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Swiss Federal Institute of Technology Zurich

DHEST
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MSUS Paradigm in Mice, an in vivo Model of Human Early Life Trauma.

Research Internship

In partial fulfilment of the requirements for the Master of Science ETH in Health Sciences and Technology (MSc ETH HST)

Submitted by:

Arianna Arpagaus

aarianna@student.ethz.ch

Supervisor:

Francesca Manuella

Brain Research Institute, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich

Tutor:

Prof. Dr. Isabelle Mansuy

Deputy head of Institute for Neuroscience, ETH Zurich

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1. Abstract

The MSUS (maternal separation and unpredictable maternal stress) is a well-established model of induced early life trauma in mice developed in the Mansuy lab. This model is able to mimic early life trauma in mammals and also shows evidence for transgenerational inheritance (mainly by epigenetic processes).

In this model, C57BL6/J mice, MSUS dams (F0) and MSUS pups (F1) are separated from postnatal day 1 to postnatal day 14 every day for three hours at unpredictable time points. During the separation the mothers are exposed under an additional stress factor (also at unpredictable time points).

During my 3 month internship I have participated in the conduction of two new breedings. F0 to F1, where the MSUS paradigm was performed and F1 to F2 to obtain offspring of an already existing batch.

Additional experiments (metabolic and behavioral) are planned to be performed in the following months.

Key Words: MSUS, early-life trauma, stress, epigenetic.

2. Introduction

Models are the best approximation of reality and help scientists answer questions, which will imply unfeasible interventions in humans. Without doubt the translation from animal model to humans is not always 1:1 but models help answer basic questions and lay the foundation for possible mechanisms that may exist in humans.

Models are widely used in science and mouse models are usually well accepted as one of the best for human related research. Mouse model are nowadays used to study human diseases as well as metabolism and biochemical mechanisms.

In the Mansuy lab the model used is C57B16/JRj mice exposed to early life trauma (MSUS, first generation). The lab has discovered many behavioral as well as metabolic alterations in the exposed mice and also in their (unexposed) offspring[2,4].

Former literature has already established that early life trauma markers are transmitted between generations (mainly via epigenetic mechanisms)[1] and strong evidence was previously provided for the fact that adverse conditions in early postnatal life can have transgenerational effects.

The MSUS paradigm can be used as a solid model of transgenerational epigenetic inheritance[4].

3. Material and Methods

3.1 Animals

C57B16/JRj Mice from Janvier were hosted in a temperature- (21°C) and humidity-controlled facility on a 12-hour reversed light–dark cycle (lights on at 09:00 am) in individually ventilated cages (SealSafe PLUS, Tecniplast, Germany) with food and water *ad libitum*. The cages contained wood chip bedding (LIGNOCEL SELECT, J. Rettenmaier & Söhne, Germany), nesting material (2 tissue papers) and a square red plastic house.

After acclimation each naïve male was put in a cage with a naïve female for one week for pairing (see Figure 1).

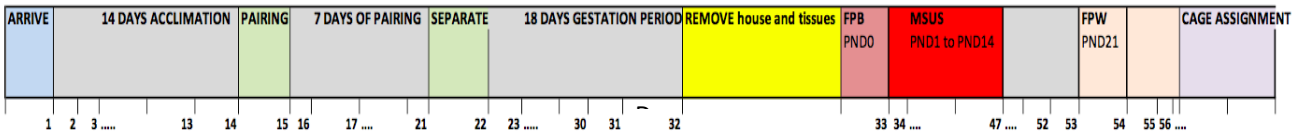

Figure 1: MSUS Paradigm timeline

3.2 Paradigm

After letting the mice mate for one week, the males are taken out of the cage and the female are single housed. It is really important that the mothers are not disturbed by noise and light during this period to achieve a good breeding outcome. Three days before the first possible birth all the nesting material is removed from the cages for better litter view (from outside the cage), as the goal is to recognize if a mother gave birth without disturbing her.

Every morning the new litters are equally distributed into MSUS or control group. Controls will stay undisturbed in the rack until weaning and get back their nesting material on PND1, whereas MSUS mice don't get any nesting material until PND 21.

The MSUS paradigm starts one day after birth, namely at PND1 (postnatal day one) and will last for 14 days. MSUS mothers (F0) and MSUS pups (F1) are physically separated allowing only olfactory and auditory recognition between them (see figure 2).

Figure 2: Physical separation during the MSUS paradigm

During the 3 hours of separation, the mothers are additionally put under physical stress. The stress can be induced by two different approaches and occur at unpredictable time points during the separation.

The first possible stress for the mother is called forced swim (see figure 3), here the mice are placed for 5 minutes in 18 °C cold water and forced to swim. It is crucial that the mothers cannot escape from the beaker and cannot touch the bottom with their tail.

Figure 3: Forced swim of the mother during the MSUS paradigm

The second possible stress induced to the mothers is called restraint and consists of placing the dams into a narrow tube for 20 minutes (see figure 4). The mothers should not be able to move freely but still be able to breathe properly.

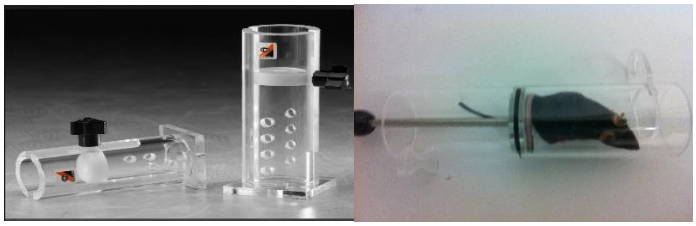

Figure 4: Restraint of the mothers during the MSUS paradigm

As you can see in table one the type of stress which will happen every day is randomly decided and the time point of the separation as well as the time point of the stress are also unpredictable.

Day	MS	Type of maternal stress (MSUS only)	Time of maternal stress (MSUS only)
1	11:15–14:15	Restraint	13:35–13:55
2	11:00–14:00	Forced swim	11:05–11:10
3	9:30–12:30	Restraint	12:00–12:20
4	11:00–14:00	Restraint	13:10–13:30
5	12:15–15:15	Forced swim	13:30–13:35
6	13:30–16:30	Restraint	14:00–14:20
7	12:15–15:15	Forced swim	14:00–14:05

Table 1: Example of a MSUS table during the first postnatal week

After three hours the mother and the pups are reunited and the cages put back into the rack. This procedure is done every single day for 14 days on all the MSUS cages (which are defined randomly at PND1) and then stopped. Please note that not all the cages will stop the paradigm at the same calendar day because not all the mothers gave birth at the same day.

After 14 days (as you can see in figure 5) the paradigm stops and the mice are left undisturbed in their cages for one more week until they reach postnatal day 21.

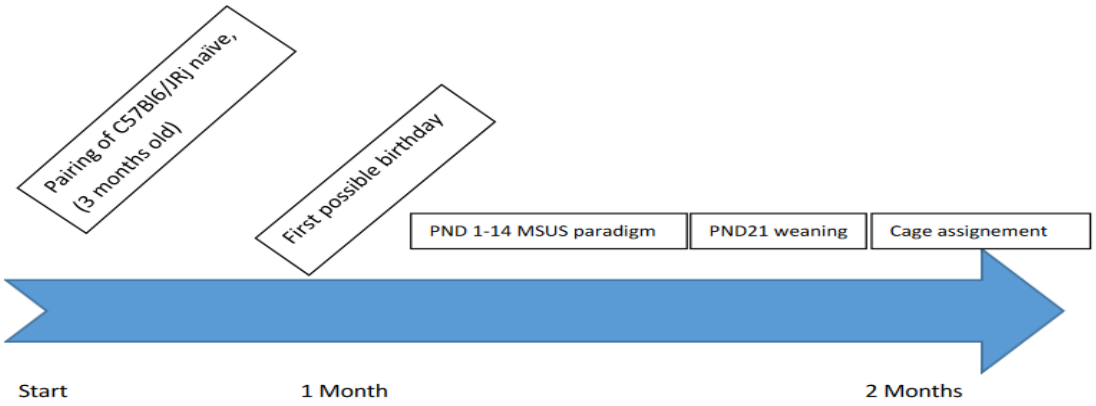

Figure 5: MSUS Timeline

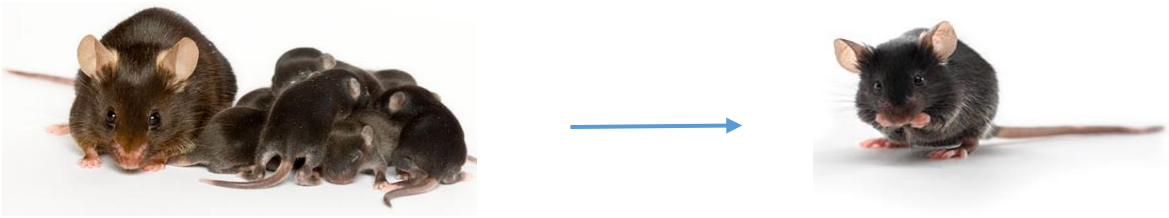

Figure 6: C57B16/JRj Mice growing, on the left PND7 with mother and on the right PND21, adapted from https://www.jax.org/news-and-insights/2017/june/what-happens-when-you-knock-out-a-gene#[3]

At postnatal day 21 mice are weaned (separated from the mothers) and placed with same-sex siblings in one cage. This cage is just a temporary one where the mice will wait until all the pups reach PND21.

When all pups are weaned (meaning they all are at least at PND21), the cage assignment can start. To avoid litter effects pups coming from the same mother are split into different cages, these will then be their final cages.

Figure 7: New cages ready for the cage assignment process

A cage assignment sheet (see figure 8) has to be prepared beforehand and cages should contain a maximum of 5 females or 4 males.

To be able to follow every single animal until adulthood the mice have to be numbered by ear holes (see figure 9) while put into the definitive cage, where they will grow (waiting for the time point at which experiments can be performed with them).

Over the whole paradigm both MSUS and control mice have to be weighed at PND1, 7, 14 and 21 (also see figure 8).

To produce a second generation, first generation (F1) males are mated with naïve primiparous C57B16/J females.

example cage assignment

GROUPED ON: 07.05.14

♂ MALES							
	iRATS #	CAGE	1	2	3	4	5
CON	332237	7	1_1	3_1	6_1	6_4	
	332238	8	1_2	3_2	6_2		
	332239	9	1_3	3_3	6_3		
MSUS		CAGE	1	2	3	4	5
	332240	10	2_1	2_3	4_1	5_1	
	332241	11	2_2	2_4	4_2	5_2	

GROUPED ON: 07.05.14

♀ FEMALES							
	iRATS #	CAGE	1	2	3	4	5
CON	332242	12	1_1	3_1	6_1	6_4	
	332243	13	1_2	3_2	6_2		
	332244	14	1_3	3_3	6_3		
MSUS	iRATS #	CAGE	1	2	3	4	5
	332245	15	2_1	4_1	4_4	5_2	
	332246	16	2_2	4_2	4_5	5_3	
	332247	17	2_3	4_3	5_1	5_4	

weight sheet example

cage nr	Birthday (DAY 0)	DAY 1		weights	weights	weights	weights	weights	weights
1	12.4.14	13.4.14	m	1.82	1.56	1.92			
			f	1.34	1.67	1.43			
2	13.4.14	14.4.14	m	1.82	1.56	1.56	1.92		
			f	1.67	1.43	1.34			
3	14.4.14	15.4.15	m	1.82	1.56	1.92			
			f	1.34	1.67	1.43			
4	15.4.15	16.4.14	m	1.82	1.56				
			f	1.56	1.92	1.34	1.67	1.43	
5	12.4.14	13.4.14	m	1.82	1.56				
			f	1.67	1.43	1.34	1.34		
6	15.4.15	16.4.14	m	1.56	1.92	1.67	1.43		
			f	1.67	1.43	1.34	1.67		

Figure 8: Cage assignment (left) and weighting (right) sheet. Into the cage assignment sheet the first number always stands for the mother whereas the second one for the pup.

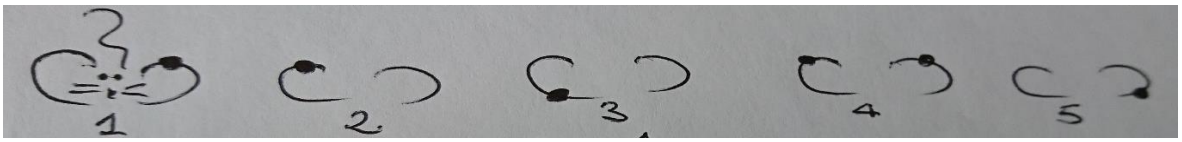

Figure 9: Ear code of mice from 1 to 5 (looking at them from the front)

4. Results

The whole paradigm was performed without any major inconvenience and the weights of the pups were assessed at PND1, 7, 14 and 21 (both MSUS and controls).

Overall the outcome of the breeding was very good. In table 2 and 3 the animal cohorts are shown.

F1	Control	MSUS
M	48	43
F	32	36

Table 2: Numbers of pups from the F1 generation divided by sex and intervention

F2	Control	MSUS
M	48	48
F	55	43

Table 3: Number of pups from the F2 generation divided by sex and intervention

The weight of the pups were assessed at PND1, 7, 14 and 21 and are represented in picture 10 for the F1 and in picture 11 for the F2.

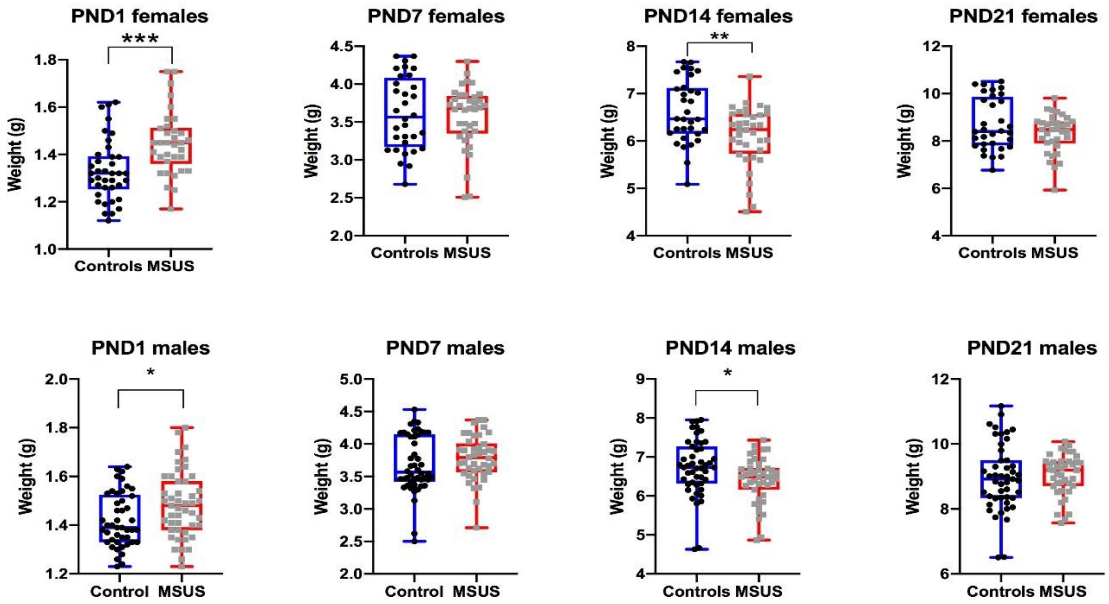

Figure 10: F1 Weights during the paradigm

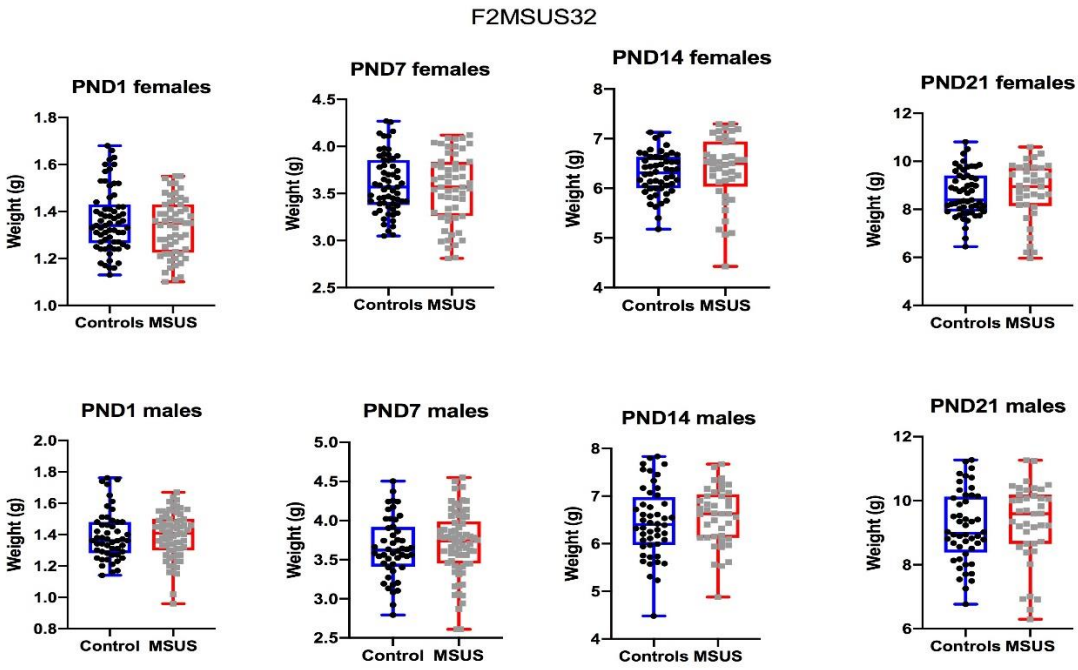

Figure 11: F2 Weights during the first 21 days of life

5. Discussion

Overall both breeding had a good outcome and lot of pups were generated. The data collected during the procedure were the body weights at PND1, 7, 14 and 21 for both genders.

Interestingly, for the F1 breeding already at PND 1 we see a significant difference in body weight between controls and MSUS, which is unexpected because at this timepoint both groups should have similar weight as they have not had any intervention yet.

By random allocation most of the litters containing bigger pups were put into the MSUS group whereas overall the control cages had lighter pups. Due to this, the expected minor change in body weight of the MSUS (as observed in the previous batches produced in the Mansuy lab) is harsh to see and at PND7 and 21 MSUS and controls seem to have comparable body weight values. However, in line with what was previously observed in the Mansuy lab, we do see a significant decrease in body weight in MSUS pups compared to the controls for both males and females at PND 14.

Considering the whole picture, we can anyways assume that the paradigm still had an effect on the body weight of the litters. Taking into account that the difference present at day 1 is no more present at day 7, 14 and 21 we can state that relatively to the birth weight, the controls gained more weight than the MSUS mice during this period of time.

In the unexposed F2 mice, which are the offspring of F1 animals (directly expose to early life trauma) we don't observe any difference in the pup's weights at any time points.

To better assess the success of the paradigm, behavioral and metabolic experiments will be performed on adult F1 mice.

6. Conclusion and Outcome

During this internship I have learned the basics of in vivo experimental procedures and was able to follow from the beginning two breeding and complete a MSUS paradigm. The mice born have now to grow up to 3 months for behavioral as well as metabolic testing.

7. References

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Zurich, September 2020

Arianna Arpagaus

9. Plagiarism

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