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BARD Project Number: IS-4199-R

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Project Title: Epigenetic Adaptation: The Regulatory Mechanisms of Hypothalamic Plasticity that Determine Stress-Response Set Point

Investigators

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Keywords: fasting conditioning, heat conditioning, histone, methylation,

Abbreviations commonly used in the report, in alphabetical order: AS-ODN - Antisense oligodeoxyribonucleotide, H3K27 - histone H3 lysine 27, enhancer of zeste 2 - EZH2, preoptic/anterior hypothalamus -PO/AH, eukaryotic translation initiation factor 2B - *Eif2b5*,

Budget: IS: \$ 150000

US: \$ 150000

Total: \$ 300000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1		1	2
Submitted, in review, in preparation	1		1	2
Invited review papers				
Book chapters				
Books				
Master theses			1 + 1 in progress	
Ph.D. theses		1	1 in progress	
Abstracts		2	8	10
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1			1
Longer Visits (Sabbaticals)				

Description Cooperation:

In order to succeed in achieving the goals of this project Dr. Meiri, Prof. D. Michael Denbow, & Dr. Cynthia Denbow shared their expertise. The shared techniques included the approach for dissecting similar brain structures, protocols and primers for identifying epigenetic marks. We met in Israel in March 2011 in a BARD workshop that we organized together to discuss our findings and to get input on research directions from other leaders in the field. We shared our results, and the papers are written together.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed

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Abstract

Our hypothesis was that postnatal stress exposure or sensory input alters brain activity, which induces acetylation and/or methylation on lysine residues of histone 3 and alters methylation levels in the promoter regions of stress-related genes, ultimately resulting in long-lasting changes in the stress-response set point.

Therefore, the objectives of the proposal were: 1. To identify the levels of total histone 3 acetylation and different levels of methylation on lysine 9 and/or 14 during both heat and feed stress and challenge. 2. To evaluate the methylation and acetylation levels of histone 3 lysine 9 and/or 14 at the *Bdnf* promoter during both heat and feed stress and challenge. 3. To evaluate the levels of the relevant methyltransferases and transmethylases during infliction of stress. 4. To identify the specific localization of the cells which respond to both specific histone modification and the enzyme involved by applying each of the stressors in the hypothalamus. 5. To evaluate the physiological effects of antisense knockdown of *Ezh2* on the stress responses. 6. To measure the level of CpG methylation in the promoter region of BDNF in thermal treatments and free-fed, 12-hour fasted, and re-fed chicks during post-natal day 3, which is the critical period for feed-control establishment, and 10 days later to evaluate long-term effects. 7. The phenotypic effect of antisense “knock down” of the transmethylase DNMT 3a.

Background: The growing demand for improvements in poultry production requires an understanding of the mechanisms governing stress responses. Two of the major stressors affecting animal welfare and hence, the poultry industry in both the U.S. and Israel, are feed intake and thermal responses. Recently, it has been shown that the regulation of energy intake and expenditure, including feed intake and thermal regulation, resides in the hypothalamus and develops during a critical post-hatch period. However, little is known about the regulatory steps involved. The hypothesis to be tested in this proposal is that epigenetic changes in the hypothalamus during post-hatch early development determine the stress-response set point for both feed and thermal stressors.

The ambitious goals that were set for this proposal were met. It was established that both stressors i.e. feed and thermal stress, can be manipulated during the critical period of development at day 3 to induce resilience to stress later in life. Specifically it was established that unfavorable nutritional conditions during early developmental periods or heat exposure influences subsequent adaptability to those same stressful conditions. Furthermore it was demonstrated that epigenetic marks on the promoter of genes involved in stress memory are altered both during stress, and as a result, later in life. Specifically it was demonstrated that fasting and heat had an effect on methylation and acetylation of histone 3 at various lysine residues in the hypothalamus during exposure to stress on day 3 and during stress challenge on day 10. Furthermore, the enzymes that perform these modifications are altered both during stress conditioning and challenge. Finally, these modifications are both necessary and sufficient, since antisense “knockdown” of these enzymes affects histone modifications, and as a consequence stress resilience. DNA methylation was also demonstrated at the promoters of genes involved in heat stress regulation and long-term resilience. It should be noted that the only goal that we did not meet because of technical reasons was No. 7.

In conclusion: The outcome of this research may provide information for the improvement of stress responses in high yield poultry breeds using epigenetic adaptation approaches during critical periods in the course of early development in order to improve animal welfare even under suboptimum environmental conditions.

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Achievements

Often poultry are raised in suboptimum environmental conditions that might reduce revenues and negatively impact the welfare of the stock. Epigenetic adaptation during critical periods in the course of early post-hatch development might have a beneficial role, increasing the resilience of the chicks. Here we investigated the hypothalamic mechanism underlying stress-response development concentrating on heat-stress and unfavorable nutritional conditions. The major achievement of this project was our ability to prove the concept that alterations in epigenetic marks at the promoters of neurogenesis-related genes are both necessary and sufficient to establish long-lasting stress resilience.

Determination of thermal-control set point is refined during a critical period of development by alterations in cellular properties in the frontal hypothalamus. These alterations in hypothalamic plasticity are achieved by alterations in the protein repertoire via activation or silencing of gene transcription, both of which are regulated by histone modifications. In this project we demonstrated induction of global histone H3 lysine 27 (H3K27) dimethylation, with no changes in its trimethylation levels in the frontal hypothalamus, as well as at the promoter of the brain-derived neurotrophic factor (BDNF) gene during thermal-control establishment. Furthermore, antisense “knockdown” of the H3K27-specific methyltransferase, EZH2, which was induced in correlation with the dimethylation of H3K27, inhibited *Bdnf* mRNA expression and disrupted the establishment of thermoregulation. This phenotypic effect was partially rescued by intracranial injection of BDNF.

The regulation of protein expression is complicated and controlled by multilevel regulatory steps. In the next stage we tested the general role of epigenetic regulation in the mechanism underlying determination of the level of transcription during stress. To this end we checked the epigenetic regulation governing the catalytic subunit of the eukaryotic translation initiation factor 2B (*Eif2b5*) which is a rate limiting protein in translation regulation. To further generalize our findings we checked an additional histone modification site - histone H3 lysine 9 (H3K9). First we demonstrated an increase in global H3K9 acetylation as well as H3K9 dimethylation in the chick PO/AH during heat conditioning at the critical period of sensory development. In contrast to the global profile of H3K9 modifications, acetylation and dimethylation patterns of H3K9 at the promoter of the catalytic subunit of *Eif2b5* were opposite to each other.

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During heat conditioning there was an increase in H3K9 acetylation at the *Eif2b5* promoter, simultaneously with decrease in H3K9 methylation?????. These alterations coincided with *Eif2b5* mRNA induction. Moreover, exposure to excessive heat during the critical period resulted in long-term effects on both H3K9 tagging at the *Eif2b5* promoter and *Eif2b5* mRNA expression. To further support the findings that dynamic changes in histone modifications take place during heat conditioning and long term stress-resilience establishment, we examined the expression profiles of several histone-acetylation modifying enzymes including SIRT1 and GCN5 and found a correlation between their expression and the histone modifications during both heat conditioning and challenge.

In parallel experiments we identified similar alterations in histone post translational modifications in an additional stressor. To this end we used unfavorable nutritional conditions at the same developmental period i.e. 3rd day post-hatch, which also cause neuronal network remodeling in hypothalamic nuclei. We demonstrated that 24h of fasting in 3 day-old chicks had a significant influence on the weight of the chicks immediately after fasting. In correlation there was a dynamic stimulatory effect on both global dimethylation and trimethylation of H3K27 in the preoptic/anterior hypothalamus (PO/AH) during the fasting period. The expression of enhancer of zeste 2 (EZH2), a H3K27-specific histone methyltransferase (HMT) which was implicated with heat conditioning, was significantly increased during fasting in the paraventricular nucleus (PVN) and PO/AH, which is consistent with the upregulation of H3K27 dimethylation and trimethylation. Furthermore, in the PVN, corticotrophin-releasing hormone (CRH) mRNA expression was significantly inhibited, while mRNA expression of thyrotropin-releasing hormone (TRH) and type 2 deiodinase (D2) were significantly stimulated by fasting. These findings highlight the potential role of H3K27 methylation status in both early feed- and heat stress responses in chicks and may be indicative of an epigenetic mechanism for later adaptation to feed intake stress.

To further understand the long-term consequences of unfavorable nutritional conditions during the neonatal critical-period. Long-term effects were evaluated by re-fasting at day 10 (D10). Fasting for 24 h at day 3 caused a long-term significant reduction in body weight that lasted for at least a week of normal feed intake. Nevertheless, preconditioning of chicks by early fasting reduced the homeostasis disturbance in the PVN caused by late fasting. While D10-fasting significantly elevated H3K27 methylation at the promoter of BDNF, D3/10-double fasting kept H3K27 methylation levels similar to that of the naïve group.

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Furthermore, it was established that the fasting at day 3 promotes long-term resilience to fasting stress. This long-term resilience was evaluated using a fasting challenge a week after the initial fasting treatment. The resilience to fasting was realized by the rescue of both the inhibition of BDNF and the activation of H3K27me2/me3 bringing them to the levels of naïve age matched chicks. To further establish these findings we showed that in D11 the specific H3K27me2/me3 methyltransferase EZH2 is not bound to the histone at the promoter of *Bdnf* in 3D-fasted chicks, which might indicate that activation of the PRC2 complex is involved in the mechanism underlying long-term resilience.

To unequivocally prove that H3K27 is crucial in long-term induction of resilience to fasting, *Ezh2* was "knocked out" using antisense. The injection of EZH2 antisense resulted in a significant inhibition of the H3K27 methylation in the FB. However, H3K27 methylation was significantly elevated in the hypothalamus as compared to the ACSF injected group probably as a feedback mechanism. Nevertheless, as a result of antisense injection in addition to fasting there was a reduction in H3K27 acetylation and elevation in methylation at the promoter of BDNF in correlation with the amount of fasting.

The expression of proteins is controlled by a multilevel epigenetic system??? To further understand the epigenetic mechanisms we added another layer of regulation studying the implication of heat stress on the CpG methylation pattern in the promoter of the BDNF coupled G-protein R-Ras3 on the 3rd day of life and as a consequence later in life. As revealed by western blot analysis, R-Ras3 expression was induced during heat conditioning and peaked 12 h into the heat treatment; at a level that was approximately 70% higher than in naïve chicks. We therefore evaluated the methylation pattern in the *R-Ras3* exon2 in both during heat conditioning and during heat challenge a week later. We found clusters of consecutive CpG that are either methylated or unmethylated along the promoter of R-Ras3 during both heat challenge and conditioning at sites which are potential binding sites for transcription factors that determine the level of expression of R-Ras3 and as a consequence heat resilience.

In conclusion, the outcome of this research may provide information for the improvement of stress responses in high yield poultry breeds using epigenetic adaptation approaches during critical periods in the course of early development in order to improve animal welfare even under suboptimum environmental conditions.

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Details of cooperation:

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List of Publication:

Kisliouk, T., Ziv, M, and **Meiri, N.** (2010)

Epigenetic control of translational regulation: Alterations in histone H3 lysine 9 post-translational modifications are correlated with expression of the translation initiation factor 2B (Eif2b5) during thermal-control establishment
Developmental Neurobiology 70:100-113.

Xu P, Denbow CJ, Meiri N, Denbow DM. (2011)

Fasting of 3-day-old chicks leads to changes in histone H3 methylation status. *Physiol Behav.* 105:276-282.

Appendix:

1. published papers
2. results and figures of papers in preparation