species (reviewed in Bertram & Hanson, 2001; Gluckman & Hanson, 2004). This seminal hypothesis has been applied successfully for decades and its core arguments have been incorporated and expanded in several more recent “mismatch” hypotheses (e.g. the predictive adaptive response and mismatch hypotheses).

IV) Predictive adaptive response (PAR)

Maternal influences during gestation and early postnatal life have frequently and independently been suggested to prepare offspring for environmental conditions (Bateson, 2001; Meylan & Clobert, 2004; Gluckman et al., 2005; Groothuis et al., 2005; Love et al., 2005; Breuner et al., 2008; Monaghan, 2008; Beery & Francis, 2011; Sheriff & Love, 2013). In a keystone paper Gluckman et al. (2005) proposed the predictive adaptive response (PAR) hypothesis to address the question “why do effects of early life environment appear after a delay and cause context dependent health and fitness outcomes?”. The PAR hypothesis states that effects of early life stress (i) can be advantageous in a consistent environment (or detrimental in an inconsistent environment) and (ii) can manifest in later developmental stages - these delayed phenotypic
changes are referred to as predictive adaptive responses (PARs). Examples of possible PARs are increasing as studies investigating the long term effects of stress in specific developmental stages are becoming more frequent – the following effects of early life stress appear only after a delay: decreased parvalbumin in prefrontal cortex interneurons and decreased synaptophysin in the hippocampus following maternal separation (Andersen & Teicher, 2004; Brenhouse & Andersen, 2011) and decreased hippocampal volume after chronic stress in adolescence (Isgor et al., 2004).

Gluckman et al. clarify their ideas by relating their hypothesis to the earlier thrifty phenotype hypothesis; Hales and Barker (1992) suggest that a nutrient-restricted fetus will undergo changes that are advantageous in a nutrient-restricted environment, but detrimental in a nutrient-rich environment - Gluckman et al. expand upon this to suggest that fetuses undergo permanent changes (PARs) in all nutrient conditions to prepare for matching nutrient conditions later in life (Table 2). Thus, two key differences that distinguish the PAR hypothesis from the thrifty phenotype hypothesis are that (1) PARs are permanent phenotypic changes that are induced in all environments (i.e. PARs can match moderate, not just extreme, conditions) and (2) the manifestation of PARs is specific to developmental stage. Gluckman et al. suggest that PARs (i) prepare an organism to thrive in a specific range of conditions through its reproductive phase, (ii) are context-dependent; the functionality of PARs is contingent upon environmental predictability and consistency, and (iii) PARs can lead to disadvantage or disease if expected environmental conditions are exceeded (in either direction).

Gluckman et al. address possible PARs for modern humans by again referencing to the thrifty phenotype hypothesis; they suggest that some adverse effects of a thrifty phenotype caused by nutrient restriction are visible only because of modern longevity. They explain that in the context of human evolutionary history, early and frequent reproduction would have been emphasized over health in later life stages (i.e. after peak reproductive age, which for modern humans is
around 30; Rothman et al., 2013). Gluckman et al. (2005) also discuss maternal constraints on offspring development that are present even in modern resource-rich environments and currently contribute to setting offspring PARs (e.g. maternal pelvis size, maternal age, and parity). It should also be noted that pregnant women frequently suffer from nutrient deprivation (not necessarily linked to caloric intake), even in modern first world countries (e.g. iron deficiency in the United States and Europe, Looker et al., 1997; Allen, 2000; Hercberg et al., 2001).

One possible limitation of the PAR hypothesis is that it does not consider maternal effects from the perspective of the mother - the ultimate goal of maternal effects is to increase the fitness of the mother (Wells, 2003; Sheriff & Love, 2013). Parent-offspring theory states that selection operates to create offspring that demand greater resources than mothers are selected to provide (Wells, 2003). Thus, the potential for mothers to influence offspring through maternal effects should be considered through this lens, rather than solely through “snapshot” views of the offspring primarily in early life (Marshall & Uller, 2007). However, if maternal effects solely functioned to modify the offspring/maternal relationship it is unclear why there would species-specific maternal effects in species with no parental care (G. aculeatus, Giesing et al., 2011) or maternal effects that manifest after dispersal (Lee & Zucker, 1988). This suggests that at least some maternal effects are for the direct benefit of the offspring, outside of the direct maternal-offspring relationship. Regardless of the proportion of maternal effects that are intended to modify the maternal-offspring relationship vs. the fitness of the offspring, an individual’s goal, and so the goal of all reproducing females, is to maximize personal fitness. Thus, when interpreting how early life conditions shape offspring responses, a greater emphasis should be placed on the possible benefits of maternal effects to the mother (Sheriff & Love, 2013). This is especially important for rats (a common model for maternal effects), given that rats have a fast pace-of-life strategy (Reale et al., 2010). As common prey animals, Rattus norvegicus prioritize fast growth rates and frequent reproductive bouts - investment in individual offspring is low and
the likelihood of an offspring reaching sexual maturity is low (Calhoun, 1952). However, a mother’s capacity for future reproductive bouts is high (particularly for the younger female rats typically studied; Reale et al., 2010). The life-history strategy of rats biases mothers to maintain resources for future reproductive bouts (Pianka & Parker, 1975). This conflict between a mother’s need to simultaneously provision for offspring and maintain resources for future reproductive bouts may complicate application of the PAR. However, overall, the PAR hypothesis is an insightful expansion of earlier theory that is being actively advanced and broadly applied in ecological and biomedical contexts (Godfrey et al., 2007; Douhard et al., 2014; Hanson & Gluckman, 2014).

V) Mismatch hypothesis

The mismatch hypothesis addresses questions similar to the PAR hypothesis, “why do animals undergo phenotypic changes in response to stress early in development and are these changes context dependent?” The mismatch hypothesis also asks “when offspring undergo phenotypic changes in response to early life conditions, who benefits?”. The mismatch hypothesis emphasizes (i) the maternal-offspring relationship and the potentially high demands of parental care, (ii) consideration of maternal inclusive fitness, encompassing both direct fitness gained across all possible reproductive bouts and indirect fitness from non-offspring kin (Sheriff & Love, 2013), and (iii) glucocorticoids as a possible mechanism by which mothers convey signals about environmental conditions to their offspring (particularly during gestation, Sheriff et al., 2009, 2010).

In emphasizing the importance of the maternal-offspring relationship, the mismatch hypothesis suggests that the match between maternal quality and offspring quality mediates fitness - females with poor body condition, or in adverse or resource-limited environments, are less able to rear “high” quality offspring and can increase fitness by producing “poor” quality offspring (i.e. low
birth weight, slow growth rate). Earlier work by Love & Williams (2008) supports this; when female European starlings with clipped feathers (low quality mothers) were paired with corticosterone-injected eggs (low quality offspring), the “match” between the maternal and offspring condition resulted in lower offspring mortality compared with a “mismatched” condition where females were impaired by tail feather clipping (low quality mothers) but offspring were not injected with corticosterone (higher quality offspring). Mismatched females also had diminished success with future reproductive bouts compared with matched females (Love & Williams, 2008). Though the mismatch hypothesis has been successfully applied to mammalian and avian species, these groups share extended maternal-offspring relationships after parturition and obligatory parental care. Generalizing the mismatch hypothesis may be mediated by species-specific levels of parental care, timing of windows of plasticity, environmental consistency (temporally and spatially homogenous environmental conditions), and environmental predictability (seasonal changes, timing of food availability, etc.) across an individual lifespan (Lee & Zucker, 1988; Sims & Holberton, 2000).

One assumption of the mismatch hypothesis is limited phenotypic plasticity in adulthood – a highly plastic animal would not need to gamble on phenotypic changes that may or may not match future environmental conditions, plasticity would allow them to continually adjust to environmental conditions. Thus, capacity for plasticity after maturation may modulate the applicability of the mismatch hypothesis for any given species; plasticity in adulthood is mediated by the costs and benefits of plasticity for that species (likely modulated by the frequency of environmental change on an evolutionary time scale, etc.). The capacity for plasticity can be shaped by natural selection; plasticity can be costly, and the timing, duration, and magnitude of phenotypic plasticity varies across species (West-Eberhard, 2003). For example, some species can adjust quickly to high-resource conditions after a period of nutrient restriction and recover from stunted growth by growing quickly to return to an average size trajectory. However, individuals
that grow quickly to compensate for previously slowed growth may sacrifice longevity (compensatory growth, Wilson & Osbourn, 1960; Birkhead et al., 1999). Less plastic species exposed to resource-rich conditions after a period of nutrient restriction may remain smaller for a longer portion of their lifespan and adjust some aspects of their phenotype, while others trait remain “optimized” for the nutrient-deprived conditions (Metcalfe & Monaghan, 2001, 2003). In addition to plasticity in developmental trajectory, it is also possible for species to have environmentally triggered phenotypic plasticity throughout their lifetimes. This can facilitate adjustment to changing social or physical conditions (e.g. socially induced sex-changes in fish, Bass & Grober, 2001, or migratory behavior, such as rapid transitions from fresh to saline water in anadromous salmonids, eels, etc.; Arai & Tsukamoto, 1998; Tsukamoto et al., 1998).

Predictive adaptive responses (PARs) may also be obviated in highly plastic species.

In the context of the mismatch hypothesis, as with the PAR hypothesis, the benefits of phenotypic changes in response to early life conditions are contingent upon environmental consistency across ontogeny. However, in contrast with the mismatch hypothesis, the PAR hypothesis does not consider reproductive strategy. In the mismatch hypothesis it is implied that all changes induced by early life conditions are immediate, making their effects dependent on the presence or duration of parental care. However, in modeling delayed effects of early life stress, the PAR hypothesis may apply equally well to species with all levels of parental care. Overall, it seems likely that some immediate effects of early stress may best interpreted with the mismatch hypothesis (particularly in relation to parental provisioning), while some changes, or changes that appear after a delay, may be best explained by the PAR hypothesis.

**VI) Allostasis**

The theory of allostasis addresses how animals deal with predictable and unpredictable stress by focusing on maintaining stability through change (McEwen & Wingfield, 2004). Allostasis,
defined as “achieving stability through change”, is similar to homeostasis but emphasizes context-specific set points. According to the allostasis model, an organism can balance energy intake and expenditure to deal with stressors by engaging allostatic processes to maintain shifting homeostatic set points (regulation of the HPA axis and catecholamine production, etc.). Allostatic processes can become hyperactive (e.g. high corticosterone in by Cushing’s disease) or hypoactive (e.g. low corticosterone in Chronic Fatigue Syndrome). A sustained change in allostatic processes can mark a change in allostatic state, defined as a combination of allostatic processes viewed at the level of the phenotype. Allostatic states can include allostatic processes that changed in opposing ways (e.g. Chronic Fatigue Syndrome is marked by both hypoactivity of corticosterone and hyperactivity of inflammatory cytokines, Patarca, 2001). If an allostatic state is maintained, allostatic overload can occur and cause pathology in two ways: (1) energy demands exceed available energy or (2) chronic conditions cause extended or continuous elevation of allostatic mediators (glucocorticoids, catecholamines, etc.), resulting in toxic or adverse effects (reviewed in Sapolsky et al., 2000). For example, chronically elevated glucocorticoids (caused by persistent social conflict, high predator density, etc.) can inhibit hippocampal neurogenesis (McEwen, 1999), cause neuronal or glial cell death (Uno et al., 1989; Rajkowska et al., 1999), decrease hippocampal volume (Starkman et al., 1992; McEwen, 1999), suppress immune responses, decrease bone minerals and muscle, and contribute to adverse or accelerated aging processes (Sapolsky et al., 1986; Lui & Mori, 1999).

One strength of the allostasis hypothesis is that it incorporates the “glucocorticoid cascade hypothesis”, which suggests that excess glucocorticoids can damage brain regions that regulate the negative feedback cycle of glucocorticoids, resulting in a feed-forward cycle of glucocorticoid production that causes adverse aging effects (Sapolsky et al., 1986) – the allostasis model terms this “wear and tear” caused by high allostatic load (McEwen, 2002, 2003). Another strength of the allostasis model is that it highlights some functions of glucocorticoids that are less frequently
considered, including the mobilization of energy stores. Further, it contextualizes glucocorticoids as mediators of energetic requirements and energy availability, which allows comparisons across diverse species and contexts and clearly links humans and non-human species.

Potential limitations of the allostasis hypothesis are that it does not address (i) the capacity for early stress exposure to modulate future stress sensitivity, (ii) “preconditioning” effects of stress that may prepare an individual to function under future stress, or (iii) individual variability in stress responses, particularly for behavioral and cognitive responses to stress. In the allostasis model, the only lasting effects of stress (after a stressor is removed) are described as wear and tear, but this represents a unilateral link between early stress and pathology, and suggests that early life stress can only impair, not enhance, resilience to subsequent stress. Thus the allostasis model, in comparison to the DST and BCST, seems to generate fewer clear predictions about the lasting effects of early stress exposure, particularly in reference to the capacity for early stress to buffer the effects of subsequent stress (see Table 1). In reference to the third possible limitation, the allostasis model does not clearly define how behavioral/cognitive responses to stress and the capacity for behavioral/cognitive stress responses to modulate physiology can fit into the model – this may be particularly important because although physiological responses to stress are highly conserved, behavioral responses to stress are more variable and can differ between even closely related species (Bonga, 1997; Wiesenthal et al., 2006; see also Box 1). For example, stress exposure can cause excess food consumption across taxonomic groups; this idea is so common that the term “stress-eating” has entered the popular lexicon (reviewed in Adam & Epel, 2007). However, the opposite response, referred to as stress-induced hypophagia (or anorexia) also occurs in multiple species – both hyper and hypophagia can affect physiological states and cause adverse health outcomes, including elevated mortality rates (Hoek, 2006). Thus, the relationship between stress and food consumption can be a two-edged sword that is influenced by individual variation and cultural context (Haworth-Hoeppner, 2000; Veugelers & Fitzgerald, 2005). It is
important to bear in mind that the effects of stress exposure on behavioral and cognitive states can be mediated by individual characteristics including developmental stage, prior experience, genotype, etc. (Francis et al., 1999).

VII) Reactive scope

The reactive scope hypothesis was proposed by Romero et al. (2009) to describe how animals deal with predictable and unpredictable stress and to rectify six limitations of the allostasis model. Briefly, Romero et al. (2009) suggest that the allostasis hypothesis is limited because (1) excessive reliance on energy budgets - energy intake and expenditure is variable and not well understood, and therefore would be challenging to measure (though this may be a weakness in our ability to assess allostatic load without being a limitation in the predictive value of the model), (2) the relationship between glucocorticoids and glucose/energy mobilization is not as straightforward as was once thought, (3) reliance on glucocorticoids over other features of the stress response, (4-5) it is unclear how to incorporate developmental effects and behavioral/cognitive responses to stress into the allostasis model, and (6) individual variation is not directly addressed (a possible weakness of the PAR and mismatch hypotheses also).

However, Romero et al. (2009) also identified three main strengths of the allostasis model that they attempted to retain in the reactive scope model: (1) acknowledgement that physiological states change over time, (2) a clear framework for defining when stress causes pathological damage (through the concept of wear and tear leading to allostatic overload), and (3) generalizability across species through a common mechanism for measuring allostatic load, energy.

The reactive scope hypothesis posits that individuals are typically in one of two states: (1) predictive homeostasis, dealing with predictable environmental changes in circadian or seasonal states, or (2) reactive homeostasis, dealing with threat or unpredictable environmental change
(Fig. 1a). When an animal is challenged and levels of a homeostatic mediator (glucocorticoids, heart rate, locomotion, etc.) exceed the range of reactive homeostasis, animals enter homeostatic overload. Conversely, an inability to maintain minimum levels of a homeostatic mediator is termed homeostatic failure. With increasing wear and tear the threshold between reactive homeostasis and homeostatic overload can decrease, reducing the range in which an animal can respond to a challenge (i.e. causing a decrease in reactive scope).

A strength of the reactive scope hypothesis is that it can readily model acute stress, with a rapid transition from predictive to reactive homeostasis, and the model makes clear predictions for the effects of acute and chronic stress over time (wear and tear). Another strength of the reactive scope model is that individual differences can be modeled by adjusting the threshold of the predictive and reactive ranges (and their proximity to either the upper “overload” and lower “failure” thresholds); Romero et al. (2009) helpfully suggest that the reactive scope model was created to be readily modifiable for application to different species and individuals.

Romero et al. (2009) suggest that the threshold between reactive homeostasis and homeostatic overload can be mediated by early life experiences, but the reactive scope model does not allow for early life conditions to affect actions of the homeostatic mediators themselves. The reactive scope model suggests that early stress can provide a “buffer” by increasing the gap between the threshold for homeostatic overload and the reactive homeostasis range (an increase in reactive scope). This increases the amount of stress required to cross the overload threshold and induce pathology. However, the reactive scope model does not address how early life conditions might cause lasting changes in levels of a mediator, which may be an important aspect of how early life conditions can shape an adult phenotype. For example, exposure to chronic stress in early life can increase levels of corticotropin-releasing hormone (CRH) in adult rat hippocampi, which may underpin lasting deficits in learning and memory that can be relieved by blocking CRH after
chronic early stress (Ivy et al., 2010). Further, it is unclear how the reactive scope hypothesis would characterize the effects of early stress on phenotype outside of the context of resistance or vulnerability to pathology (particularly in the context of behavioral and cognitive changes). A potential modification of the reactive scope hypothesis for the effects of early life stress, that addresses the effects of early life stress on circadian rhythms, basal production of mediators, and responsivity to subsequent stressors, is depicted in (Fig. 1b). It should be noted, however, that all reactive scope models are specific to a single mediator, and the proposed modification still cannot capture behavioral and cognitive effects of early stress such as those discussed in Table 1.

One possible limitation of the reactive scope hypothesis, relative to the allostasis hypothesis, is that the reactive scope hypothesis models mediators individually while allostatic states describe the relationship of multiple mediators, which can help define a specific state or pathology (Singh & Rose, 2009). By modeling homeostatic mediator separately, the reactive scope model may gain greater accuracy for some homeostatic mediators but sacrifice a more integrative phenotypic view. Modeling mediators individually may also lead to an overly simplistic view of mediator function; mediators may not move linearly, even within “physiological systems” identified by the reactive scope model (e.g. central nervous system, behavior, HPA axis). For example, the model suggests that animals in the reactive homeostatic range exhibit increases in learning and memory, but if animals reach homeostatic overload learning and memory processes will be reduced. However, different types of learning and memory can independently relate to stress, for example, exposure to early stress can cause lasting increases in contextual fear learning but decreases in spatial learning, though both are hippocampal-dependent tasks (Champagne et al., 2008; Oomen et al., 2010).

The concept of homeostatic failure can also be unclear in the reactive scope model; homeostatic failure is said to occur when levels of a mediator are too low to maintain life such that “death
usually follows”, which differentiates it from homeostatic overload when mediators are overproduced, possibly resulting in pathology but not “immediate death” as with homeostatic failure (Romero et al., 2009). Complex states like PTSD and lethargy are suggested to be homeostatic failure in the reactive scope model - PTSD is characterized by chronic decreases in glucocorticoid levels, but chronic elevation of corticotrophin-releasing factor and norepinephrine (suggesting opposing threshold effects in the reactive scope model; Yehuda et al., 1990; de Kloet et al., 2008; Wingenfield et al., 2015). In PTSD, these opposing relationships are so strong that successful pharmaceutical interventions for PTSD function to increase glucocorticoid levels or inhibit norepinephrine via blockade of the α₁ adrenergic receptor (Taylor & Raskind, 2002; Taylor et al., 2006). Opposing effects of stress can also be detected at the level of whole brain regions; PTSD is marked by hyperactivity of the amygdala but hypoactivity of the medial prefrontal cortex (reviewed in Shin et al., 2006). Thus, when considering multiple mediators in the context of the reactive scope hypothesis, it is unclear when animals should be categorized as in the reactive or overload range. If homeostatic overload thresholds cannot be universally identified, and are specific to the mediator being considered, then comparisons between species and contexts will be difficult at best.

In the reactive scope model, an ideal phenotype for dealing with stress exposure has a maximized reactive scope (or physiological range of production of homeostatic mediators). In the allostasis model an ideal phenotype can always rebound back to set points (is impervious to wear and tear). This is in contrast to the PAR and mismatch hypotheses, which suggest that ideal phenotypes for dealing with stress exposure are shaped during gestation, but that these phenotypes are context-specific and will be ideal only if the predictions made during gestation (or early life) remain accurate estimates of ultimate environmental conditions.

VII) Arousal-shift hypothesis
The arousal-shift hypothesis, proposed by the current authors in Chaby et al., 2015b, attempts to marry PAR/mismatch lines of thought with earlier models of performance and stress set forth by Yerkes and Dodson (1908) in order to explain the effects of early stress on cognitive performance (Box 2), a particularly important challenge because cognitive responses to stress are omitted from most prior stress models (Romero et al., 2009; Chaby et al., 2015b). The arousal-shift hypothesis does not contradict crucial early hypotheses that predict that prenatal stress can prepare an individual for an adverse environment, but suggests that developmental stress may manifest though a shift in the relationship between performance and arousal. Application of the arousal-shift hypothesis may inform our understanding of the role of changes in plasticity across development and the capacity for transformative change during adolescence, but requires more empirical evidence and mechanistic grounding before it can be applied more broadly and may be limited to cognitive and behavioral responses to stress within a single lifespan.

**Box 2: Yerkes-Dodson law**

The Yerkes-Dodson law has been in use for over a century and is based on a series of visual discrimination experiments in which mice are exposed to weak, moderate, and strong electrical stimulation (Yerkes & Dodson, 1908). Yerkes and Dodson found a linear relationship between the strength of the electrical stimulation and acquisition of a simple discrimination task, but a curvilinear relationship for a task of moderate difficulty. The Yerkes-Dodson law states that for more challenging tasks, i) moderate arousal can enhance performance (Ni, 1934; Salehi et al., 2010) in part by modulating motivation (Diamond et al., 2007), but ii) high levels of arousal can decrease performance through processes such as a reduction in the amount of information that can be processed (Diamond et al., 1999; Kim & Diamond, 2002), as described in the Easterbrook hypothesis (Easterbrook, 1959; Anderson & Revelle, 1982). Yerkes and Dodson’s findings have been replicated in numerous taxa with modern techniques and statistical analyses and have been
The arousal-shift hypothesis emphasizes the importance of context on behavior and performance, and the importance of understanding the relationship between testing environment and early environment. The proposed model for stress during adolescence to cause a shift in the relationship between arousal and performance, the “arousal-shift hypothesis”, proposes that the lasting cognitive effects of early stress could be underpinned by a shift in the curvilinear relationship between arousal and performance on higher-demand cognitive tasks (Fig. 2).

Such a shift would allow adolescent-stressed animals to perform better at higher levels of arousal that exceed the optimal range of arousal for unstressed animals (the peak of the Gaussian curve). Under this framework, exposure to adolescent-stress would cause an increase in optimal arousal range, but adolescent-stressed animals would still show a decline in performance after arousal exceeds their optimal level. It would follow that adolescent-stressed rats would maintain a performance advantage over threat-naïve animals throughout the decline until their level of arousal becomes too high to permit completion of a moderately challenging task regardless of rearing environment. In this model, the effect of adolescent-stress on performance is minimal in the absence of threat (low arousal conditions). As arousal increases in the positive slope of the Gaussian curve, both unstressed and adolescent-stressed animals increase performance; no difference in performance may be detected until near the optimal arousal level of unstressed animals.

To understand the lasting effects of early stress on cognition as animals age and threats in their environment change we must investigate cognition in “matched” and “mismatched” environments (threat vs. safe), and performance in a task-dependent manner (simple vs. complex). Chaby et al.
(2015a) investigated whether the lasting effects of stress during adolescence could be explained by mismatch hypotheses by exposing laboratory rats to chronic variable stress in adolescence and comparing their problem solving ability under high and low threat to rats reared in standard laboratory conditions. We found that under high-threat conditions control, unstressed animals decreased their performance by an average of 28% ± SE 9% (number of rewards obtained) compared to their performance in a prior low-threat test ($\left[\frac{\text{final-initial}}{\text{initial}}\right] \times 100$). Interestingly, high-threat conditions did not detract from the performance of animals that had experienced adolescent-stress, on average adolescent-stressed rats showed a small increase in performance (2% ± SE 16%) compared to their performance in the prior low-threat test. Previous studies have also shown cognitive enhancements under high-threat conditions following adverse conditions in early life, particularly in fear learning assays (Champagne et al., 2008; Oomen et al., 2010), while others have shown unaffected or impaired cognition in the absence of threat (McCormick et al., 2012; reviewed in McCormick & Mathews, 2010).

4) **How can we understand lasting phenotypic changes from early stress?**

In this review, we compare models of stress in early development to (i) facilitate discourse between those using biomedically-driven models of early stress and those using evolutionary/ecological models, (ii) discuss how well current models explain the lasting effects of exposure to stress early in development, and (iii) address recent evidence suggesting that stress in early development can have beneficial effects later in life. We demonstrate that scientists from a myriad of fields are working to understand the role of transformation from stress, and their views align on a number of important ideas, particularly related to the potential adaptive value of the lasting changes induced by prenatal, neonatal, and adolescent stress. Many models discuss the potential adaptive value of phenotypic changes resulting from early stress in an evolutionary context, which has the capacity to greatly further our understanding of the stress response in an
ecological context and our own stress response. The hypotheses presented here have made vital advances and can have great explanatory value for many aspects of developmental stress with careful consideration of species and context. The applicability of the hypotheses discussed here will likely depend upon the environmental stability a species, or perhaps even an individual, experiences in their lifetimes. For example, the mismatch hypothesis may be best suited for animals that live in relatively consistent, stable conditions (island populations, etc.) whereas the DST/BSCT may better model fitness in conditions that vary, or where offspring must disperse to variable environmental conditions. However, the hypotheses presented here are not mutually exclusive. For example, in the PAR/mismatch hypotheses all individuals exposed to early life stress will undergo phenotypic changes to prepare for adverse conditions later in life (e.g. PARs), but it is possible that individual variation in the ability to undergo PARs can be described by the DST/BSCT.

In the future, models of stress should (i) account for developmental changes in stress sensitivity and responsivity (Spears, 2000; Suri et al., 2013), (ii) acknowledge differences in coping with stress, not just “ability” but also in strategy, across species and between individuals (determined by age, sex, etc., Box 1), (iii) differentiate between acute and chronic stress exposure (which can drive ‘states’ to ‘traits’; Obradovic et al., 2010). Additionally, it should be remembered that the ultimate goal of all individuals is maximizing personal fitness and so greater emphasis should be placed on benefits of maternal effects to the mothers than is currently in the literature (Sheriff & Love, 2013). Finally, it is difficult but necessary to empirically address the fitness impacts of early stress effects; thus far the few studies that have done this have not been consistent with current lines of thinking (Douhard et al., 2014; Meylan & Clobert, 2005). While there are clear benefits to marrying function with mechanism, many challenges remain in understanding developmental stress but these are very likely to benefit from interdisciplinary collaboration and communication.
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Figures

**Fig. 1:** Basic reactive scope model (A) adapted from Romero et al. (2009). Panel (B) shows a model of the lasting effects of early life stress on glucocorticoid production. Early (prenatal) stress can cause a phase shift in daily rhythms of corticosterone production in rats (Koehl et al., 2009), but may or may not affect basal HPA axis function (reviewed in Welberg & Seckl, 2008). The “acute stress response bars” in (B) are longer for the early stressed animals (gray) to reflect that exposure to early stress can increase responsivity to low-threat stimuli (i.e. causing less differentiation between high and low threat; reviewed in Ellis et al., 2011). It should be noted that for the reactive scope model, each mediator requires its own model.
Fig. 2: Context-dependent relationship between cognition and early stress, adapted from Chaby et al., 2015b. Based on earlier the Yerkes-Dodson Law, which proposes a curvilinear relationship between arousal and cognitive performance for moderately or highly difficult tasks (Box 2).
### Table 1: Studies reporting lasting effects from stress in early development that appear to enhance functioning

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Stress exposure</th>
<th>Effect type</th>
<th>Stage at testing</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meylan et al., 2004</td>
<td>Lizard (Lacerta vivipara)</td>
<td>Exogenous corticosterone exposure during gestation</td>
<td>Behavioral</td>
<td>Juvenile</td>
<td>Corticosterone-exposed offspring were more likely to disperse from small/young moms but less likely to disperse from large (more experienced) moms</td>
</tr>
<tr>
<td>Avital &amp; Richter-Levin, 2005</td>
<td>Laboratory rats (Rattus norvegicus)</td>
<td>Exposure to elevated platform stress in juvenile and adult stages, compared to stress exposure only in adulthood</td>
<td>Behavioral</td>
<td>Adulthood</td>
<td>Faster swimming and more exploration in a Morris water maze</td>
</tr>
<tr>
<td>Mathis et al., 2008</td>
<td>ringed salamanders (Ambystoma annulatum)</td>
<td>Eggs exposure to chemical predation cues</td>
<td>Behavioral</td>
<td>Larvae</td>
<td>Exhibit antipredator behaviors from birth; low activity; increased shelter-seeking</td>
</tr>
<tr>
<td>Mathis et al., 2008</td>
<td>wood frog (Rana sylvatica)</td>
<td>Egg exposed to predation cue and/or alarm cues</td>
<td>Behavioral</td>
<td>Tadpole</td>
<td>Tadpoles classically conditioned to respond to newt predation cue</td>
</tr>
<tr>
<td>Tsoory et al., 2009</td>
<td>Laboratory rats (Rattus norvegicus)</td>
<td>Variable stress during juvenile development</td>
<td>Behavioral</td>
<td>Adulthood</td>
<td>Enhanced auditory fear conditioning compared with unstressed controls</td>
</tr>
<tr>
<td>Oomen et al., 2010</td>
<td>Laboratory rats (Rattus norvegicus)</td>
<td>Maternal separation</td>
<td>Behavioral</td>
<td>Adulthood</td>
<td>Maternally separated rats show enhanced synaptic plasticity following corticosterone exposure; enhanced fear memory</td>
</tr>
<tr>
<td>Chaby et al., 2013</td>
<td>Laboratory rats (Rattus norvegicus)</td>
<td>Chronic variable stress in adolescence (physical, social)</td>
<td>Behavioral</td>
<td>Adulthood</td>
<td>Adolescent-stressed rats showed accelerated decision making compared with unstressed rats</td>
</tr>
<tr>
<td>Chaby et al., 2015</td>
<td>Laboratory rats (Rattus norvegicus)</td>
<td>Chronic variable stress in adolescence (physical, social, predation cues)</td>
<td>Behavioral</td>
<td>Adulthood</td>
<td>Adolescent-stressed rats showed enhanced foraging performance in the presence of novel threat (but unaffected foraging performance in the absence of threat) compared with unstressed rats</td>
</tr>
<tr>
<td>Shine &amp; Downes, 1999</td>
<td>Lizard (Pseudemonia pagenstecheri)</td>
<td>Gestating females exposed to snake scent</td>
<td>Morphological</td>
<td>Neonate</td>
<td>Offspring had increased body mass; long tails (potentially to enhance tail autotomy – an antipredator behavior); high</td>
</tr>
<tr>
<td>Study Authors, Year</td>
<td>Species/Species Characteristics</td>
<td>Behavioral/Physiological Changes</td>
<td>Stage(s)</td>
<td>Summary of Findings</td>
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<tr>
<td>Buwalda et al., 2013</td>
<td>Laboratory rats (<em>Rattus norvegicus</em>)</td>
<td>Social defeat in adolescence</td>
<td>Behavioral/Physiological</td>
<td>Adulthood: Exposure to social defeat in adolescence caused faster attack latency and fewer received attacks in adulthood compared with rats reared in control conditions.</td>
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<tr>
<td>Giesing et al., 2011</td>
<td>Three-spine sticklebacks (<em>Gasterosteus aculeatus</em>)</td>
<td>Gestating females chased with predator model</td>
<td>Morphological/Physiological/Behavioral</td>
<td>Juvenile: Larger eggs; higher cortisol in eggs; more tight shoaling (antipredator behavior).</td>
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<tr>
<td>Crino et al., 2014</td>
<td>Zebra finches (<em>Taeniopygia gutta</em>)</td>
<td>Male nestlings fed corticosterone</td>
<td>Behavioral</td>
<td>Adulthood: Increased parental provisioning (female driven); higher body condition in offspring; greater number of genetic offspring.</td>
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<td>Champagne et al., 2008</td>
<td>Laboratory rats (<em>Rattus norvegicus</em>)</td>
<td>Pups exposed to low licking mothers (compared with pups exposed to high licking mothers)</td>
<td>Neurological/Behavioral</td>
<td>Adulthood: Enhanced long-term potentiation following corticosterone exposure (but impaired under basal conditions); enhanced contextual fear memory.</td>
<td></td>
</tr>
<tr>
<td>Meylan &amp; Clobert, 2005</td>
<td>Lizard (<em>Lacerta vivipara</em>)</td>
<td>Exogenous corticosterone exposure during gestation</td>
<td>Survival</td>
<td>Juvenile: Corticosterone-treated male offspring had greater survival after release (but female survival was unaffected).</td>
<td></td>
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</table>
Table 2: Ideal phenotypes to respond to acute stressful event(s) according to current models of early life stress.

<table>
<thead>
<tr>
<th>Theory</th>
<th>Description</th>
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<tbody>
<tr>
<td>Differential susceptibility theory</td>
<td>Individuals born less environmentally responsive (i.e. “alternative strategists”) are most resistant to the negative effects of stress.</td>
</tr>
<tr>
<td>Biological sensitivity to context theory</td>
<td>Individuals that experienced moderate conditions (low-stress) in early life will be most resistant to the negative effects adverse conditions later.</td>
</tr>
<tr>
<td>Predictive adaptive response hypothesis</td>
<td>If an individual is exposed to developmental stress, and is then exposed to an adverse environment later in life that matches the range of the developmental environment, then the adaptive responses caused by early stress should enhance fitness, compared to unstressed individuals. (Exposure to stress can prepare an individual for later stress).</td>
</tr>
<tr>
<td>Mismatch hypothesis</td>
<td>If an individual is exposed to developmental stress, and is then exposed to an adverse environment later in life (independent of severity), then the phenotypic changes caused by early stress should increase the likelihood of successful rearing to reproductive age and enhance maternal and personal fitness, compared to unstressed individuals. (Exposure to stress can prepare an individual for later stress).</td>
</tr>
<tr>
<td>Allostasis/ Reactive scope</td>
<td>The ideal phenotype to deal with stress is an individual with low wear and tear (no prior stress) or an individual with a wider gap between predictive homeostasis and homoeostatic overload (due to individual differences in resilience, seasonal changes, food availability, etc).</td>
</tr>
</tbody>
</table>
References


Chaby, L. E., Sheriff, M. J., Hirrlinger, A. M., & Braithwaite, V. A. (2015a). Can we understand how developmental stress enhances performance under future threat with the Yerkes-
Dodson law? *Communicative & Integrative Biology, 8*(3), e1029689.
http://doi.org/10.1080/19420889.2015.1029689


http://doi.org/10.1523/JNEUROSCI.0526-08.2008


http://doi.org/10.1017/S0954579400007318


http://doi.org/10.1016/j.bbr.2008.02.004


http://doi.org/10.1016/S0026-0495(03)00295-6


Neel, J. V. (1962). Diabetes mellitus: A “thrifty” genotype rendered detrimental by “progress”?

http://doi.org/10.1037/h0075629

http://doi.org/10.1161/01.RES.0000258460.41160.ef


http://doi.org/10.1016/0031-9384(94)00308-R

http://doi.org/10.1111/j.1467-8624.2009.01394.x

http://doi.org/10.1523/JNEUROSCI.0247-10.2010


http://doi.org/10.1016/S0006-3223(99)00041-4

http://doi.org/10.1056/NEJM197608122950701


http://doi.org/10.1016/j.earlhumdev.2006.07.001

http://doi.org/10.1016/j.fertnstert.2013.02.040


http://doi.org/10.1016/j.psyneuen.2014.10.023


http://doi.org/10.1002/cne.920180503

http://doi.org/10.1074/jbc.273.50.33741


Chapter 2

Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence

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Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence

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INTRODUCTION

Negative life experiences can have long-term effects on behavior and physiology (Sheriff et al., 2009; Archard et al., 2012). Stressful events (e.g., stressors) come in a variety of forms, but in vertebrates they are often considered to be unpredictable aversive stimuli that provoke a glucocorticoid hormone response mediated by the hypothalamic-pituitary-adrenal (HPA) axis (Spear, 2000; Koolhaas et al., 2011). Stages of development differ in sensitivity to stress, certain life stages have specific vulnerabilities that can lead to different, permanent changes in future responses to adverse events (McCormick and Mathews, 2008; Vidal et al., 2011). For example, in zebra finches (Taeniopygia guttata) exposure to excess heat during early-life enables the birds to modify their response to subsequent heat exposures in adulthood to minimize oxidative damage (Costantini et al., 2012). Similarly, rodent pups that experience isolation at different stages of development exhibit contrasting hormonal responses to stress in adulthood; rat pups separated from their mothers for 2 h a day at postnatal days 2–14 develop a hyper-responsive HPA axis, whereas pups isolated at postnatal days 15–16 develop a hypo-functioning HPA axis (Plotsky and Meaney, 1993; Sánchez et al., 1998; reviewed in Sánchez et al., 2001).

During the adolescent stage, glucocorticoid production in response to a stressor exceeds the adult hormone response in duration and intensity (McCormick et al., 2010). In comparison to adult rats, adolescent rats exposed to an acute stressor show a higher increase in both adrenocorticotrophic hormone (ACTH) and glucocorticoids (reviewed in Romeo and McEwen, 2007; Foilb et al., 2011). Additionally, during adolescence various neural structures involved in stress and reward processing are still immature (Spear, 2000; McCormick and...
Mathews, 2008). These characteristics of the adolescent life stage suggests that this period may be particularly vulnerable to effects from chronic glucocorticoid exposure (Romeo and McEwen, 2007; McCormick et al., 2010). Chronic exposure to elevated levels of glucocorticoid hormones has numerous effects on the brain including suppressed neurogenesis and enhanced dendritic pruning in the hippocampus, dendritic shortening in the medial prefrontal cortex, and enhanced dendritic growth in the amygdala, the fear center of the brain (reviewed in McEwen, 2005).

Adverse experiences during adolescence can impact the maturation of the central nervous system, shape future reward responses, and influence endocrine and behavioral function in adulthood (Romeo, 2003; McCormick et al., 2004; Andersen and Teicher, 2008; McCormick and Green, 2012). The changes that occur following stress exposure during adolescence are dynamic; some are immediate, some are short in duration, and some are long-term but only become apparent after an acute stressor is applied (McCormick and Green, 2012; McCormick et al., 2012; Saul et al., 2012). McCormick et al. (2012) found that exposure to unpredictable social instability and isolation in adolescent rats resulted in learning deficits in adulthood, but these effects were only apparent after an acute stressor was applied (McCormick et al., 2012). Others have reported that behavioral effects of stress during adolescence can be transient and fade over the lifetime of an animal (e.g., unpredictable isolation and novel social partner stressors during adolescence induce temporary changes in boldness, Mathews et al., 2008). Despite these important early studies, the long-term effects of stress during adolescence on emotion and cognition are not well-characterized. Yet, if we are to understand how animals cope with stress during development, and how early adverse experiences can prepare an animal to deal with subsequent stressors, we need to determine the long-term impacts of stress during the adolescent stage (Romeo, 2010).

A number of studies have demonstrated that stress during adolescence, including unpredictable chronic social and physical stress, can impact HPA axis function and glucocorticoid production in adulthood (McCormick and Mathews, 2008; Buwalda et al., 2011). The long-term consequences of stress during adolescence on cognition and behavioral coping response, however, remains unknown. A method to assess behavioral coping response and reward loss sensitivity, as mediated by glucocorticoid production, is the successive negative contrast (SNC) test (Mitchell and Flaherty, 1998; Gomez et al., 2009). SNC has been used for over 3 decades to evaluate an animal’s response to the unexpected downshift of a familiar high-value reward to a novel low-value reward (Lombardi and Flaherty, 1978; Flaherty and Rowan, 1989). Recently SNC has been used as a measure of coping response to infer background emotional state in non-human animals (Burman et al., 2008; Gomez et al., 2009).

In humans, background emotional state can affect decision-making through a cognitive bias in stimulus interpretation that impacts stimulus perception, attention, and processing (Winkielman et al., 2007). Increasingly, measures of cognitive bias are being used as indicators of background emotional state in non-human animals (Burman et al., 2009; Brydges et al., 2011). Unlike most behavioral and physiological measures, cognitive bias tests can measure the valence of affect (positivity vs. negativity) rather than just arousal (Mendl and Paul, 2004).

Prior studies have shown that adult rats can exhibit a negative cognitive bias, marked by an increased propensity to interpret ambiguous stimuli as threatening or aversive that can start during stress exposure and last up to several days after an aversive event (Harding et al., 2004; Burman et al., 2009). The potential longevity of a negative cognitive bias following exposure to stress, however, remains unclear (Mendl et al., 2009; but see Brydges et al., 2012). A previous focus on short-term changes in cognitive bias has meant that long-term changes have so far been underexplored (Brilot et al., 2010). In the current study, we addressed the long-term effects of chronic unpredictable stress during adolescence on behavior and cognition by evaluating changes in cognitive bias, decision-making, associative learning rate, coping response, and motivation to consume a reward in adulthood.

A range of behavioral tests were used to examine the consequences of stress during adolescence: (1) sucrose preference (Strekalova et al., 2004), (2) exploration of a novel object (Van Dijken et al., 1992; Cavigelli et al., 2009), (3) successive negative contrast (SNC), and (4) ambiguous judgment cognitive bias (Harding et al., 2004; Doyle et al., 2011). We measured exploratory behavior and motivation to consume a reward because alterations in these fundamental traits could potentially affect the interpretation of more complex reward or activity based tests including the cognitive bias and SNC tests. Stress can alter both the motivation to consume a reward and exploratory behavior; the magnitude of effects from stress are dependent upon the type and duration of the stressors and traits intrinsic to the animal (Zurita et al., 2000; Strekalova et al., 2004; Brilot et al., 2010). We hypothesized that stress during adolescence would induce a negative cognitive bias and stronger sensitivity to reward loss, both suggestive of a long-term negative background emotional state. Additionally, we predicted that stress during adolescence would result in altered decision-making, impaired associative learning, and decreased exploratory behavior in adulthood.

**METHODS**

**SUBJECTS AND HOUSING**

Sixteen male Long-Evans rats (Harlan Laboratory in Fredrick, Maryland, USA) were obtained at 21 days of age. Following transport, rats were given 7 days to settle before handling and behavioral testing commenced. A full timeline of all manipulations and behavioral tests is provided in **Figure 1**. Animals were pair-housed in plastic cages, 20 × 26 × 46 cm, with corn cob bedding and basic enrichment items: two 7.6 cm diameter PVC tubes hanging from the wire cage lid and two 2.5 × 2.5 × 8 cm pine wood blocks. Rats were kept on a 12:12 reversed light/dark cycle at 20–21°C and 41–42% relative humidity. Standard rat chow (LabDiet®) and tap water were available ad-libitum unless otherwise noted. To minimize disturbance, the experimenter was not in the room during data collection. Work was approved by the Pennsylvania State University IACUC committee, protocol #35761.
ADOLESCENT CHRONIC UNPREDICTABLE STRESS

Four cages of pair housed rats \( (n = 8) \) were randomly assigned to the control condition and four cages \( (n = 8) \) to the stress treatment. For the latter group, stressors were presented daily from 30 to 70 days of age, with 8 days of rest occurring intermittently. Prior studies of adolescent-stress have varied in the duration of stress exposure, due in part to the large window of time during which adolescent ontogenetic changes occur. These changes are thought to conclude at approximately 55–60 days of age in male rodents (Spear, 2000). To cover the entirety of the ontogenetic window of adolescence, studies have included a postpubertal “sub-adult” period (Schmidt et al., 2007). Studies of adolescent-stress have used stress exposure periods spanning from 28 to 80 days of age (Spear, 2000; Sterlemann et al., 2010). As the current study evaluated behaviors mediated by the prefrontal cortex (i.e., decision-making, coping), and this region is still developing in early adulthood, the duration of stress exposure (30–70 days of age) included a postpubertal period in early adulthood (van Eden et al., 1990; Spear, 2000).

For the chronic unpredictable stress procedure both physical and social stressors were presented randomly across the light/dark cycle to maximize unpredictability. An average of three physical and three social stressors were presented between each rest day. Stressors noted to induce short-term changes in cognitive bias were used (e.g., cage tilt, damp bedding; Harding et al., 2004; e.g., crowding, confinement: Doyle et al., 2011; see Table 1). An additional stressor, isolation, was chosen because it has been associated with long-term changes in behavior following exposure during adolescence (McCormick et al., 2012).

To control for the influence of circulating corticosterone on tests mediated by glucocorticoid levels, such as the SNC, we controlled for daily rhythms in glucocorticoid production by avoiding testing during peak corticosterone production; all tests were completed within 6 h of the start of the test (days 85–134). Studies mediated by glucocorticoid levels, such as the SNC, we associated with long-term changes in behavior following exposure during adolescence (Harding et al., 2004). An additional stressor, isolation, was chosen because it has been associated with long-term changes in behavior following exposure during adolescence (McCormick et al., 2012).

SUCCESSIVE NEGATIVE CONTRAST (SNC)

Coping response was evaluated from 166 to 184 days of age with an SNC test measuring response to an unexpected downshift in reward value (Burman et al., 2008; Gomez et al., 2009; see Figure 2). During the SNC test, individual animals were tested daily in an opaque, plastic container, 30.5 × 30.5 × 30.5 cm, for 5 min. A plastic bottle of sucrose solution was attached to the center of one wall. Motivation to consume sucrose solution was measured with a basic electronic device attached to a computer that registered each lick through the closing of a circuit, the computer then provided a record of licking rates. After an initial 12 days of trials with a 32% sucrose (w/v) reward, the solution concentration was decreased without warning to 4% (w/v). The lower concentration was administered for 7 days to monitor the recovery of lick rates. To ensure reward salience, 2 h of food deprivation preceded each trial. We defined animals as having learned the SNC task upon registering 10 licks in one session; pre-shift data were evaluated from the first day that more than 60% of the animals had learned the task (day 4) to the last day of 32% sucrose solution presentation (day 12).

COGNITIVE BIAS, DECISION-MAKING, AND ASSOCIATIVE LEARNING

The ambiguous judgment task was used to assess the long-term impacts of stress during adolescence on cognitive bias, decision-making, and associative learning. Using a paradigm similar to

Table 1 | Chronic unpredictable stressor descriptions.

<table>
<thead>
<tr>
<th>PHYSICAL</th>
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<tbody>
<tr>
<td>Smaller cage</td>
<td>Rat pairs were housed for 4 h in a cage with a 25% reduction in volume from the 20 × 26 × 46 cm standard home cage (Doyle et al., 2011).</td>
</tr>
<tr>
<td>Damp bedding</td>
<td>While rats were temporarily in an empty transfer cage, 200 ml of water was mixed into 2/3 of the bedding of the home cage. After 6 h in the damp bedding, pairs were transferred to a clean home cage (Zurita et al., 2000; Harding et al., 2004).</td>
</tr>
<tr>
<td>Cage tilt</td>
<td>Home cages were tilted at a 30° angle for 6 h (Zurita et al., 2000; Harding et al., 2004).</td>
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<table>
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<tr>
<th>SOCIAL</th>
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<tbody>
<tr>
<td>Isolation</td>
<td>Rats were housed individually for 1.5 h in a clean cage (20 × 26 × 46 cm) with a 76 cm diameter PVC tube and a 2.5 × 2.5 × 8 cm pine wood block (Zurita et al., 2000; McCormick et al., 2012).</td>
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<tr>
<td>Crowding</td>
<td>Sets of 2 rat pairs were combined into one clean cage (20 × 45 cm) for 4 h; iterations of pair combinations were balanced (Zurita et al., 2000; Harding et al., 2004; Doyle et al., 2011).</td>
</tr>
<tr>
<td>Foreign bedding</td>
<td>Experimental pairs were housed in the empty home cage of a pair of older conspecifics for 12 h. (Harding et al., 2004).</td>
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</table>
Brydges et al. (2011), animals were trained to associate a conditioned stimulus, a type of sandpaper (rough or smooth), with the location and color of a bowl containing an available food reward. To do this, individuals were placed in a 30 \times 40 \times 45 \text{ cm} opaque plastic start box that was connected to a goal box by an 80 cm PVC pipe (see Figure 3). The goal box contained a white bowl and a black bowl separated by an opaque partition to ensure that the rats made a choice between the two bowls upon exiting the PVC tunnel. Of the two available bowls, one was associated with a high-value reward (3 Cheerios), the other with a low-value reward (1 Cheerio). To balance the scent cues, each bowl always contained three Cheerios, but the accessibility of the rewards varied depending on the trial condition. For a high-reward trial, 3 Cheerios were available in the high-reward bowl while the low-reward bowl contained 3 inaccessible Cheerios. For a low-reward trial, there was 1 accessible Cheerio (with two mesh covered inaccessible Cheerios) in the low-reward bowl, and all 3 Cheerios were inaccessible in the high-rewarded bowl.

A tactile cue lining the PVC tunnel and goal box indicated which bowl had an available reward; one of two grades of silicon carbide waterproof sandpaper, coarse (P60) or fine (P1200), was paired with a specific reward type (e.g., coarse sandpaper signaled a low-reward in the black bowl on the left; Brydges et al., 2011). All sandpaper were of the same brand and were black in color. Pairings of sandpaper grade, bowl color, bowl side, and reward passing a learning criterion, animals were presented with a novel cue ambiguous in its equal distance from the two trained sandpaper cues. To indicate an interpretation of the ambiguous cue as closer to either a high or low-value reward the animal moved to either the high or low-reward location in the testing chamber. Interpretation of the ambiguous paper as closer to either the cue for the high or low-value reward conveyed a positive or negative value assignment from properties intrinsic to the animal, i.e., a positive or negative cognitive bias in the interpretation of ambiguity.
value were counterbalanced. All elements of the testing chamber were cleaned with 70% ethanol between each trial.

To study the stress-stimulus associations rats were exposed to daily training sessions that consisted of 2 high-reward trials and 2 low-reward trials; the order of the 4 trials was randomized. Animals moved from the start box through the PVC tunnel into the goal box, and chose either the “correct” rewarded or “incorrect” unrewarded bowl. A choice was defined as a rat moving its nose or paw inside the bowl or touching the outside of the bowl with its nose or paw. If an animal chose the rewarded bowl first, the trial was counted as correct and the rat was allowed to consume the reward. If an animal chose incorrectly it was allowed to move to the correct bowl and consume the reward during the first 5 days of training. Decision-making was measured during the first 8 trials, after the rats had consumed at least one reward in the test chamber, by timing the latency between the incorrect selection of an inaccessible reward and the switch to choose the rewarded bowl. Starting the 6th day of training, the rat was removed immediately if it chose the incorrect side.

A learning criterion was set at 3 out of 4 trials with a correct first bowl choice for 4 out of 5 days. The number of days to reach the learning criterion was evaluated to determine if stress during adolescence impacts adult associative learning (Hammond et al., 2009). In both the stressed and control group 2 rats did not pass the learning criterion. After passing the criterion, probe trials were conducted where ambiguous/intermediate grade silicon carbide waterproof sandpaper (P220) was placed in the PVC tube connecting the start and goal boxes. On each day of probe testing a total of 5 trials were run; in addition to the 4 standard trials, one probe trial using ambiguous sandpaper was randomly inserted into the normal sequence, but the last trial was never a probe trial. A total of 5 probe trials were run over 5 days following the same design as Brydges et al. (2011). During probe testing all animals maintained the learning criterion.

After choosing a bowl during the ambiguous probe trial, the bowl choice was noted as either a high or a low-reward categorization of the ambiguous sandpaper cue and the animal was allowed to consume the reward. A number of studies using unrewarded probe trials found that animal stop responding during repeated probe trials, interpreted as a consequence of the animals learning that probes are not reinforced (Bateson and Matheson, 2007; Brilot et al., 2010; Doyle et al., 2010). To circumvent this, in the current study both high and low-rewards were present during probe trials to avoid cessation of response. A potential limitation of this design is that an initial probe interpretation may be misleading that probes are not reinforced (Van Dijken et al., 1992; Cavigelli et al., 2009). To minimize the potential for the second set of behavioral tasks to be influenced by the first, the two test iterations were separated by 55 days and new stimulus objects were used during each novel object test. All tests were run in a 122 × 122 × 46 cm opaque Plexiglas arena. Each task involved 5 min of free exploration during which latency to leave a 7.6 cm diameter PVC tube shelter was measured. All animals started both exploratory tests inside the PVC tube shelter; the tube was placed along the base of one arena wall in the same position and orientation for all tests.

**EXPLORATORY BEHAVIOR**

Rats were given two tests to assess exploratory behavior, an open field and a novel object test. Both tests were administered at two time points, one before and one after stress exposure (Van Dijken et al., 1992; Cavigelli et al., 2009). To minimize the potential for the second set of behavioral tasks to be influenced by the first, the two test iterations were separated by 55 days and new stimulus objects were used during each novel object test. All tests were run in a 122 × 122 × 46 cm opaque Plexiglas arena. Each task involved 5 min of free exploration during which latency to leave a 7.6 cm diameter PVC tube shelter was measured. All animals started both exploratory tests inside the PVC tube shelter; the tube was placed along the base of one arena wall in the same position and orientation for all tests.

**Exploratory task 1: open field**

To compare activity levels between adolescent-stress and control animals, activity in the arena was quantified with a video-recorded open field assay at two time points (28 and 84 days of age) pre and post chronic unpredictable stress. During video analysis an 8 × 8 grid was used to quantify activity by counting the number of squares crossed on the grid. Crossing of grid squares along the walls of the arena and the proportion of time spent in squares along the arena walls were quantified as indicators of thigmotaxis, a positive correlate of anxiety (Simon et al., 1994).

**Exploratory task 2: novel object**

Response to novelty was evaluated before and after chronic unpredictable stress (at 29 and 85 days of age) with two behavioral measures: time to leave the PVC shelter (i.e., when all 4 feet were touching the arena floor) and latencies to physically contact the two novel objects in the arena with either a paw or nose. The novel objects varied in texture, color, and size. Several plastic objects were used including a translucent red triangle, an opaque matt yellow bowl, a shiny yellow cylinder, and a translucent shelter.

**DATA ANALYSIS**

Sucrose preference and cognitive bias data conformed to the assumptions for parametric analyses. SNC data were square root transformed to achieve normality. Exploratory behavioral data from the novel object and open field assays were natural log transformed to achieve normality. To test whether
chronic unpredictable stress during adolescence affected sucrose preference or exploratory behavior over time (pre and post chronic unpredictable stress), we used 2 factor (time and stress condition) repeated measures ANOVAs to compare across the 2 tests. For post-hoc analysis, independent samples two-tailed t-tests were used to compare the stress and control groups within the 2 test iterations. Only significant post-hoc findings are reported.

To evaluate ambiguity interpretations in the cognitive bias assay, we tested the first two ambiguous probe exposures separately using univariate general linear models because response to the ambiguous probe changes with repeated exposures; initial exposures are more reliable measures of affect (Bateson and Matheson, 2007; Brilot et al., 2010; Doyle et al., 2010). Following individual analysis of the first and second probes, all 3 probe exposures were evaluated with a repeated measures general linear model as in Brydges et al. (2011) and Burman et al. (2009). To determine whether activity or motivation to consume a reward impacted performance in the cognitive bias test, we included activity in the open field and sucrose preference as covariates in the ambiguous probe general linear models. Neither activity nor sucrose preference were significant factors in explaining variation in the data, so they were removed from the model [activity: F(1,12) = 0.929, P = 0.36; sucrose preference: F(1,12) = 0.590, P = 0.46]. The associative learning and decision-making data were analyzed with independent samples two-tailed t-tests. To determine whether animals that experienced adolescent stress had a stronger response to reward devaluation than control animals, repeated measures ANOVAs were used to assess behavior for pre-shift days 4–12 and post-shift days 13–18. To assess the impacts of activity and motivation to consume a reward on SNC scores locomotion in the open field and sucrose preference were included as covariates in the repeated measures ANOVAs; neither factor significantly explained variation in the data and were subsequently removed from the model [Pre-shift: activity: F(1,12) = 0.065, P = 0.81; sucrose pref: F(1,12) = 0.092, P = 0.77; Post-shift: activity: F(1,12) = 1.959, P = 0.20; sucrose pref: F(1,12) = 1.984, P = 0.20]. To evaluate response to the reward devaluation, lick numbers on the first day of post-shift were subtracted from the average of the last 3 days of pre-shift. These difference scores were tested with a two-tailed t-test. Analyses were run in SPSS; values are reported as means ± standard deviation.

RESULTS
SUCCESSIVE NEGATIVE CONTRAST
Response to the reward downshift was greater in the adolescent-stress group than in the control animals [T(1,14) = 2.216, P = 0.04, d = 1.02, see Figure 4]. No differences were found in the pre-shift lick rates of the adolescent-stress and control animals [RM ANOVA, F(1, 6) = 0.092, P = 0.77] or over time [F(1, 6) = 4.022, P = 0.37], nor was there an interaction [F(1, 6) = 1.173, P = 0.61]. Post-shift lick rates changed over time as rats returned to pre-shift licking rates [RM ANOVA, F(1, 6) = 9.911, P = 0.01], but stress and control animals did not differ in their post-shift lick rates [RM ANOVA, F(1, 6) = 0.003, P = 0.95], nor was there an interaction [F(1, 6) = 0.644, P = 0.70].

COGNITIVE BIAS ASSAY
All stressed animals interpreted the ambiguous cue as negative on the first day of probe testing, demonstrating a negative cognitive bias that differed from the control animals whose interpretations were half positive and half negative [F(1, 12) = 5.000, P < 0.05, R2 = 0.33, see Figure 5]. This difference in the interpretation of the ambiguous probe was not significant in subsequent trials [Day 2: F(1,12) = 0.000, P = 1.00; GLM 5 days: F(1,12) = 0.471, P = 0.508]. The total number of positive and negative probe interpretations from each group are depicted in Figure 6. Within the first two days of training, adolescent-stressed animals were faster to correct wrong decisions by abandoning the wrong bowl, reorienting, and choosing the correct bowl [t-test T(1,14) = 3.245, P = 0.01, d = 1.62, see Figure 7]. However, animals stressed during
adolescence showed no difference in the number of days to learn the associative task compared with controls [t-test, stress: 26 ± 3 vs. control: 25 ± 5; T(1, 10) = 0.419, P = 0.68].

**Sucrose Preference**
Sucrose preference decreased over time [RM ANOVA effect of time: \( F(1, 7) = 5.680, P = 0.04 \)], but there was no effect of stress [RM ANOVA effect of stress: \( F(1, 7) = 0.417, P = 0.53 \)] and no interaction between stress condition and time [RM ANOVA stress × time interaction: \( F(1, 21) = 0.631, P = 0.42 \); time 1: stress 83 ± 7% vs. control 82 ± 7%; time 2: stress 78 ± 7% vs. control 74 ± 8%].

**Open Field Activity Scores**
Activity increased over time, which is consistent with previous studies indicating that age influences exploratory behavior in an open field [RM ANOVA effect of time: \( F(1, 7) = 8.454, P = 0.01 \); Bronstein, 1972]. There was no effect of stress [RM ANOVA effect of stress: \( F(1, 7) = 0.093, P = 0.77 \)], nor was there an interaction between stress condition and time [RM ANOVA stress × time interaction: \( F(1, 7) = 1.423, P = 0.25 \); time 1, stress 244 ± 30 squares crossed vs. control 233 ± 86 squares crossed; time 2, stress 282 ± 29 squares crossed vs. control 303 ± 40 squares crossed].

Thigmotaxis decreased over time in all animals [RM ANOVA effect of time: \( F(1, 7) = 97.685, P < 0.00 \)]. There was no effect of stress condition [RM ANOVA effect of stress: \( F(1, 7) = 0.110, P = 0.75 \)], and no interaction between stress and time [RM ANOVA stress × time interaction: \( F(1, 7) = 0.359, P = 0.56 \); time 1, stress 283 ± 12(s) vs. control 282 ± 10(s); time 2, stress 233 ± 15(s) vs. control 238 ± 21(s)].

**Novel Object**
The latency to approach a novel object decreased in animals exposed to stress during adolescence [RM ANOVA effect of stress: \( F(1, 7) = 4.682, P < 0.05 \)]. In the second test iteration rats exposed to adolescent-stress were faster to approach a novel object than control animals [latency to approach novel object at time 2: stress 4.6 ± 2(s) vs. control: 16 ± 13(s); \( T(1, 14) = 2.419, P = 0.03, d = 1.23 \)] with no baseline difference in the approach latency prior to stress exposure [latency to approach novel object at time 1, stress: 15.4 ± 19(s) vs. control: 16.7 ± 13(s); \( T(1, 14) = 0.136, P = 0.88 \)]. While this difference appears to be a real biological effect, the variance between the groups was high which may explain the lack of interaction between time and treatment [RM ANOVA stress × time interaction: \( F(1, 7) = 1.544, P = 0.23 \)]. Latency to leave the PVC shelter decreased over time in all animals, which is congruous with previous findings that behavior in a novel object test changes as animals reach adulthood [RM ANOVA effect of time: \( F(1, 7) = 11.179, P = 0.01 \); Saul et al., 2012]. During the novel object test, 15 days after the completion of the chronic unpredictable stress treatment, rats exposed to stress during adolescence left the PVC shelter faster than control animals [exit latency at time 2, stress: 2.3 ± 0.6(s) vs. control: 7.3 ± 1.8(s); \( T(1, 14) = 2.240, P = 0.04, d = 3.73 \)]. There was no baseline difference in the latency to leave the PVC shelter prior to stress exposure [exit latency at time 1, stress: 2 ± 0.5(s) vs. control: 1.7 ± 1(s); \( T(1, 14) = 0.344, P = 0.74 \)].

**Discussion**
Our results show that chronic unpredictable stress during adolescence has long-term effects on coping response, cognitive bias, and decision-making. Associative learning and sucrose preference, however, were not affected by stress exposure during adolescence. The novel object test showed increased boldness behaviors 15 days after completion of the chronic unpredictable stress paradigm. Activity and thigmotaxis in the open field were not affected by prior adverse experience. Stress-exposed rats were faster to leave a familiar shelter in an environment containing novelty and approached novel objects more quickly than control animals. The successive negative contrast test demonstrated that stress during adolescence induces a stronger response to the devaluation of an expected reward in adulthood. The sucrose preference test demonstrated that stress during adolescence does not alter motivation to consume a reward, confirming that the altered response to reward devaluation exhibited by animals exposed to stress during adolescence was not due to a difference in reward salience, but was a reaction to the downshift in reward value.
Exposure to stress during adolescence also decreased the latency to correct a choice and locate a food reward after an incorrect decision. In an early phase of training for the cognitive bias assay, adolescent-stressed animals were faster at abandoning, reorienting, and switching their choice of food bowl after encountering a bowl with an inaccessible reward than the control animals. The results from the sucrose preference test exclude the possibility that the shorter latency to find the reward after an incorrect decision is due to a difference in motivation to obtain the reward, as the preference test demonstrates that motivation to consume a reward is unchanged by stress during adolescence. Thus, the expediency of decision-making in stressed animals could be the result of decreased behavioral inhibition or increased impulsivity when compared to the control animals. Animals exposed to exogenous corticosterone during adolescence show a form of impulsivity marked by an increased preference for an immediate, small reward rather than a larger reward delivered after a variable delay (Torregrossa et al., 2012). It is possible that the decreased latency to abandon a first choice and transition to a second choice demonstrated by adolescent-stress animals also reflects increased impulsivity. Long-term changes in impulsivity behaviors may be underpinned by stress-induced changes in the brain. Stress may impair maturation processes that typically occur during adolescence, such as myelination in the prefrontal cortex, thereby prolonging an immature-like state of top-down connectivity into adulthood (McCormick, 2007). An immature-like state in prefrontal cortex could maintain increased impulsivity behaviors characteristic of the adolescent stage, and alter behavioral inhibition and decision-making in adulthood.

Our results showed that stress during adolescence induces a long-term negative cognitive bias. This finding, along with the SNC results demonstrating increased sensitivity to reward loss, indicate that stress during adolescence generates a long-term negative background emotional state (Burman et al., 2008; Mendl et al., 2009). A negative background emotional state can bias decision-making and expectations for the future; humans with a negative emotional state exhibit biases in attention (e.g., greater attention to threatening stimuli), memory (e.g., enhanced negative memory retrieval), and judgment (e.g., risk and ambiguity aversion, Paul et al., 2005). It is important, however, to keep in mind the potential ecological context of a negative cognitive bias induced by stress. For example, in sites of high predation, traits like threat bias and risk aversion may serve an adaptive function. If threat is prevalent in an environment, it may be advantageous to more readily treat ambiguity as negative or a potential threat (Mendl et al., 2009). Stress induced programming during adolescence for a long-term threat bias may serve to prepare an individual to cope with future exposure to a dangerous environment. Human studies suggest that the consequences of a negative cognitive bias are far reaching, but the full impacts of a negative cognitive bias in non-human animals are not yet clear (Winkielman et al., 2007).

The ambiguous judgment cognitive bias task used here captured differences in the interpretation of ambiguity as a result of stress during adolescence. The results of the 5 probe trials evaluated together, however, highlight a limitation of the ambiguous judgment test. During the first exposure to the ambiguous probe, animals interpret the novel ambiguous stimulus based only on their own biases and life history, whereas subsequent exposures to the ambiguous probe are influenced by previous interpretations of the probe. Thus, repeated probe tests can be subject to effects from learning (Doyle et al., 2010). In the current study the initial ambiguous probe trials were analyzed separately from subsequent probe trials similar to Brilot et al. (2010). Future studies that use the ambiguous judgment task should analyze initial probe exposures separately, as the use of repeated probe tests allows for learning and can yield misleading results (Brilot et al., 2010; Doyle et al., 2010).

The current study found that sensitivity to reward loss in adulthood is intensified by exposure to stress during adolescence, suggesting that animals exposed to adverse events during this period can undergo a long-term change in coping with challenge. This result could help explain an interesting phenomenon documented in previous studies: adolescent-stressed animals can appear to have unaltered behavior, temperament, and learning in adulthood, until they encounter a challenge, at which point behavioral differences become apparent (Watt et al., 2009; Vidal et al., 2011; McCormick et al., 2012). Our results suggest that the altered response to challenge demonstrated by adult animals exposed to stress during adolescence could arise from a long-term change in coping response that has behavioral and cognitive consequences that only become apparent upon subsequent exposure to stress.

Immediately following exposure to isolation and unpredictable housing during adolescence, exploratory behavior in an elevated plus maze is increased, however, a month following stress exposure exploratory behavior is decreased relative to controls (McCormick et al., 2008). Our results expand upon this finding to demonstrate that 2 weeks following physical and social stress during adolescence, male rats are faster to approach novelty, suggesting increased exploratory behavior. The contrast in effects of closely related stress paradigms emphasizes the need for longitudinal studies that evaluate the consequences of specific stress paradigms and span multiple life stages in order to more completely understand how resilience and vulnerability to stress change over the lifetime of an organism.

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REFERENCES


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Chapter 3

Chronic stress during adolescence shapes learning and memory in adulthood

Chapter 3 is in peer-review and is included on the following pages.

Author contributions: LEC conceived of and designed the studies, lead data acquisition, analyzed and interpreted the data, and wrote the manuscript.
Chronic stress during adolescence shapes learning and memory in adulthood

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Abstract

Exposure to acute stress can cause a myriad of cognitive impairments, but whether negative experiences continue to hinder individual as they age is not well understood. We determined how chronic unpredictable stress during adolescence affects multiple learning and memory processes in adulthood. Using male Sprague Dawley rats, we measured learning (both associative and reversal) and memory (both reference and working) starting 110 days after completion of the adolescent-stress treatment. We found that adolescent stress affected adult cognitive abilities in a context-dependent way. Compared to rats reared without stress, adolescent-stressed rats exhibited enhanced reversal learning, an indicator of behavioral flexibility, but showed no change in associative learning and reference memory abilities. Working memory, which in humans is thought to underpin reasoning, mathematical skills, and reading comprehension, was enhanced by exposure to adolescent stress. However, when adolescent-stressed animals were tested after a novel disturbance, they exhibited a 5-fold decrease in working memory performance while unstressed rats continued to exhibit a linear learning curve. These results emphasize the capacity for stress during adolescence to transform the cognitive abilities of adult animals, even after stress exposure has ceased and animals have resided in safe environments for the majority of their lifespans.
Keywords:
Adolescence; Chronic unpredictable stress; Learning; Memory; Reversal learning; Rattus norvegicus; Laboratory rat

Highlights:
• This study tested the effects of adolescent stress on adult learning and memory.
• Adolescent stressed rats had enhanced reversal learning compared to unstressed rats.
• Adolescent stress exposure also enhanced working memory in adulthood.
• However, adolescent stress made working memory more vulnerable to disturbance.
• Adolescent stress did not affect adult associative learning or reference memory.
1. Introduction:

During adolescence, mammals are remarkably sensitive to their environment and can undergo changes in behavior, physiology, and cognition that persist into adulthood (Romeo et al., 2005; Toledo-Rodriguez & Sandi, 2011; McCormick et al., 2012; Caruso et al., 2014; reviewed in Brown & Spencer, 2013; Green & McCormick, 2013). Why animals undergo this phase of plasticity remains unclear, but modifications during this transitional period may facilitate colonization and acclimation to new environments (Crone & Dahl, 2012). Important development changes in biological systems controlling reproduction, cognition, and the ability to respond to adversity typically occur during adolescence (Spear, 2000; Romeo & McEwen, 2006; Tanner, 1962). Stress can disrupt these developmental trajectories, and can cause lasting phenotypic alterations that may impact fitness, including reduced motivation for social interactions (Green et al., 2012) and exacerbated age-related cognitive decline (Sterlemann et al., 2010). Yet it appears that adolescent-stress can also enhance adult foraging-related problem solving abilities under threat (Chaby et al., 2015) and cause longer lasting threat associations compared with unstressed animals (Toledo-Rodriguez & Sandi, 2007), which could be advantageous in a dangerous environment. Given that experiences during adolescence can influence adult cognition, determining how exposure to stress during adolescence affects learning and memory processes remains a key goal for understanding developmental plasticity and the potential for developmental stress to shape life-long outcomes.

Adolescents may be more sensitive to stress for at least three reasons (sensu Romeo, 2013, 2015); (1) they produce higher levels of glucocorticoid “stress” hormones in response to aversive physical and psychological stimuli compared with adults (McCormick et al., 2005; Romeo, 2010), (2) adolescents may be more sensitive to the effects of glucocorticoids on gene regulation (Lee et al., 2003), and (3) adolescent brain areas involved in stress regulation, learning, and memory (e.g. prefrontal cortex (PFC), hippocampus, and amygdala) are still developing and
maturing during adolescence (Spear, 2000; Dahl, 2004). In particular, brain structures integral in learning and memory processes, including the PFC and the hippocampus, undergo numerous maturational processes during adolescence that include the pruning and loss of large numbers of glutamatergic cells and increases in white matter density (Insel et al., 1990; Jolles et al., 2011; Scherf et al., 2006). It is suggested that stress may alter the maturation of these structures and affect their functioning later in life (Spear, 2000).

Changes in cognitive ability might affect fitness in at least two ways. First, cognitive ability can be a target of mate selection, and thereby affect reproductive output (Keagy et al., 2009; Verzijden et al., 2012). Second, increased cognitive abilities, such as learning and memory, can allow animals to maximize resource use in changing and complex environments (Papaj & Prokopy, 1988; Papaj & Vet, 1990; Dukas & Duan, 2000). Associative learning – i.e. establishing a link between predictive stimulus or location and reinforcer (De Houwer et al., 2014) – is well-conserved across taxa and can facilitate exploitation of an environment by decreasing time spent searching for resources or by enhancing prediction and avoidance of threat (Dukas & Duan, 2000). Animals can cope with changes in the environment through reversal learning – i.e. abandoning previously established associations for alternative associations that were not previously reinforced (Clark et al., 2004). Reversal learning is often linked to behavioral flexibility, which in humans involves the dorsomedial striatum and the orbitofrontal cortex (comparable to the medial frontal cortex in rats; Berendse et al., 2004; Ragozzino, 2007). In adult rats, reversal learning can be impaired shortly after stress exposure (Cerqueira et al., 2007).

Memory can also be affected by stress (Luine et al., 1994; Kirschbaum et al., 1996). Working memory, defined as holding information in memory for temporary use or manipulation (Hitch, 2002), is thought to constrain cognitive abilities including reasoning, reading comprehension, and mathematical skills (Hitch & Baddeley, 1976; Carretti et al., 2009; Alloway & Passolunghi, 2011) and is a more accurate predictor of academic success than IQ (Alloway & Alloway, 2010).
Exposure to stress can impair working memory (Diamond, 1996, 1999). Deficits in working memory can reduce quality of life (Alptekin et al., 2005). Thus, determining if adolescent stress has long-lasting effects on adult learning and memory is important to understand the effects of developmental stress on fitness and well-being.

Prior studies have shown that adult stress exposure can affect learning and memory processes (Luine et al., 1994; Kirschbaum et al., 1996; Diamond 1996, 1999). For example, exposure to stress impairs reference memory, or the ability to retrieve information after a delay (Nadel & Hardt, 2011). Over time, the effects of stress can accrue and cause lasting changes in both stress hormone production and memory (Hutchinson et al., 2012; Lupien et al., 2009). In adult rats, spatial reference memory in an 8-arm water maze is reduced by chronic stress, but recovers after not being exposed to stress for 3 weeks (Hoffman et al., 2011). In contrast, chronic stress during adolescence (28-56 days of age) does not have an immediate effect on reference memory in the open Morris water maze, but impairs reference memory after a 3 week delay (Isgor et al., 2004).

Despite the importance of these cognitive processes, and the potential influence of stress on these processes, it is unclear whether adolescent-stress causes lasting changes in these systems. Daily isolation during an earlier developmental window in rats, from weaning (21 days of age) to early adolescence (34 days of age), impairs adult reversal learning (Han et al., 2011). However, stress exposure during an earlier juvenile phase (27-29 days of age) can have lasting effects on adult behavior that differ from the lasting effects of adolescent stress exposure; stress during both stages can cause poor avoidance learning, but learned helplessness is only affected by earlier juvenile-stress, and not adolescent-stress (Tsoory & Richter-Levin, 2006). The effects of adolescent-stress on cognitive performance in adulthood, however, can be dependent upon whether or not aversive stimuli are present in the adult environment (Chaby et al., 2015). It remains unknown whether chronic stress in adolescence has lasting effects on learning and
memory under low threat conditions. Here, we examine the effects of stress during adolescence on multiple learning and memory processes in radial maze tasks to assess associative and reversal learning, as well as working and reference memory. We predicted that associative learning, a simple form of learning, would not be affected by adolescent-stress but that reversal learning, which may require behavioral flexibility (Bond et al., 2007), would be impaired. Similarly, we predicted that working memory and reference memory would be decreased by exposure to adolescent-stress.

2. Methods

2.1. Subjects and housing

Male Sprague-Dawley rats (24) were obtained at 21 days of age from Harlan Laboratory (Frederick, Maryland). Animals were pair-housed in plastic cages, 20 cm x 26 cm x 45 cm, according to the National Institute of Health (NIH) recommendations described in the Guide for the Care and Use of Laboratory Animals. Enrichment items were added to all cages at 23 days of age (two 7.6 cm diameter PVC tubes hanging from the wire cage lid and two 2.5 cm x 2.5 cm x 8 cm pine blocks). All cages were kept at 20-21°C and 40-45% relative humidity and cleaned weekly. Enrichment items were changed when soiled. Rats were kept on a 12:12 reversed light:dark cycle to accommodate testing during the dark phase when rats are most active; all testing began at least 2 hours after the start of the dark phase and was completed within 6 hours. Standard rat chow (LabDiet® 5001, 23% protein) and tap water were available ad libitum except preceding rewarded tests food was removed 2 hours beforehand to motivate rats to complete the tests. A timeline of manipulations is given in Fig. 1. To minimize disturbance the experimenter was not in the room during testing and experiments were video-recorded. Test chambers were sprayed with 70% ethanol solution and wiped clean between trials. Experiments were approved by the Pennsylvania State University IACUC, protocol #44459.
2.2. Chronic unpredictable stress

Pair-housed rats were randomly assigned to the adolescent-stress treatment (n=12) or the unstressed control group (n=12). Each week between 30-70 days of age adolescent-stress rats encountered six stressors, three between 000-1200 h and three between 1200-2400 h. The three stressor types (physical, social, and predation) and order of stressor presentation varied, but were balanced so that each type of stressor was represented twice per week.

**Physical stressors:** (1) Housed in a cage 25% smaller than the home cage for 4 hours, (2) housed in damp bedding for 6 hours, (3) home cage tilted 30° for 6 hours.

**Social stressors:** (1) Individually-housed for 1 hour, (2) crowded by combining 2 rat pairs for 4 hours, (3) exposed to bedding from older conspecifics for 12 hours.

**Predation stressors:** Exposed for 30 minutes to (1) a continuously moving taxidermied bobcat (Blumstein et al., 2004), (2) house cat (*Felis catus*) fur, (3) large cat vocalizations.

This stress paradigm has previously induced long-term behavioral changes and is described in more detail in Supplementary Table 1 and Chaby et al., 2015. To account for handling and cage changes during the stressors, rats in the unstressed group were handled and transferred to clean cages approximately twice per week and coincided with stressors that required a new cage. All rats were weighed weekly during the stress treatment, and every second week thereafter to monitor health. The duration of the stress treatment (30-70 days of age) included a short postpubertal period in early adulthood (55-70 days of age) to cover the entire ontogenetic window of adolescence (Schmidt et al., 2007; Sterlemann et al., 2010) and to evaluate behaviors mediated by the prefrontal cortex, which continues to develop into early adulthood (Spear, 2000).

2.3.1. Radial Maze: Habituation
At 176 days of age, rats were placed in the center of the radial maze individually and allowed to explore for 5 minutes to familiarize rats with the testing environment (depicted in Supplementary Fig. 1). We quantified entries of the radial arms as an indicator of baseline activity in the testing conditions. An arm entry was defined as crossing all four feet into an arm.

2.3.2. Radial Maze: Associative learning shaping

Rats underwent two shaping sessions prior to associative learning trials in order to familiarize rats with consuming rewards in a single arm in the maze (the “correct” rewarded arm for the associative learning experiment). During shaping, a Cheerio® reward was placed halfway down one of the five arms of the radial maze. The rewarded arm was counterbalanced across treatment. In the first shaping session, rats were placed in the center of the maze and allowed to explore freely. In the second shaping session, rats began in the start chamber for three trials separated by inter-trial intervals of 30 seconds. Rats were separated into two groups of 12, balanced by treatment. One group of 12 underwent shaping trials at 177 and 179 days of age, the second at 178 and 180. During all shaping trials, rats were removed 1 minute after consuming the Cheerio or after 5 minutes had elapsed.

2.4. Associative learning

From 181 to 198 days of age rats underwent 12 days of associative learning trials (with 6 days of rest). Conditions of the associative learning trials were similar to the shaping trials; rats were given 3 trials per day with 30-second inter-trial intervals, and were removed 1 minute after consuming the reward or after 5 minutes. Rats began each trial in the start chamber, but could not enter the radial maze until 20 seconds elapsed and an opaque plastic barrier was removed via pulley. To control for visual cues, Cheerio rewards were located in a dish recessed into the maze floor so that rewards were not visible until a rat was standing over the reward dish at the end of the arm. To control for olfactory cues, Cheerios were placed alongside the arms on the outside of
the maze. To assess associative learning, we recorded latency to enter the radial maze (all four feet inside radial maze), latency to find the food reward (rat’s head dipped inside reward dish), whether a trial was “correct” (rewarded arm entered first), reference memory errors (enter an unrewarded arm), and working memory errors (re-enter an unrewarded arm in the same trial).

2.5. Long-term reference memory

Rats underwent long-term reference memory probes at 10, 20, and 55 days after the last training day. Memory probes consisted of a single trial identical to associative learning trials. We recorded latency to enter the radial maze, latency to find the food reward, arms entries, and reference and working memory errors.

2.6. Retraining & reversal learning

Rats were tested for reversal learning at 258 or 259 days of age. For the reversal learning test, and all subsequent parts of the experiment, rats were tested in two groups of 12, balanced by treatment and tested on alternate days. Prior to the reversal learning test, rats underwent two days of retraining (identical to the associative learning trials). After retraining, reversal learning was tested by moving the reward to a previously unrewarded arm and measuring latency to enter the radial maze, latency to find the food reward in the novel arm, and number of arms entered during each of two reversal trials (de Bruin et al., 1994). Conditions in reversal trials were identical to retraining, except for the position of the reward. We conducted a second reversal trial to determine whether behavior was consistent across both trials, indicating a potential motivational difference.

2.7. Working memory & novel disturbance

The effect of adolescent-stress on adult working memory and the ability to maintain a working memory after a novel disturbance was tested at 261 and 262 days of age. Rats were exposed to a novel reward arm, distinct from the arms used in the associative and reversal learning
experiments, over the course of 3 trials to create a working memory of a new reward location (Kesner, 2000; Cerqueira et al., 2007). These trials were identical to the associative learning trials except for the novel reward location. Following the third working memory trial, rats were exposed to a novel environment for 20 minutes to disrupt memory for the new reward location (Diamond et al., 1996). The novel environment was a circular grey plastic chamber (diameter 29 cm, height 36 cm) which had been wiped with a citrus orange cleaner. Citrus scents can be aversive (Amiri et al., 1998) and exacerbate stress responses in laboratory rats (Komori et al., 2003). Rats were then returned to the radial maze for two additional trials with the reward remaining in the same location as the earlier working memory trials. To determine whether any differences in behavior following exposure to the chamber could be explained by changes in activity, each arm was divided into quadrants and the number of crosses between quadrants was measured from video recordings.

2.7. Consummatory extinction

At 314 days of age, motivation for a reward was tested, as this could mediate behavior in reward-based learning and memory tasks. We used a modified successive negative contrast test where animals are first familiarized with a reward, then the reward is made inaccessible and the degree of persistence to obtain the absent reward is quantified (Flaherty, 1979; Chaby et al., 2013). In the first phase of the test, rats were given daily access to a 32% sucrose solution for 5 minutes for 9 days in an opaque, plastic chamber (30.5cm³). In the second phase of the test, rats were given 5 minutes in the same chamber for two additional days, but the solution was made inaccessible by a layer of plastic at the seam of the spout that was not visible when the bottle was positioned for the test. An open bottle of sucrose solution outside the opaque testing chamber provided olfactory cues similar to the reinforcing cues present in the first phase of testing. To quantify persistence we used an electronic device that registered each time a rat contacted the metal spout to obtain the reward (see Chaby et al., 2013; Flaherty et al., 1979).
2.9. Data analysis

To determine the amount of time spent engaged in the task, latency to enter the maze was subtracted from the total latency to locate the reward. To conform to the assumptions for parametric analyses, we used natural log transformation of latency to locate the reward and activity difference scores. To determine whether adolescent-stress affected the number of arm entries during habituation to the radial maze, we used a two-tailed t-test. The effect of adolescent-stress on associative learning was tested by averaging the latency to locate the reward across the three trials per day, and using a repeated-measures general linear model (RMGLM) with stress condition and time as fixed factors. To assess associative learning we also tested the total number of correct trials each day with a RMGLM with stress condition and time as fixed factors. The effect of adolescent-stress on reference memory was tested in the three memory probes (each consisting of a single trial), using the latency to locate the reward and the number of errors in separate RMGLMs with stress condition and time as fixed factors. Performance just prior to the reversal trials, was tested using the latency to locate the reward and the number of arm entries in the three trials on the last day of re-training, using RMGLMs with stress condition and time as fixed factors. Number of arm entries was used because 5 adolescent-stressed and 6 unstressed rats made no errors by the completion of retraining. For the reversal learning trials, because only the first reversal trial was novel, measures from the first trial were analyzed using GLMs with stress condition as a fixed factor. Measures from the subsequent reversal trials were analyzed with RMGLMs with stress condition as fixed factors. For the working memory trials before the novel chamber, latency to locate the reward and the number of arm entries were analyzed with RGLMs with stress condition and time as fixed factors. To assess whether the novel chamber induced changes in behavior (and to account for group differences in performance in the working memory trials), we subtracted latency to locate the reward in the two
trials after the novel chamber from the latency to locate the reward in last working memory trial. The same procedure was used for the number of arm entries and the activity measure. The resulting “difference scores” were analyzed with RMGLMs with stress condition and time as fixed factors. One rat from the adolescent-stressed group became distressed in the novel chamber and repeatedly attempted to jump out of the chamber. This rat was returned to his home cage, and was not included in re-exposure trials. No other rat exhibited signs of distress. To compare performance during the consummatory extinction test, we used a RMGLM with stress condition and time as fixed factors. Analyses were run in IBM® SPSS® Statistics v 21; values are reported as means ± standard error (SE). Statistical significance is assigned when p ≤ 0.05.

3. Results

3.1. Habituation

Adolescent stress did not affect the number of maze arm entries during the habituation task (stress average: 16.5 ± 0.8, unstressed average: 15.3 ± 0.9; T_{22} = 1.04, P = 0.31).

3.2. Associative learning

Across the 12 trials, latencies to locate a reward decreased (F_{1,22} = 96.94, P < 0.001) and number of correct trials per day increased (F_{1,22} = 7.52, P < 0.001). Stress during adolescence did not affect associative learning; there were no differences between adolescent-stressed and control animals in (a) latency to locate the reward (F_{1,22} = 1.00, P = 0.33; Fig. 2A) or (b) total number of correct trials each day (F_{1,22} = 0.20, P = 0.66; Fig. 2B). Adolescent-stress did not affect the rate of improvement in the latency to locate the reward (stress x time interaction: F_{1,22} = 0.70, P < 0.74; Fig. 2A). However, adolescent-stressed rats increased the number of correct trials over time more slowly than unstressed rats (stress x time interaction: F_{1,22} = 3.63, P < 0.01; Fig. 2B).
3.3. Long-term reference memory

In the three memory probes, starting 10 days after the associative learning trials, all rats exhibited an increase in latency to locate the reward over time ($F_{1,22} = 12.10, P < 0.00$) but remained constant in number of errors ($F_{1,22} = 0.90, P = 0.41$; Supplementary Fig. 2). Adolescent-stress did not affect reference memory (Supplementary Fig. 2) – either latency to locate the reward ($F_{1,22} = 0.17, P = 0.69$) or number of arm entry errors ($F_{1,22} = 1.25, P = 0.28$). On average, rats made less than one mistake in each of the three memory probes. Adolescent-stress did not affect the rate of change in reference memory (stress x time interaction) in either latency to locate the reward ($F_{1,22} = 0.30, P = 0.75$) or the number of arms entered ($F_{1,22} = 0.04, P = 0.96$).

3.4. Re-training & reversal learning

Performance improved during retraining for all rats; latency to locate the reward ($F_{1,22} = 4.33, P = 0.02$) and number of arm entries ($F_{1,22} = 6.37, P = 0.01$) decreased over time. In the final retraining day, adolescent-stress did not affect latency to locate reward ($F_{1,22} = 0.12, P = 0.73$; stress average: 11 ± 6 seconds, unstressed average: 13 ± 7 seconds) or number of arm entries ($F_{1,22} = 0.28, P = 0.61$; stress average: 1.5 ± 0.2, unstressed average: 1.4 ± 0.2). There were no stress x time interactions (latency to locate the reward, $F_{1,22} = 0.18, P = 0.84$, number of arms entered, $F_{1,22} = 0.67, P = 0.52$).

Adolescent-stress enhanced reversal learning (Fig. 3A); adolescent-stressed rats located the food reward 45% faster than unstressed rats in the first reversal trial ($F_{1,22} = 5.10, P = 0.04$). By the second reversal trial this effect had abated, after only a 30 second inter-trial interval, suggesting that motivation to obtain the reward was the same for both groups ($F_{1,22} = 0.02, P = 0.90$). The number of arm entries did not differ between groups in the first or second reversal trial ($F_{1,22} = 0.07, P = 0.79$; $F_{1,22} = 0.40, P = 0.53$).
3.5. Working memory & novel disturbance

All rats exhibited a decreased latency to locate the reward across trials ($F_{1,22} = 9.40, P < 0.001$; data in Supplementary Table 2). Across the three working memory trials, before the novel chamber exposure, adolescent-stressed rats found the reward faster than unstressed rats ($F_{1,22} = 4.22, P = 0.05$; Fig. 3B). The number of arm entries was not affected by adolescent-stress ($F_{1,22} = 0.86, P = 0.37$; data in Supplementary Table 2) or time ($F_{1,22} = 2.19, P = 0.13$; data in Supplementary table 2). There were no stress x time interactions for latency to find the reward ($F_{1,22} = 1.46, P = 0.24$) or number of arm entries ($F_{1,22} = 2.23, P = 0.12$).

In both re-exposure trials, adolescent-stress increased the effect of the novel chamber on performance (effect of stress: $F_{1,21} = 9.39, P < 0.01$, stress x time: $F_{1,21} = 0.99, P = 0.32$). In the first trials after exposure to the novel chamber, adolescent-stressed rats showed a greater than 500% increase in latency to find the reward while unstressed rats decreased their latency by 30%.

In the second trial after the novel chamber, adolescent-stressed rats were still $5 \pm 7$ seconds slower to find the reward compared to their performance before the chamber, while the unstressed rats located the reward an average of $36 \pm 15$ seconds faster. Adolescent-stress did not affect number of arm entries in the re-exposure trials ($F_{1,21} = 1.27, P = 0.27$). The difference in latency to locate the reward following exposure to the novel chamber was not explained by a change in activity (effect of stress: $F_{1,21} = 3.38, P = 0.10$, stress x time: $F_{1,21} = 2.38, P = 0.16$, data in Supplementary table 3).

3.6. Consummatory extinction

Stress during adolescence did not affect persistence to obtain a reward ($F_{1,22} = 0.45, P = 0.51$). Adolescent-stressed and unstressed rats exhibited a similar number of licks during the first trial with the inaccessible sucrose solution (stress average: $53 \pm 7$, unstressed average $47 \pm 7$) and during the second trial (adolescent-stressed: $48 \pm 9$, unstressed: $60 \pm 13$).
Exposure to chronic unpredictable stress during adolescence was found to shape adult cognition; stress exposure had beneficial effects on some learning and memory processes, and detrimental effects on others, and no effect on yet other aspects of learning and memory in adulthood. Stress during adolescence did not affect associative learning or reference memory tested 10, 20, and 55 days after learning. Despite this, adolescent-stressed animals showed enhanced reversal learning more than 6 months after stress exposure, compared to unstressed rats. Further, the ability to maintain a novel reward location in working memory in adulthood was enhanced by adolescent-stress. However, after a disruption (exposure to a novel chamber), adolescent-stressed rats increased their latency to locate the reward more than 5-fold. This decrease in performance was so strong that adolescent-stressed rat performance dropped to the level of the first trial when they were naïve to reward location. Unstressed rats, however, continued to exhibit a linear learning curve even after the novel disturbance, suggesting a more robust working memory of the reward location. These changes in learning and memory could last the lifespan of Rattus norvegicus; the differences in working memory and reversal learning described here were detected shortly after the median lifespan of male Norway rats outside of captivity, approximately 250 days (Davis, 1948, 1953).

Exposure to the novel chamber to disrupt working memory for the reward location had opposite effects on the two treatment groups; latency to locate the reward decreased by 30% in unstressed rats but increased more than 500% in adolescent-stressed rats. However, after all rats had been re-exposed to the reward location, in the second trial after the chamber (following only a 30-second inter-trial interval), adolescent-stressed rats matched unstressed rats in latency to locate the reward. The short delay between the trials suggests that novelty-induced motivational differences
cannot account for the increase in latency to locate the reward, but rather that adolescent-stress increases vulnerability to disturbance in working memory (Diamond et al., 1996). To further determine whether these were cognitive or motivational differences, we tested persistence to obtain a familiar, inaccessible reward using a consummatory extinction task (Flaherty, 1996; Cuenya et al., 2012). Exposure to adolescent-stress did not affect persistence for a reward, suggesting that reversal learning and working memory effects should not be attributed to motivational differences, but rather reflect changes in cognitive function. Activity could not account for differences in performance because the groups did not differ in baseline activity or in activity following the novel chamber. Furthermore, in both trials after the novel chamber on average the adolescent-stressed rats increased activity levels compared to pre-chamber activity, indicating that inactivity does not explain the increase in vulnerability to disruption of working memory caused by adolescent-stress.

Our results highlight the importance of context when considering long-term effects of stress (e.g. Chaby et al., 2015). The importance of context is further demonstrated by the juxtaposition of our results with those described in Toledo-Rodriguez & Sandi (2007), which showed that adolescent-stress can enhance fear learning, a type of associative learning. In fear learning an innocuous stimulus is associated with an aversive stimulus, such as a shock or predator cue. Fear learning in adulthood can also be enhanced by isolation stress during early life (Lukkes et al., 2009). Animals exposed to early stress may have an advantage in fear learning assays, but not reward-based associative learning tasks, relative to unstressed animals, because the testing environment in fear learning tasks is more consistent with a stressful developmental environment, compared with reward-based learning environments (Breuner, 2008; Love et al., 2005; Sheriff & Love, 2013). Exposure to stress during adolescence can also inhibit extinction when a trained cue is presented repeatedly without the corresponding aversive stimulus, adolescent-stressed rats do not alter their behavior while unstressed rats decrease their response to the cue (Toledo-Rodriguez & Sandi,
2007). The differences in performance exhibited by adolescent-stressed animals in aversive vs. appetitive (reward-driven) learning environments suggest that environmental conditions in adulthood shape cognition, but also that rearing environment, and whether an adult testing environment is consistent with an animal’s early environment, also acts to shape cognitive processes.

Adolescence is characterized by heightened plasticity and behavioral flexibility (reviewed in Crone & Dahl, 2012). It is suggested that behavioral flexibility in adolescence may facilitate integration into novel social or environmental contexts following dispersal from natal environments (Crone & Dahl, 2012). Increased flexibility might also be advantageous later in life in unpredictable, stressful environments. It is possible that exposure to aversive or unstable conditions during adolescence could program an animal to maintain behavioral flexibility into adulthood. Heightened plasticity in adolescence is central to hypotheses that adolescence is a period of vulnerability and an opportunity for “programming” of future behavioral and physiological responses (reviewed in McCormick et al., 2010; Romeo, 2015). The capacity of adolescent-stress to have programming effects that persist throughout life is supported by lasting changes in the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the hormonal response to stress (Seckl, 2001; Pohl et al., 2007) and persistent behavioral changes, such as those documented here. The effects of adolescent-stress on the HPA axis vary by sex, temperament, and the frequency of stressor presentation (Pohl et al., 2007; Schmidt et al., 2007; Sterlemann et al., 2008; McCormick et al., 2008; Caruso et al., 2014). It remains unclear why some models of adolescent-stress cause lasting changes in glucocorticoid production while others do not (e.g. Overmier & Murison, 1991; McCormick et al., 2005; Chaby et al., 2015; reviewed in McCormick et al., 2010), but these differences may be important for understanding the role of persistent changes resulting from stress.
Although it is difficult to predict how results from laboratory models might translate to free-living animals, it is important to note that without intervention the consequences of adolescent-stress can have lasting effects on behavior (Green et al., 2013), cognition (McCormick et al., 2012), and physiology (Isgor et al., 2004) in adulthood. Following early life stress, however, exposure to environmental enrichment (toys, group housing) can reverse some lasting changes in behavior and physiology (Francis et al., 2002; Bredy et al., 2003, 2004; discussed in Romeo & McEwen, 2006). Although rescue effects from enrichment can be substantial, some effects of early stress persist, including changes related to learning (e.g. decreases in hippocampal long-term potentiation, Bredy et al., 2003) and future processing of stress (e.g. changes in corticotropin-releasing factor (CRF) gene expression, Francis et al., 2002). In the current study, adolescent-stressed rats were exposed to adverse unpredictable stimuli for 40 days. Following this, to assess the lasting effects of adolescent-stress, rats were housed without any manipulations in standard laboratory conditions for 106 days. Contrastingly, in naturalistic environments, it is likely that animals are continually exposed to dynamic stimuli that can exacerbate or ameliorate the lasting effects of adversity in adolescence. Future studies are needed to determine how the effects of adolescent-stress on cognition manifest in naturalistic environments and how these effects might impact performance and fitness.

5. Conclusions
Exposure to chronic unpredictable stress during adolescence was found to have both beneficial and detrimental effects on learning and memory processes in adulthood. Compared to rats reared without stress, adolescent-stressed rats exhibited enhanced reversal learning 188 days after stress exposure, suggesting adolescent-stress may increase behavioral flexibility in adulthood. Exposure to adolescent-stress also enhanced working memory 191 days later, which is suggested to underpin reasoning, mathematical skills, and reading comprehension in humans. However,
working memory in adolescent-stressed animals was highly vulnerable to disturbance. The differences in working memory and reversal learning described here were seen shortly after the median lifespan of male Norway rats outside of captivity, approximately 250 days (Davis, 1948, 1953), suggesting that the influence of adolescent stress on adult cognition are quite long-lived

**Conflict of Interest Statement**

The authors declare that no commercial or financial relationships affected the research and that the research was conducted in the absence of conflicts of interest.

**Acknowledgements**

We thank the Huck Institute of Life Sciences and the Eberly College of Science, and the Pennsylvania Department of Health using Tobacco CURE Funds for support. The Department specifically disclaims responsibility for any analyses, interpretations, or conclusions. We thank Erin Platz, Hannah Cooper, Weiyuan Tian, Kaitlyn Grubb, Nicholas Russell, and Carl Hirrlinger. We also thank David Diamond for his guidance.
References


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Verzijden, M. N., ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., and
Evol. 27*, 511–519. doi:10.1016/j.tree.2012.05.007
Figures:

Fig. 1: Timeline of adolescent-stress manipulations and experiments.
Fig. 2: The effect of stress during adolescence on associative learning in adulthood, measured by the latency to obtain the reward (A) and the number of correct trials out of three trials (B) in a radial arm maze, means ± SE.
Fig. 3: The effect of stress during adolescence on reversal learning (A) and working memory (B) in adulthood, measured by the latency to obtain the reward. Asterisks indicates $p < 0.05$; means $\pm$ SE.
Supplementary materials

**S Table 1:** Chronic unpredictable stressor descriptions

<table>
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<td><strong>Cage tilt</strong></td>
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<th>Social Stressors</th>
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<td><strong>Isolation</strong></td>
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<td><strong>Crowding</strong></td>
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<td><strong>Foreign bedding</strong></td>
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<th>Predation Stressors</th>
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<td><strong>Taxidermied bobcat</strong></td>
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<td><strong>Cat fur</strong></td>
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<td><strong>Feline vocalizations</strong></td>
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**S Table 2:** The effect stress in adolescence on arm entries in a working memory task.

<table>
<thead>
<tr>
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<th>Adolescent-stressed rats: number of arms entered</th>
<th>Unstressed rats: number of arms entered</th>
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<tbody>
<tr>
<td>First working memory trial</td>
<td>3.3 ± 0.5</td>
<td>3.2 ± 0.4</td>
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<tr>
<td>Second working memory trial</td>
<td>2.8 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Third working memory trial</td>
<td>1.8 ± 0.6</td>
<td>3.2 ± 0.6</td>
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</tbody>
</table>

*depicts means ± standard error
**S Table 3:** The effect stress in adolescence on activity in a working memory task.

<table>
<thead>
<tr>
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<th>Adolescent-stressed rats: activity (quadrants crossed)</th>
<th>Unstressed rats: activity (quadrants crossed)</th>
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<tbody>
<tr>
<td>Last trial before chamber</td>
<td>15 ± 5</td>
<td>24 ± 5</td>
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<tr>
<td>First trial after chamber</td>
<td>21 ± 3</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Second trial after chamber</td>
<td>17 ± 3</td>
<td>10 ± 2</td>
</tr>
</tbody>
</table>

*denotes means ± standard error

**S Fig. 1:** Radial arm maze schematic (not drawn to scale).
**S Fig. 2:** The effect of chronic stress during adolescence on reference memory at three time points in adulthood, measured by the latency to obtain the reward and the number of entries into incorrect arms, means ± standard error.
Supplementary references


Chapter 4

Chronic unpredictable stress during adolescence causes long-term anxiety

Chapter 4 was published in the journal *Behavioural Brain Research* and is included on the following pages with citation information in reprint form.

Author contributions: LEC conceived of and designed the studies, lead data acquisition, analyzed and interpreted the data, and wrote the manuscript
Short Communication

Chronic unpredictable stress during adolescence causes long-term anxiety

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HIGHLIGHTS

- Stress during adolescence causes a long-term increase in anxiety.
- Increased hyponeophagia is evident 196 days after exposure to unpredictable stress.
- Behavioral changes are not mediated by altered basal corticoid “stress” hormones.

ABSTRACT

Exposure to stress during adolescence can cause long-term changes in behavior and cognition. Anxiety diagnoses rise during adolescence and are increased by adverse experiences. Currently, it is unknown how long stress during adolescence alters anxiety in adulthood. We found that rats exposed to chronic unpredictable stress during adolescence expressed altered behavior 6.5 months later; showing increased anxiety in a feeding test in a novel environment. Although behavioral changes indicative of anxiety were detected in late adulthood, the basal levels of fecal corticoid metabolites in prior-stressed rats did not differ from unstressed, control rats.

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Both laboratory and clinical studies indicate that adolescence is a stage of particular vulnerability to stress exposure [1,2]. Trauma during adolescence appears to increase anxiety rates more than other forms of mental illness, making anxiety an important target for research [3,4]. Anxiety diagnoses increase during adolescence, and can be amplified by adverse conditions [2]. For example, adolescent survivors of the shipwreck of “Jupiter” in 1988 in Greek waters had a 40.7% chance of developing an anxiety disorder, compared to only 18.4% of a demographic-matched control population [3].

In addition to increased psychological vulnerability, laboratory studies with rodents suggest that adolescents are more physiologically vulnerable to stress [5]. During adolescence, the hypothalamic-pituitary-adrenal (HPA) axis that regulates the hormonal stress response is still immature [5,6]. Compared to adults given the same aversive stimuli, adolescents produce glucocorticoid “stress” hormones for a longer duration, thus increasing the adolescent’s overall exposure to glucocorticoids [1]. Chronic exposure to glucocorticoids can cause changes in both the brain and behavior that may persist for several months, including altered neural development and modified dendritic branching [5,7]. Currently, however, it is unclear how long changes in anxiety persist after animals experience stress during adolescence (see SI for a summary of current studies). This ambiguity is due, in part, to the challenge of defining the adolescent phase in rodents; studies vary in timing and duration of stress exposure, making cross-study

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comparisons difficult [8]. Additionally, there are numerous laboratory measures of anxiety that vary in face validity, cost, and ease-of-execution, again making direct comparisons of such assays challenging [9].

The current study investigated behaviors in a novelty suppressed feeding test, which has been used as an assay for anxiety-like behavior in animals for more than seven decades [10]. This test was selected because it has better face validity than other rodent behavioral assays; at least 2 weeks of daily selective serotonin reuptake inhibitor (SSRIs) treatment are required to alter behavior in the novelty suppressed feeding test, which is more consistent with timelines of SSRI effects and human therapies, whereas a single exposure to SSRIs has been shown to alter behavior in forced swim or tail suspension tests [10,11]. Additionally, novelty-suppressed feeding tests are based on natural rodent behavior, potentially making their interpretation more reliable [12].

In a previous study of the effects of adverse social conditions during adolescence and early adulthood (from 32 to 77 days of age) on novelty suppressed feeding in mice, Sterleman et al. [13] found increased anxiety-like behavior immediately following social unpredictability, but, 12 months later there was no observable difference from unstressed controls. Using a similar approach, we investigated novelty suppressed feeding in adult rats after exposure to a diverse, chronic unpredictable stress paradigm during adolescence that included social, physical, and predation stress (Table 1). A variation of this chronic unpredictable stress paradigm has previously been shown to induce long-term behavioral changes including enhanced reward loss sensitivity, accelerated decision-making, and a negative cognitive bias [19]. The current stress regimen included physical stress, which appears to induce longer-term changes in behavior and physiology compared with treatments that use only social stress [20], and also included a short period of early adulthood. We assessed anxiety-like behavior 6.5 months after completion of the chronic unpredictable stress paradigm (Fig. 1).

Male Sprague-Dawley rats (n = 30) were obtained at 21 days of age from Harlan Laboratory in Fredrick, Maryland, USA. Animals were pair-housed and maintained on a reverse 12/12 light–dark cycle to allow for behavioral testing during the dark phase when rats are most active. Nine days after rats arrived in the lab, a subset of rats (n = 14) began the chronic unpredictable stress paradigm that lasted throughout the adolescent period and into early adulthood from 30 to 78 days of age, based on Sterleman et al. [13]. Stressors were presented at variable times between 06:00 h and 01:00 h for 6 days per week; although presentation order was randomized, on average rats were exposed to each of the three types of stress twice per week. After completion of the chronic stress procedure, all rats were maintained in standard housing with no further exposure to stressors for the remainder of the experiment. The additional 16 rats were maintained in standard pair housing throughout development and served as unstressed controls. All experiments were approved by the Pennsylvania State University IACUC committee, protocol #35761.

At 274 days of age, 196 days after the chronic stress procedure, anxiety levels were assessed using a novelty suppressed feeding test. In this test, rats were exposed to a familiar food reward in a novel environment; a longer latency to consume the reward is indicative of behavioral inhibition and increased anxiety. To familiarize animals with the food reward, an almond slice was placed in a petri dish in their home cage in the same manner in which the animals would encounter the reward in the novel context [13]. Three days later, the rats were tested by placing them in a fixed starting position along the base of a wall in a novel 122 cm × 122 cm × 46 cm opaque Plexiglas arena. The latency of each rat to pick up and consume the almond slice in the center of the arena was measured. The arena and petri dish were cleaned with 70% ethanol between trials.

Ten days after the novelty suppressed feeding test, production of glucocorticoid “stress” hormone (corticosterone) was estimated from fecal corticosterone metabolites at 287 days of age. It has been suggested that long-term behavioral changes could be a result of altered circulating levels of glucocorticoids [13]. Currently, there is conflicting evidence whether long-term changes in circulating corticosterone occur after exposure to adolescent stress [6,7,13,22]. In addressing this question, to our knowledge only plasma-based measures of corticosterone have been used after such a long delay following exposure to adversity. Recently, it was demonstrated that male rats exposed to either novel or no social partners during adolescence exhibited decreased basal corticosterone using a fecal measure at 110 days of age [22]. The use of a fecal measure later in life may shed light on whether stress during adolescence causes long-term changes in glucocorticoid production because fecal measures quantify corticoid metabolites, which represent only free corticoids. Biologically active, free corticoids are the subset of corticoids available to respond to challenge because they are unbound to corticosteroid-binding globulin (CBG) [21]. Plasma measures typically quantify all corticoids both bound and unbound to CBG. Consequently, measures of fecal corticoid metabolites are suggested to more accurately represent the ability of an animal to physiologically respond to challenge than plasma measures [21]. Fecal sample collection is also non-invasive, which may provide a more accurate measurement of unstressed, basal corticosterone production [21,23]. It should also be noted, however, that fecal measures often require a greater difference in circulating corticosterone to

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Table 1
Chronic unpredictable stressor descriptions.

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- Rat pairs were housed for 4 h in a cage 25% smaller than their home cage [15].
- Rat pairs were housed for 6 h with 200 ml of water mixed into 2/3 of the bedding of their home cage [16].
- Home cages were tilted at a 30° angle for 6 h [16].
- Rats were housed individually for 1 h in a clean cage with a 7.6 cm diameter PVC tube and a 2.5 cm × 2.5 cm × 8 cm pine wood block [6].
- Two pairs of rats were combined in one clean cage (20 cm × 45 cm) for 4 h [15,16].
- Rat pairs were housed in the soiled home cage of older conspecifics for 12 h [16].
- An adult male taxidermied bobcat was placed on a wheeled cart and pushed in front of rat home cages for 30 min [17].
- Tin’s Red Fox’s fur was sprayed onto cotton balls, concealed in plastic container with 6 small holes for air flow, and placed into the rat home cages for 30 min [18].
- Felis catus fur, inside of mesh, was placed into the rat home cages for 30 min [19].
- Bobcat (Lynx rufus), mountain lion (Panthera concolor), domestic cat (Felis catus), lion (Panthera leo), and tiger (Panthera tigris) vocalizations were played for 30 min.

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detect a difference between groups compared to plasma measures [22].

Fecal corticoid metabolites were extracted and quantified using a commercially available radioimmunoassay (RIA) kit (MP Biomedical, Solon, OH) using procedures described in Wasser et al. [23] and Cavigelli et al. [24]. To extract corticosterone metabolites, fecal pellets were dried in a centrifugal evaporator, crushed, and mixed with ethanol before boiling in a water bath. Samples were then centrifuged, and the supernatant was evaporated and reconstituted in methanol. For the radioimmunoassay (RIA), aliquots were diluted 1:50 (using steroid diluent from the RIA kit) to achieve antibody binding along the linear portion of a binding curve, between 20 and 80% binding. Replicates were run for all samples; samples with percent error above 6% were reanalyzed. ‘High’ and ‘low’ control samples (at 60 and 30% binding) were included to verify accuracy. Corticosterone metabolite levels are presented as concentration (ng/g) relative to dry fecal weight. We controlled for circadian rhythms by collecting all fecal samples 2 h into the dark phase of the light cycle [24]. Fecal samples were collected within 20 min of separating rat pairs in clean cages identical to their home cage. Corticosterone and behavioral data were assessed using Levene’s test for equality of variances. After natural log transforming the corticosterone metabolite data, all data met the assumptions for parametric analysis.

The results showed that chronic unpredictable stress during adolescence increased anxiety; rats exposed to stress in adolescence took longer to initiate eating in the novel environment (initiate eating: $t_{28} = 2.58$, $p = 0.02$, Fig. 2). Adolescent-stress did not affect the latency to touch the food reward ($t_{20} = 0.49$, $p = 0.63$), implying that stress during adolescence may specifically increase anxiety without impacting the latency to approach objects, an indicator of boldness [25]. Exposure to adolescent stress did not affect the time spent eating; once eating was initiated, the time to complete eating was the same for prior-stressed rats (15.4 ± 2 s) and unstressed control rats (13.1 ± 2 s; $t_{20} = 0.86$, $p = 0.40$). The finding that adolescent-stress did not affect the rate of reward consumption supports the postulation that the delay to begin eating exhibited by prior-stressed rats was not caused by differences in motivation to consume the reward, but by hyponeophagia.

The current results demonstrate the longest-retained increase in anxiety-like behavior that has been documented following stress during adolescence [51]. It does not appear that differences in glucocorticoid production underpin these behavioral changes ($t_{20} = 0.49$, $p = 0.63$; Fig. 3). However, differences in total fecal production were observed, complicating the comparison between groups, and suggesting that adolescent-stress may influence metabolic processes later in life (total dry fecal weight: prior-stressed: 4.06 ± 0.3 g, control: 2.85 ± 0.4 g, $t_{20} = 2.60$, $p = 0.02$). While we did not find evidence that changes in circulating glucocorticoids underpin long-term behavioral changes, excess glucocorticoid production occurring during adolescent-stress exposure may induce structural changes in the brain that are retained into adulthood, such as altered glucocorticoid receptor density or neuronal structure [13]. However, it should also be noted that although there was no detectable difference in fecal corticoids, that does not necessarily mean that there are not physiologically significant differences in circulating corticosterone between prior-stressed and non-stressed animals. The conflicting accounts of whether stress during adolescence causes long-term changes in glucocorticoid production may be due, in part, to differences in methodology. Models of adolescent stress vary dramatically in both the duration of stress exposure [8] and the type of stressful stimuli presented (e.g. physical, social, or predation). Although predation stimuli can elicit a physiological stress response, it is difficult to determine whether these stimuli are interpreted as indicating the
presence of a predator, differentiating them from other models of chronic stress, or whether they are merely aversive [17].

Differences between the current results and those described in Sterleman et al. [13], where social stress exposure did not cause changes in anxiety after 12 months, may be caused by differences in the types of stimuli used in the chronic stress procedure [13], as well as the longer delay to testing in Sterleman et al. [13]. A better understanding of what underpins long-term effects of stress during adolescence on adult behavior may be gained by investigating factors related to the functioning of glucocorticoids, such as the proportion of free and bound corticosterone and corticosterone-binding globulin (CBG), as well as downstream targets of corticosterone including glucocorticoid and mineralocorticoid receptors. Additionally, direct comparisons of the effects of stress exposure during adolescence with stress exposure during other life stages could help to determine whether the numerous long-term effects of adolescent-stress exposure, documented here and in previous studies [4,8,19], are due to vulnerabilities unique to adolescence or whether similar long-term effects would also result from exposure to stress during other life stages. Such comparisons would help to resolve not only the capacity for adolescents to be shaped by exposure to stress, but also would address how adolescents may be uniquely affected by adversity.

Acknowledgements

We thank the Huck Institute of the Life Sciences and the Eberly College of Science for support. This project was also funded, in part, under a grant with the Pennsylvania Department of Health using Tobacco CURE Funds. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions. We would also like to thank the members of the Braithwaite group, with a special thanks to Carl Hirrlinger for his patience and assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2014.09.003.

References


Chapter 5

Stress during adolescence improves adult foraging under future threat in rats

Chapter 5 was published in the peer-reviewed journal *Animal Behaviour* and is included on the following pages with citation information in reprint form.

Author contributions: LEC conceived of and designed the studies, lead data acquisition, analyzed and interpreted the data, and wrote the manuscript
Does early stress prepare individuals for a stressful future? Stress during adolescence improves foraging under threat

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b Center for Brain, Behavior, and Cognition, Pennsylvania State University, University Park, PA, U.S.A.
c Department of Ecosystem Science & Management, Pennsylvania State University, University Park, PA, U.S.A.
d Department of Biology, Pennsylvania State University, University Park, PA, U.S.A.

Adolescent exposure to adverse environmental conditions can cause lasting changes in behaviour, cognition and physiology. One explanation for why such changes occur is that they allow organisms to adjust aspects of their phenotype to enhance function in an unfavourable environment. This concept has been investigated for stress during gestation (e.g. thrifty phenotype hypothesis, maternal mismatch hypothesis). Here, we apply these ideas within an individual’s lifetime as a possible explanation for long-term phenotypic changes in response to stress during adolescence. To test whether stress during adolescence can cause phenotypic changes that prepare an animal for future threat, we exposed laboratory rats to either chronic stress or unstressed control conditions during adolescent development. After a 5-week delay, rats were assessed in a timed-foraging task under both low-threat and high-threat conditions. Chronic stress during adolescence caused long-term changes in foraging behaviours and foraging performance. In low-threat conditions, stress-exposed rats had a longer latency to begin foraging but consumed the same number of rewards as unstressed rats. However, under high-threat conditions, rats exposed to stress during adolescence began foraging sooner, made more transitions between foraging patches and consumed more rewards than unstressed rats. These results indicate that stress exposure enabled rats to forage more effectively under later novel threat, and that phenotypic changes resulting from stressful experiences during adolescence may enhance function in future high-threat environments.

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alter decision making (Chaby, Cavigelli, White, Wang, & Braithwaite, 2013; Irwin, 1989; Torregrossa, Xie, & Taylor, 2012), change HPA axis function (McCormick et al., 2010) and increase anxiety (Green, Barnes, & McCormick, 2012; Schmidt et al., 2007). Several studies of the lasting effects of stress during adolescence have reported negative functional outcomes, such as impairments to memory of object locations (McCormick et al., 2012) and poor reversal learning (Han, Wang, Xue, Shao, & Li, 2011). These changes have been attributed to disruptions of development and subsequent abnormal functioning of brain regions that mature during adolescence (Toledo-Rodriguez & Sandi, 2007). However, other studies have reported phenotypic responses to adolescent stress that appear to be beneficial, including enhanced auditory fear conditioning (Toledo-Rodriguez & Sandi, 2007) and accelerated decision making (Chaby et al., 2015). To understand the alternative outcomes from these studies, it is important to consider the context in which these responses were assessed.

The concept that organisms can adjust aspects of their phenotype to enhance function in an unfavourable environment has been investigated for stress during gestation (e.g. thrifty phenotype: Hales & Barker, 1992; maternal mismatch hypothesis: Sheriff & Love, 2013). According to the thrifty phenotype and mismatch hypotheses, early exposure to an adverse environment will prepare individuals for a high-threat environment, but may detract from performance under low-threat conditions. The disadvantages of a mismatch between gestational and later life environment have been demonstrated using cross-fostering designs (Hales & Ozanne, 2003; reviewed in Hales, 1997), natural population cycles in snowshoe hares, Lepus americanus (Sheriff, Krebs, & Boonstra, 2009, 2010), and manipulations of glucocorticoid exposure during embryonic development (Love, Chin, Wynne-Edwards, & Williams, 2005; Love & Williams, 2008). Here we apply these ideas within the span of an individual lifetime as a possible explanation for long-term phenotypic changes resulting from exposure to stress during adolescence (Fig. 1). We hypothesized that adolescent-stress exposure would shape adult phenotype in a context-dependent way, by preparing animals to perform better under future threat, but decreasing performance in a mismatched, low-threat environment when compared to animals reared without stress. To our knowledge this is the first study to test the effects of early exposure to stress on performance using the same assay in different environmental conditions.

To test these ideas we exposed laboratory rats to stress during adolescence or to unstressed control conditions. After a 5-week delay, we screened adult foraging behaviours (latency to engage in foraging, number of patch visits) and foraging performance (number of rewards eaten) in both low-threat and high-threat conditions. Wild rodents adjust foraging behaviours when cues of predators are present (O’rock, Danielson, & Brinkerhoff, 2004), and foraging performance can be influenced by predation conditions (Pintor & Sih, 2009; Sih, 1982; Werner & Hall, 1988). Foraging performance can affect fitness through the ability to mitigate exposure to threat (Morris & Davidson, 2000), attract mates (Keagy, Savard, & Borgia, 2009, 2011) and provision offspring (Schwagmeyer & Mock, 2008). Foraging was evaluated both with and without cues of predation, using a foraging task that permitted animals to access a familiar reward by manipulating a novel object; similar assays have been used with captive and wild animals (e.g. humans and chimpanzees, Pan troglodytes: Herrmann, Hernandez-Lloreda, Call, Hare, & Tomasello, 2009; spotted hyaenas, Crocuta crocuta: Benson-Amram, Weldele, & Holekamp, 2013; satin bowerbirds, Ptilonorhynchus violaceus: Keagy et al., 2009; house sparrows, Passer domesticus: Bokony et al., 2013).

We predicted that stress during adolescence would prepare animals for future threat in a context-dependent way such that, under high-threat conditions, adolescent-stressed rats would show more active foraging behaviours (begin foraging faster, move between patches more) and enhanced foraging performance (consume more rewards) relative to unstressed rats, whereas under low-threat conditions, adolescent-stressed rats would show reduced foraging behaviours and performance. Alternatively, if stress during adolescence results in a negative functional phenotype, adolescent-stressed rats should show reduced foraging behaviours and performance under both high- and low-threat conditions.

METHODS

Animals and Housing

Male Sprague–Dawley rats (N = 24) were obtained at 21 days of age from Harlan Laboratory in Fredrick, Maryland, U.S.A. Following transport, rats were given 7 days to acclimate before handling and experimental procedures began. Animals were randomly assigned to pair-housing in plastic cages (20 × 26 × 45 cm) with wood chip

Figure 1. Framework of hypothesized performance in environmental conditions that were consistent and inconsistent with rearing (treatment) conditions. The effect of chronic unpredictable stress during adolescence on foraging performance of laboratory rats in adulthood was quantified using a seven-patch open arena foraging task under low-threat (standard conditions, under dim red light) and high-threat (visual and auditory cues of avian predation, bright white light) stimuli used to create the high-threat testing environment were novel to both groups of rats.
bedding, two pine wood chews, and two 7.6 cm diameter PVC tubes that were suspended from the cage lid. All cages were changed weekly; wood chews and PVC tubes were replaced when visibly soiled. Standard rat chow (LabDiet® 5001, 23% protein) and tap water were available ad libitum unless otherwise noted. Rats were kept at 20–21 °C and 40–45% relative humidity on a 12:12 h reversed light:dark cycle; the dark phase was 0900–2300 hours.

To minimize disturbance, all trials were video recorded and the experimenter was not in the room during testing. To control for circadian rhythms, tests were performed a minimum of 2 h after the beginning of the dark cycle and completed within 6 h. Testing order was randomized within the adolescent-stressed and unstressed groups, and alternated between groups to control for changes in circadian rhythms. Equipment was sprayed with 70% ethanol solution and wiped clean between all trials and subjects.

**Chronic Unpredictable Stress and Body Mass**

Pair-housed rats were randomly assigned to either the adolescent-stress treatment (N = 12) or the unstressed control group (N = 12). Each week adolescent-stress rats between 30 and 70 days of age encountered six stressors, three during 0000 and three during 1200 group (Chronic Unpredictable Stress and Body Mass). Equipment was sprayed with 70% ethanol so that the reward was palatable but not exhausted during the test. Testing order was randomized within the adolescent-stressed and unstressed groups, and alternated between groups to control for changes in circadian rhythms. Equipment was sprayed with 70% ethanol solution and wiped clean between all trials and subjects.

### Table 1

<table>
<thead>
<tr>
<th>Stressor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical</strong></td>
<td></td>
</tr>
<tr>
<td>Smaller cage</td>
<td>Rat pairs were housed for 4 h in a cage with a 25% reduction in volume (Doyle et al., 2011)</td>
</tr>
<tr>
<td>Damp bedding</td>
<td>Rat pairs were housed for 6 h with 200 ml of water mixed into 2/3 of the bedding of the home cage (Harding et al., 2004)</td>
</tr>
<tr>
<td>Cage tilt</td>
<td>Home cages were tilted at a 30° angle for 6 h (Harding et al., 2004)</td>
</tr>
<tr>
<td>Social</td>
<td></td>
</tr>
<tr>
<td>Isolation</td>
<td>Rats were housed individually for 1 h in a clean cage with a 7.6 cm diameter PVC tube and a 2.5–2.5 × 8 cm pine wood block (McCormick et al., 2008)</td>
</tr>
<tr>
<td>Crowding</td>
<td>Two pairs of rats were combined into one clean cage (20–45 cm) for 4 h (Harding et al., 2004; Doyle et al., 2011)</td>
</tr>
<tr>
<td>Forehead</td>
<td>[two pairs of rats were combined into one clean cage (20–45 cm) for 4 h (Harding et al., 2004; Doyle et al., 2011)](two pairs of rats were combined into one clean cage (20–45 cm) for 4 h (Harding et al., 2004; Doyle et al., 2011)</td>
</tr>
<tr>
<td>Predation</td>
<td></td>
</tr>
<tr>
<td>Taxidermic bobcat</td>
<td>An adult male taxidermic bobcat, Lynx rufus, was placed on a wheeled cart and moved continuously in front of rat cages for 30 min (Blumstein, Daniel, &amp; Springett, 2004)</td>
</tr>
<tr>
<td>Fox urine</td>
<td>Tink’s Red Fox®P® was sprayed onto cotton balls and placed into home cages for 30 min (Fendt &amp; Endres, 2008)</td>
</tr>
<tr>
<td>Cat fur</td>
<td>Domestic cat, Felis catus, fur was placed into the home cages, inside of mesh, for 30 min (Kendig, Bowen, Kemp, &amp; McGregor, 2011)</td>
</tr>
<tr>
<td>Feline vocalizations</td>
<td>Bobcat, mountain lion, Puma concolor, domestic cat, lion, Panthera leo, and tiger, Panthera tigris, vocalizations were played for 30 min (Chaby et al., 2015)</td>
</tr>
</tbody>
</table>

**Ethical Note**

Animals were housed according to the National Institutes of Health (NIH) recommendations described in the Guide for the Care and Use of Laboratory Animals (8th ed.). Food restriction is common in laboratory rodent studies and is advocated by NIH in order to increase longevity and decrease rates of obesity, metabolic disease, cardiovascular disease and cancer (Keenan et al., 1994; reviewed in Anderson, Shammanuyagam, & Weindruch, 2009). During the stress treatment, no signs of pain or aggression were observed, but behavioral changes relating to the type of stressor were noted. For example, during predation stressors, increases in escape and burrowing behaviors were observed. During physical and social stressors, some avoidance behaviors were noted. For example, during exposure to damp bedding, rats spent little time in contact with the bedding and instead spent time in the PVC tubes. Following exposure to the stressors, there were no changes in aggression or health. Experiments were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC protocol number 44459).

**Motivation to Consume a Reward**

We evaluated whether stress during adolescence influenced motivation to consume a reward in adulthood, in order to dissociate between the ability and the motivation to complete the foraging tests. Motivation was assessed at three time points: the first test was conducted for 5 min at 98 days of age, the subsequent two tests were conducted for 10 min at 99 and 104 days of age. To test motivation, rats were deprived of food for 5 h, then placed individually into empty clean cages and presented with 15 Cheerios® in a petri dish. Any Cheerio that was partially eaten or bitten was considered ‘eaten’ because rats often sample food items rather than consuming them whole, potentially to avoid ingesting large quantities of contaminated food (Clark, 1982). The proportion of Cheerios that were partially and completely consumed did not vary between treatment groups. All rats ate 2–12 Cheerios, indicating that the reward was palatable but not exhausted during the test, thus providing a time frame for the subsequent foraging tests. Repeated exposure to the Cheerio rewards during the motivation...
tests also served to familiarize the rats with Cheerios before the foraging tests, thus minimizing the potential effects of differences in novelty seeking caused by the adolescent-stress treatment (Toledo-Rodriguez & Sandi, 2011).

Foraging Tests

To determine whether stress during adolescence had long-term effects on foraging, we conducted three foraging tests, two in low-threat conditions (at 108 and 111 days of age) and one under high-threat (at 144–145 days of age). Outside of captivity, the median life span of male Norway rats is approximately 250 days (Davis, 1948, 1953). The low-threat foraging test was given twice to determine whether adolescent stress interfered with the ability to learn the foraging task. The second low-threat foraging test also screened for potential stress-induced decreases in neophobia (Chaby et al., 2013). To allow for habituation to the testing environment, 3 days prior to the first foraging test rats were placed individually in the white Plexiglas testing arena (122 × 122 × 46 cm) without rewards for 5 min. Rats were deprived of food in their home cage for 5 h before the habituation and foraging tests.

The high-threat condition was tested last because rodents often show altered behaviour when re-exposed to an environment where they have encountered a predator, even after the predator is removed (reviewed in Maren, 2001). For example, California ground squirrels, Spermophilus beecheyi, show similar or greater rates of vigilance and antipredator behaviour (e.g. tail flagging, aerial leaps) in an environment where they have previously seen a rattlesnake but the snake is no longer visible relative to when a snake is present (Putman & Clark, 2013). The effects of encountering a predator can be persistent; a single predator encounter can cause lasting increases in anxiety in laboratory rats (Adamec & Shallow, 1993). To minimize the effects of the low-threat tests on the high-threat test, the two testing conditions were separated by 34 days.

During the foraging tests, rats moved freely between seven objects that each concealed zero to three Cheerios to simulate a multipatch foraging scenario. The total number of Cheerios available in all foraging tests was 15. Rats consumed Cheerios ad libitum for 12 min in the first low-threat test and for 10 min in the second low-threat test and the high-threat test. Rats obtained Cheerios by manipulating objects in the arena; some objects required forepaw manipulations, such as inversion or pushing, while others could be accessed by nose poking. Certain objects required whole-body manipulations, such as climbing under an inverted bin. Objects varied in texture, colour, shape and size, and in the manipulation required to obtain the reward (Supplementary Fig. S1). Objects included green, blue and yellow plastic sand toys, plastic bins, semicircular mesh domes and a plastic pinwheel. Given that the arrangement of objects was novel in the first low-threat test (with the second low-threat test intended only to look at learning), we used a novel arrangement of objects in the high-threat test in order to compare foraging performance across novel foraging contexts. In addition, the use of novel object arrangements in the low- and high-threat tests controlled for possible differences in spatial or object memory, traits that can be affected by adolescent stress (Jigor et al., 2004; McCormick et al., 2012). Within the two low-threat and the high-threat test, object position, orientation and the number of available rewards were the same for all animals. Given that objects covered potential food rewards, we use the term ‘patch’ below to discuss the combination of object and potential food reward.

Foraging behaviours

Exposure to stress can either increase (Archard & Braithwaite, 2011) or decrease rates of activity depending on context (Faraday et al., 2002). To understand activity during the foraging task, we measured switches between patches (Heithaus, Opler, & Baker, 1974; Meyhöfer, 2001). A switch between patches was defined as visiting two patches sequentially. Patch switches likely serve many functions including foraging and exploration.

Stress can increase both vigilance (Diamond & Lazarus, 1974; Liley & Creel, 2008) and the latency to eat a familiar food (Sterlemann et al., 2008); to determine whether stress during adolescence increased the latency to forage, we measured the latency to the first patch visit. Visiting a patch was defined as physically contacting an object in the arena, or the Cheerios it concealed, with either a paw or nose. Foraging behavioural data were obtained from video recordings by an experimenter naïve to treatment.

Foraging performance

Foraging performance was defined as the number of Cheerios eaten during the timed foraging test (using the same operational definition of eaten as the motivation test). Performance was determined immediately after each foraging test by an experimenter naïve to treatment.

Low-threat and high-threat conditions

The conditions in the low- and high-threat foraging tests differed only in the addition of predation cues and brighter lighting conditions in the high-threat test. The low-threat test was conducted in dim, red light, whereas the high-threat test was conducted in standard laboratory light conditions (430 lx). Light levels as low as 60 lx can be aversive to nocturnal rodents (Bueno, Zangrossi, & Viana, 2005) and can signal heightened predation risk (Clarke, 1983; Kotler, Brown, & Hasson, 1991). Cues of avian predators were used in the high-threat test because they were novel to both groups. Hawk vocalizations were used as acoustic predation cues (e.g. Cooper’s hawk, Accipiter cooperii, red-tailed hawk, Buteo jamaicensis) and were played from an audio recorder approximately 1.5 m above the arena floor. A hawk silhouette moved over the foraging arena in a pendulum motion as a visual cue of predation. When the momentum of the silhouette stopped, it was positioned over a perch 1.5 m above the arena. The silhouette was congruent in size with the hawk predators used for the acoustic predation cues (wing span: length × width = 47 × 95 cm; Cabe, 1993). The acoustic and visual cues began as soon as the experimenter left the testing room.

The stimuli used in the high-threat test are known to be aversive to laboratory rats (loud noise: Pearl, Walters, & Chris, 1964; suddenly moving objects: Blanchard, Mast, & Caroline, 1975; Bronstein & Hirsch, 1976; bright light: Crozier & Pincus, 1927; Keller, 1941). These cues were used to create a high-threat environment, but whether rats interpret these cues, or other simulated predators, as signals of predation or merely as aversive is debated (Griffith, Evans, & Blumstein, 2001; reviewed in Blumstein, 2006).

Data Analysis

To meet the assumption of normality, data for latency to visit a patch and number of Cheerios eaten were in transformed. All analysed data were evaluated using Levene’s test for equality of variances and conformed to the assumptions for conducting parametric analyses. Body weight data were analysed using a repeated measures analysis of variance (RMANOVA) with stress condition and time as fixed effects. Data from the three motivation tests were also analysed with a RMANOVA with stress condition and time as fixed effects. Data from the three foraging tests (latency to visit a patch, switches between patches, number of Cheerios consumed) were analysed with RMANOVAs with stress condition and time as fixed effects. If time or interaction effects were detected in the
RMANOVA, each time point was analysed individually using univariate ANOVA. One data point from the control group was omitted from the latency to visit a patch analysis because it was greater than 12 standard deviations away from the mean. Analyses were run using IBM® SPSS® Statistics (Version 21); values are reported as means ± SE. Partial eta-squared values, expressing the proportion of variance explained, are provided as effect size estimates.

RESULTS

Chronic Unpredictable Stress and Body Mass

Adolescent stress did not affect body mass ($F_{1,22} = 1.59$, $P = 0.220$, $n_g^2 = 0.081$). Body mass increased with age in all rats ($F_{1,22} = 4045$, $P < 0.001$, $n_g^2 = 0.995$). Just before the beginning of the adolescent-stress treatment, the adolescent-stressed group weighed on average 83.1 ± 2.3 g and the unstructured group weighed 81.0 ± 1.6 g. The rate of change in body mass was accelerated by stress treatment; between 30 and 75 days of age, body mass of adolescent-stressed rats increased to 346.4 ± 3.8 g while that of unstressed rats increased to 332.5 ± 6.6 g (stress × time interaction: $F_{1,22} = 2.84$, $P = 0.008$, $n_g^2 = 0.106$). The groups diverged most in early adulthood, but adolescent-stressed and unstressed rats did not differ significantly in body mass at any time point (ANOVAs: $P > 0.05$). Body mass of groups converged as time from the adolescent-stress treatment increased.

Motivation to Consume a Reward

Adolescent stress did not affect motivation to consume rewards across the three motivation tests (stressed average: 6.2 ± 0.4; unstressed average: 5.2 ± 0.5; $F_{1,22} = 1.59$, $P = 0.221$, $n_g^2 = 0.070$). Consumption remained constant over time ($F_{1,22} = 0.90$, $P = 0.416$, $n_g^2 = 0.041$). Stress did not affect the rate of change in motivation to consume a reward (stress × time interaction: $F_{1,22} = 0.46$, $P = 0.636$, $n_g^2 = 0.021$). Reward consumption in the adolescent-stressed and unstressed rats increasingly converged across the three motivation tests and did not differ significantly at any point (ANOVAs: $P > 0.05$).

Foraging Behaviours

Across the three foraging tests, the latency to visit a patch was not affected by adolescent-stress exposure ($F_{1,21} = 0.66$, $P = 0.451$, $n_g^2 = 0.012$) but did change over time ($F_{1,21} = 3.24$, $P = 0.039$, $n_g^2 = 0.138$). No interaction was detected (stress × time interaction: $F_{1,21} = 1.15$, $P = 0.346$, $n_g^2 = 0.074$). When the foraging tests were evaluated individually, we found that during the first low-threat foraging test, adolescent-stressed rats had a 17% longer latency to visit a patch than unstressed rats ($F_{1,21} = 4.88$, $P = 0.042$, $n_g^2 = 0.268$; Fig. 2a); the stress treatment did not affect the latency to visit a patch during the second low-threat test or during the high-threat test ($P > 0.05$; means in Table 2).

The number of switches between patches across the three tests was not affected by stress exposure ($F_{1,22} = 1.87$, $P = 0.205$, $n_g^2 = 0.073$) but did change over time ($F_{1,22} = 110.55$, $P < 0.001$, $n_g^2 = 0.729$). There was a stress × time interaction ($F_{1,22} = 4.06$, $P = 0.035$, $n_g^2 = 0.181$; Fig. 2b). During the first low-threat test, rats exposed to stress during adolescence visited 9% fewer patches than unstressed rats, but under high-threat, adolescent-stressed rats visited 20% more patches than unstressed rats. When the foraging tests were evaluated individually, no treatment effects were detected in low-threat conditions (low-threat test 1: $F_{1,22} = 3.19$, $P = 0.092$, $n_g^2 = 0.158$; low-threat test 2: $F_{1,22} = 0.38$, $P = 0.542$, $n_g^2 = 0.017$). During the high-threat test, however, adolescent-stressed rats switched patches more than unstressed rats ($F_{1,22} = 4.86$, $P = 0.039$, $n_g^2 = 0.188$).

Foraging Performance

The number of Cheerios eaten was affected by stress exposure across the three tests ($F_{1,22} = 5.36$, $P = 0.035$, $n_g^2 = 0.135$; Fig. 3) and changed over time ($F_{2,22} = 9.02$, $P = 0.001$, $n_g^2 = 0.230$). We found no stress × time interaction ($F_{1,22} = 1.13$, $P = 0.338$, $n_g^2 = 0.094$). When the foraging tests were evaluated individually, we found that adolescent-stressed and unstressed animals did not differ in reward consumption in low-threat conditions (test 1: $F_{1,22} = 2.68$, $P = 0.121$, $n_g^2 = 0.023$; test 2: $F_{1,22} = 0.61$, $P = 0.442$, $n_g^2 = 0.27$). In high-threat conditions, however, adolescent-stress rats consumed 43% more Cheerios than unstressed rats ($F_{1,22} = 4.24$, $P = 0.050$, $n_g^2 = 0.171$).

DISCUSSION

We found that chronic stress exposure during adolescence and early adulthood had long-term effects on foraging under low- and high-threat conditions. Motivation to consume a reward was not altered by adolescent stress, suggesting that differences detected in the foraging task could be attributed to the ability to perform the task, rather than motivation to consume food rewards. Under low-threat conditions, exposure to adolescent stress affected foraging behaviours but not foraging performance; during the first low-threat foraging test, adolescent-stressed rats took 106% longer to visit a patch but consumed the same number of food rewards as unstressed rats. During the second low-threat foraging test, both groups improved foraging performance at the same rate, suggesting that stress during adolescence does not affect the ability to learn a foraging task. In the high-threat environment, rats previously exposed to stress during adolescence visited more patches and consumed more of the available rewards compared to unstressed rats. This suggests that stress during adolescence enhanced foraging-related problem solving under threat and supports our hypothesis that prior stress prepares adolescent-stressed animals to function better under future threat.

The effects of adolescent stress on foraging behaviours may be related to managing threat. In the first low-threat foraging test, adolescent-stressed rats showed prolonged vigilance before visiting a patch and tended to visit fewer patches compared to unstressed rats, possibly to reduce exposure to open areas (although the latter effect was nonsignificant). This suggests that stress during adolescence may heighten threat avoidance behaviours even in the absence of a direct threat. Arcia and Desor (2003) found that, under low-threat conditions, rats made foraging decisions based primarily on safety, rather than food density. Prior studies have also shown that exposure to stress during adolescence can increase the latency to eat a familiar food in a novel environment (hyponeophagia; Chaby et al., 2015; Sterleman et al., 2008) and decrease time in open areas in an elevated plus maze in adulthood (McCormick, Smith, & Mathews, 2008; Schmidt et al., 2007; Sterleman et al., 2008; Wilkin, Waters, McCormick, & Menard, 2012). The current results are consistent with the possibility that long-term behavioural changes resulting from adolescent stress may function to maximize threat avoidance even under low-threat conditions. Whether or not behavioural changes resulting from adolescent stress functionally deter threat, however, requires further investigation.

Animals exposed to stress during adolescence tended to maintain more consistent foraging behaviours and foraging performance across the testing conditions. The change in foraging behaviours between the first low-threat test and the high-threat test was
habituation to stress (adjust to functioning in a high arousal state more easily, similar to being more familiar with the effects of the stress response, and so can match the quality of a challenging environment where provisioning is difficult or dangerous (Love et al., 2005; Love & Williams, 2008)).

The current design, however, did not reveal the mechanism by which stress affected foraging. At least three possible explanations could account for the apparent increased resilience in foraging behaviours and performance of adolescent-stressed rats following introduction of threat. First, following extensive exposure to stress (from 30 to 70 days of age), adolescent-stressed rats may be more familiar with the effects of the stress response, and so can adjust to functioning in a high arousal state more easily, similar to habituation to stress (Natelson et al., 1988) or physiological acclimation to temperature or oxidative stress (Arens & Cooper, 2005; Grim, Miles, & Crockett, 2010; reviewed in Romeo, 2015).

Another explanation is that rats compared the high-threat cues with prior aversive stimuli they encountered, and because the adolescent-stressed rats were exposed to more extreme aversive stimuli, they interpreted the high-threat cues as less threatening. This would be similar to the psychological phenomenon of contrast effects, where exposure to high-intensity stimuli biases the perception of subsequent related stimuli as less intense (Moskowitz, 2005).

During the high-threat foraging test, unstressed rats obtained 2.8% of their daily nutrient requirements, while adolescent-stressed animals obtained 4.1% (based on 100 calories (418.58 J) per day; National Research Council (US) Subcommittee on Laboratory Animal Nutrition, 1995). Were rats to continue foraging at this pace, unstressed rats would obtain their daily nutrient requirements in 5.88 h; adolescent-stressed rats would satiate faster, in 4.12 h, potentially reducing their exposure to threat. When tested in a context consistent with their adolescent environment, adolescent-stressed rats performed better than threat-naïve rats, supporting both the mismatch and thrifty phenotype hypotheses. The increased foraging performance of adolescent-stressed rats under high-threat conditions could be explained by expanding the maternal mismatch hypothesis to address the effects of stress during development (Sheriff & Love, 2013). According to the mismatch hypothesis, maternal stress during gestation, or shortly thereafter, can cause phenotypic adjustments in offspring that are adaptive if the maternal environment predicts the offspring environment. For example, developing European starlings, Sturnus vulgaris, exposed to high levels of stress hormones hatch smaller and require less provisioning, and so match the quality of a challenging environment where provisioning is difficult or dangerous (Love et al., 2005; Love & Williams, 2008).

### Table 2
Mean ± SE latency to visit a patch by rats foraging under low-threat and high-threat conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Latency to visit a patch (s)</th>
<th>Control ± SE</th>
<th>Adolescent-stressed ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-threat test 1</td>
<td>1.7 ± 0.3</td>
<td>3.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Low-threat test 2</td>
<td>3.5 ± 0.9</td>
<td>2.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>High-threat test</td>
<td>5.6 ± 1.1</td>
<td>5.1 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

### Figure 2
Effect of stress during adolescence on foraging behaviours of laboratory rats. (a) Latency to approach an object during the first low-threat test for unstressed (control) rats and adolescent-stressed rats. Plotted values are raw data, but analyses were performed on ln-transformed data. (b) Number of visits to food patches by adolescent-stressed and control rats in the first low-threat test and in the high-threat test. Values are means ± SE. *P < 0.05.

### Figure 3
Effect of stress during adolescence on foraging performance of adolescent-stressed and unstressed (control) rats in low-threat tests 1 and 2, and in the high-threat test. Plotted values are raw data, but analyses were performed on ln-transformed data. Values are means ± SE. *P < 0.05.
It has been suggested that adolescence is a sensitive period, like gestation, when programming can occur to prepare an individual for a specific environment (reviewed in McCormick et al., 2010). Thus, it seems that programming during adolescence may better match an individual to a future environment that is consistent with their adolescent environment. We did not find evidence that exposure to adolescent stress decreases foraging performance in mismatched, low-threat conditions (as might be predicted by extensions of both the mismatch and thrifty phenotype hypotheses). This could indicate that rats maintain foraging performance by accepting trade-offs in the performance of other systems not evaluated here. Alternatively, stress during adolescence could affect foraging performance on a longer timescale than was evaluated here, or the design of the foraging task may not have captured existing impairments caused by stress. It is noteworthy that although the effect of stress on foraging performance was not significant under low-threat conditions, on average the adolescent-stressed rats consumed more rewards in both low-threat tests. The results from low-threat conditions do not contradict crucial early hypotheses addressing the effects of prenatal stress, but suggest that the consequences of stress during adolescent development, and their potential role in preparing for later environments, may not function by the same mechanisms as prenatal stress. This emphasizes that both the immediate and lasting effects of stress are highly dependent upon ontogenetic stage at exposure (Lupien et al., 2009).

The presence of long-term behavioural changes following adversity during adolescence may imply an expectation of environmental consistency. In the wild, Norway rats, Rattus norvegicus, can have relatively constant habitat conditions; they are suggested to be philopatric beyond independence (Waser & Jones, 1983), they tend to have relatively small home ranges (0.066 ha; Taylor, 1978; 0.024 ha: Villafañe, Muschetto, & Busch, 2008), and they are vulnerable to the same predators throughout ontogeny (Childs, 1986). These characteristics may contribute to the capacity for temporary periods of stress to have long-lasting effects in rats. In a species where early environment does not predict adult environment we would expect only temporary changes in phenotype following stress during development. For example, adult wood frogs, Rana sylvatica, require different habitats than juvenile tadpoles, and adults do not retain antipredator responses from earlier stages of development (Relyea, 2005; Relyea & Auld, 2005). Thus, it seems possible that long-term changes resulting from stress during development may be more likely in species with high environmental consistency. Evaluation of species with environmental needs that vary throughout ontogeny could reveal whether the lasting changes documented here will generalize across contexts and whether environmental consistency and the duration of behavioural changes are causally related. Furthermore, given the limited ability of laboratory systems to inform free-living systems (Koolhaas, de Boer, & Buwalda, 2006), investigation of species outside of captivity could help elucidate the biological significance of the behavioural changes described here.

The possibility that individuals can adjust phenotypic traits during adolescence in order to adjust to a persistent high-threat environment remains an attractive but incomplete story. Our results demonstrate that stress during adolescence can affect behaviour long after direct exposure to stressors has ceased and that these effects are context specific, emphasizing the importance of careful consideration of testing conditions and their relationship to environmental conditions throughout development. These findings have implications for understanding the potential functional role of stress-induced behavioural changes. They also broaden our understanding of how stress-induced phenotypic plasticity may interact with the consistency of threat across ontogeny. Our results support the idea that adolescence can be a transformative developmental stage during which environmental pressures can generate long-term behavioural changes that prepare an individual for a specific environment.

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Supplementary Material

Supplementary Material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2015.03.028.

References


Supplementary materials

S Fig 1: Depiction of object arrangement, orientation, and colour in the low-threat and high-threat foraging tests. Objects concealed between 0-3 Cheerios; the numbers within each object indicate the number of Cheerio rewards available at that patch. Arrows indicate which motor action was most often used to extract rewards at each object. Not drawn to scale.
Chapter 6

Stress during adolescence shapes performance in adulthood: Context-dependent effects on foraging and vigilance

Chapter 6 is in peer-review and is included on the following pages.

Author contributions: LEC conceived of and designed the studies, lead data acquisition, analyzed and interpreted the data, and wrote the manuscript
Stress during adolescence shapes performance in adulthood: Context-dependent effects on foraging and vigilance

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Abstract

Exposure to chronic stress during adolescence can alter adult behaviour, cognition, and physiology, but the consequences of these changes remain unclear. Prior studies reporting altered performance following adolescent stress exposure have generally interpreted lasting changes as impairments. However, we have recently shown that exposure to chronic unpredictable stress during adolescence (from postnatal days 30-70) can actually enhance functioning while foraging in a context-dependent manner. Such increases in foraging performance, measured by the number of rewards obtained, are often associated with tradeoffs in other behaviours, such as vigilance. Here, we examined the effect of stress exposure in adolescence on adult foraging across low and high-threat conditions in male Sprague-Dawley rats to determine i) whether the increase in foraging performance exhibited by adolescent-stressed animals is balanced by a decrease in vigilance, and ii) whether stress in adolescence alters the time allocation between foraging and vigilance behaviours. We found no evidence of a tradeoff between foraging and vigilance; under low-threat conditions stress exposed rats spent more time being vigilant than unstressed rats, suggesting that adolescent-stress enhances anticipation of threat in adulthood. Under high-threat conditions, adolescent-stressed and unstressed rats did not differ in time allocation or the frequency of foraging and vigilance behaviours. Given that we have previously found that adolescent-stressed rats nearly double food intake under high-threat, and we now show that high-performing rats do not spend more time foraging, it appears that stress exposure in adolescence may enhance efficiency (food consumed/time) under high-threat conditions. We also examined the relationship, at the level of the individual, between foraging and vigilance behaviour and foraging.
performance; the change in foraging performance across threat conditions was independent of behavioural changes (i.e. both highly and poorly performing rats were equally active and contacted a similar number of patches). This suggests that the ability to obtain many rewards under high-threat conditions may be related to efficiency or cognitive differences, rather than the frequency of foraging and effort related behaviours.

**Keywords**

Adolescence, Chronic stress, Foraging, Laboratory rodent, Risk, Risk-sensitive

**Highlights**

- We compared adult foraging and vigilance in adolescent-stressed and unstressed rats.
- In low-threat, adolescent-stress increased time spent foraging and being vigilant.
- In high-threat, adolescent-stress did not affect foraging or vigilance behaviours.
- Thus adolescent-stress may increase foraging efficiency but not effort under threat.
1. Introduction

Adolescence is a developmental transition from dependence in early life to independence in adulthood that occurs in many taxonomic groups (Crone & Dahl 2012; Spear 2000). During this phase, multiple physiological and neural systems mature, making adolescence a period of both plasticity and vulnerability to stress exposure (Gogtay et al. 2004; Romeo & McEwen 2006). Adversity during adolescence can cause lasting changes in behaviour (Green et al. 2013; Toledo-Rodriguez & Sandi 2011), cognition (Chaby et al. 2013), and physiology (Caruso et al. 2014; Romeo 2010), and can affect performance in adulthood (McCormick et al. 2010). Determining the role of these outcomes may be aided by understanding the life history context in which the experience takes place (Clinchy et al. 2011), or by understanding the function of behavioural changes from adolescent-stress in free-living animals. Further, it is important to understand how the consequences of early life stress can manifest in context-dependent manner (Breuner 2008; Sheriff & Love 2013).

Early stress can result in a unitary phenotype that performs differently in varying environmental conditions (Oomen et al. 2010; Sheriff & Love 2013; Sheriff 2015). For example, environmental context can affect the ability to manipulate novel stimuli to obtain food, which is an indicator of problem solving ability that can affect fitness by mediating exposure to threat (Keagy et al. 2009; Morris & Davidson 2000). Previously, we found that exposure to stress in adolescence enhanced the ability of rats to manipulate novel objects to obtain food (foraging performance) in high-threat conditions, but that foraging performance in low-threat conditions was unaffected by chronic stress during
adolescence (Chaby et al. 2015a). In environments with varying threat level, the amount of food consumed in a specific context correlates negatively with the amount of fear or apprehension an animal experiences in that context (Brown 1999; Kotler et al. 2004).

Here, we compare time allocation and the frequency of foraging and vigilance behaviours in high-threat (using a simulated predator and bright lighting conditions) and low-threat (standard testing conditions) in unstressed and adolescent-stressed rats.

While foraging, animals must balance time spent obtaining resources and time spent monitoring their environment for threats through vigilance (Lima & Dill 1990; Brown 1999; Brown et al. 1999; Favreau et al. 2014). To determine the full impact of chronic stress in adolescence on adult foraging ability in high-threat conditions, it is necessary to understand both foraging performance and vigilance behaviour. It is possible that adolescent-stressed rats, relative to unstressed rats, may increase foraging performance through an opportunity cost of reducing competing behaviours, such as vigilance (Bachman 1993). Although this is somewhat counterintuitive given the general assumption that stress will increase vigilance behaviours, it is in line with predictions of the predation risk allocation hypothesis (Lima & Bednekoff 1999). This hypothesis states that after extended exposure to high stress, if patchy resources become available, animals will become more active and forage more even under high threat conditions because the need for resources is too great and there are no “better” conditions in which to forage (Sih & McCarthy 2002). Thus, we hypothesized that compared to rats reared without stress, exposure to chronic stress during adolescence would (a) result in an increase in time spent foraging during a pulse of high-value resource availability in both low and high-
threat conditions, and that (b) in high-threat conditions, the opportunity cost of increasing efforts towards resource acquisition would include a decrease in vigilance compared with unstressed rats. In low-threat conditions, however, foraging performance is not affected by exposure to stress in adolescence (Chaby et al., 2015a), suggesting that such tradeoffs might not occur and vigilance behaviours might be unaffected by adolescent-stress. It should be noted that these predictions are specific to a pulse of resource availability and are distinct from predictions for conditions where resources are uniform or unavailable (Ford 1983; Arditi & Dacorogna 1988). Understanding whether extended exposure to stress in adolescence can affect time allocation may help explain the context-specific consequences of exposure to stress in adolescence.

In addition to effects from balancing time spent foraging with time spent being vigilant, foraging performance may also be mediated by differences in effort or efficiency (food consumed/time). We found that in high-threat conditions, some rats exposed to stress in adolescence decreased the number of food rewards obtained as much as unstressed rats (by up to 43%), while other adolescent-stressed rats increased their intake (by up to 300%), greatly exceeding the performance of all unstressed animals (Chaby et al. 2015a). We investigated foraging and vigilance behaviours at the level of the individual to understand the why some rats performed well under high-threat conditions while others exhibited a decrease in the number of rewards obtained. We hypothesized that decreased performance in high-threat conditions would be driven by reduced foraging and effort-related behaviours (reduced activity, fewer patches contacted, etc.) while an increase in performance would be driven by increases in foraging and effort-related behaviours, such
that rats that exhibited minimal change in foraging behaviour across low and high-threat conditions would show minimal change in foraging performance, while animals exhibiting a decrease in foraging effort should obtain fewer rewards in the high-threat environment. Similarly, we predicted that an increase in energy and time spent on vigilance across the threat conditions would also result in less foraging and fewer rewards obtained in the high-threat environment.

2. Methods

2.1. Animals and housing

Male Sprague-Dawley rats (n=24) were procured at postnatal day 21 from Harlan Laboratory in Maryland. Rats were randomly assigned to pair-housing in plastic cages that contained wood chip bedding, two pine wood chews, and two 7.6cm diameter PVC tubes. Standard rat chow (LabDiet® 5001, 23% protein) and tap water were available ad libitum, except just before behavioural trials as described below. Rats were kept on a 12:12 hr reversed light:dark schedule to facilitate testing during the dark phase when rats are most active. Foraging performance data from these rats was previously reported in Chaby et al. 2015a. In the current study, we report novel data on foraging and vigilance time allocation, as well as novel behavioural frequency data, in order to understand whether the effects of adolescent stress on foraging performance are mediated by tradeoffs between foraging and vigilance.

2.2. Chronic unpredictable stress
Pairs of rats were randomly assigned to the adolescent-stress group (n=12) or the unstressed control group (n=12). Unstressed rats were reared in standard laboratory conditions with no exposure to stress while adolescent-stressed rats were exposed to three types of stressors (physical, social, and predation; Table 1) from 30 to 70 days of age, using procedures described in Chaby et al. 2014, 2015. This chronic stress paradigm has previously been shown to cause lasting behavioural and cognitive changes (Chaby et al. 2013, 2014). Briefly, rats were exposed to 6 stressors each week. Stressor order was randomized, but on average each type of stressor was presented twice per week. Within each week, rats were exposed to three stressors between 0-1200 h and three stressors between 1200-2400 h; within these blocks the specific hour of stress exposure and stressors presented was randomized. To control for handling and cage changes during the stressor procedures, rats in the unstressed group were given additional handling and cage changes approximately twice per week while adolescent-stressed rats were handled approximately once per week (Kabbaj et al. 2002; Isgor et al. 2004). Adolescent-stressed rats were given new cages after all predation stressors as well as foreign and wet bedding stressors. All rats were weighed weekly during the stress treatment, and every second week thereafter because body mass is an indicator of health which can be decreased by exposure to stress in laboratory rats (Pare 1965; Shimizu et al. 1989; Bhatnagar et al. 2005). The duration of the stress treatment (30-70 days of age) included a short postpubertal period (approximately days 55-70 of treatment; Spear et al. 2000) to cover the entire ontogenetic window of adolescence (Schmidt et al. 2007; Sterleman et al. 2010) and because we wanted to evaluate foraging and decision making behaviours
mediated by the prefrontal cortex (Seamans et al. 1995), a region which continues to develop into early adulthood (Spear 2000; van Eden et al. 1990).

2.3. Ethical note

Housing conditions conformed to the National Institute of Health (NIH) recommendations described in the Guide for the Care and Use of Laboratory Animals, 8th edition. To ensure motivation to participate, rats were food deprived for 5 hours before the foraging tests. Food restriction in laboratory rodents is advocated by the NIH to increase longevity and decrease rates of obesity, metabolic disease, cardiovascular disease, and cancer (Keenan et al. 1994; reviewed in Anderson et al. 2009). During the stress treatment, no signs of pain, aggression, or changes in health were observed. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC), protocol #44459.

2.4. Foraging tests

Foraging was evaluated in low-threat conditions, at 108 days of age, and in high-threat conditions, at 144-145 days of age. To control for circadian rhythms, tests began at least 2 hours after the beginning of the dark cycle and were completed within 6 hours. Three days prior to the first foraging test rats were placed in an empty white Plexiglas foraging arena (122cm x 122cm x 46cm) for 5 minutes to allow for habituation to the arena and testing room. During the foraging tests, the arena contained 15 Cheerios that were concealed by seven objects. Each object had 0-3 available Cheerios. We have previously
shown that exposure to this adolescent-stress paradigm does not affect motivation to consume Cheerios (Chaby et al. 2015a).

Objects in the arena varied in texture, colour, shape, and size (examples: green and blue plastic bins, semicircular mesh domes). Objects also varied in the manipulation required to obtain the reward (e.g. forepaw manipulations, nose-poking, and whole-body manipulations such as climbing under an inverted bin). To refer to the combination of object and potential food, we use the term “patch” below. Rats freely consumed Cheerios in the foraging arena for 12 minutes in the low-threat test and 10 minutes in the high-threat test (to account for increases in performance related to learning across the tests). Given that the arrangement of objects was novel in the low-threat test, we used a second novel object arrangement in the high-threat test in order to compare performance across novel foraging contexts. The use of novel object arrangements in both the low and high-threat tests also controlled for possible differences in spatial or object memory, traits that can be affected by exposure to stress in adolescence (Isgor et al. 2004; McCormick et al. 2012). Within the low and high-threat tests object position, orientation, and the number of available rewards were the same for all animals (see Chaby et al. 2015a sec. 2.5.1. for further discussion).

The high-threat condition was tested last because rodents often exhibit altered behaviour when re-exposed to an environment where they encountered a predator, even after the predator is removed (reviewed in Maren 2001). For example, California ground squirrels exposed to an environment in which they previously saw a rattlesnake (but the snake is
no longer visible) exhibit vigilance and antipredator behaviours at the same rate or
greater compared to when a snake is present (e.g. tail flagging, arial leaps; Putman &
Clark 2014). The effects of encountering a predator can be persistent; a single predator
encounter in a laboratory rat can cause lasting increases in anxiety (Adamec & Shallow
2014). To minimize the effects of the low-threat tests on the high-threat test, the two
testing conditions were separated by 34 days; although rats were well into adulthood for
all foraging tests, but not approaching old age for a rat (approximately 400-750 days after
the final test; Pietrelli et al., 2012; Richardson et al., 2013), it should be noted this delay
may affect comparisons between low and high-threat tests.

All other conditions in the low and high-threat foraging tests were the same, except for
the addition of acoustic and visual cues of predation in the high-threat test and differences
in lighting conditions. Predator cues that were novel to both the adolescent-stressed and
unstressed rats were used in the high-threat test to avoid potential sensitization or
habituation effects from the adolescent-stress treatment. The low-threat test was
conducted in dim, red light, whereas the high-threat test was conducted in standard
laboratory light conditions (430 lux). Light levels as low as 60 lux can be aversive to
nocturnal rodents (Bueno et al. 2005) and can increase the perception of predation risk
(Clarke 1983; Kotler et al. 1984). During the high-threat test, acoustic predation cues
(e.g. vocalizations from Cooper’s Hawk, Red-tailed Hawk) were played from an audio
recorder approximately 5ft above the arena floor and a visual predation cue, a hawk
silhouette (47cm length x 95cm wingspan), was moved over the foraging arena in a
pendulum motion. Although it is unclear whether the rats interpreted the hawk calls and
silhouette as indicators of predation or merely as aversive (Apfelbach et al. 2005), it is common to use predator models and recordings, and the stimuli used in the high-threat test are inherently aversive to laboratory rats (loud noise: Pearl et al. 1964; suddenly moving objects: Blanchard et al. 1975; Bronstein & Hirsch 1976; bright light: Crozier & Pincus 1927; Keller 1941). All trials were video-recorded and the experimenter was not in the room during testing. Equipment was cleaned with 70% ethanol between all trials and subjects.

2.4.1. Foraging & vigilance behaviours

Recordings of all foraging trials were analyzed for foraging behaviours: the number of active foraging bouts, time spent foraging, and vigilance behaviours: number of rearing bouts, time spent rearing, number of stretch attends, and number of head scans (operational definitions in Table 2, Quenette 1990). Recordings were analyzed by an experimenter blind to stress condition using EthoLog® v. 2.2.5. (Ottoni 2000). Data on consumption of Cheerios were obtained immediately after the foraging tests and were previously reported in Chaby et al. 2015a. To compare behaviour between the low and high-threat tests, we calculated the percent of total test time spent foraging and being vigilant by dividing the measures of time spent foraging and rearing by the length of the test.

2.4.2. Individual behaviour during foraging tests

To compare individual-level changes in behaviour and performance between the low and high-threat conditions; we calculated the change in foraging and vigilance behaviours
(described in 2.5) for each rat using the percent change formula \( \frac{(\text{high-threat} - \text{low-threat})}{\text{low-threat}} \times 100 \). To quantify foraging effort, we measured activity (Eilam et al. 1999; Snaith & Chapman 2005), object touches (Klaassen et al. 2006), and number of entries and time spent in the middle of the foraging arena (where the concentration of patches is highest; Valone & Brown 1989), in low and high-threat conditions (Table 2). Time spent in the middle of the arena has been used as an inverse-index of anxiety (Simon et al. 1994; Harris et al. 2009). We also analysed, at the level of the individual, whether foraging success correlated to these foraging and vigilance behaviours within the low and high-threat tests. Following this, we related changes in these behaviours across the low and high-threat conditions to changes in foraging performance (the number of Cheerios eaten during each foraging test).

2.5. Data analysis

To meet the assumption of normality, the number of active foraging bouts and the percent of time spent rearing were natural log transformed. Less than 5% of the rats exhibited stretch attends or head scans in either the low or high-threat condition, so these behaviours were not included in subsequent analyses. To confirm that the data met the assumptions of parametric analyses, all data were required to pass Levene's Test for Equality of Variances. We tested the effect of adolescent-stress at the population level on the number of active foraging bouts, percent of time spent foraging, the number of rearing bouts, and percent of time spent rearing in each threat condition (out of a standardized length of time) using a multivariate analysis of variance tests (MANOVAs) with stress condition as a main effect for each threat condition. MANOVAs were
evaluated with Box’s Test of Equality of Covariance Matrices and conformed to assumptions for parametric analyses. Dependent variables in the MANOVAs were assessed for multicollinearity. If significant treatment effects were detected in the MANOVA, we used discriminant analysis to determine how the dependent variables interacted (Field 2013; Borgen & Seling, 1978). To understand whether performance related to changes in behaviour between the low and high-threat tests (independent of stress condition), we investigated the relationship between foraging success and changes in behaviour using Pearson’s correlation analyses with a Benjamini Hochsberg correction for multiple comparisons. Analyses were run using SPSS® Statistics V. 21; values are reported as means ± standard error.

3. Results

3.1. Foraging & vigilance behaviours

Under low-threat conditions, adolescent-stressed and unstressed rats differed in foraging and vigilance behaviours (MANOVA main effect: $F_{4,18} = 3.51$, $p = 0.031$; Figure 1). The two vigilance variables, number of rearing bouts and percent of time spent rearing, were significantly correlated ($R = 0.86$, $p < 0.000$). The significant correlation between the two vigilance variables makes it difficult to isolate the effect of exposure to stress in adolescence on these two variables individually and suggests that the two variables provide similar information. No other variables in the MANOVA for the low-threat condition were correlated. Discriminant analysis revealed one discriminant function encompassing all four predictors (number of active foraging bouts, percent of time spent
foraging, rearing bouts, and time spent rearing), canonical $R^2 = 0.47$, which significantly differentiated the grouping variable, stress condition, $\Lambda = 0.83, \chi^2 (4) = 10.71, p = 0.030$, depicted in S Figure 1. This significant discriminant function indicated that separation between the unstressed and adolescent-stressed rats was driven primarily by increased vigilance behaviours exhibited by the adolescent-stressed animals, including greater time spent rearing (canonical variate correlation coefficients ($r = 0.81$) and a greater number of rearing bouts ($r = 0.51$), which suggests that despite a close relationship between time spent rearing and the number of rearing bouts, the time spent rearing better distinguishes the effects of the stress treatment than does the number of rearing bouts. Exposure to stress in adolescence had an opposite effect on the two foraging measures; compared to unstressed animals, adolescent-stressed rats exhibited fewer foraging bouts ($r = -0.47$), but spent a greater amount of time eating ($r = 0.34$), though these effects were weaker than the effects of stress on vigilance. Under high-threat conditions, adolescent-stress exposure did not affect foraging and vigilance behaviours (MANOVA main effect: $F_{4,18} = 0.45, p = 0.770$; Figure 1). No effect of adolescent-stress exposure was detected in the high-threat test, on either time spent foraging or being vigilant, or the number of foraging bouts or rears. The two vigilance variables, number of rearing bouts and percent of time spent rearing, were also significantly correlated in the high-threat condition ($R = 0.69, p < 0.000$). No other variables in the MANOVA for the high-threat condition were correlated.

3.2. Individual behaviours during foraging tests
During the low-threat foraging test, several behaviours affected foraging performance (food consumption), including activity and strong trends for entries in to the middle and time spent eating (see Table 3). During the high-threat test, however, only the amount of time spent eating related to foraging success ($r = 0.70$, $p < 0.00$). An individual’s change in food consumption from the low to the high-threat test only correlated to change in percent of time spent eating, and not to changes in vigilance or effort-related behaviours (see Table 4). However, changes in effort-related foraging behaviours between the low and high-threat tests were interrelated (see Table 4). For example, rats were likely to decrease the number of patches they touched if they reduced either entries into the middle ($r = 0.56$, $p = 0.005$) or time spent in the middle of the arena ($r = 0.63$, $p = 0.002$). Similarly, animals that decreased activity across the threat conditions also decreased their entries into the middle ($r = 0.58$, $p = 0.003$). Despite these relationships, there was no correlation between the change in effort related behaviours and the change in food consumption, which suggests that effort related behaviours do not mediate food consumption under threat (see Table 4). This suggests that effort-related behaviours do not mediate food consumption under threat.

4. Discussion

Early stress can shape a phenotype such that it performs differently depending upon the environmental context (Sheriff & Love 2013; Chaby et al. 2015b). In the current study, we tested the effects of stress in adolescence on time spent being vigilant and manipulating novel objects to obtain food, an indicator of problem solving ability (Keagy
et al. 2009), in adulthood in low and high-threat conditions. Under low-threat conditions, adolescent-stressed rats exhibited greater time spent being vigilant and foraging, as well a greater number of rearing bouts, the most prevalent vigilance behaviour, and a decrease in the number of foraging bouts compared to unstressed rats. Under high-threat conditions, exposure to stress in adolescence did not affect time spent being vigilant or foraging, or the number of bouts of rearing or foraging. Previously we found that exposure to chronic stress in adolescence nearly doubled the number of food rewards they obtained when tested in high-threat conditions compared with unstressed controls (Chaby et al. 2015a), while the current study showed no effect of adolescent-stress on time allocation between foraging or vigilance or the frequency of foraging bouts in high-threat conditions. This suggests that in high-threat conditions, prior exposure to stress in adolescence increases foraging efficiency (food consumed/time) rather than affecting behavioural decisions on whether or not to forage. At the level of the individual, we found that foraging behaviours are related to foraging success in low-threat conditions, but not under-high threat. This indicates that it is possible for an animal in high-threat conditions to exhibit high foraging effort (e.g. with frequent foraging bouts and high activity) but poor performance (obtain few rewards). Similarly, changes in foraging behaviours (activity, contact with patches, entries in the middle of the arena, etc.) between low and high-threat are independent of changes in the amount of food consumed. Given that differences in foraging performance cannot be attributed to changes in foraging behaviours, and our adolescent-stress paradigm does not affect motivation to consume rewards in adulthood (Chaby et al., 2015a), it follows that differences in performance might be attributed to cognitive or emotional processes that
interfere with the ability of a threat-naïve animal to consume food in high-threat conditions. For example, animals in anxiety-like states caused by exposure to a novel arena can exhibit hypophagia and delay consumption even after locating a desirable food item (Merali et al. 2003; Samuels & Hen 2011).

In low-threat conditions, adolescent-stressed rats spent more time foraging and being vigilant than unstressed rats, refuting our prediction that exposure to stress in adolescence would decrease vigilance in order to increase foraging. The finding that stress experienced during adolescence increases the time spent foraging, but decreases the number of foraging bouts, suggests that adolescent-stress may cause a change in strategy reflected by fewer, longer bouts of foraging in low-threat conditions. In Chaby et al. (2015a) we proposed that adolescent-stress could cause context specific changes in foraging by (a) increasing familiarity with and functioning in high stress states, (b) heightening anticipation of future threat even under low-threat conditions, or (c) biasing the perception of subsequent stressful stimuli as less intense (i.e. contrast effects; Moskowitz 2005). Both explanations (a) and (c) suggest that differences between adolescent-stressed and unstressed rats would be observed primarily when high-threat was present. The current findings support explanation (b); in low-threat conditions adolescent-stressed rats showed greater vigilance, suggesting that stress experienced during adolescence may cause lasting changes in cognition that function to increase anticipation of threat. We have previously shown that the adolescent-stress paradigm used in the current study causes lasting increases in anxiety-like behaviour, tested 274 days after exposure to adversity had ceased (Chaby et al. 2014).
A persistent increase in anxiety, or fearfulness, could account for the increase in vigilance that adolescent-stressed rats exhibit under low-threat conditions; if such an anxious-like state were intense enough to chronically mimic the presence of a threat, it could also explain why aspects of adolescent-stressed animal’s response to an increase in threat are reduced. The potential role of persistent anxiety, or heightened fearfulness, following stress experienced during adolescence is unclear; it is possible that persistent anxiety could function in the processing of threat related information (Macleod et al. 1986; Macleod & Mathews 1988; reviewed in Shechner et al. 2012), or in early threat detection and evasion to prepare animals for an adult environment where threats are common (Kaviani et al. 2004; Melzig et al. 2008; Buwalda et al. 2013; reviewed in Davis 1990). Increased anticipation of threat induced by an anxiety-like state could explain why adolescent-stress enhances fear learning, as shown by Toledo- Rodriguez and Sandi (2007). Anxiety can affect threat detection processes; highly anxious rats and humans have a startle response with a larger amplitude (Kaviani et al. 2004; reviewed in Davis, 1990), increased activity of the autonomic nervous system during anticipation of threat (Melzig et al. 2008), and elevated resting levels of norepinephrine, which serve as vital components of the early stress response (Mathew et al. 1981; Sevy et al. 1989). Most importantly, compared to individuals with low trait anxiety, high trait anxiety individuals (that experience pervasive anxiety outside of the context of a challenge) are biased toward threat (Macleod et al. 1986; Macleod & Mathews 1988) and identify threats faster (Byrne & Eysenck 1995). Exposure to threatening conditions during adolescence may trigger phenotypic changes that allow faster identification of threats in adulthood. This
advantage in threat detection may be mediated by a pervasive anxiety-like state that is present outside of the context of a challenge.

In high-threat conditions, exposure to stress in adolescence did not affect time allocation or the number of foraging and vigilance behaviours, although adolescent-stressed rats have been shown to increase performance in high-threat conditions (Chaby et al. 2015a). This suggests that for the risk allocation hypothesis, it is possible for extended exposure to threat to increase the number of resources acquired without an opportunity cost to vigilance. It should be noted that these effects differ markedly from changes in performance induced by acute stress, which typically decreases foraging performance (Watson et al. 2004; Graham et al. 2010), suggesting that extended exposure to stress can prepare an individual for subsequent stress (e.g. Match-Mismatch hypothesis, Buwalda et al. 2013; Sheriff & Love 2013), and that these effects depend on the developmental stage at exposure (e.g. Predictive Adaptive Response theory, Gluckman et al. 2005).

When behaviours were analyzed at the level of the individual, we found that activity and the number of interactions with patches are independent of changes in food consumption across the low and high-threat testing conditions. Both this result and the finding that unstressed rats engage in the same number of foraging behaviours but consume fewer rewards compared with adolescent-stressed rats (Chaby et al. 2015a), suggest that performance under threat may relate to efficiency rather than effort and differences in performance may be attributed to cognitive processes. The capacity for chronic stress during adolescence to have lasting effects on cognition has been documented for spatial
learning (Isgor et al. 2004), reversal learning (Han et al. 2011), object memory (McCormick et al. 2012), and cognitive bias (Chaby et al. 2013; reviewed in McCormick & Mathews 2010). How differences in cognition might “scale-up” to affect performance, and how changes in performance might impact fitness, requires further investigation.

The results suggest that stress exposure during adolescence enhances anticipation of future threat by increasing vigilance behaviours in the absence of threat. Further, in high-threat conditions, the results show that adolescent-stress does not affect time allocation between foraging and vigilance. This advances our previous findings showing that stress exposure in adolescence enhances foraging performance to suggest that adolescent-stress enhances foraging efficiency under threat while maintaining vigilance (Chaby et al. 2015a). Our results emphasize the importance of contextualizing stress-induced changes in behaviour (sensu Sheriff & Love 2013), whether in conditions consistent with an animal’s developmental environment or in conditions that reflect ecologically relevant challenges, such as predation threat or competing stimuli. The current findings also inform our understanding of the role of developmental plasticity and the capacity for phenotypic change in adolescence to cause lasting changes in the ability to perform asks that require problem-solving or divided-attention (such as monitoring threat and obtaining resources).

Conflict of interest statement
The authors declare no conflict of interest, nor any financial or personal relationships that could inappropriately influence or bias the content of this paper.

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References


Figures:

**Low-threat test**

- A: Foraging behaviours
  - Number of foraging bouts
  - Percent of time spent foraging

- B: Vigilance behaviours
  - Number of rearing bouts
  - Percent of time spent rearing

**High-threat test**

- C: Foraging behaviours
  - Number of foraging bouts
  - Percent of time spent foraging

- D: Vigilance behaviours
  - Number of rearing bouts
  - Percent of time spent rearing

**Figure 1**: The effect of chronic stress during adolescence on foraging and vigilance behaviours in low and high-threat environments; means + SE. Under low-threat conditions, adolescent-stressed rats allocated time between foraging and vigilant behaviours differently compared to unstressed rats (MANOVA, p = 0.031). Under high-threat conditions, despite previous work showing that in high-threat adolescent-stressed rats obtained more food compared to unstressed rats, the stressed and unstressed rats did not differ in foraging or vigilance behaviours (MANOVA, p = 0.770).
Tables:

Table 1: Descriptions of chronic unpredictable stressors.

<table>
<thead>
<tr>
<th>Physical Stressors</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller cage</td>
<td>4 hrs</td>
</tr>
<tr>
<td>Housed in a 25% reduced volume cage</td>
<td></td>
</tr>
<tr>
<td>(Doyle et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Damp bedding</td>
<td>6 hrs</td>
</tr>
<tr>
<td>Housed with 200ml water mixed into</td>
<td></td>
</tr>
<tr>
<td>bedding (Harding et al., 2004)</td>
<td></td>
</tr>
<tr>
<td>Cage tilt</td>
<td>6 hrs</td>
</tr>
<tr>
<td>Home cage tilted at a 30° angle</td>
<td></td>
</tr>
<tr>
<td>(Harding et al., 2004)</td>
<td></td>
</tr>
</tbody>
</table>

Social Stressors

<table>
<thead>
<tr>
<th>Social Stressors</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>1 hr</td>
</tr>
<tr>
<td>Individually housed with 7.6cm</td>
<td></td>
</tr>
<tr>
<td>diameter PVC tube and 2.5cm x 2.5cm</td>
<td></td>
</tr>
<tr>
<td>x 8cm pine wood block (McCormick et</td>
<td></td>
</tr>
<tr>
<td>al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Crowding</td>
<td>4 hrs</td>
</tr>
<tr>
<td>Two rat pairs combined into one</td>
<td></td>
</tr>
<tr>
<td>clean cage (Harding et al., 2004;</td>
<td></td>
</tr>
<tr>
<td>Doyle et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Foreign bedding</td>
<td>12 hrs</td>
</tr>
<tr>
<td>Pairs housed in the empty home cage</td>
<td></td>
</tr>
<tr>
<td>of a pair of older conspecific pair</td>
<td></td>
</tr>
<tr>
<td>(Harding et al., 2004)</td>
<td></td>
</tr>
</tbody>
</table>

Predation Stressors

<table>
<thead>
<tr>
<th>Predation Stressors</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxidermied bobcat</td>
<td>½ hr</td>
</tr>
<tr>
<td>Adult male taxidermied bobcat (Lynx</td>
<td></td>
</tr>
<tr>
<td>rufus) was placed on a wheeled cart</td>
<td></td>
</tr>
<tr>
<td>and pushed in front of rat cages</td>
<td></td>
</tr>
<tr>
<td>(Blumstein et al., 2004)</td>
<td></td>
</tr>
<tr>
<td>Fox urine</td>
<td>½ hr</td>
</tr>
<tr>
<td>Tink's Red Fox-P® was sprayed onto</td>
<td></td>
</tr>
<tr>
<td>cotton balls and placed into the</td>
<td></td>
</tr>
<tr>
<td>rat home cages (Fendt &amp; Endres, 2008)</td>
<td></td>
</tr>
<tr>
<td>Cat fur</td>
<td>½ hr</td>
</tr>
<tr>
<td>Felis catus fur was placed inside</td>
<td></td>
</tr>
<tr>
<td>mesh into the home cages (Kendig et</td>
<td></td>
</tr>
<tr>
<td>al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Feline vocalizations</td>
<td>½ hr</td>
</tr>
<tr>
<td>Bobcat (Lynx rufus), mountain lion</td>
<td></td>
</tr>
<tr>
<td>(Puma concolor), domestic cat</td>
<td></td>
</tr>
<tr>
<td>(Felis catus), lion (Panthera leo),</td>
<td></td>
</tr>
<tr>
<td>and tiger (Panthera tigris) vocali-</td>
<td></td>
</tr>
<tr>
<td>zations.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Operational definitions for behaviours measured during the low and high-threat foraging tests.

<table>
<thead>
<tr>
<th>Behaviour measured</th>
<th>Operational Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foraging &amp; Vigilance behaviours</td>
<td></td>
</tr>
<tr>
<td><strong>Foraging behaviours</strong></td>
<td></td>
</tr>
<tr>
<td>Time spent eating</td>
<td>Time extracting, manipulating, or consuming food rewards.</td>
</tr>
<tr>
<td>Number of active foraging bouts</td>
<td>Active foraging was defined as eating or moving between patches. To qualify as a single bout, foraging behaviours were separated by at least 5 seconds of vigilance or inactivity.</td>
</tr>
<tr>
<td><strong>Vigilance behaviours</strong></td>
<td></td>
</tr>
<tr>
<td>Time spent rearing</td>
<td>Time standing on two rear legs scanning the environment (Blumstein, 1996; Vásquez, 1997; Vásquez et al., 2002; Quenette, 1990).</td>
</tr>
<tr>
<td>Number of rearing bouts</td>
<td>To qualify as a single bout, rearing behaviours were separated by at least 5 seconds of vigilance or inactivity.</td>
</tr>
<tr>
<td>Stretch attends*</td>
<td>Stretching of the body towards a stimulus while keeping rear legs immobile (Ribeiro-Barbosa et al., 2005).</td>
</tr>
<tr>
<td>Head scans*</td>
<td>Moving the head back and forth while keeping the body stationary (Whishaw et al., 1992; Wishaw &amp; Lethbridge, 2004).</td>
</tr>
<tr>
<td><strong>Effort-related behaviours in the foraging test</strong></td>
<td></td>
</tr>
<tr>
<td>Object touches</td>
<td>Contacting an object in the arena with either a paw or nose.</td>
</tr>
<tr>
<td>Entries into the middle of the arena</td>
<td>During analysis of the video recorded trials, a transparent 8x8 grid was superimposed to separate the arena into 64 equally-sized squares. When a rat moved all four feet from grid squares bordering the arena walls into grid squares in the center, it was counted as an entry into the middle.</td>
</tr>
<tr>
<td>Time spent in the middle of the arena</td>
<td>Time in squares in the middle of the arena (Grønli et al., 2005).</td>
</tr>
<tr>
<td>Activity</td>
<td>Using the 8x8 video analysis grid, the number of squares crossed with all four feet was quantified as a measure of activity (Candland &amp; Nagy, 1969).</td>
</tr>
</tbody>
</table>

* Less than 5% of the rats exhibited stretch attends or head scans in either threat condition, so these behaviours were not included in subsequent analyses.
Table 3: Correlations between behaviours and performance in the low and high-threat tests.

<table>
<thead>
<tr>
<th></th>
<th>Time spent eating</th>
<th>Foraging bouts</th>
<th>Time spent rearing</th>
<th>Rearing bouts</th>
<th>Object touches</th>
<th>Entries into the middle</th>
<th>Time in the middle</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-threat foraging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>performance</td>
<td>R = 0.48 p = 0.05¹</td>
<td>R = -0.14 p = 0.59</td>
<td>R = 0.06 p = 0.81</td>
<td>R = -0.35 p = 0.15</td>
<td>R = 0.44 p = 0.07¹</td>
<td>R = 0.51 p = 0.03¹</td>
<td>R = -0.15 p = 0.53</td>
<td>R = -0.70 p = 0.001*</td>
</tr>
<tr>
<td>High-threat foraging</td>
<td>R = 0.70 p &lt; 0.00*</td>
<td>R = 0.22 p = 0.30</td>
<td>R = -0.14 p = 0.53</td>
<td>R = -0.13 p = 0.54</td>
<td>R = 0.28 p = 0.18</td>
<td>R = -0.08 p = 0.71</td>
<td>R = -0.21 p = 0.35</td>
<td>R = -0.07 p = 0.74</td>
</tr>
</tbody>
</table>

¹Indicates significant at p > 0.05, with Bonferroni correction for multiple comparisons.  
*Indicates a trend, with Bonferroni correction for multiple comparisons.
Table 4: Percent change in behaviors across low and high-threat conditions.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time in the middle</th>
<th>Time spent eating</th>
<th>Time spent rearing</th>
<th>Number of foraging bouts</th>
<th>Number of rearing bouts</th>
<th>Object touches</th>
<th>Entries into the middle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foraging performance</td>
<td>$R = 0.75$</td>
<td>$p = 0.000^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigilance</td>
<td></td>
<td>$R = 0.13$</td>
<td>$p = 0.584$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effort-related behaviors in the foraging test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indicates a trend, with Bonferroni correction for multiple comparisons.

*Indicates significant at $p > 0.05$, with Bonferroni correction for multiple comparisons.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Time spent eating (sec)</th>
<th>Time spent rearing (sec)</th>
<th>Object touches</th>
<th>Entries into the middle</th>
<th>Object eating (sec)</th>
<th>Time spent foraging (sec)</th>
<th>Unstressed adolescents</th>
<th>Low-threat behaviors</th>
<th>Unstressed adolescents</th>
<th>Low-threat behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adol. stressed rats</td>
<td>104 ± 6</td>
<td>104 ± 5</td>
<td>3 ± 3</td>
<td>12 ± 2</td>
<td>23 ± 10</td>
<td>23 ± 10</td>
<td>51 ± 10</td>
<td>16 ± 6</td>
<td>94 ± 10</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Low-threat</td>
<td>276 ± 11</td>
<td>276 ± 10</td>
<td>15 ± 6</td>
<td>15 ± 6</td>
<td>33 ± 11</td>
<td>33 ± 11</td>
<td>35 ± 9</td>
<td>35 ± 9</td>
<td>183 ± 10</td>
<td>183 ± 10</td>
</tr>
<tr>
<td>Adol. stressed rats</td>
<td>183 ± 11</td>
<td>183 ± 10</td>
<td>41 ± 7</td>
<td>41 ± 7</td>
<td>22 ± 9</td>
<td>22 ± 9</td>
<td>57 ± 11</td>
<td>57 ± 11</td>
<td>183 ± 10</td>
<td>183 ± 10</td>
</tr>
<tr>
<td>Unstressed adolescents</td>
<td>276 ± 11</td>
<td>276 ± 10</td>
<td>15 ± 6</td>
<td>15 ± 6</td>
<td>33 ± 11</td>
<td>33 ± 11</td>
<td>35 ± 9</td>
<td>35 ± 9</td>
<td>183 ± 10</td>
<td>183 ± 10</td>
</tr>
</tbody>
</table>

Supplementary Table 1: Foraging behaviours data in the low and high-threat tests (mean ± standard error).
Chapter 7

The lasting effects of stress in adolescence

The capacity for exposure to challenges to cause changes in behavior and cognition, and for these effects to accrue over time and have adverse consequences, has been recognized for centuries. Even Shakespeare wrote about such situations around the turn of the 17th century; Macbeth tells the story of increasingly dire circumstances that drive a noblewoman to madness and eventual suicide. Indeed, the ways in which internal strife can have severe external consequences has been a facet of literary interest for centuries. However, the internal processing of adverse stimuli is a relatively recent concept in science. The term “stress” was introduced in 1936 by a Hungarian medical doctor, Hans Selye, to describe “the non-specific response of the body to any demand for change” (Selye, 1950). Following this, Selye introduced the term “stressor” to refer to the stimulus provoking the stress response and “eustress” to describe the possible positive effects of stress (Selye, 1976). However, since this time, application of the term stress has broadened and currently the definition is contested; “stress” is currently used to refer to three distinct processes: (i) stimuli in the environment that are aversive or challenging (also called stressors), (ii) the emergency physiological and behavioral responses to challenge (also called the stress response), and (iii) the potentially pathological consequences of over-stimulation of the emergency response (sensu Romero et al., 2009). Additionally, these processes can be species-specific, making it challenging to devise a definition that can apply to all systems. For example, some species go through developmental stages where their physiological response to stress is hypo or hyperactive. Studies in rodents have shown that shortly after parturition young
rats are hyporesponsive to stressors, whereas in adolescence rats can be hyperresponsive to stressors (Sapolsky et al., 2010). Though many aspects of the mammalian stress response are undergoing maturational changes during adolescence, this period has only recently received scientific attention.

Adolescence

Exposure to stress in adolescence, an inherently plastic stage in development, can cause lasting changes in behavior, cognition and physiology (Caruso et al., 2014; McCormick et al., 2010; Sterlemann et al., 2008; Weintraub et al., 2010; reviewed in: Lupien et al., 2009; Romeo, 2010a). Adolescents may be more sensitive to stressful environments for at least three reasons (Romeo, 2013, 2015); (1) adolescents produce higher levels of glucocorticoid “stress” hormones in response to aversive physical and psychological stimuli compared with adults (McCormick et al., 2005, Romeo, 2010a,b), (2) adolescents may be more sensitive to the effects of glucocorticoid hormones on gene regulation (Lee et al., 2003), and (3) many regions in the brain mature during adolescence, including some that both function in the stress response and are sensitive to stress (e.g., the prefrontal cortex (PFC), hippocampus, and amygdala [Spear, 2000; Dahl, 2004]). These maturational processes include the pruning and loss of large numbers of glutamate cells and the insulation of neuronal axons in fatty myelin sheaths called white matter (Insel et al., 1990, Harburger et al., 2007; Jolles et al., 2011; Scherf et al., 2006). The PFC shows pronounced increases in white matter density during adolescence, and plays a key role in the transition from short-range neural connections characteristic of early life to the long-range neural connections in adult brains (Gogtay et al., 2004). It is suggested that stress may interfere with these maturational processes and affect brain function later in life (Spear, 2000), including changes in regions that
are important for cognitive processes, such as the PFC and hippocampus (Thompson, 1986; Puig et al., 2014). Understanding whether exposure to stress during adolescence can cause lasting changes in phenotype is important both for our understanding of (i) developmental plasticity and (ii) how stress during development can affect Darwinian fitness and human health. The goal of this thesis was to investigate the role of phenotypic changes resulting from developmental stress and to explore how these changes can be explained by current theory.

The research described in this thesis tested the effects of chronic unpredictable stress exposure throughout adolescent development in male rats (see Chapter 3, Supplementary table 1). Though I chose chronic stress, it is important to note that stress during development can come in many forms: chronic unpredictable, repeated acute, or acute, and the consequences of these can differ considerably. Like the term stress, definitions of these terms are debated, but some distinctions can be made:

- **Chronic unpredictable (or variable) stress** – repeated exposure to several different stressors that are typically unpredictable in frequency or timing; less likely for animals to habituate to a specific stressor
- **Repeated acute stress** – repeated exposure to the same stressor (can follow temporal patterns); more likely for animals to habituate
- **Acute stress** – a single or few exposures to a stressor

There are a number of challenges with these definitions, and with comparisons across different patterns of stress, even if developmental timing is held constant. Some of these challenges include differences in (a) duration, (b) intensity, (c) opportunity for habituation/sensitization, (d) resemblance to natural patterns of stress (which may be species-specific and specific to the type
of stress), and (e) individual experiences (though this may only be an issue if the animals were free-living). An example of a discrepancy in duration that can complicate comparisons across these stress patterns is an acute stress exposure, such as food restriction, that lasts 23 hours (Toth et al., 2008), vs. repeated acute stress where animals are exposed to physical restraint on 10 occasions for only 1 hour at a time (Thorsell et al., 1999). Although the term acute may imply temporally limited, in this case a single acute stressor has a longer duration than the 20 instances of repeated stress. This particular case is further complicated by the fact that some animals, including male rats, can habituate to restraint, but some stressors are less likely to be subject to habituation effects (e.g. predation stress). Thus, the types of stressors presented are also important to consider for cross-study comparisons; not only are some stressors more frequently habituated to, but there is evidence to suggest that some types of stressors may be more likely to cause lasting effects than others (Lupien et al., 2009). Importantly, exposure to these different stress paradigms, even within the same developmental window, can have different results (Tsoory & Richter-Levin, 2006). Though discrepancies in the pattern and type of stressor can hinder cross study comparisons, and therefore complicate literature synthesis, for the purposes of this thesis, it should be noted that generalizing results from the studies I present, which all use the chronic unpredictable stress paradigm, should be considered carefully in relation to studies or contexts with differing patterns of stress exposure.

Chronic unpredictable stress

The chronic unpredictable stress procedures used in Chapters 2-6 were initially designed to amalgamate existing stress protocols previously found to cause behavioral and cognitive changes consistent with the hypotheses described in Chapter 2. For all subsequent chapters the chronic
stress procedures were replicated because the chapters predicted similar cognitive changes and to facilitate comparisons across my experiments. For the chronic stress procedure, adolescent-stressed rats encountered 6 stressors each week between 30-70 days of age; 3 between 000-1200 h and 3 between 1200-2400 h. The order of stressor presentation and type of stressor and varied, but were balanced so that each type of stressors (physical, social, predation) was represented twice per week. Following exposure to chronic stress in adolescence (or unstressed conditions for the control animals), rats were retained in standard, unstressed housing conditions and were not manipulated until they reached mid-late adulthood. After this delay, I used assays that measured behavior, cognition, emotion, and physiology. This chronic stress paradigm induced long-term behavioral and cognitive changes summarized in Table 1.

Predictive Adaptive Response (PAR) theory

The Predictive Adaptive Response (PAR) theory states that some advantages of a maternally-stressed phenotype, referred to as predictive adaptive responses, manifest in later developmental stages, making them contingent upon environmental predictions and consistency (Gluckman et al., 2005). For example, in meadow voles (*Microtus pennsylvanicus*) coat thickness is determined during gestation, but does not have a functional role until after weaning (Lee & Zucker, 1988). It is important to note that this can be applied to high-threat conditions, but also unstable future environments – suggesting that chronic unpredictable stress in adolescence should prepare animals not just for aversive conditions later in life, but also for changing conditions. A primary benefit of applying the Predictive Adaptive Response theory to the capacity for stress in adolescence to cause lasting phenotypic change is that PAR can provide an explanation for both transient effects (e.g. Sterlemann et al., 2008) and delayed effects (e.g.
Andersen & Teicher, 2004). Additionally, PAR theory specifies that not all traits induced by early stress that primarily function within an explicit developmental window are eliminated after that developmental window (e.g. unless selection is strong and conditions are met to eliminate the trait, the PAR trait will likely endure into later life stages even after its primary usefulness is complete). Thus, it is important to bear in mind that persistent changes in phenotype detected in late adulthood resulting from stress in adolescence, such as those I have reported in the Chapters 2-6, may primarily function earlier in development rather than when they have been detected experimentally. This would make it challenging to interpret behavioral and cognitive changes in order to understand the role of altered traits based on observations solely in later developmental stages. Thus, both the PAR theory and the current results emphasize the importance of longitudinal studies and studies that span multiple life stages.

Mismatch hypothesis

The Mismatch Hypothesis, as proposed by Sheriff & Love (2013), emphasizes the match between maternal and offspring environmental conditions. It suggests that females with poor body condition, or occupying adverse/resource-limited environments, are less able to rear offspring in “good” condition (i.e. high mass, fast growth rate) and that “poor” condition females can increase fitness by matching their reduced rearing potential with offspring quality by producing “poor” quality offspring (i.e. low birth weight, slow growth rate). The mismatch hypothesis makes clear predictions about how stress in early development can affect response to stress later in life, and applies well to the results in Chapter 5 where rats exposed to stress during adolescence were tested in high-threat conditions. Under these conditions, which were consistent with their adolescent environment, their foraging performance exceeded that of animals naïve to
threat (supporting both the mismatch and PAR hypotheses). In Chapter 3, I found cognitive changes that contrasted with the mismatch hypothesis in two ways: (1) adolescent-stressed rats outperformed unstressed rats under control conditions (though this was the adolescent-stressed rats “inconsistent” environment, suggesting that their performance should be impaired) and (2) the introduction of stress (in the form of an aversive novel chamber) was more disruptive to the cognitive performance of the adolescent-stressed animals compared with the unstressed rats. This is in direct opposition to the predictions of the mismatch hypothesis, which suggests that prior experience with stress during development should prepare animals for dealing with stress later in life. I also showed in Chapter 5 that in low-threat conditions, adolescent-stressed rats took twice as long to initiate foraging, but consumed the same amount of food as unstressed rats, which contrasts with prior hypotheses predicting decreased functionality in low-threat conditions (Breuner, 2008; Sheriff & Love, 2013). To try and explain some of these discrepancies, I proposed the arousal shift hypothesis, discussed below.

**Arousal-shift hypothesis**

I investigated whether stress during adolescence could prepare an organism to better function under threat in the future in Chapters 1 and 5, an idea predicted by an extension of a wealth of theory related to prenatal stress (Hales & Barker, 1992; Breuner, 2008; Beery & Francis, 2012; Sheriff & Love, 2013). In Chapter 5 in high-threat conditions, rats that had been exposed to stress in adolescence began foraging sooner, made more transitions between foraging patches, and consumed more food compared with unstressed rats, but in low-threat conditions, adolescent-stressed rats took longer to initiate foraging, but consumed the same amount of food as unstressed rats (Chaby et al., 2015a). I considered these results in the framework of the
The Yerkes-Dodson law was proposed to help explain a series of experiments where mice performed visual discrimination tasks under weak, moderate, and strong electrical stimulation (Yerkes & Dodson, 1908). Yerkes and Dodson found a linear relationship between learning a simple discrimination task and the strength of electrical stimulation, but a curvilinear relationship between learning a task of moderate difficulty and stimulation strength. Yerkes and Dodson’s observations have been repeated in numerous taxa with modern techniques and statistical analyses (Telegdy & Cohen, 1971; Anderson, 1994; Dickman, 2002) and across many contexts including athletic training (Stinson & Bowman, 2014), workplace conditions (Chang et al., 2013; Gidding et al., 2013), and video games (Jeong & Biocca, 2012). The Yerkes-Dodson law states that for more challenging tasks, i) moderate arousal can enhance performance (Broadhurst, 1957; Ni, 1934; Salehi et al., 2010) in part by modulating motivation (Diamond et al., 2007), but ii) high levels of arousal can decrease performance (Diamond et al., 1999; reviewed in Kim & Diamond, 2002) through processes such as a reduction in the amount of information that can be processed.

In Chapter 5, I tested rats in a moderately challenging, problem-solving foraging task that required varying motor actions and object manipulations under both high-threat conditions (auditory and visual predator cues, bright light) and low-threat conditions (standard laboratory conditions, dim red light). Under high-threat conditions control, unstressed animals decreased the number of rewards they obtained compared to their performance in a prior low-threat test.
Interestingly, high-threat conditions did not detract from the performance of animals that had experienced adolescent-stress, on average adolescent-stressed rats showed a small increase in performance compared to their performance in the prior low-threat test, though this was not statistically significant. These differences in performance and threat condition could be underpinned by a shift in the curvilinear relationship between performance and arousal as described by the Yerkes-Dodson law. Such that adolescent-stressed animals perform better at higher levels of arousal that exceed the optimal range of arousal for unstressed animals (the peak of the Gaussian curve). Under such a framework, exposure to adolescent-stress would cause an increase in the optimal arousal range, but adolescent-stressed animals would still show a decline in performance after arousal exceeds their optimal level. Adolescent-stressed rats are therefore expected to maintain an advantage over threat-naïve animals throughout their decline until the level of arousal becomes too high to permit completion of a moderately challenging task regardless of the rearing environment. In this model, the effect of adolescent-stress on performance is minimal in the absence of threat (low arousal conditions). As arousal increases in the positive slope of the Gaussian curve, both unstressed and adolescent-stressed animals increase performance; the difference in performance may only be detected when at the near optimal arousal level of unstressed animals.

My results emphasize the importance of context on behavior and performance, and the need to understand the relationship between testing and rearing environment. The proposed model of the capacity for stress during adolescence to cause a shift in the relationship between arousal and performance, the “arousal-shift hypothesis”, could guide future studies of the long-term effects of early stress as animals age and threats in their environment change. In addition to
understanding how performance is dependent on environmental context (threat vs. safe), performance should be assessed in a task-dependent manner (simple vs. complex). In Chapter 5, I investigated whether the lasting effects of stress during adolescence could be explained by expanding well supported hypotheses predicting the effects of prenatal stress (e.g. thrifty phenotype, maternal mismatch). I now suggest the arousal-shift hypothesis to explain the effects of early life stress on cognition later in life. The arousal-shift hypothesis does not contradict crucial early hypotheses that predict how prenatal stress can prepare an individual for an adverse environment, but it suggests that developmental stress may manifest though a shift in the relationship between performance and arousal.

**Conclusions**

Despite numerous attempts at multiple levels of inquiry (e.g. hormonal, morphological), described both in this thesis and in prior studies of adolescent-stress (McCormick et al., 2010), no unifying mechanism for lasting phenotypic changes resulting from exposure to stress in adolescence has been identified. It would seem likely that there is no single system that underpins all changes resulting from adolescent-stress, but rather that there are a number of physiological and neurological changes resulting from exposure to stress in adolescence.

For example, the hippocampus has been suggested to underpin many changes resulting from adolescent stress, which seems likely given that the hippocampus is maturing during adolescence (McCormick et al., 2010; McCormick & Mathews, 2010), but some of the lasting changes documented in my thesis can be hippocampal independent (e.g. associative learning).
In addition to investigating unifying mechanistic explanations for the lasting effects of developmental stress, understanding the teleological causes might illuminate the relationship between the myriad of phenotypic changes resulting from stress in adolescence (see Table 1). As prior hypotheses addressing maternal stress have suggested, insights may be gained by understanding the lasting effects of stress in different environmental contexts – both in terms of the cognitive demand (and recruited brain structures) and environmental context (threat, consistency, distractions/overall stimulation). For example, under low threat conditions unstressed rats engage in an appetitive task faster by visiting rewarded patches more quickly compared with adolescent-stressed rats (Chapter 5), however, in an aversive task unstressed rats are slower than adolescent-stressed rats to engage in entering an arm to find an escape platform (Chaby et al., *in review*). This contrasts with prior hypotheses about developmental stress and calls for further investigation into how exposure to early stress may differentially prime animals for later physiological and cognitive responses to stress.
### Table 1: Lasting effects of chronic unpredictable stress in adolescence

<table>
<thead>
<tr>
<th>Adolescent-stress exposed rats</th>
<th>Unstressed, control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lasting behavioral changes</strong></td>
<td></td>
</tr>
<tr>
<td>- Accelerated decision making (correction of 2-bowl choice) 1</td>
<td>- Slower decision making (correction of 2-bowl choice) 1</td>
</tr>
<tr>
<td>- Neophilia (faster to approach novel object) 1</td>
<td>- Slower to approach novel object 1</td>
</tr>
<tr>
<td>- Increased anxiety (increased hyponeophagia) 3</td>
<td>- Decreased anxiety (hyponeophagia) 3</td>
</tr>
<tr>
<td>- No change in sucrose preference 1 or motivation to consume a reward 1,2,4</td>
<td>- No change in sucrose preference 1 or motivation to consume a reward 1,2,4</td>
</tr>
<tr>
<td>- More vigilant under low threat conditions, equally vigilant under high threat conditions 4</td>
<td>- More vigilant under low threat conditions, equally vigilant under high threat conditions 4</td>
</tr>
<tr>
<td>- Longer to visit a patch under low-threat conditions 4</td>
<td>- Faster to visit a patch under low-threat conditions 4</td>
</tr>
<tr>
<td>- No change in general activity/exploratory patterns 2,4</td>
<td>- No difference in general activity/exploratory patterns 2,4</td>
</tr>
<tr>
<td>- Engage in resolving aversive task faster (finding platform in water maze) 6</td>
<td>- Slower to engage in resolving aversive task (finding platform in water maze) 6</td>
</tr>
<tr>
<td><strong>Lasting cognitive changes</strong></td>
<td></td>
</tr>
<tr>
<td>- Negative cognitive bias 1</td>
<td>- Equally likely to exhibit positive or negative cognitive bias 1</td>
</tr>
<tr>
<td>- Better problem solving/foraging performance under high threat; no change under low threat 4 (independent of changes in behavior) 5</td>
<td>- Reduced problem solving/foraging performance under high threat; no change under low threat 4 (independent of changes in behavior) 5</td>
</tr>
<tr>
<td>- Better working memory, but more disrupted by exposure to novel/adverse environment 2</td>
<td>- Poorer working memory, but more robust following exposure to novel/adverse environment 2</td>
</tr>
<tr>
<td>- No change in associative learning, reference memory (in high 6 or low threat 2)</td>
<td>- No change in associative learning, reference memory (in high 6 or low threat 2)</td>
</tr>
<tr>
<td>- Enhanced reversal learning in appetitive radial arm maze task 2 but no change in an aversive water maze 6</td>
<td>- Poorer reversal learning in appetitive radial arm maze task 2 but no change in an aversive water maze 6</td>
</tr>
<tr>
<td>- Less improvement in spatial learning performance over time (did not refine their performance when given additional information) 6</td>
<td>- Greater improvement in spatial learning performance over time (refine their performance when given additional information) 6</td>
</tr>
<tr>
<td><strong>Lasting emotional changes</strong></td>
<td></td>
</tr>
<tr>
<td>- Negative emotional state (increased response to reward negative contrast) 1</td>
<td>- Lesser response to reward negative contrast 1</td>
</tr>
<tr>
<td><strong>Lasting physiological/morphological changes</strong></td>
<td></td>
</tr>
<tr>
<td>- No change in body mass 4</td>
<td>- No difference in body mass 4</td>
</tr>
</tbody>
</table>
- No change in basal plasma corticosterone, adrenal weight, amygdala weight, or hippocampus weight (unpublished data)
- No difference in fecal corticosterone
- No difference in basal plasma corticosterone, adrenal weight, amygdala weight, or hippocampus weight (unpublished data)

**References:**


5) Chapter 6: Chaby, L.E., Sheriff, M.J., Hirrlinger, A., Lim, J., Braithwaite, V.A., in review. Stress during adolescence affects rates of foraging and vigilance in adulthood?

References


*Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 29, 1988–1993. doi.org/10.1038/sj.npp.1300528


*Hormones and Behavior* 66, 517–524. doi:10.1016/j.yhbeh.2014.07.010

Chaby, L.E., Sheriff, M.J., Hirrlinger, A., Lim, J., Fetherston, T.B., and Braithwaite, V.A., in review.

Does chronic unpredictable stress during adolescence affect spatial cognition in adulthood?

Chaby, L.E., Sheriff, M.J., Hirrlinger, A.M., Braithwaite, V.A., 2015a. Can we understand how developmental stress enhances performance under future threat with the Yerkes-Dodson law?

*Communicative & Integrative Biology* 8, e1029689. doi:10.1080/19420889.2015.1029689


Yerkes, R.M., Dodson, J.D., 1908. The relation of strength of stimulus to rapidity of habit-formation. Journal of Comparative Neurology and Psychology 18, 459–482. doi:10.1002/cne.920180503
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Chaby, L.E., Sheriff, M.J., Hirrlinger, A., Lim, J., and Braithwaite, V.A. Stress during adolescence shapes performance in adulthood: Context-dependent effects on foraging and vigilance.
Chaby, L.E., Sheriff, M.J., Hirrlinger, A., Lim, J., Fetherston, T.B., Tian, W., and Braithwaite, V.A. Does chronic variable stress during adolescence affect spatial cognition and spatial strategies in adulthood?
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