Committee for Aid and Education in Neurochemistry (CAEN)
CATEGORY 1B: Research supplies for use in the applicant's home laboratory, International Society for Neurochemistry, June, 2014

Contact details of the applicant
Dr. D. Chand Basha Davuljigari
Young Scientist (SERB)
Department of Zoology
Sri Venkateswara University
Tirupati-517502, A.P. India
Email: drchandbasha2012@gmail.com

REPORT:

Postnatal Neurochemical and Behavioral Perturbations in Rats Following Prenatal Consumption of High Fat Diet: Protective effect of Calcium.

BACKGROUND OF THE PROJECT: Diets rich in fats have been shown to be detrimental to cognitive performance. Consumption of High fat (HF) diet and associated obesity has influence on cognition. It is assumed that HF diet during pregnancy and lactation is detrimental to the future health of offspring, irrespective of the maternal diet prior to conception. A very limited number of studies indicated that maternal HF diets might be an important trigger that alter brain development and increase the cognitive dysfunctions in the offspring. However, the epidemic of maternal obesity is still relatively new and we don't have the longitudinal data to know how maternal HF diets affect late-life health of offspring. Investigations into the precise mechanisms behind these changes in offspring are now required. With the current prevalence of maternal obesity throughout world, it is vital to examine the impact of the same on the behaviour and physiology of future generations. Recent data showed that it is not simply HF diet that is damaging the brain, but the nutritional status also influence. Recently, low calcium intake has been identified as a potential contributing factor to obesity. New evidence and review of earlier studies supports the view that calcium also plays a role in adipocyte lipid kinetics at the cellular level and in moderating fatness at the population. However, very few studies have investigated the protective role of calcium supplement against HF diet induced neurological disorders especially in early life.

Hypothesis to be tested: Therefore, the present study will investigate the hypothesis that protective effect of calcium on late life alterations in brain chemistry and neurobehavioral functions in rats following consumption of HF diet during prenatal period.

MATERIALS AND METHODS:

Female and male albino rats (Wistar) were acclimated for one week before mating. Two females and one male were placed overnight in a cage, and the presence of a vaginal sperm plug was recorded. Pregnancies determined by the presence of sperm in the vaginal smear were considered a gestational day (GD) 1. On GD 6, dams were randomly assigned to three experimental groups (Group I- *Control,* Group II- *High fat diet fed rats* and Group III- *0.02% calcium supplement+ High fat diet fed rats*) and housed individually. Pregnant animals were fed in the laboratory with high fat and control diets from gestational day 6 (GD 6) to postnatal day (PND) 21 and stopped at weaning. Calcium was supplemented as 0.02% in drinking water (calcium chloride dissolved in distilled water) to the HF diet fed dams from GD 6 to PND 21. All pups in each experimental group, 24 h after birth (PND1) were pooled and the new litters consisting of eight males were randomly selected and placed with each dam.

Body weight of dams and offspring: The differences in body weight of dams were noted on GD 6 and GD 20 and of the male offspring were recorded on PNDs 1, 7, 14, 21, 28 and 3 months in control and experimental rats.

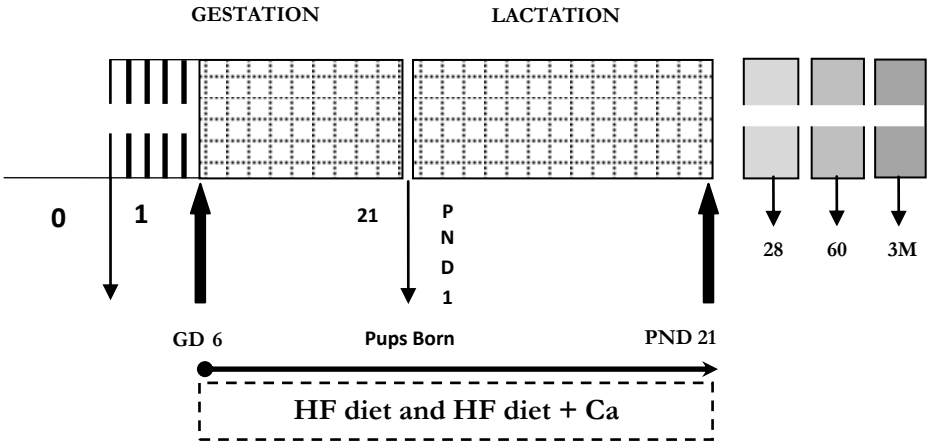


Fig.1 Study design: A schematic representation of the exposure period and the parameters evaluated in different age groups of rats.

Developmental Landmark Studies: Beginning on PND 1, the male pups were weighed and observed for developmental land marks, such as fur development (the appearance of fur sufficient to cover skin), incisor teeth eruption (the first appearance of the upper and lower incisors), pinnae detachment (both ears completely unfolded from the head), eye slit formation, eye opening (both eyes fully open) and crown rump length.

Early postnatal behavioral studies: During lactation period, male pups of each dam were evaluated for reflex development and neuro-musucular maturation *[Righting reflex (PNDs 4 - 7); Slant board behavior (PNDs 8 - 10); Forelimb hang (PNDs 12 - 16)].*

Lipid Profiles: Serum cholesterol and triglycerides was evaluated by using the commercially available kits.

Estimation of neurotransmitters and mitochondrial antioxidant enzymes:

- Acetylcholine (ACh); Acetylcholinesterase activity (AChE); Superoxide dismutase activity (SOD); Catalase activity (CAT) and Lipid peroxidation were estimated by using standard protocols.

Behavioural studies:

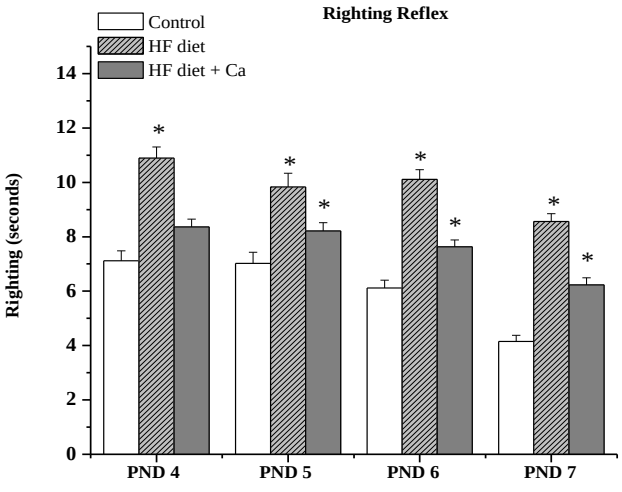
- *Morris water maze test:* The water maze is a circular water tank measuring 1.85 m in diameter and 0.7 m deep constructed according to a basic design similar to that of Morris (1984). *Total locomotor activity:* Locomotor activity of control, and experimental was measured using OPTO-VARIMEX (Columbus instruments, USA) on the designated time periods (PNDs). The activity was presented as counts/min.
- *Exploratory Behavior:* Exploratory behavior was measured in a box with a hole board bottom (90 X 90 cm) containing three equally spaced holes (3cm in diameter) in the floor. Each rat was placed in the centre of the arena for 5 min. during which time the number of head dips and head-dipping duration (in seconds) were recorded. A head dip was scored if both eyes disappeared into the hole.

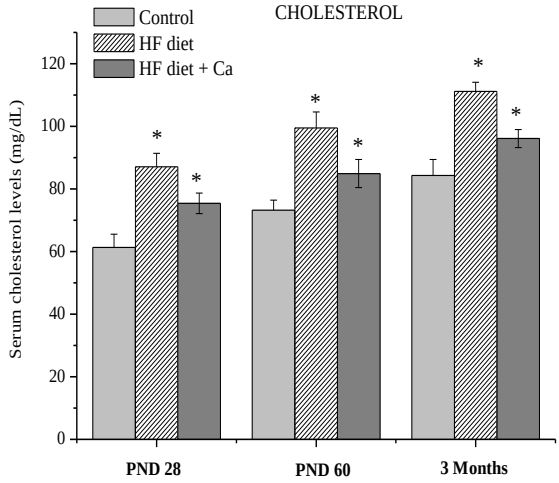
Analysis of Data: The data were subjected to one way analysis of variance (ANOVA) followed by student Newman-Keuls (SNK) post hoc test using SPSS to compare the effects among various groups.

RESULTS & DISCUSSIONS:

In the present study, we evaluated the protective effect of calcium supplement against maternal HF diet-induced effects on the development of offspring, and neurobehavioral functions at different postnatal age groups of rats. The rats were observed daily for the development of clinical signs of toxicity throughout the treatment period. No mortality and abnormal behavioural patterns were observed either in control or in HF diet fed rats. The body weights of the rat dams significantly increased from GD 6 to GD 20 ($p<0.001$) whereas body weight of male offspring from HF diet fed mothers showed marginal increased on postnatal days (PND) 1, PND 7, PND 14, PND 21, PND 28 but 3 months age group rats showed significant ($p<0.001$) changes in body weight when compared to controls. Several clinical and experimental studies have shown that prenatal HF diet consumption has significant roles in the bodyweight of offspring (Sullivan et al., 2014; Tamashiro et al., 2009). Interestingly, our results showed significant increase in body weight later in life and similar findings have been reported in other studies (Mazzuco et al., 2015; Giriko et al., 2013). Other reports showed no changes in birth weight possibly due to differences in study designs, length and timing of maternal HF diet feeding.

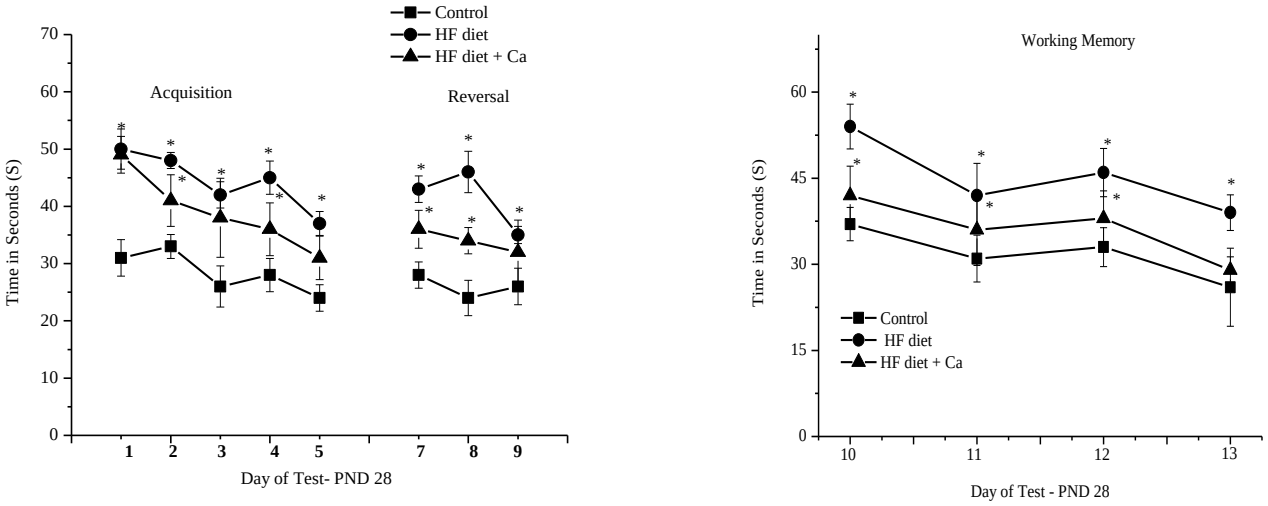
Beginning on PND 1, all retained male pups were observed daily for the occurrence of developmental landmarks such as pinnae unfold, fur development, lower and upper incisor eruption, eye slit formation and eye opening but no significant effects of HF diet were found in these developmental land marks. Rapid brain growth occurs during the third trimester in humans, where as in rats the last week of prenatal life and the first three weeks of postnatal life would be critical for continued neural development. Therefore, we investigated that the effects of HF diet on reflex tests which useful in the assessment of developmental disturbances and reflecting on the functioning of the brain regions. However, currently there is very limited number of studies indicated that maternal obesity might be important trigger that altered brain development of the offspring. In this study, we examined early postnatal neurobehavioral studies such as righting reflex (PND 4 to PND 7), slant board behavior (PND 8 to PND 10) and forelimb hang performance (PND 12 to PND 16) were observed in control and HF diet fed rats ((p<0.001). Significant increase (p<0.01) was observed in latency to turn in righting reflex and slant board behavior in offspring of HF diet fed rats. HF diet fed rats also showed decrease in the forelimb hang performance (p<0.01) recorded from PND 12 to PND 16. Significant recovery was observed in development of offspring and neuromotor maturation of rats when HF diet fed rats was supplemented with calcium.





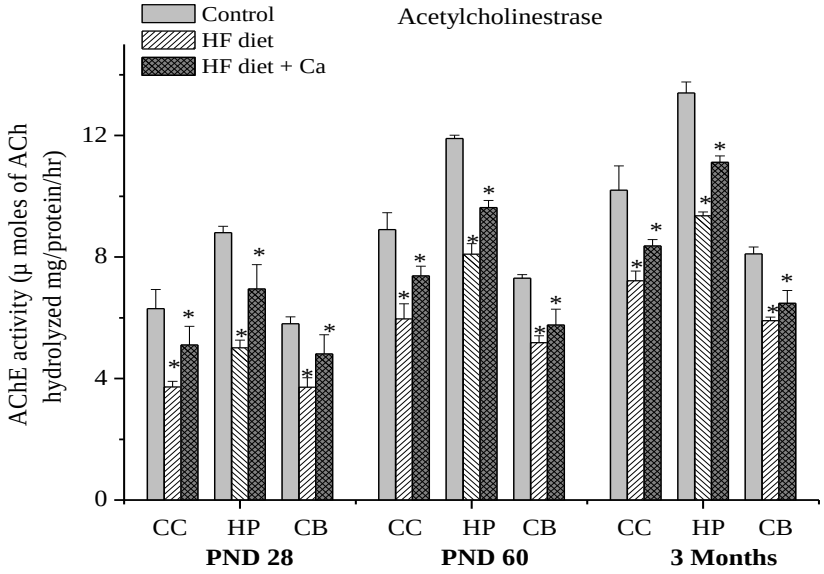
Maternal HF diet consumption during lactation caused significant increase in cholesterol and triglyceride levels in the placenta as well as in plasma of the fetus (Laker et al., 2013). Our results clearly showed significant increase in serum cholesterol and triglycerides levels in offspring (PND 28, PND 60 and 3 months) of HF diet fed rats (p<0.001). The increased lipids in the fetal circulation has the potential to alter gene expressions during development and may play a role in the cellular signalling process responsible for increased susceptibility of later disease. The link between dyslipidemia and risk of neurological functions or dementia is unclear. Indeed, rats fed with higher amount

of dietary fat showed widespread cognitive deficits on various tasks of learning and memory. The results of present study showed HF diet fed rats took longer time to find hidden platform and caused impairments in acquisition, reversal phases and working memory in Morris water maze test at PND 28, PND 60 and 3 months age groups of rats. Rats used in the reference and working memory test reached a latency criteria of ≤ 10s. HF diet fed rats never found the platform in under 20s during the reference memory, though the latencies to reach the submerged platform significantly ($p<0.001$) increased in reference memory following consumption of HF diet when compared with controls. Similarly, HF diet consumption significantly increased ($p<0.01$) the latency to find hidden platform in working memory also. The increase in escape latency was greater in reference memory than working memory. Although, alterations in reference and working memory in water maze task was more pronounced at PND 28 than PND 60 and 3 months age group rats however calcium supplement significantly reversed these HF diet induced alterations in reference and working memories. The alterations in exploratory behavior were examined in open field chamber. Significant decrease was observed in head dip counts and head dip duration in selected age groups of HF diet fed rats. Further, no significant changes were observed in locomotor activity in selected age groups of HF diet fed rats.



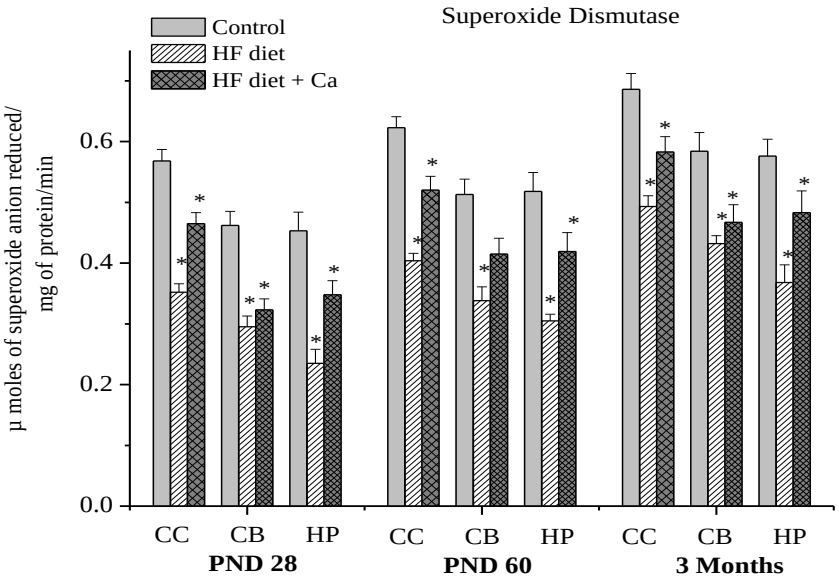
Cognitive dysfunctions are characterized by a substantial loss of cholinergic system in different brain regions of rat. However, few studies were concentrated on whole brain evaluation and missed the presence of brain region specific elevations in response to HF diet consumption. In this connection, present study evaluated the brain region specific alterations in cholinergic system (ACh and AChE) in different age groups of rats. The specific activity of synaptosomal acetylcholinesterase enzyme (AChE) and levels of acetylcholine (ACh) were determined in cortex, cerebellum and hippocampus at PND 28, PND 60 and 3 months age groups of control and experimental rats. The activity of AChE and ACh levels in general increased from PND 28 to 3 month's age rats. Hippocampus

exhibited higher levels of ACh and activity of AChE than cortex and cerebellum. HF diet fed rats showed significant decrease in AChE activity and accompanied by increase in ACh levels in cortex, cerebellum and hippocampus at various time points of postnatal development and young adult age group rats ($p<0.001$). However, the alterations in different brain regions indicate that HF diet influences the cholinergic system in brain region specific manner and contributing to the observed changes in spatial learning, memory and exploratory behaviors of rats. The mechanisms by which HF diet induce alterations in cholinergic system are not clearly known. HF diets increase oxidation of fatty acids through the peroxisomal oxidation pathway, that is associated with increased generation of free radicals and reduce the antioxidant enzyme activities. In the present study, we also estimated the mitochondrial SOD and CAT enzyme activities and tissue MDA levels in cortex, cerebellum and hippocampus regions at PND 28, PND 60 and 3 months age groups in control and experimental rats.



HF diet fed rats showed decrease in the activities of SOD, CAT and increase the tissue MDA levels in selected age groups of rats, compared to control ($p<0.001$). The observed alterations in synaptosomal cholinergic system and mitochondrial SOD, CAT and tissue MDA levels were greater in hippocampus than cortex and cerebellum further these alterations increased with age in HF diet fed rats. However, Ca supplementation significantly reversed the HF diet induced alterations in brain region specific neurochemistry in all selected age groups of rats. HF diet caused oxidative damage in brain by enhancing peroxidation of membrane lipids due to the generation of ROS and decreases the activity levels of antioxidant enzymes leading to oxidative stress. Our results clearly showed that an increased MDA level has been accompanied by reduction in the activities of antioxidant enzymes in different brain regions of rats. The decreased antioxidant enzymes activities in HF diet fed rats are indicative of the oxidative stress and a response to the

cholinergic system perturbations. The major findings of the present study are that, consumption of HF diet during prenatal period can lead to late life impairments in spatial learning, memory and brain cholinergic and antioxidant systems in rats.



One of the objectives of this study was to determine whether calcium supplementation would reduce late life neurotoxicity of HF diet in rats. In the present study, we found that the calcium supplementation may potentially be beneficial in treating HF diet induced impairments in neurological functions during development. It has been reported that calcium intake can lead to a reduction of body weight and body fat in both animal and human studies (Parra et al., 2008). Recent evidence indicates that elevation of fecal fat excretion in response to increased calcium intake should be considered especially in individuals with an excessive fat intake. Another possible explanation is reduced absorption of fat in the gut, decrease in lipid profiles, another that intracellular calcium has a regulatory role in fat metabolism by influencing lipolyis, fat oxidation (Torres et al., 2013; Jacobsen et al., 2005). However, our results showed that the co-administration of calcium supplementation along with HF diet reduced the HF diet induced perturbations in neurobehavioral functions suggesting that adequate calcium intake may be potentially beneficial in treating the HF diet induced toxicity.

Conclusions:

- Our results suggest that consumption of HF diet during pregnancy and lactation causes long-term deficits in cognitive and exploratory behaviours, and produces alterations in cholinergic and antioxidant systems in different brain regions.
- The increases observed in serum cholesterol and triglycerides and the changes in cholinergic system and oxidative stress markers in brain regions may have contributed to the observed changes in cognitive and exploratory behaviours. Further, there are no

significant changes observed in motor functions of offspring following consumption of HF diet during prenatal period.

- From the results it is also clear that age of the animal and duration of HF diet consumption are major factors determining the vulnerability of the brain to the detrimental effect of HF diet.
- The major findings of the present study are that, adequate calcium intake in an early life may be potentially beneficial in treating the HF diet induced toxicity during development.
- Based on the above conclusions, we extend our future studies to examine the influence of maternal high fat diet on cognitive functions in adult and old age groups of rats and further develop suitable therapeutic strategies with essential nutrient metal supplements.

References

Giriko CÁ, Andreoli CA, Mennitti LV, Hosoume LF, Souto Tdos S, Silva AV, Mendes-da-Silva C. Delayed physical and neurobehavioral development and increased aggressive and depression-like behaviors in the rat offspring of dams fed a high-fat diet. Int J Dev Neurosci. 2013; 31(8):731-9.

Jacobsen R , Lorenzen J K, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion International Journal of Obesity 2005; 29: 292–301.

Mazzucco MB, Fornes D, Capobianco E, Higa R, Jawerbaum A, White V. Maternal saturated-fat-rich diet promotes leptin resistance in fetal liver lipid catabolism and programs lipid homeostasis impairments in the liver of rat offspring. J Nutr Biochem. 2015; S0955-2863(15)00220-X.

Rhianna C. Laker , Mary E. Wlodek, Jessica J. Connelly, Zhen Yan, , Epigenetic origins of metabolic disease: The impact of the maternal condition to the offspring epigenome and later health consequences Food Science and Human Wellness 2013; 1–11

Sullivan EL, Nousen EK, Chamlou KAMaternal high fat diet consumption during the perinatal period programs offspring behavior. Physiol Behav. 2014;123:236-42.

Tamashiro KL, Terrillion CE, Hyun J, Koenig JI, Moran TH. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. Diabetes. 2009; 58(5):1116-25.

Torres MR, Sanjuliani AF. Effects of weight loss from a high-calcium energy-reduced diet on biomarkers of inflammatory stress, fibrinolysis, and endothelial function in obese subjects. Nutrition. 2013; 29(1):143-51.

Parra P, Bruni G, Palou A, Serra F. Dietary calcium attenuation of body fat gain during high-fat feeding in mice. J Nutr Biochem. 2008; 19(2):109-17.

Acknowledgements:

This research work was supported by a grant from **International Society for Neurochemistry (ISN)** under the scheme of the Committee for Aid and Education in Neurochemistry **(CAEN).** I express my sincere thanks to Dr. Roberto Cappai (Chairman) and committee members of CAEN for providing me financial support to carry out the above research work.

Conferences/ Presentations

- D. Chand basha and G. Rajarami Reddy. Late life impairments in brain chemistry and behaviour in rats following prenatal consumption of high fat diet: protective effect of calcium. **14th meeting of the Asian-Pacific Society for Neurochemisty,** 27-30th August, 2016, Selangor, Malaysia (Submitted for Travel Award).
- D. Chand Basha and M. Usha Rani. High fat diet induced age related alterations in liver histology and oxidative stress in rats. National seminal on molecular and genetics basis of neurological disorders. Dept. of Zoology, SVSSC College, Sullurpeta, Nellore. **(Presented as Invited talk & received Young Scientist Award).**

Publications

- Late life impairments in brain chemistry and behaviour in rats following prenatal consumption of high fat diet: protective effect of calcium, **Nutrition Research.** (Manuscript in preparation).

- The fund was spent on the obtaining the chemicals and glassware's, behavioural apparatus, micro pipettes, commercial kits for biochemical assays, rats, rat food, high fat diet etc..
- Part of financial support was used to collaborative work with Department of Psychology.