



Research Article

Behavior, Memory, Anxiety, and Curiosity are Affected by Weaning Time in a Rat Model

Yonatan Crispel^{1,2*}, Oren Katz², Geula Klorin²

¹Department of Hematology, Thrombosis and Hemostasis Unit, Rambam Health Care Campus, Haifa, Israel

²Rappaport Family Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

***Corresponding author:** Yonatan Crispel, Department of Hematology, Hematological Institute, Rambam Health Care Campus, Hospital of Haifa, Haifa, Israel Rambam Medical Center, POB 9602, Haifa 31096, Israel

Citation: Crispel Y, Katz O, Klorin G (2025) Behavior, Memory, Anxiety, and Curiosity are Affected by Weaning Time in a Rat Model. Ann Case Report. 10: 2441. DOI: 10.29011/2574-7754.102441

Received: 03 November 2025; **Accepted:** 07 November 2025; **Published:** 10 November 2025

Abstract

Introduction

The transition between growth stages is critical for physiological and psychological development. In this study, we examined the psychological effects, including anxiety, memory, behavior, and curiosity, of early and late weaning from breastfeeding.

Materials and Methods

To investigate the psychological issue, we used several tests, mainly an open field test that tests motor movement and anxiety versus curiosity, an object recognition test that tests the ability to identify new objects compared to old ones, and in our case, this tested short-term memory.

Results

In this study, we examined three generations and compared early weaning on day 16 to day 26 late weaning. In the first generation, the result was not significant. In second and third generation the results were significant ($p < 0.05$), between early weaning on day 16 to late weaning on day 26.

Conclusion

In conclusion, early weaning affects the plasticity of the brain, causing epigenetic changes to manifest in a different psychological, behavioral, and physiological personality structure than late weaning.

Introduction

This study is part of a larger study that has been going on for many years and discusses growth and transition from infancy to childhood and juvenal. This research is unique and examines the effect of weaning on the physiology and biopsychology of rats. In previous studies, we examined the effect of weaning on growth, on Structural changes in the Willi of the small intestine, and in reproduction [1-3]. In the current study, we focused whether early, late, and regular weaning have an effect on behavior in male rat's

models. The study examined whether the effect is maintained over several generations and will also appear in the 2nd and 3rd generations that were weaned normally, while the change in breastfeeding occurred only in the first generation of parents. The assumption is animals that are weaned early from suckling will develop behavior of an unsafe world, compared to animals that are weaned late or compared to the control group that was weaned normally. At the physiological level, we examined eye opening, ear separation, fur formation, and more [1]. At the bio-psychological

level, we performed tests that examined self-confidence and fear of objects as an effect of separation from the mother early before the end of weaning and later than the normal weaning of the control group.

Research Objective

We hypothesize that weaning from breastfeeding is a marker of the transition from infancy to childhood. Therefore, we want to examine whether weaning from breastfeeding at different stages affects critical transitional stages in growth, physiological development, sexual maturation, behavior, and cognition, and whether the changes are transmitted across generations. Hypothesis 1: Early weaning from suckling will accelerate development towards adaptation to environmental conditions. Hypothesis 2: Rats that are weaned from suckling early will develop unsafe behavior compared to rats that are weaned late.

Materials and Methods

All animal procedures were approved by the Technion Animal Use and Care Committee and were performed under the supervision of an experimental animal veterinarian surgeon.

To test the above hypotheses, we performed three main tests: a test to examine physiological development, with an emphasis on development that occurs during the period when mammals are still infants. An open field test that tests motor movement and anxiety versus curiosity, an object recognition test that tests the ability to identify new objects compared to old ones. In our case, this tested short-term memory. The tests were carried out inside a special cardboard box. Dimensions of the box Length 60 Width 60 Height 40 cm The box is divided into 16 equal squares in an area of 15 cm each, a total of 4 squares in the center and 12 adjacent to the walls of the box. An innovation we introduced in this study is the monitoring of the different life stages of open field behaviors (Chart 1 and Chart 2). From age 22 days, weekly to age 60 days we examined eight males from each group to assess differences between groups and to see behavioral changes as a function of life stage development as reflected from this test. Will these changes be passed from the F1 to F2 generation and from F2 to F3 generation? Will early or late weaning cause changes in behavior, and will these changes continue across stages?



Chart 1: Open field test



Chart 2: Object recognition test

Statistical Analysis: Physiological data were analyzed using SAS 6.12, utilizing two-way ANOVA. Histomorphometric data were analyzed using SPSS 6.0 (Chicago, IL). Comparison between cases used the nonparametric Kruskal-Wallis ANOVA followed by a corrected Mann-Whitney U test for multiple comparisons. Differences with 2-tailed P values < 0.05 were considered significant.

Results

In this study, we examined three generations and compared early weaning on day 16 to day 26 late weaning. In the first generation, the result was not significant. second generation and third generation the results were significant ($p < 0.05$), between early weaning on day 16 to late weaning on day 26. We present in graphs only the result of the second and third generation. The current study was conducted on males only due to concerns about endocrine abnormalities; research into changes in females will be conducted separately.

First generation - Open Field and Open Field Anxiety

Open field test, no significant differences were found between groups at 16 days and 26 days, at 22 days two groups are less active until 36 days of age they reach already the peak of motor activation, and from there is a decline that lasts until 60 days of age. In a test that examined differences between anxiety versus curiosity no significant differences were found in males weaned on day 16 and males weaned on day 26, Both groups showed more anxiety at 22 days of age and it was evident that they preferred to be in the periphery of the box. At the age of 29-36, they dared to enter the center more. From the age of 36-60, they entered the center less, perhaps because there was also a decrease in general activation.

Second and Third Generation - Open Field - Total frequency

In the second generation, there are significant differences between males from the 16-day group and the 26-day group. Males who were weaned earlier and began to develop earlier were able to perform motor movement earlier - they crossed 71 lines in three minutes compared to 58.4 in males who were weaned later ($p < 0.05$). These gaps narrowed at age 29 days, but recurred and remained significant throughout all tests until age 60 days (Figure 1 A1). In the third generation, there are significant differences between males from the 16-day group and the 26-day group. Males who were weaned earlier were able to perform motor movement earlier - they crossed 72 lines in three minutes compared to 60 in males who were weaned later ($p < 0.05$). These gaps narrowed at age 29 days but returned to be significant at age 43 - 50 and 60 days (Figure 1 B1).

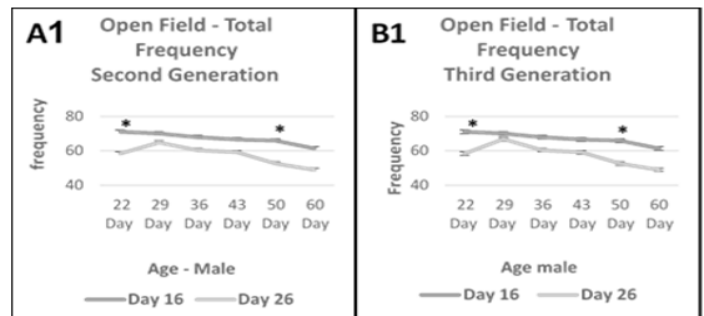


Figure 1: Males were weaned earlier and began to develop earlier were able to perform motor movement earlier differences between groups, A1-B1, day 16 & day 26 Second and third generation * $p < 0.05$

Second and Third Generation - Open Field - Anxiety and Curiosity

In a test that examined anxiety versus curiosity, significant differences were found between males in group 16 and males in a group 26 days. In the first two tests - age 22 and 29 days early weaned rats stayed in the center of the board longer than late weaned rats the difference was significant ($p < 0.05$). from the age 36 the gaps narrowed and did not remain significant, but returned to be significant at age 43 (Figure 2 A2). In a third generation, first test age of 22-29 days, the gaps were significant rats weaned early stayed in the center of the board longer than rats weaned late, the difference was significant ($p < 0.05$). And from 36 days of age, the gaps narrowed and were not significant, but returned to be significant at age 43 (Figure 2 B2).

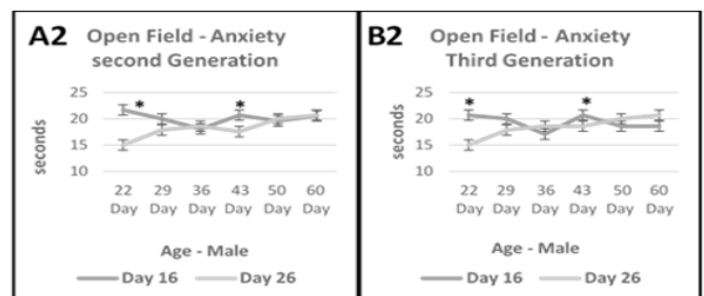


Figure 2: Anxiety versus curiosity, differences between groups, male A2-B2 day 16 and day 26 second and third generation * $p < 0.05$.

Second and Third Generation - Object Recognition - Time near new object A1

In a test for the detection of new objects, that tests short-term memory in males, significant differences were found between the 16-day group and the 26-day group. At age 29, the gap narrowed and reopened at age 43 until day 60. Even at these ages, the gap was significant (Figure 3 A3). In a test for the detection of new objects, that tests short-term memory in males, significant differences were found between the 16-day group and the 26-day group. all the test from day 22 to day 43 was significant at age 50 day the gap narrowed until age 60 day (Figure 3 B3).

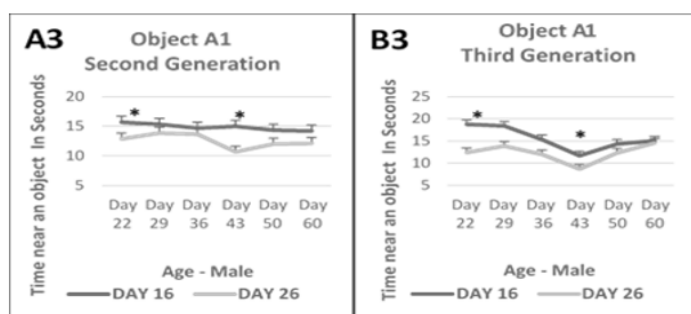


Figure 3: Time near same object A1 in seconds, differences between groups A3-B3 Male, day 16 & day 26 second and third generation *p<0.05.

Second and Third Generation - Object Recognition - Time near same object A2

In a test for the detection of the same objects that test short-term memory in males, significant differences (p<0.05) were found between the 16-day group and the 26-day group. from age 22 to age 29 the gap narrowed and was not significant, and reopened at age 36 until age 60 day and was significant (Figure 4 A4). In a third-generation significant differences (p<0.05) were found between the 16-day group and the 26-day group. from age 22 to age 29 the gap narrowed and was not significant, and reopened at age 36 until age 60 day (Figure 4 B4).

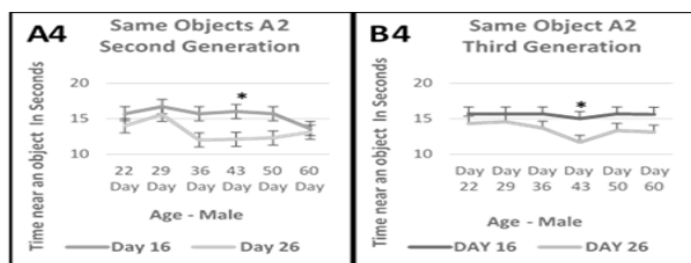


Figure 4: Time near same object A2 in seconds, differences between A4-B4 Male groups, day 16 & day 26 second and third generation *p<0.05.

Second and Third Generation - Object Recognition - Time near different object B2

In a test for the detection of different objects, that tests short-term memory in males, significant differences (p<0.05) were found between the 16-day group and the 26-day group. from age 22 the gap was significant, at age 29 the gap narrowed and reopened at age 36 until age 60 day, even at these ages, the gap was significant (Figure 5 A5). In a third-generation significant differences were found between the 16-day group and the 26-day group. on the first test on day 22, the gap was significant from age 29 and age 36 day the gap narrowed and from day 43 the gap reopened until age 50 day (Figure 5 B5).

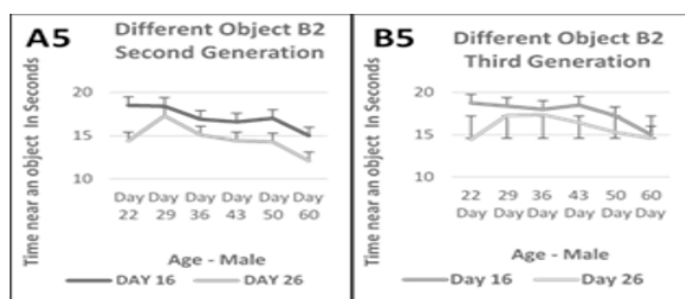


Figure 5: Time near different object B2 in seconds, differences between groups A5-B5 day 16 & day 26 second and third generation *p<0.05.

Discussion

In the current study, we expected that animals that were weaned earlier would be insecure, because they are in a less safe world, and therefore would be more anxious, and would tend to be more introverted with less mobility and curiosity, to our surprise, we got the opposite results, in all parameters so animals that were weaned earlier were more curious, more mobile and safety, compared to animals weaned later. This process could partially explain why the early-weaned animals were larger, leaner with better BMI, and less prone to diabetes. The animals weaned later were shorter and fatter in length with less mobility with high BMI and tendency to diabetes [1].

Growth and development in mammals and infants in particular, depend on the brain's transition phase. in humans, there are four phases, and in other mammals, there are three phases, the transition from phase to phase depends on many factors including environmental processes, stressful situations, nutrition, and the duration of weaning from breastfeeding [1,4,8].

The explanation behind this change is the hypothesis that weaning age programs the tempo of development. animals weaned earlier feel they are in a less safe world and do earlier programming of the transition from infancy to the juvenile in the brain. Early weaning

forces the puppies to go out and look for food on their own in order to survive, so they are more active, more fertile, and strive to leave offspring faster since their environment is less safe compared to animals weaned later [1,4,5].

In this study, it can be seen that early weaning affects behavior, anxiety, and memory as shown in the graphs and results. We hypothesize that the main finding is an epigenetic biological mechanism. This comparison was found to be significant over four generations and the assumption is that the cause is an epigenetic process that preserves the changes over a limited number of generations without affecting the structure of the genome itself.

The transition from infancy to childhood is cross-cultural and passed down from generation to generation. DNA methylation in the various stages of pregnancy is well documented in the professional literature, what happens in the critical stages of postpartum life is a mystery; the reason for this is the common assumption that DNA methylation during pregnancy is a process that defines the genome in such a way that determines A lifelong path. Newer studies show that DNA undergoes additional methylations after birth observed changes in DNA methylation during infancy, and shows that the methylation is very sensitive and changes in relation to the treatment from the mother [5].

This mechanism is passed down from generation to generation by the manipulation of early weaning from breastfeeding and passed on to future generations. This explanation is based on the hypothesis that examines changes and epigenetic differences as well as changes made through the mediation of growth and development hormones [6].

We, therefore, hypothesize that the transition from one stage in life history to another involves new epigenetic programming of genome functions possibly this mechanism is the mechanism of the transition. We hypothesize that social and fiscal circumstances in our environment reshape the epigenome, and this is an adaptive mechanism of the genome during the transition period from infancy to childhood. This process reflects on the mental and physical health of the baby in adulthood, understanding this process is important to understand how life events, especially in the early years, shape and affect us and our quality of life in adulthood [7].

The transition from one stage to another in the history of life requires a certain mechanism that will allow the next stage to exist, so we have raised epigenetics as a mechanism and weaning as a catalyst. An important and basic premise in the study is that the traditional definition of weaning from infancy is that weaning from breast milk is a physiological and metabolic process and the transition from infancy to childhood has a crucial evolutionary and environmental impact [6].

Each genotype has the ability to create a different phenotype in changing environmental conditions it is the plasticity trait of the organism that causes it to change, depending on the environment, and thus have a higher chance of surviving and producing successful offspring. Expression of genes in the transition stages from infancy to childhood and in the early stages of development is responsible for the creation of plasticity and from it the various structures that adapt to each organism. The environmental conditions experienced by the organism in the early years of its life are crucial conditions that will affect a person's biology, health, and behavior in the future [9-11].

Conclusion

In conclusion, early weaning affects the plasticity of the brain, causing epigenetic changes to manifest in a different psychological, behavioral, and physiological personality structure than late weaning. This study is another step in the series of studies we have carried out that shows the importance of weaning from breastfeeding and the importance of the duration of breastfeeding.

References

1. Crispel Y, Katz O, Ben-Yosef D, Hochberg Z. (2013). Effects of breastfeeding on body composition and maturational tempo in the rat: *BMC Medicine Journal*. 11: 114.
2. Crispel Y, Shaoul R, Khamaise R, Sabo E, Hochberg Z. (2019). Effect of weaning age on the small intestine mucosa of rats. *Appl Physiol Nutr Metab*. 44: 985-989.
3. Crispel Y, Danin Y, Klorin G, Hochberg Z. (2021). Effect of weaning age on morphological and histological changes that occur in a rat's testis. *Int J Vet Sci Res*. 7: 024-028.
4. Hochberg Z, Albertsson-Wikland K. (2008). Evo-devo of infantile and childhood growth. *Pediatric Research*. 64: 2-7.
5. Hochberg Z. (2008). Juvenility in the context of life history theory. *Archives of Disease in Childhood*. 93: 534-539.
6. Levy-Shiff R, Lerman M, Har-Even D, Hod M. (2002). Maternal adjustment and infant outcome in medically defined high-risk pregnancy. *Dev Psychol*. 38: 93-103.
7. Little MT, Hahn P. (1990). Diet and metabolic development. *FASEB J*. 4: 2605-2611.
8. Kavushansky A, Leshem M. (2004). Role of oxytocin and vasopressin in the transitions of weaning in the rat. *Dev Psychobiol*. 45: 231-238.
9. Robinson SM, Marriott LD, Crozier SR, Harvey NC, Gale CR, et al. (2009). Variations in infant feeding practice are associated with body composition in childhood: a prospective cohort study. *J Clin Endocrinol Metab*. 94: 2799-805.
10. Janus K. (1987). Effects of early separation of young rats from the mother on their open field behavior. *Physiol Behav*. 40: 711-715.
11. Hall C, Ballachey EL. (1932). A study of the rat's behavior in a field. A contribution to method in comparative psychology. University of California Publications in Psychology. 6: 1-12.