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Epigenetic Modifications of Brain and Behavior: Theory and Practice

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Abstract

Evolutionary change is a product of selection. Selection operates on the phenotype, and its consequences are manifest in representation of the genotype in successive generations. Of particular interest to both evolutionary and behavioral biologists is the newly emerging field of epigenetics and behavior. Two broad categories of epigenetic modifications must be distinguished. Context-Dependent epigenetic change can be observed if the environmental factors that bring about the epigenetic modification persists (e.g., the frequency and quality of maternal care modifying the brain and future behavior of the offspring each generation). Because the environment induces epiallelic change, removing the causative factor can reverse a Context-Dependent epigenetic state. Germline-Dependent epigenetic change occurs when the epigenetic imprint is mediated through the germline. Such effects are independent of the causative agent and there is no evidence at present that a Germline-Dependent epigenetic state can be reversed. Finally, only Germline-Dependent epigenetic modifications can be truly transgenerational. Although an individual's life history is progressive and continuous, it might usefully be viewed as the cumulation of divisions; each period emerging from what has gone before and, at the same time, setting the stage for what follows. These life history stages are somewhat arbitrary, with many traits spanning conventional divisions, but each period tends to have its own characteristic ethologies and particular contribution to neural and behavioral phenotypes. To understand how these episodes 'fit' together, it is necessary to deconstruct early life events and study each period both in its' own right and how it interacts with the preceding and subsequent stages. Lastly, it seems intuitive that Germline-Dependent and Context-Dependent epigenetic modifications interact, resulting in the individual variation observed in behaviors, but until now this hypothesis has never been tested experimentally.

INDEX WORDS

transgenerational; individual variation; life history; prenatal; postnatal; adolescence; litter

Introduction

An individual's phenotype is influenced by the experiences that accumulate throughout its life (Insel and Fernald, 2004). Early experiences in particular shapes how individuals will respond to later experiences, and later experiences modify the effects of earlier experiences

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(Champagne, 2008; Champagne and Curley, 2010; Curley et al., 2009; Korosi and Baram, 2009; Moriceau et al., 2009; Romeo et al., 2010). Studies of the role of experience in behavioral development can be divided into those that focus on the parental and other social influences vs. physical/biotic (e.g., storms that demolish nests early in the reproductive cycle of seasonally breeding birds/appropriate day length and temperature) influences. The former has been well characterized in avian and mammalian species while the latter is best observed in species that depend upon environmental factors to establish gonadal sex or alternative mating strategies within a sex.

Experiences during sensitive periods of development such as embryonic, neonatal, and adolescence can act together or independently to modify the genome without altering DNA structure. These effects, referred to as epigenetic, can lead to an epigenetic inheritance, such that the environment can affect the transcriptome of the individual during its development and potentially that of its descendants. Epigenetic modifications to an individual can either be at the gross or molar level (influencing the individual's interactions with its biotic and physical environment through time) or at the fine or molecular level (altering gene expression at transcriptional and translational levels during development) (Crews, 2008). An example at the molar level would include the emergent properties of progressive changes in development on behavioral outcomes can be found in precocial birds, where the difference in timing is a major factor in differentiating filial imprinting from sexual imprinting (Bateson, 1991; Gottleib, 1997). An example of molecular epigenetics would be the finding (see below) that pups of mothers who exhibit high levels of licking and grooming are less reactive to stress as adults as a consequence of increased serotonin tone and DNA methylation within exon 17 of the promoter of the glucocorticoid receptor in the hippocampus (Weaver et al., 2004).

Molar and molecular epigenetic modifications interact. Thus, changes at various levels (e.g., pattern of gene expression, physiological systems, and the organization and activation of brain circuits) bring about functional differences in brain and behavior that result in molar epigenetic changes. These then modify how individuals respond to conspecifics and their environment, bringing about changes at higher levels of biological organization. Ultimately these can lead to molecular epigenetic modifications that support the new trajectory in life history.

In considering the nature of epigenetic modifications, it is important to distinguish between Context-Dependent and Germline-Dependent epigenetic changes. The processes involved in these two categories of epigenetic modification are fundamentally different (Crews, 2008) yet can be interactive in shaping behavioral development. I next discuss the distinction between these types of epigenetic effects and their parallels with the concept of ultimate and proximate causation, followed by an illustration of the influences of contextual factors on development (that likely involve epigenetic variation) and studies of Germline-Dependent epigenetic effects. The interaction of Context- and Germline-Dependent epigenetic effects may have real-world consequences for reproduction. I conclude with a discussion of the implications of these experience-driven non-genomic changes.

A. Context-Dependent vs. Germline-Dependent Epigenetics

Context-Dependent epigenetic change can be observed if the environmental factors that bring about the epigenetic modification is maintained through time. For example, if the temperature, diet, behavior, or environmental toxicant, etc. continues to persist in the environment, then the epigenetic modification will manifest itself in each generation. Importantly, Context-Dependent epigenetic modifications can be reversed by removing the factor(s) from the environment (e.g., by removing heavy metals from the environment) or by providing methyl donors or histone deacetylase inhibitors. Although there is no evidence for

how long such changes may occur (e.g., chemically induced epigenetic modifications presumably will last as long as the environment is contaminated), there is no evidence that behaviorally induced epigenetic changes (e.g., those produced by the nature and quality of maternal care) last beyond a single generation without continued exposure. For example, transmission from mother to young can be said to “cross a generation”, but it is not *truly* transgenerational in nature; that is, it will not perpetuate itself without further exposure each and every generation. Thus, Context-Dependent epigenetic modifications deal with transmission *within* a generation (within an individual’s own lifetime) and are propagated through somatic cells (= *mitotic* epigenetic inheritance).

Germline-Dependent epigenetic change occurs when the epigenetic imprint is mediated through the germline and is transferred to subsequent generations (= *meiotic* epigenetic inheritance) (Davies et al., 2008; Keverne and Curley, 2008). In such instances the modification (e.g., DNA methylation of heritable epialleles) is passed through to subsequent generations rather than being erased as occurs normally during gametogenesis and shortly after fertilization. Since the effect is manifest in each generation in the absence of the causative agent, only Germline-Dependent epigenetic modifications are transgenerational, involving transmission *across* generations. This rigorous definition of Germline-Dependent epigenetic change is necessary since, in the case of chemically induced epigenetic modifications, it is not until the third generation from exposure that the body burden of chemical is no longer detectable in the descendants (Skinner, 2008). Although there is still only limited evidence in vertebrates for environmentally induced epigenetic modifications, in plants such effects are known to last for hundreds of generations, if not in perpetuity (Crews, 2008).

The division between Context-Dependent and Germline-Dependent effects has a direct parallel to the conceptual distinction between ultimate and proximate factors in evolutionary biology and reproductive biology. For example, in a classic treatise on the evolution of breeding seasons, Baker (1936) suggested that ultimate factors determine when young can be most efficiently raised, while proximate enable individuals to adjust or synchronize reproductive processes so that individuals are in breeding condition at the appropriate time. Thus, ultimate factors are responsible for the adaptation of breeding seasons while proximate factors keep the adapted organism synchronized with its environment. Examples of ultimate factors controlling reproductive seasonality would include: Quality and quantity of food; Adequate nesting material and sites; Predation pressure; Competition between species. Examples of proximate factors controlling reproductive seasonality would include any environmental stimulus or cue that is reliably and predictably connected to the same environmental change (e.g., photoperiod, temperature, rainfall, behavior, etc.). As in the case of behavioral development, it is rare that a reproductive cycle is dependent on a single factor; usually the timing of the reproductive process depends on a suite of cues with different stimuli regulating its onset, maintenance and termination. Within the context of this essay on epigenetics and behavior, Germline-Dependent epigenetic modifications could be considered analogous to ultimate causation, while Context-Dependent epigenetic modifications would be analogous to proximate causation.

B. Early Context-Dependent Epigenetic Modifications Shape Brain And Behavior

In mammals and birds the formative environment for social and anxiety-related behaviors is the family unit; in the case of laboratory rodents, the mother and her litter are typically isolated while in nature, communal breeding may be more typical. It is important to keep in mind certain precepts that are specific to studies of behavioral development. Paraphrasing Alberts and Shank (2010), these principles are: (1) Ontogeny is not a single process but comprises a sequence of interacting processes that operate at different levels of biological organization (genetic, physiological, morphological, and neurological). Behavior then is a

dynamic property emerging from lower-level interactions. (2) Development is sustained and constrained by context, or the immediate physical and biotic elements of the individual's environment at each life stage. When applied to species having collectives of young such as mammals and birds, both the study of the development of behavior as well as the regulation of patterns of gene expression are most appropriately investigated when also considering the individual within the context of family or social groups. (3) Such family or social groups in turn are the result of how the participating individuals interact to produce specialized functional outcomes.

A deciding factor in this early environment for laboratory rats and mice is the sex ratio of the litter. Usually the sex ratio *in utero* and following birth are similar, but this does not mean that each period has an equivalent contribution to behavior. There is abundant evidence that the prenatal environment (the sex of fetal neighbors) and the postnatal period (the nature and quantity of maternal care) affect the adult behavioral phenotype (Ryan and Vandenbergh, 2002; Fleming et al., 2002; Meaney, 2001; Moore, 1995). However, in none of these studies have these two periods (prenatal and postnatal) been disassociated. Specifically, research demonstrating that the intrauterine sex ratio influences adult behavior has not controlled for sex ratio of the litter postnatally. Similarly, research demonstrating litter sex ratio influences on maternal behavior has not taken into account the prenatal sex ratio of the pregnant mother. Thus, it is not known the extent to which the former (prenatal) affects the latter (postnatal), or vice versa.

It is possible to re-assemble the litter at birth and in that manner deconstruct this usually continuous process into its component life phases. When this is done we find the surprising finding that neither intrauterine position or maternal behavior have significant effects on the performance and attractiveness of males in adulthood (deMedeiros et al., 2010). Rather, it is the sex ratio of the litter postnatally that has the greatest effect on sexuality. That is, regardless of prenatal sex ratio, males raised in female-biased (2 males: 6 females) litters exhibit less mounting compared to males raised in litters of equal sex ratio (4 males: 4 females) or in male-biased (6 males: 2 females) litters. Further, males from female-biased litters are less attractive to sexually receptive females. These differences are not erased by sexual experience, suggesting that the effects of the sibling environment are permanent. Surprisingly, these males compensate for their lower attractiveness by being more efficient copulators.

With the advent of genetically-modified model organisms came an additional confound factor of various offspring genotypes and how this may complicate study of early life sexual differentiation/development. This is particularly important in model systems that involve the mating of heterozygotes (HTZ) to yield varying numbers of wildtype (WT), HTZ, and knockout (KO) young. For example, in the case of mice and rats lacking functional copies of gene(s), the ratio of the various genotypes within the litter is as important as litter sex ratio. Typically, researchers using KO mice do not control for the early social environment of their experimental animals. However, this early social environment has a powerful effect on shaping the adult behavioral and neural phenotypes. A common model in this type of research is the estrogen receptor α (ER α) knockout mouse (here designated as KO). Both HTZ and KO individuals lack a functional copy of this important sex steroid receptor and research has revealed the role of this gene in differentiation of morphology and physiology, as well as distinct behavioral phenotypes (Ogawa and Pfaff, 2000; Rissman et al., 1999).

The question becomes, to what extent are the behavioral phenotypes due to the absence of the gene versus the sex and genotype ratios in the litter in which the individual develops. It is possible to reconstitute litters soon after birth to control for these two factors (Crews et al., 2004, 2006, 2009). Using this approach recent work has revealed that in both males and

females the Sex and Genotype of siblings affect aggressive behaviors as well as patterns of metabolic activity in limbic nuclei later in adulthood. For example, WT females spend significantly more time in social contact in a Resident-Intruder test compared to KO females when raised in same-sex, same-genotype litters (Crews et al., 2009). Further, it appears that female WT siblings are able to compensate for this deficit, just as KO siblings cause a deficit in WT females. The neural networks that subserve sociosexual behavior vary in different ways. First, there is a significant genotype difference in the neural network of WT and KO mice; the compensation/deficit in the behavior are reflected in the metabolic activity of the neural circuit. Second, the type of sibling (sex as well as genotype) influences the neural network exhibited in adulthood. The relative effects of sex independent of genotype, and of genotype independent of sex, are striking. Taken together, these findings indicate that in studies with genetically modified mice, the litter composition during the preweaning period must be considered as it can effect the development of behavior and the neural network responsible for the regulation of emotional behaviors.

Finally, developmental psychobiologists and behavioral endocrinologists traditionally have focused on the sexual differentiation of behavior, virtually ignoring the problem of individual differences (Crews, 1998, 1999). This in part is due to the organisms studied most (typically inbred birds and mammals) and, as a consequence, the perspective engendered is that variation is categorical (male vs. female) rather than continuous. However, sex and sexuality are conceptually distinct but often confused; gonadal sex is a discrete character that categorizes the individual, whereas sexuality is a suite of continuously variable traits that is unique to each individual. Sexuality therefore resides in the brain, not in the gonads, and the sexual differentiation of mating behaviors, their underlying mechanisms, and the relation to individual variation involves more than just the type of gonad and pattern of circulating sex steroid hormones.

Mammals and birds have sex chromosomes, and, as a consequence, genetic sex and gonadal sex (hormones) are inextricably linked. The very nature of genotypic sex determination (GSD) makes it difficult to distinguish non-genomic yet heritable (epigenetic) factors from genetic contributions to sexuality. Consider, for example, aggressive and sexual behaviors displayed by both sexes, but at different frequencies and often in different contexts and situations. To what extent are differences observed between adult males and females due to their differences in sex chromosomes, differences in the nature and pattern of past and present endocrine history, differences in environmental influences or sex-typical experiences? Examples exist for all of these. Although research with transgenic mice suggests that the sex-specific gene SRY may play a role in behaviors, these effects are not robust and it is doubtful that in wild species they play a role in sexually dimorphic behaviors.

The leopard gecko has temperature-dependent sex determination (TSD) and thus does not have the complications arising from genotypic sex determination. In this species high and low incubation temperatures produce only females while intermediate incubation temperatures produce different sex ratios. That is, extreme incubation temperatures (26 °C or 34 °C) are female-producing incubation temperatures, whereas intermediate temperatures result in different sex ratios: 30 °C (Tf) produces a female-biased sex ratio (25:75), and 32.5 °C (Tm) a male-biased sex ratio (75:25). Despite this temperature determination of sex, there is continuous within-sex variation in both sexes that with few exceptions overlap. Incubation temperature accounts for much of the within-sex variation observed in the morphology, growth, endocrine physiology, and aggressive and sexual behavior of the adult (Crews, 1998; Crews and Groothuis, 2004; Crews et al., 1998; Sakata and Crews, 2002).

Temperature during embryogenesis is one type of experience. Another type is sexual experience during adulthood. How might experiences early in life interact with experiences later in life to affect sexual behavior and their underlying neural circuits? One indication is seen in mate preference (Putz and Crews, 2006). Sexually experienced Tf and Tm males both show strong preferences in a Y-maze apparatus to females or their odors, but the type of female they choose depends upon their incubation history. Among females, Tm females are less attractive to males than are Tf females and also exhibit male-typical patterns of aggression.

For neurobehavioral studies, cytochrome oxidase (CO) histochemistry is a particularly useful tool for studies of the effects of significant life-history events. The abundance and activity of CO activity in a brain area is a measure of the metabolic capacity of that brain region. In other words, the CO abundance not only reflects the metabolic history of an area but also determines the amount of ATP available in a neuron, constraining the amount of activity a neuron can sustain (Sakata et al., 2005).

Incubation temperature also influences the metabolic capacity of specific forebrain nuclei, in adult leopard geckos (Coomber et al., 1997). Sexual experience in females has a different effects depending upon the individuals incubation temperature (Crews et al., 1997). Both sexually naïve and sexually experienced females show a significant increase in most, but not all, nuclei relative to overall brain activity occurs, but importantly not in the same manner.

Although outside the scope of this essay, an often unappreciated distinction is that of experience versus age. An individual ages as it gains experience, but it is possible for an animal to age without acquiring certain experience; for example, a socially subordinate male may never have breeding experience. There is clear evidence that age and experience have separate, and at times, opposing effects on behavioral and neural phenotypes (Crews et al., 1997). For example, CO activity is higher in the anterior hypothalamus of males, in the ventromedial hypothalamus of both males and females from the Tm incubation temperature, and in the preoptic area of females from both incubation temperatures. These differences were not paralleled by differences in circulating levels of sex hormones; only plasma androgen levels differed as a function of experience in males. These data suggest that the volume and metabolic capacity of specific brain regions change as animals age and gain sociosexual experience, but the nature and degree of change depends upon prenatal events.

C. Germline-Dependent Epigenetic Modifications Shape Brain And Behavior

Michael Skinner and colleagues have developed a rat model in which the male germline bears a permanent epigenetic imprint, thereby creating a truly transgenerational epigenetic phenotype (Anway and Skinner, 2005; Anway et al., 2005, 2006; Guerrero-Bosagna and Skinner, 2009). Exposing gestating Sprague-Dawley female rat to the endocrine disrupting pesticide methoxychlor or the fungicide vinclozolin during the period of embryonic sex determination reprograms the germline in a sex-specific manner. This modification is evident in every generation without further exposure to the chemical for five generations (the number of generations studied to date). As noted above, the F3 generation is the first generation that the body burden from the exposure is completely absent and any effects cannot be attributed to the chemical exposure. Remarkably, males in each generation show accelerated onset of adult diseases such as cancer, prostate disease, kidney disease and immune defects; these diseases develop spontaneously at about 240 days of age in normal males, but in the transgenerationally modified males, they begin to be observed around 120 days of age. The appearance of a series of new imprinted-like genes that transgenerationally transmits this altered epigenome to promote disease phenotypes appear not only in the sperm epigenome but also in the brain epigenome (Anway et al., 2006; Skinner et al., 2008).

Using the Vinclozolin model system we have established the behavior of individuals is also epigenetically modified. Females discriminate and prefer male descendants of the line that were not exposed to the chemical, whereas similarly epigenetically imprinted males do not exhibit such a preference (Crews et al., 2007). Specifically, in a partner preference test, F3 generation females of both the Vinclozolin- and Control-lineages discriminate and prefer males who do not have a history of exposure; males do not exhibit such a preference. Odor preference tests rule out possible differences in the odor discrimination ability of epigenetically modified animals; males and females of both lineages explore odors of the opposite sex much more than familiar (self) odors or novel odors of the same sex, and all animals explore novel odors of the same sex more than own odors.

D. Do Context-Dependent Epigenetic Modifications and Germline-Dependent Interact and, If So, How?

It seems intuitive that Germline-Dependent and Context-Dependent epigenetic modifications would interact, and thereby underlie the individual variation observed in traits. That is, events in past generations (heritability) influence how an individual responds to events in their own life history (experience). The possible combinations are detailed in Table 1. Skinner and I have been investigating the effects of chronic restraint stress on brain and behavior using the Vinclozolin model system described above. This work builds on the large literature demonstrating that chronic restraint stress experienced early in life in rats modifies an individual's behavioral and neural phenotypes as an adult. Specifically the hypothesis is that chronic stress during adolescence interacts (either additively or synergistically) with the transgenerational epigenetic imprint.

Initially we have focused on a well-studied trait, body weight gain through life. Figure 1 illustrates that there is no difference in body weight between Vinclozolin- and Control-lineage males at birth or at weaning, but Vinclozolin-lineage males become heavier. This difference is greatest in non-stressed animals. Chronic restraint stress has an immediate effect on body weight regardless of lineage. Weight gain (average % gain relative to prior weight) in stressed animals is half that of non-stressed animals within two days of onset of stress. Following restraint stress the rate of change is increased in stressed animals regardless of lineage. This 'catch-up' in the rate of change occurs in both lineages. This finding is consistent with other studies showing that chronic restraint stress can have a long-lasting effect on traits (e.g., Marquez et al., 2002; Wood et al., 2003; Klein et al., 2010).

It is of particular interest that within the dyads, chronic restraint stress attenuated weight differences the Vinclozolin-lineage individual tend to be larger, and this difference is minimized in stressed animals, suggesting that restraint stress prevents the transgenerational Germline Dependent epigenetic imprint from being expressed. Weight differences within pairs of Vinclozolin- and Control-lineage males revealed that chronic restraint stress attenuated weight differences, suggesting that the shared experience might mitigate individual differences in feeding and/or metabolism (does stress prevent the transgenerational imprint from being expressed?); in general the Vinclozolin-lineage male was both heavier and exhibited dominant behavior regarding access to food to the Control-lineage male.

As indicated in the Introduction, the concept of ultimate vs. proximate factors is long-standing in reproductive biology. It also is used as a concept in evolutionary biology to distinguish between the influences of heredity and the environment (sometimes referred to as nature vs nurture). Ultimate factors refer to how selection (either natural, sexual, or artificial) gives rise to population and species differences, while Proximate factors encompass those experiences that an individual has within its own unique life history. In

both reproductive and evolutionary biology, the ultimate factors operate in evolutionary time, while proximate factors operate in life history time.

Inherent in this idea is that in real life heredity and the environment interact to shape the adult phenotype. For example, inherited factors predispose the individual to respond in different ways to proximate factors. While this is a commonly held that this how real life operates, there are remarkably few studies to test this intuitive conclusion. How can this hypothesis be tested?

First, it is important to define “interaction”. Conventionally this term means “mutual or reciprocal action” in that “two or more objects have an effect upon one another”. There is no mention as to the nature of this relationship and it could be additive or synergistic.

Another feature that must be clarified is the linearity of the interaction and, in particular, is the interaction general or specific. For example, is this interaction global in the sense that the alteration in the germline modification alters the individual’s responsiveness to all challenges in its life history? Importantly, the change in responsiveness is not specific to the type of challenge but posits that the change in sensitivity is general in nature. If this is true, then historical exposure to an estrogen-mimicking endocrine disruptor should change the individual’s response to chronic stress. Alternatively, the nature of the heritable modification might restrict the type(s) of challenges that the individual is sensitive to. For example, historical exposure to an estrogen-mimicking endocrine disruptor changes the individual’s sensitivity only to estrogen-mediated responses.

Our evidence on body weight does not support the global hypothesis, but the study was not designed to test the restrictive hypothesis. Our evidence suggests that the historical (Germline-Dependent) and proximate (Context-Dependent) epigenetic modifications as studied do not interact, but that each has effects independent one another. That is, there is no evidence that the vinclozolin-induced transgenerational epigenetic imprint modifies the long-term responses of chronic restraint stress experienced during adolescence. Finally, this suggests that the different effects (i.e., Germline- and Context-dependent epigenetic modifications) are mediated by different epigenetic mechanisms. Several possibilities suggest themselves. For example, Vinclozolin may operate via DNA methylation (e.g., *Comt*, *Bdnf*, *Drd2*) (Skinner et al., 2008), while the chronic restraint stress may operate via histone modifications or through other key genes that regulate HPA function (e.g., *CRH*, *POMC*, and *11 β -HSD2*) (Meaney, 2001).

Summary

Epigenetics is the new frontier in research in the development of behavior. Adult behavioral phenotypes are affected by multiple factors, some beginning in generations past while others originate during sensitive periods or life stages. There is clear evidence that such experiences can interact with genetic predispositions to lay the foundation for an individual’s behavior as an adult. The question becomes whether this truism can be applied to epigenetic modifications.

This essay describes some of the mechanisms by which factors influence adult behavioral responsiveness and their underlying neural substrates, particularly how the environment can produce significant individual variation in social behaviors. The recent discovery that the environment can affect the genome of future generations without changing the DNA sequence has particular relevance to understanding brain and behavior. Two distinct epigenetic modifications are described: Context-Dependent modifications are similar to proximate environmental effects while Germline-Dependent modifications are equivalent to ultimate environmental effects in shaping brain and behavior.

Different ‘experiences’ during sensitive life stages produce variation among individuals that markedly influence how the individual responds to social and sexual cues later in adulthood. This variation is the substrate on which evolution can act. I have presented several studies that deconstruct various confounds inherent in research in developmental psychobiology. For example, in the ERαKO mouse, the sex and genotype ratios of the litter have separate and distinct effects on the nature and quality of the individual’s behavior later in adulthood, as well as on the metabolic activity within networks of brain nuclei that underlie these behaviors. The finding that functional neural systems can be re-organized depending upon the composition of the litter in which the individual develops is startling yet yields a deeper understanding of how neural systems are organized early in life.

How these ultimate and proximate events might interact to influence how an individual responds to events in their own life history now can be addressed. In the only study to date, we find the surprising result that chronic restraint stress during adolescence of male rats does not interact with germline-dependent epigenetic modifications laid down in previous generations. This suggests that different types of experiences can result in different epigenetic modifications that are independent, but together influence the phenotype.

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Figure 1.

Effects of germline-dependent and context-dependent epigenetic modifications on body weight in F3 Sprague-Dawley rats whose grandmothers had been treated with either Vinclozolin-Lineage or Vehicle-Lineage while pregnant. All pups were produced at Washington State University and shipped to the University of Texas for experimental use. The pups were weaned at postnatal day (PND) 21 and shipped to the University of Texas the following day. On receipt (PND 24) individuals were paired (one each from a Vinclozolin- and Vehicle Control-lineage) and housed together. Thus, all animals were subjected to the stress of shipment, but after assignment half of the pairs were subjected to a daily regimen of restraint stress lasting for 6 hours each day for 21 days (beginning at PND 25); the other half of the pairs were not stressed. Animals were fed *ad lib* (Purina rodent chow (#5LL2) Prolab RMH 1800 diet) and body weight monitored twice weekly. The experiment was replicated and the sample size of each cohort shown in insert; combined data is shown as means $\pm$ SEM. Within the dyads (insert showing moving averages ($n = 3$) of weight differences expressed as differences of Vinclozolin-Vehicle) shows that Vinclozolin-lineage individuals are larger in the non-stressed dyads, but this difference is attenuated in dyads that were stressed. CNS-Control Non-Stressed; VNS-Vinclozolin Non-Stressed; CS-Control Stressed; VS-Vinclozolin Stressed.

Table 1

Epigenetic modifications can either be Germline-Dependent or Context-Dependent, referring to the necessity of the causal stimulus being present in the life of the individual; in the case of Germline-Dependent modifications the exposure is historical, occurring in a generation in the distant past while in Context-Dependent modifications the exposure is experienced during the individual's life history. Four types of scenarios are depicted. The first does not exist in nature or in the laboratory. An example of Life History 2 would be studies of the effects of selection (natural, sexual, or artificial) for a particular trait. Life History 3 would be studies in which individuals are exposed to a challenge(s) during its life history. This is characteristic of most experimental studies of behavior today. Life History 4 is believed to reflect how real life operates, but the extent to which the heredity and experience interact is rarely studied experimentally.

Life History	Epigenetic Modification		Phenomenon	Examples
	Germline	Context		
Life History 1	--	--	No Heredity or Experience	Does not exist
Life History 2	+	--	Heredity only	Selection
Life History 3	--	+	Experience only	Exposure
Life History 4	+	+	Heredity X (?) Experience	Real life