Application for Support for the
Committee for Aid and Education in Neurochemistry (CAEN)

CATEGORY 1B: Research supplies for use in the applicant's home laboratory

Research Report Alberto Camacho Morales

Award started: April, 2016.

Award finished: April, 2017.

The CAEN award support was highly beneficial to tackle two experiments in two research projects, one of them was accepted for publication in the Behavioral Brain Research Journal and the second one was submitted to the PLOSone for publication and we are still waiting the reviewer´s response.

Project 1:

Title: Role of maternal programming on synaptic plasticity defects in rat offspring

Background to project: Hypercaloric diet intake during pregnancy and lactation programs metabolic failure in rat offspring and also in humans. Initial studies have shown that obesity during pregnancy leads to metabolic derangements such as hyperphagia, adiposity, hyperlipidemia, and glucose intolerance in offspring[1-2]. Also, new evidence from animal models has shown that a hypercaloric diet during pregnancy modulates behavior including anxiety and sensitivity to positive rewards

such as drugs and palatable foods in offspring of rats. Behavior alterations during selection of hypercaloric food choices has also been demonstrated in animals showing a conditioned place preference (CPP) to high-caloric palatable foods in marmoset monkeys and rats[3]. These evidences suggest that context selection for food choices coordinate the decision and their intrinsic reinforcing properties to engage in palatable eating behavior. Conditioning preference for reward choices are regulated by changes in the synaptic plasticity of glutamatergic signalling pathways, including a-amino-3-hydroxy-5- methylisoxazole-4-propionate receptors (AMPA), N-methyl-d-aspartic acid (NMDA) and metabotropic glutamate receptors[4-5]. However, it is unknown if glutamatergic neurotransmisión depending on AMPA, NMDA and metabotropic glutamate receptor expression during conditioned fat food seeking might be programmed during pregnancy leading to addiction susceptibility in offspring.

Here we evaluated in a murine model if the conditioning preference and motivation for highly palatable food leads to alteration of glutamatergic synaptic plasticity protein expression and if these changes are transmitted to their offspring by maternal programming.

In a brief summary of our results, data showed that rats displayed preference for palatable fat food and an increase in caloric intake when compared to a chow diet. Notably, 74% of rats showing a preference for fat food intake correlate with a positive HFD-paired score whereas 26% failed to get HFD-conditioned. Also, male rats trained under an operant training response schedule (FR1, FR5 and PR)

showed high and low responder groups to work for food. Notably, hypercaloric nutritional programing of female rats leads to exacerbation for reinforcers in female offspring compared to offspring from chow diet. Finally, we found that an operant training response to palatable reinforcers correlates with upregulation of mGlur 2,3 in the NAc shell and PFC of male rats and female offspring. Also, we found selective Nr1 upregulation in NAc shell and the PFC of female offspring. Our data suggest that nutritional programing by hypercaloric intake leads to incentive motivation to work for food and synaptic plasticity alteration in the mesolimbic system.

References:

1. Chen, H., Simar, D. & Morris, M. J. Hypothalamic neuroendocrine circuitry is programmed by maternal obesity: Interaction with postnatal nutritional environment. PLoS One 4, (2009).
2. Rajia, S., Chen, H. & Morris, M. J. Maternal overnutrition impacts offspring adiposity and brain appetite markers-modulation by postweaning diet. J. Neuroendocrinol. 22, 905–914 (2010).
3. Duarte, R. B. M. et al. Consumption of a highly palatable food induces a lasting placeconditioning memory in marmoset monkeys. Behav. Processes 107, 163–166 (2014).
4. van Huijstee, A. N. & Mansvelder, H. D. Glutamatergic synaptic plasticity in the mesocorticolimbic system in addiction. Front. Cell. Neurosci. 8, 466 (2014).
5. 17. Scofield, M. D. et al. The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. Pharmacol. Rev. 68, 816–71 (2016)

In particular, CAEN funding was helpfull to perfom western blot analysis in *in vivo* and *in vitro* models.

Reference: Camacho A, Montalvo-Martinez L, Cardenas-Perez RE, Fuentes-Mera L, Garza-Ocañas L. Obesogenic diet intake during pregnancy programs aberrant synaptic plasticity and addiction-like behavior to a palatable food in offspring. Behav Brain Res. 2017. 330:46-55.

Project 2:

Title: Effect of hypercaloric diets intake on hypothalamic ER stress response and mitochondria function leading to insulin resistance in rats

Background to project: The hypothalamus is an important target of lipids toxicity during obesity (1). High fat diet (HFD) intake in mice promotes lipotoxicity by activating ER stress and mitochondrial dysfunction resulting in insulin resistance and type two diabetes mellitus (T2DM) (1). We and others have shown that saturated lipid stimulation, palmitic acid, promotes mitochondrial deregulation, oxidative stress and JNK and NF-kB activation in muscle cells resulting in insulin resistance (2). Also, palmitic promotes endoplasmic reticulum (ER) stress activation in different cell types including neurons (2-4). These evidences suggest that ER and mitochondria are primary targets of lipids leading to insulin resistance states and the development of metabolic disorders including obesity and T2DM.

The ER and mitochondria are functionally and metabolically associated through a region known as mitochondria-associated membranes (MAMs) (5). Mitochondria and ER crosstalk into MAMs domains are maintained in part by mitofusin 2 (Mfn2) protein. We found recently that in contrast to unsaturated fatty acid, palmitic acid stimulation of hypothalamic neurons and high fat diet intake of mice four months old decrease Mfn2 expression in hypothalamus which correlates with insulin resistance generation (6), an effect previously documented in skeletal muscle cells (2). These evidences suggest that Mfn2 seems to play a fundamental role in the ER-mitochondria crosstalk in metabolic relevant organs coordinating metabolic

homeostasis by insulin or leptin sensitivity in adult mice, however, the role of lipototoxicity insult at earlier development stages (pregnancy and/or lactation) on Mfn2 expression and metabolic compromise have not been address. Here we tested the hypothesis that hypercaloric diets intake during pregnancy and lactation decrease hypothalamic Mfn2 expression leading to ER stress, mitochondrial dysfunction and diabetes.

References:

1. Williams LM (2012) Hypothalamic dysfunction in obesity. The Proceedings of the Nutrition Society 71(4):521-533.
2. Nie Q, et al. (2014) Mitofusin 2 deficiency leads to oxidative stress that contributes to insulin resistance in rat skeletal muscle cells. Molecular biology reports 41(10):6975-6983.
3. Kwon H, et al. (2014) Adipocyte-specific IKKbeta signaling suppresses adipose tissue inflammation through an IL-13-dependent paracrine feedback pathway. Cell reports 9(5):1574-1583.
4. Yuzefovych LV, Solodushko VA, Wilson GL, & Rachek LI (2012) Protection from palmitate-induced mitochondrial DNA damage prevents from mitochondrial oxidative stress, mitochondrial dysfunction, apoptosis, and impaired insulin signaling in rat L6 skeletal muscle cells. Endocrinology 153(1):92-100.
5. Giorgi C, et al. (2015) Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. Antioxidants & redox signaling 22(12):995-1019.
6. Diaz B, et al. (2015) Saturated lipids decrease mitofusin 2 leading to endoplasmic reticulum stress activation and insulin resistance in hypothalamic cells. Brain research 1627:80-89.

In particular, CAEN funding was helpfull to perfom the molecular characterization of ER stress activation and Mfn2 expression evidenced by western blot analysis. The results of this paper has been submited to PLOSone journal and is under revisión.

ISN
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Financial Status:

		US Dollars	
	Funding	$ 5,000.00	
Expenses	Reagents for celular and molecular biology		$ 4,239.59
	Congress (Registration and Hotel)		$ 759.84
	Total expenses :		$ 4,999.42