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The serotonin transporter and early life stress: do they interact to induce a depressive-like phenotype?

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In the research of Caspi et al. (2003) it was found that in humans the short version of the serotonin transporter linked polymorphic region (5-HTTLPR) and adverse early life stress (ELS) interact to induce a depressive-like phenotype. To study this interaction in animals, animals with a diminished serotonin transporter (SERT) expression can be used. Especially the heterozygous SERT knockout (SERT$^{+/-}$) rodents seem to show similarities to the short version of the serotonin transporter linked polymorphic region. There is a lot of discussion if this SERT x ELS interaction really exists, especially because this interaction is hard to find in rodents. In addition, studies using females are scarce. Therefore the aim of this study is to investigate the possible interaction between the SERT genotype and different early life stressors and to find the right animal model to induce depression by ELS.

Female wildtype (SERT$^{+/+}$), heterozygous (SERT$^{+/-}$) and homozygous (SERT$^{-/-}$) SERT knockout rats were subjected to predictable or unpredictable maternal separation from postnatal day 2-15. From post-natal week 12 behavioural tests were performed to indicate depressive-like behaviour.

Multiple effects were found in the behavioural tests. The social recognition test indicates that SERT$^{-/-}$ rats display higher sociability, social motivation and affiliation compared to the other genotypes in the sociability part of the test. SERT$^{-/-}$ rats also show more depressive-like behaviour in the forced swim test. Though maternal separation affected the genotypes differently, not one consistent effect could be found during the behavioural tests.

The results do not indicate an interaction between the SERT genotype and early life stress in the form of maternal separation. Therefore the link Caspi et al. (2003) found couldn't be recreated.

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Introduction

From the major neurotransmitters, noradrenaline, serotonin (5-HT), acetylcholine, and dopamine in the central nervous system that have been linked to depression, 5-HT seems to have the most eminent role (Vergne, Nemeroff 2006). 5-HT is a modulatory neurotransmitter, which is important for emotion, motivation and cognition. It also has a function in the gut and neuroendocrine system (Murphy, Lerner et al. 2004). The 5-HT homeostasis is regulated by the Na^+/Cl^--dependent serotonin transporter (SERT), which regulates the reuptake of serotonin in the synaptic cleft (Segal, Schenkel et al. 2009).

It is known that when the serotonin system is disturbed that it contributes to the psychopathology of many psychiatric disorders, for example major depressive disorder (MDD) (Andrew, Bharwani et al. 2015). MDD is also known as unipolar depressive order or major depression. The National Institute of Health estimates that in 2016 in the United States 16,1 million suffered from MDD, that's 6,7 % of the US population. The diagnostic criteria for major depression from DSM IV are depressed or irritable mood, decreased interest or pleasure, significant change in weight, appetite, sleep and/or activity, fatigue or loss of energy, guilt/worthlessness, concentration and suicidality (American Psychiatric Association 2013). At least five of these symptoms have to persist for a minimum of 2 weeks to diagnose the patient with depression. MDD can become a life-threatening disease, particularly when a patient has been suffering from severe MDD for a long time. Eventually MDD can even lead to suicide, affecting approximately 800.000 people each year.

One of the most common theories for the pathophysiology of MDD is the 'serotonin hypothesis'. This theory is now almost 50 years old. This hypothesis proposes that a decreased activity of the 5-HT pathways plays a role in the pathophysiology of MDD (Cowen, Browning 2015). The best evidence for a deficiency in 5-HT transmission in patients with MDD is a reduced plasma tryptophan concentration (Cowen, Browning 2015), tryptophan is the precursor for 5-HT. Also decreased levels of 5-HT have been found in the cerebrospinal fluid of depressed patients (Cowen, Browning 2015). However, in healthy participants a reduced plasma tryptophan concentration does not produce symptoms of depression. This suggests that an impaired 5-HT function plays a role in the pathophysiology of depression in some circumstances (Cowen, Browning 2015).

Both genetic and environmental factors, especially very early in life, increase the risk of a MDD. Especially the influence of serotonin transporter gene (*SERT*, 5-HTT, or SLC6A4) variation seems to have its effects on a major depressive disorder. Humans carry a polymorphic region in the promoter region of the SERT gene (5-HTTLPR). This region includes a common 44-base pair insertion/deletion of a repetitive sequence (Homberg et al. 2007). The dominant short (S) allele comprising 14 repeats in the 5-HTTLPR is associated with lower transcriptional efficiency of the SERT and reduced 5-HT uptake. The long (L) allele comprising 16 repeats has higher transcriptional efficiency of the SERT and higher 5-HT uptake compared to the S allele. Furthermore, the L allele is associated with less SERT mRNA in the brain, less SERT binding sites, and a 40 % diminution in the re-uptake of 5-HT in blood platelets (Homberg et al. 2007, Caspi et al. 2003). Also increased amygdala activity and higher anxiety traits are associated with s/s and s/l genotypes (Vergne, Nemeroff 2006).

This polymorphism has been linked to multiple psychiatric disease states (Homberg et al. 2007). It seems that the SERT gene interacts with (early) life events to increase the risk of depression symptoms, diagnosis and suicidality (Caspi et al. 2003). Animal models of developmental psychopathology suggest that even stressful situations early in life are factors for the

development of behavioural and emotional disorders. In humans it was found that mistreated children often develop depression, but not all maltreated children do. Therefore it is extremely important to find the processes and mechanisms that play a role in the development of a depression and internalizing psychopathology in mistreated children (Cicchetti, Rogosch 2014).

Caspi et al. (2003) examined the possible connection between mistreatment and depression, they were the first to report a G x E interaction, specific for the SERT genotype, in depression and suicide. They found that the genetic variation in the polymorphism in the promotor region of the 5-HTT gene plays a moderating role. Adults who carry the S allele show more depressive symptoms and suicidality in response to stressful life events than individuals carrying the L/L allele. Wilhelm et al. (2006) replicated these findings by demonstrating a significant interaction between the 5-HTTPLR genotype and detrimental events in determining the chance of getting MDD in a longitudinal cohort. Furthermore, Kaufman et al. (2004) found that mistreated children with the S/S genotype had higher depression levels, these levels were almost twice as high as the depression levels of children with the S/L or L/L genotypes.

Although a lot publications have been made about the G x E interaction between the 5-HTTLPR variation and the sensitivity of individuals to life events, there were also studies that failed to confirm these findings. For example in the study of Risch et al. (2009) it appeared that the number of stressful life events was significantly associated with MDD, but that there was not a significant link between the 5-HTTLPR genotype and MDD and no interaction between genotype and stressful life events and MDD. Zammit and Owen (2006) also doubt the interaction between the 5-HTTLPR variation and early life stress. In their review they found multiple studies which didn't find this interaction (Surtees, Wainwright et al. 2006, Gillespie, Whitfield et al. 2005).

To study the SERT x ELS interaction animal models are often used, though it appears to be difficult to find a clear interaction. Mother-infant separation may be an evolutionary model for early life stress. Studies often use different durations of maternal separation and therefore the reports on its effects are often varied. Because the duration of the separation differs so much between studies, in this study we will use different models to find the optimal model for early life stress in rats.

Not only MS can have its effects on developing a depression later in life, also maternal care seems to be an important factor. The level of maternal care, which is usually measured by maternal licking and grooming of the pups, could significantly regulate the effect of the 5-HTT knockout mutations on depressive behaviour during the first 2 weeks of life (Carola, Frazzetto et al. 2008). In another study it was shown that adult females that received less maternal licking as pups showed more anxiety-like behaviour in the open field test (Pedersen, Vadlamudie et al. 2011).

Designing an appropriate rodent model of the 5-HTTLPR x life stress risk factor for MDD is not straightforward. Therefore we will use heterozygous, wildtype and knockout SERT rodents, which is comparable to the human situation, as regards to SERT expression. Heterozygous SERT knockout rodents (SERT$^{+/-}$) show similarities to the human S-allele carriers (Carola, Gross 2012, Houwing et al. 2017, Bengel, Murphy et al. 1998).
The aim of this research is to find the optimal combination of the SERT genotype and different early life stressors to create the optimal model for depressive-like behaviour.

Our hypothesis is that we will find an interaction between the SERT genotype and the different early life stressors. For the different maternal separation treatments we expect that the MSUS180 treatment will have the most negative effect. Furthermore, we expect the SERT$^{-/-}$ rats to show an increased level of depressive-like behaviour.

Methods and materials

Experimental animals
Male and female heterozygous SERT knockout rats (F$_0$) were crossbred to create wildtype SERT knockout rats (SERT$^{+/+}$), heterozygous SERT knockout rats (SERT$^{+/-}$) and homozygous SERT knockout rats (SERT$^{-/-}$). The pups were born on postnatal day 0 (PND0). The pups were housed socially in groups of three or four from postnatal day 21. The rats housed together always had the same treatment, but not the same genotype. Rats were given standard laboratory chow from the brand altromin (3.2 kcal/g, 11% fat, 65% carbohydrate, 24% protein) and water ad libitum in a room maintained at 22 ± 1 °C on a 12 h : 12 h light/dark cycle (lights on at 21:00).

Maternal separation
The maternal separation (MS) procedure started on PND2 and stopped on PND15. There were four different groups of maternal separation and one control group (CTR), where the pups were only separated for 15 minutes from their mothers. The MS groups can be seen below:
- MS180: These pups were maternally separated from 09:30 till 12:30 each day.
- MS360: These pups were maternally separated from 09:30 till 15:30 each day.
- MSU180: These pups were maternally separated for 180 minutes at a unpredictable time each day.
- MSUS180: These pups were maternally separated for 180 minutes at a unpredictable time each day. In this group the mothers were stressed during the separation, this consisted of either 20-min restraint in a Plexiglas tube or 5 minutes in the forced swim test at 18 C. This was applied unpredictably and randomly during the MS (Franklin et al. 2010)

During the MS the pups were placed with the whole litter in another room on a heating mat During PND2-8 temperature was set at 32±1° C, while temperature was set at 28±1° C during PND9-15. The number of rats per group and genotype can be found in table 2. The schedule of the MSU/MSUS can be found in table 1 in the supplementary.

	CONTROL	MS180	MS360	MSU180	MSUS180
WT	10	9	9	7	8
HET	9	9	9	9	8
KO	10	9	9	7	8

Table 1. Number of rats per group.

During the maternal separation period the mothers were weighed as well. The pups were weighed once a week from week 1 until week 10.

Maternal Care
The maternal care of 43 mothers was scored for 30 minutes at 09:00, 11:30, 14:30 and 17:00 each day from PND1 till PND16 in the wintertime. When the daylight saving started maternal care was scored at 10:00, 12:30, 15:30 and 18:00, so the time for the rats would stay the same.

Presence of the mothers and the pups on the nest was scored. The behaviours licking and grooming of the pups, self-grooming, eating, drinking, nest building, carrying pups and if the pups were together with their mothers were scored as well.

Behavioral tests

Female offspring (F1) were tested when adult in several behavioral tests for depressive-like behavior. All the tests were performed in a test chamber, away from the other rats. The tests would take place between 09:00 and 18:00 o'clock, during their night cycle, when they would be most active.

Social recognition and sociability test

The social recognition test, otherwise known as the three-chamber test, is widely used as a test to study neuropsychiatric disorders such as depression (Kaidanovich-Beilin, Lipina et al. 2011). Normally a rat would spent more time with an unfamiliar rat over an empty chamber, which indicates normal sociability, social motivation and affiliation. If this doesn't happen it could indicate impaired sociability and thus depressive-like behaviour. Usually when a second unfamiliar rat is added to the other chamber the rest rat tends to spend more time with the new rat, indicating social memory and curiosity for novel experiences. If this doesn't happen it could indicate decreased social motivation and interest for novelty (Kaidanovich-Beilin et al. 2011).

The social recognition test was done in an apparatus (120 x 80 x 40cm, l*b*h), which was separated into one middle chamber and two side chambers (40*80cm). The rooms used for the social recognition test had an illumination of ~2.5 lux and were spared from strong smells and sounds. The three-chambered apparatus was cleaned with 70 % ethanol before and between the social tests. The rats were placed in the middle chamber, with the doors closed. When the doors were opened they could freely explore all 3 chambers for 10 minutes (habituation). Afterwards the rat was put in the middle chamber again with the doors closed. An unfamiliar animal (stimulus 1) then was randomly placed in the left or right grid, next the doors were opened and the test rat could explore again for 10 minutes. This part is also called the sociability part. Thereafter the rat would be placed in the middle chamber again with doors closed. Following the sociability part is social recognition part. Another unfamiliar animal (stimulus 2) was placed in the other grid. When the doors were opened the test animal could explore for 10 minutes.

The stimulus rats were 5 weeks old SERT$^{+/+}$ female Wistar rats obtained from Envigo (TNO Institute, the Netherlands). They were used 2 or 3 times a day, each time in combination with a different stimulus rat.

The performance of the rats was recorded using a digital camera operated by Ethovision version 11.5 (Noldus, Spink et al. 2001). Which acquired the following variables, total distance travelled and time and spent in each room and the time spent interacting with the stimulus rats.

Sucrose preference test

The sucrose preference test is used as an indicator of anhedonia, which can be a symptom of depression (Winderbaum Fernandez, Grizzell et al. 2014).
In week 15 the rats were transferred to individual cages, with two bottles, to test the sucrose preference. The rats were habituated for four days with water. On the fifth day the sucrose preference test would start. There were four sucrose preference testing days alternated with water days. Each sucrose preference testing day would have a different sucrose solution with increasing sucrose percentages. The different sucrose percentages were 0.25%, 0.5%, 0.75%

and 1%. Sucrose and water bottles were placed randomly assigned to a side of the cage, which alternated on each sucrose day to prevent a preference to one side of the cage.

Forced swim test

The forced swim test (FST) was executed following the procedure of Porsolt et al. (1978). Porsolt, et al. (1978) were the first to use the FST to screen for andidepressiva. They found that when rats with antidepressants were less immobile then rats without the antidepressants.

The test is divided in to two sessions, separated over two days. In the first session, the habituation part, each rat was placed in a cylindrical glass tank (18 cm), containing 30 cm of water of 22 (±1) °C, for 15 minutes. Afterwards the rats were placed on a heat mat to dry. The second session, the test phase, took place exactly 24 hours later then the first. The test phase takes 5 minutes.

Four types of behaviour were scored:
1. Immobility – rats were rated as immobile when they would float passively in the water;
2. Swimming – rats were rated to be swimming if they were making swimming movements and held their heads above the water;
3. Climbing – rats were rated to be climbing when they were actively trying to get out of the cylinder by crawling against the walls with their forepaws and pattering with their hind legs in the water;
4. Diving – rats were rated to be diving when they were swimming actively to the bottom of the cylinder.

The FST was recorded by a camera and then analysed in the The Observer XT 13 (Noldus 1991).

Statistics

Statistical analysis were performed using IBM SPSS version 24.0. First, data was checked for parametric distribution. If non-parametric, data was transformed to fit a normal distribution. Statistical significance was assessed using a two way multivariate ANOVA (genotype x treatment) for the FST and the maternal care followed by a Fishers LSD post hoc to correct for multiple comparisons. For the sucrose preference test a repeated measures ANOVA was used. Significance was set at $p < 0.05$ for all tests. All data are expressed as mean ± SEM.
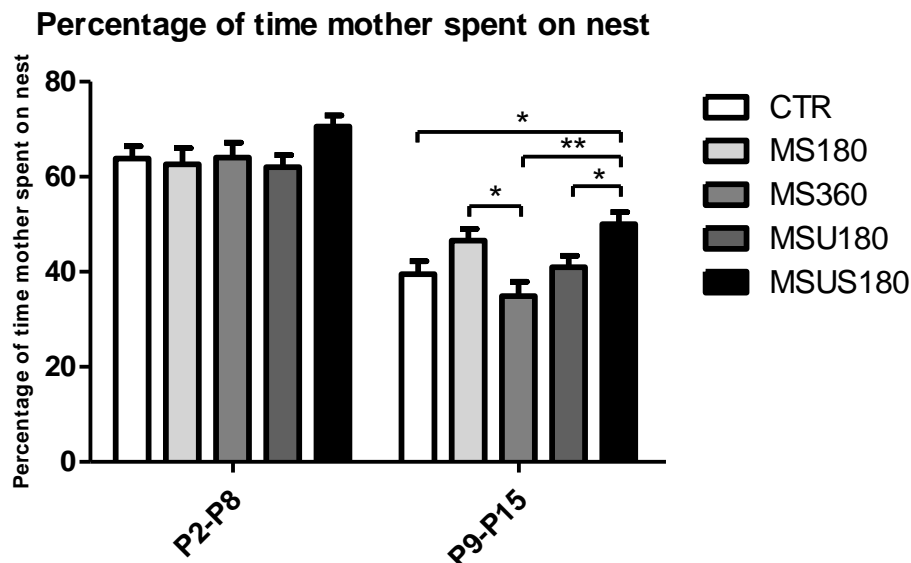
Results

Maternal care

Figure 1. Time mother spent on nest.

Mothers spent more time on the nest in the first week post-delivery (PND 2-8). The overall effect of resulting deficit in maternal care was apparent in the second postnatal week. MSUS180 mothers (n = 9) spent more time on the nest in the second week post-delivery (PND 9-15) compared with the CTR mothers [CTR, n = 9; $F_{(4, 4.249)}$ = 3.729, p = 0.017], the MS360 mothers [MS360, n = 8, $F_{(4, 4.379)}$) 3.729, p = 0.001] and the MSU180 mothers [MSU180, n = 9; $F_{(4, 4.249)}$ = 3.729, p = 0.040]. Furthermore, the MS180 mothers (n = 8) spent more time on the nest the MS360 mothers [MS360, n = 8; $F_{(4, 4.506)}$ = 3.729, p = 0.013]. In the first week post-delivery there was no difference found between the different treatment groups (*figure 1*). There was no significant effect found in any of the other behaviours.

Forced Swim Test

Deletion of the serotonin transporter gene profoundly affected forced swim test behaviour. There was increased time spent immobile in SERT$^{-/-}$ rats (SERT$^{-/-}$; n = 33) compared with SERT$^{+/+}$ rats [SERT$^{+/+}$, n = 38.; $F_{(2, 3.92)}$ = 19,74, p = 0.000] and SERT$^{+/-}$ rats [SERT$^{+/-}$, n = 36; $F_{(2, 3.97)}$ = 19,74, p = 0.000] (*figure 2*). This confirms the assumption that knocking out the serotonin transporter gene induces depressive-like behaviour. No significant treatment effects were found.
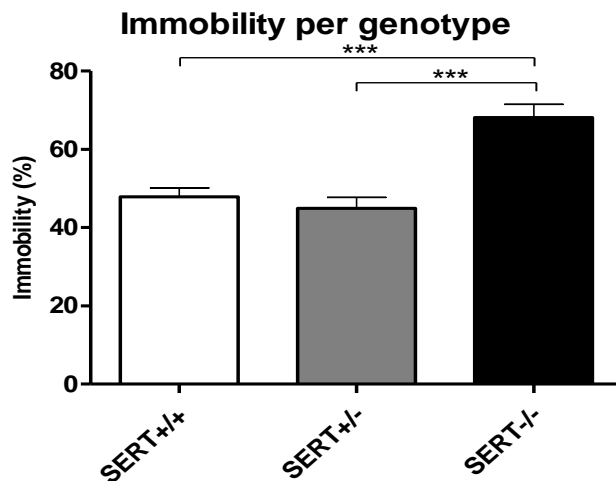
Immobility per genotype

Figure 2. Immobility per genotype in the forced swim test.

In the FST there was increased time spent immobile in the CTR SERT$^{-/-}$ rats (SERT$^{-/-}$; n = 7) vs. CTR SERT$^{+/+}$ rats [SERT$^{+/+}$, n = 10; F(2, 8.84) = 4.05, p = 0.010]; MS180 SERT$^{-/-}$ rats (SERT$^{-/-}$; n = 6) compared with MS180 SERT$^{+/+}$ rats (SERT$^{+/+}$) [SERT$^{+/+}$, n = 9; F(2, 6.43) = 14.91, p = 0.000] and compared with MS180 SERT$^{+/-}$ rats (SERT$^{+/-}$, n = 8; F(2, 6.59) = 14.91, p = 0.000]; and MS360 SERT$^{-/-}$ rats (SERT$^{-/-}$; n = 5) compared with MS360 SERT$^{+/+}$ rats (SERT$^{+/+}$) [SERT$^{+/+}$, n = 6; F(2, 6.22) = 24.92, p = 0.004] and compared with MS360 SERT$^{+/-}$ rats (SERT$^{+/-}$, n = 7; F(2, 6.02) = 24.92, p = 0.000]. This falls in line with the previous results shown in figure 2. There were no differences found in the MSU180 group and the MSUS180 group (*Figure 3*).
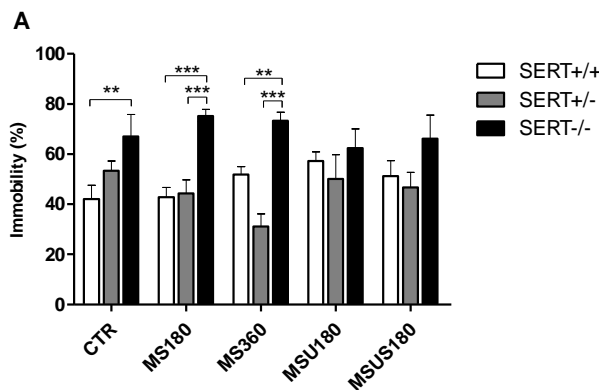

Figure 3. Immobility per genotype per group in the FST.

Therefore the results of the forced swim test show that the SERT$^{-/-}$ rats have an enhanced level of depressive-like behaviour compared with SERT$^{+/+}$ rats and SERT$^{+/-}$ rats.

Sucrose Preference Test
The preference for sucrose solution above water was calculated, as well as the intake of milligram sucrose per gram bodyweight. For the 0.25% sucrose solution, sucrose preference appeared to be significantly higher in the MSU180 treatment group (*n* = 25) compared with the MS360 treatment group [MS360, *n* = 26; F(4, 0.179) = 2.02, *p* = 0.008]. For the 1.0 % sucrose solution, the sucrose preference appeared to be significantly higher for the MSUS180 group (*n* = 24) compared to the MS180 group [MS180, *n* = 27; F(4, 0.137) = 2.241, *p* = 0.039] and compared to the MS360 group [MS360, *n* = 25; F(4, 0.139) = 2.241, *p* = 0.007].

No genotype or genotype*concentration interaction were found. Furthermore, a concentration effect was found. Rats showed a higher preference to the sucrose solution at higher concentrations of sucrose in the solution.
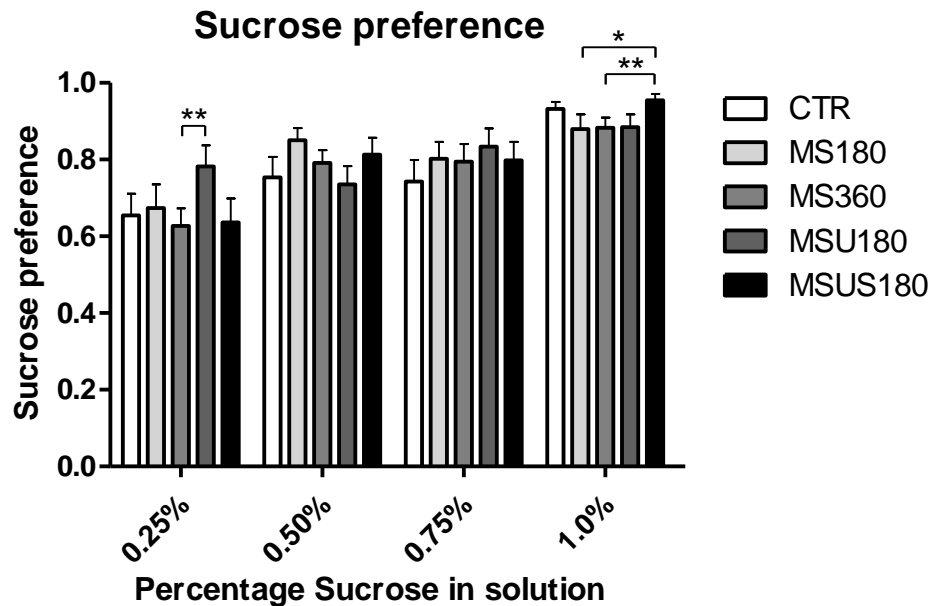

Figure 4. Sucrose preference test.

A significant treatment effect was observed for the mg sucrose per gram rat in the 1% sucrose solution (*Figure 5*). Mg sucrose per gram rat appeared to be significantly higher in the MSUS180 treatment group (n = 25) compared with the CTR treatment group [CTR, n = 26; $F_{(4, 0.165)} = 3.330$, p = 0.003], the MS180 treatment group [MS180, n = 27; $F_{(4, 0.164)} = 3.330$, p = 0.009] and the MSU180 treatment group [MSU180, n = 25; $F_{(4, 0.167)} = 3.330$, p = 0.004]. No significant genotype or genotype*treatment effects were found.
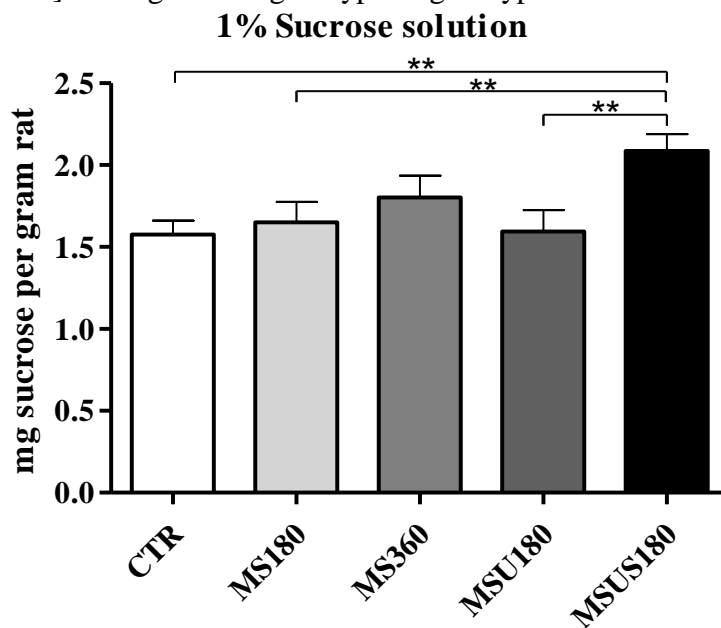

Figure 5. Mg sucrose per gram rat per group in the 1% sucrose solution part of the sucrose preference test.
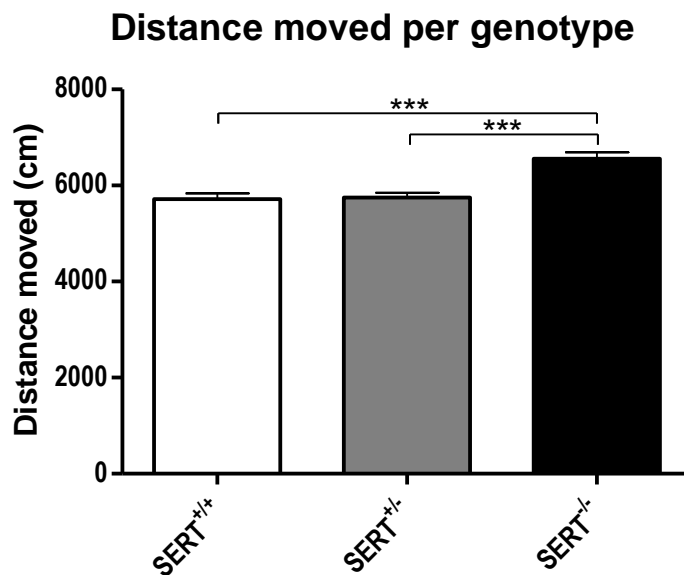
Habituation

Distance moved per genotype

Figure 6. Distance moved per genotype.

A p*ost hoc* showed a significant difference between the SERT$^{-/-}$ rats ($n = 41$) and the SERT$^{+/+}$ rats [SERT$^{+/+}$, $n = 40$; $F(2, 168.801) = 15.867$, $p = 0.000$] and SERT$^{+/-}$ rats [SERT$^{*/-}$, $n = 44$; $F(2, 165.794) = 15.867$, $p = 0.000$].The SERT$^{-/-}$ rats appeared to move significantly more than the SERT$^{+/+}$ rats and the SERT $^{+/-}$ rats, which indicates that the SERT $^{-/-}$ rats are more active (*figure 6*).

Sociability

Sociability was measured by the sociability index (SI), that is, the quotient of time the test rat spent around the novel rat (stimulus 1) divided by the sum of the time spent around the novel rat (stimulus 1) and the empty grid during the sociability session. A post hoc showed a significant difference between the CTR rats ($n = 25$) and the MS180 rats [MS180, $n = 26$; $F(4, 0.042) = 2.125$, $p = 0.026$] and the MS360 rats [MS360, $n = 25$; $F(4, 0.043) = 2.125$, $p = 0.014$] (*figure 7A*). Between the genotypes a significant difference was found between the SERT$^{+/-}$ ($n = 44$) and the SERT$^{-/-}$ rats [SERT$^{-/-}$, $n = 41$; $F(2, 0.033) = 3.694$, $p = 0.008$]. A trend was found between the SERT$^{+/+}$ ($n = 40$) and the SERT$^{-/-}$ rats [SERT$^{-/-}$, $n = 41$; $F(2, 0.034) = 3.694$, $p = 0.096$] (*Figure 7B*). This was calculated by the LSD post-hoc test following the two way ANOVA (multivariate). This could indicate that the SERT-/- rats are more social compared to the SERT+/- rats. No genotype*treatment effects was found.
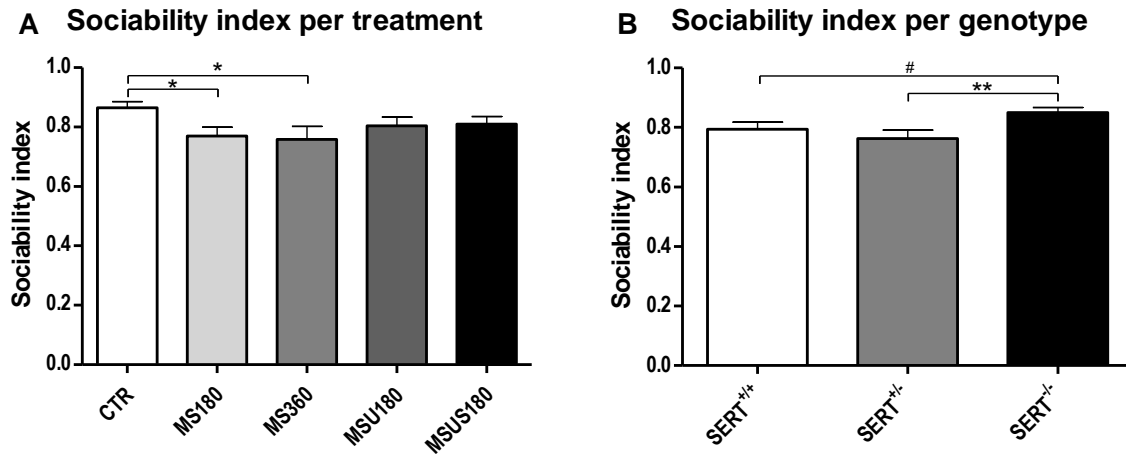
Figure 7. Sociability index per group **(A)** and per genotype **(B)**.

A *post hoc* showed a significant difference between the CTR rats ($n = 25$) and the MS180 rats [MS180, $n = 26$; $F(4, 18.255) = 2.553$, $p = 0.005$], between the MS180 and the MS360 rats [MS360, $n = 25$; $F(4, 18.255) = 2.553$, $p = 0.047$] and the MSUS180 rats [MSUS180, $n = 25$; $F (4, 18.255) = 2.553$, $p = 0.010$] (*figure 8A*). This corresponds to the fact that the CTR also have a higher sociability index compared to the MS180 rats (*figure 7A*). Between the genotypes a significant difference was found between the SERT$^{-/-}$ rats ($n = 41$) and the SERT$^{+/+}$ rats [SERT$^{+/+}$, $n = 40$; $F(2, 14.484) = 11.951$, $p = 0.000$] and the SERT$^{+/-}$ rats [SERT$^{+/-}$, $n = 44$; $F(2, 14.147) = 11.951$, $p = 0.000$] (*figure 8B*). This also corresponds to the fact that the sociability index of the SERT-/- rats is higher than the sociability index of the SERT+/+ rats and the SERT+/- rats (*figure 7B*).

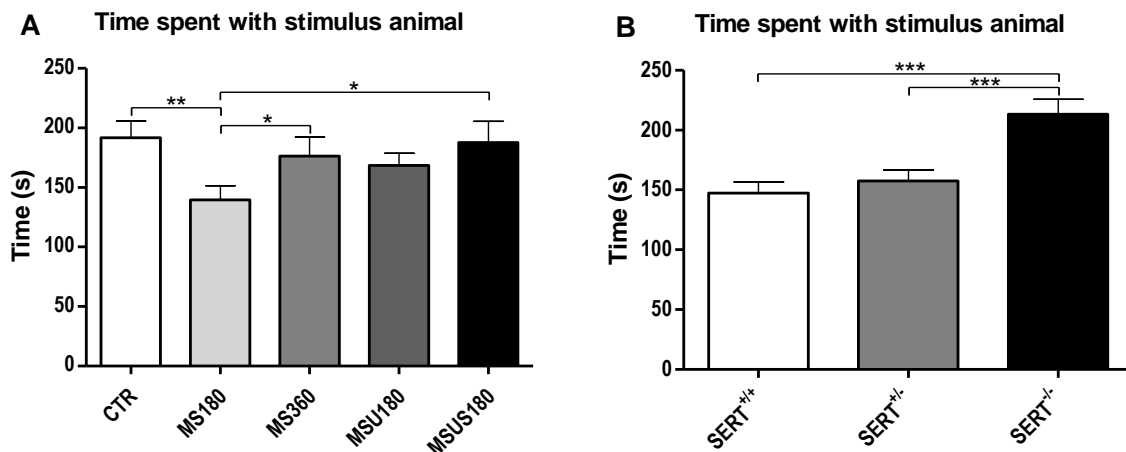*Figure 8.* Time spent with stimulus animal per group **(A)** and per genotype **(B)**.

Social recognition

Social recognition was measured by the recognition index (RI), that is, the quotient of time the test rat spent around the novel rat (stimulus2) divided by the sum of the time spent around the familiar rat (stimulus 1) and novel rat (stimulus 2) during the social recognition session. The recognition index was calculated for the first five minutes of the social recognition session. Rats who behaved out of the ordinary, as indicated by exploring the results in SPSS, were excluded from the results

A one-sample T-test showed a significant difference between 0.5 and CTR SERT$^{+/+}$ rats [n = 9; t(8) = 2.981, p = 0.018], MS180 SERT$^{+/+}$ rats [n = 8; t(7) = 3.092, p = 0.018], MS180 SERT$^{-/-}$ rats [n = 7; t(7) = 4.308, p = 0.004], MS360 SERT$^{+/+}$ rats [n = 7; t(6) = 6.878, p = 0.000], MS360 SERT$^{+/-}$ rats [n = 9; t(8) = 2.852, p = 0.021], MS360 SERT$^{-/-}$ rats [n = 6; t(5) = 3.054, p = 0.028], MSU180 SERT$^{+/+}$ rats [n = 6; t(5) =3.757, p = 0.013], MSU180 SERT$^{-/-}$ rats [n = 7; t(6) = 3.514, p = 0.13], MSUS180 SERT$^{+/+}$ rats [n = 7; t(6) = 2.535, p = 0.044] and MSUS180 SERT$^{+/-}$ rats [n = 9; t(8) = 3.586, p = 0.007] (*figure 9*). If a group has a significantly higher social recognition index than 0.5, it indicates that the group has significantly more interest in the novel rat.
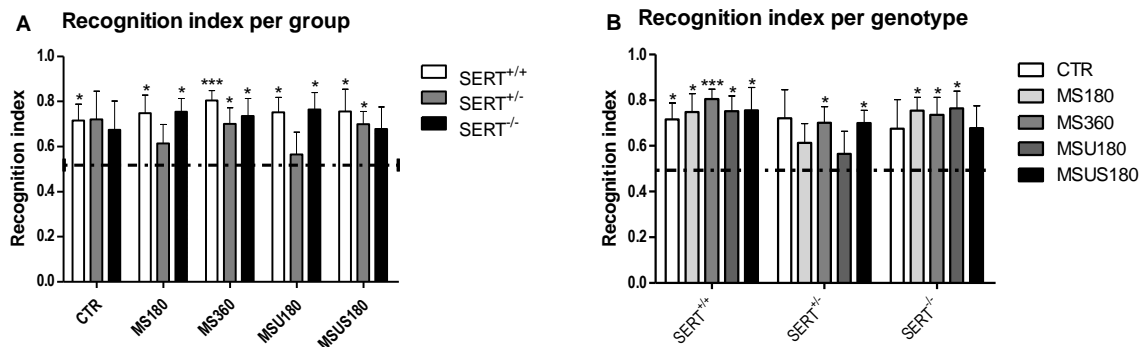

Figure 9. Recognition index per group (**A**) and per genotype (**B**).

A post hoc showed a significant difference between the SERT$^{+/+}$ rats (n = 37) and the SERT$^{+/-}$ rats [SERT$^{+/-}$, n = 42; F(2, 4.621) = 3.221, p = 0.013] in the social interaction with grid 1 (*figure 9*).
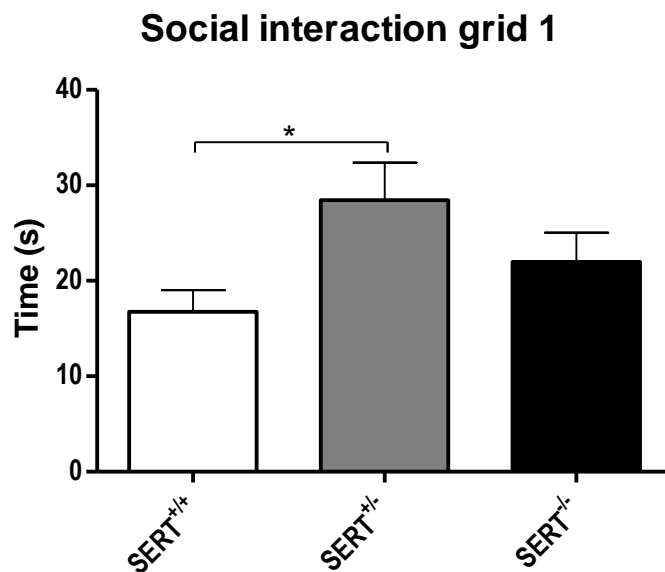

Figure 10. Social interaction with grid stimulus 1.

Discussion

In the present study, depressive-like behaviour in the SERT knockout rat model, with additional focus on early life stress, by means of maternal separation was examined . The results show several contradictory results. The results that were found for the genotype or the treatment or the genotype*treatment interaction do not always point the same way and this leads to a lot of discussion.

In the maternal care the MSUS180 mothers spent more time on the nest in the second week post-delivery compared with the CTR mothers. But the other treatments were not significantly different from the CTR mothers. However, there was a difference between the MSU180 and the MSUS180 rats, this could indicate that stressing the mothers has an effect on maternal care and separating the pups from the mothers doesn't. It could be that MSUS180 mothers spent more time on the nest because they don't know when their pups will return, but this can't explain why the MSU180 mother don't spent more time on the nest.
However, it is interesting that Franklin et al. (2010) found that the MSUS mothers spent significantly more time off the nest and less time on the nest compared to the compared to the control mothers. These results are contradictory with our results. Next to that they found these results in post-delivery week one and they didn't find any differences in post-delivery week two, which is again very contradictory to our results because here we found differences in postnatal week two. These differences could be due to the fact that Franklin et al. (2010) perform the maternal separation on PND 1-14 and in our research maternal separation was performed on PND 2-15. Furthermore, Franklin et al. (2010) measured maternal care three times a day, where we measured four time a day. These small differences could lead to different results.

On the other hand, there weren't any results found of the other maternal behaviours. Also the scoring of the maternal care was found a bit problematic, because the people who scored couldn't see all of the behaviour very well. The rats would often hide in their nests, thereby making it difficult to see them, this way it was difficult to score behaviours like licking and grooming.

In the forced swim test it was observed that, in the different genotype groups, where the results of the treatment groups were being counted together, the SERT$^{-/-}$ rats spent significantly more time in the immobile state compared to the SERT$^{+/+}$ rats and the SERT$^{+/-}$ rats, suggesting depressive-like behaviour. These differences were also seen in the MS360 and the MS180 group, in the CTR group there was only a difference between the SERT$^{+/+}$ rats and the SERT$^{-/-}$ rats. In the MSU180 and the MSUS180 treatment groups there were no differences between the different genotypes. So it seems that the different genotypes lose their effect in the rats with the MSU180 and MSUS180 treatments. An overall treatment effect wasn't found. This is contradictory with the results of Franklin et al. (2010), where they found that the MSUS180 treatment group spent significantly more time in the immobile state compared to the CTR treatment group. But this test was performed in male rats which could explain the difference. Huang and Lin (2006) also state that maternal separation causes rats to spent more time in the immobility state. They did test their rats earlier in life, in post-natal week 4 till week 8, and there maternal separation lasted for only 60 minutes per day.
Dimatelis et al. (2015) who also separated their rats for 180 minutes per day from PND 2-14 stated that female rats are resistant to developing a depressive phenotype induced by MS. They didn't find any difference between the CTR group and the MS180 group in the forced swim test. This is interesting because our research was also performed in female rats and there weren't found any differences between the different treatment groups. So the explanation of

Dimetalis et al. (2015) that female rats are resistant to developing a depressive-like phenotype induced by MS could be an explanation for our results as well.

In the sucrose preference test it was observed that the MS180 and the MS360 treatment groups had a significantly lower sucrose preference for the 1% sucrose solution compared to the MSUS180 treatment group. No differences were found between the CTR group and the other treatment groups. In the article of Shalev and Kafkafi (2002) also no differences were found for the sucrose preference for the 1% sucrose solution between the CTR group and the MS180 group. However, this research was performed in male rats and the maternal separation took place from PND 3-14, these differences make it difficult to compare these results with our results.
No genotype effect was found for the sucrose preference. This was found peculiar, because the expectation was that there would at least be a difference between the SERT$^{+/+}$ rats and the SERT$^{-/-}$ rats. This expectation was based on the article of Olivier et al. (2008), where they found that the SERT$^{-/-}$ rats had a significantly lower preference for the sucrose solution compared with the SERT$^{+/+}$ rats in female and male rats. On the other hand are the results of this research not completely comparable, because Olivier et al. (2008) used higher sucrose percentages for their solutions.

Yet there was found a difference in the mg sucrose per gram rat. Namely, the mg sucrose per gram rat appeared to be significantly higher in the MSUS180 treatment group compared with the CTR, the MS180 and the MSU180 group. This would mean that the MSUS180 group consumed significantly more sucrose than the CTR, MS180 and the MSU180 group. Because in this research a decrease in sucrose intake reflects anhedonia and thus depressive-like behavior, these results would indicate that the MSUS180 group isn't showing depressive-like behavior and do not suffer from anhedonia as was expected. However, in the research of Franklin et al. (2010) they found a contradictory results compared to the results of this research. Namely, it was found that the MSUS180 males consumed less sucrose solution compared to the CTR group. But the females didn't show any differences.
A genotype effect or genotype*treatment effect wasn't found. In the research of Olivier et al. (2008) a genotype effect was found. Namely, sucrose intake was lower in female SERT$^{-/-}$ rats compared with SERT$^{+/+}$ rats, but this result was found at higher sucrose solutions than the sucrose solutions that were used in this research.
These results aren't in line with the results of the forced swim test, where SERT$^{-/-}$ showed more depressive-like behavior compared with the SERT$^{+/+}$ group and the SERT$^{+/-}$ group. In this sucrose preference test there weren't any differences between the different genotypes.

The fact that differences were only found in the 1% sucrose solution and not in the other sucrose solutions, could be blamed on the fact that the other solutions had lower sucrose percentages. It could be that there was no effect because the rats just tasted the sucrose less and therefore had less interest in the sucrose solution. In other researches higher sucrose percentages were used and seemed to have more of an effect (Olivier et al. 2008).

For the habituation phase in the social recognition and sociability test it was found that the distance moved was significantly higher in the SERT$^{-/-}$ rats compared with the SERT$^{+/+}$ rats and the SERT$^{+/-}$ rats. This indicates that the SERT$^{-/-}$ rats are more active. Which can be found peculiar if compared with results for distance moved in other researches. For example, in the research of Olivier et al. (2008) they found no differences in the distance moved between the different genotypes in the open field test in female rats. In the research of Kalueff et al. (2007) it was found that the SERT$^{-/-}$ had a smaller distance moved compared with the SERT$^{+/+}$ group.

For the sociability part of the social recognition and sociability test it was found that the sociability index was significantly higher than 50% for all of the genotypes and all of the treatment groups. The sociability index is, the quotient of time the test rat spent around the novel rat (stimulus 1) divided by the sum of the time spent around the novel rat (stimulus 1) and the empty grid during the sociability session. All the rats had a preference for the social stimulus, which indicates that the all the rats had normal sociability, social motivation and affiliation. However, the CTR group spent significantly more time with the social stimulus compared to the empty grid compared with the MS180 and MS360 treatment group. So it seems here that these treatments do have an effect on the sociability behaviour. Furthermore, the SERT$^{-/-}$ group had a significantly higher sociability index compared with the SERT$^{+/-}$ group. Also a trend was found between the SERT$^{-/-}$ group and the SERT$^{+/+}$ group, in which the SERT$^{-/-}$ had a higher sociability index. These results seem to be comparable with the results of the time the test animal spent with the stimulus animal. Namely, the SERT$^{-/-}$ rats spent significantly more time with the stimulus animal compared with the SERT$^{+/+}$ rats and the SERT$^{+/-}$ rats. These results indicate that the SERT$^{-/-}$ rats show more sociability behaviour. This doesn't follow the expectations. It was expected that the SERT$^{-/-}$ would show less sociability behaviour, because it was expected that they would show more depressive-like behaviour.

These results are also a bit contradictory with the results of Sakakibara et al. (2014). In their research no differences were found between the different genotypes. Though it was found that all the animals spent significantly more time with the stimulus animal compared to the empty grid. However, this research was performed in male rats, which makes it difficult to compare to the results of our female rats.

Furthermore, the CTR group spent significantly more time with the stimulus animal compared to the MS180 treatment group, which is in line with the results of the sociability index as described above. Also the MSUS180 and MS360 group spent significantly more time with the stimulus animal compared with the MS180 group. So the MS180 treatment group seems to show less sociability.

In the social recognition part of the test it was found that the SERT$^{+/+}$ group had a significantly higher social recognition index than 50%. In the SERT$^{+/-}$ group only the MS360 and the MSUS180 group had a higher social recognition index. And in the SERT$^{-/-}$ group only the MS180, MS360 and the MS180 group had a higher social recognition index than 50%. It seems here that the SERT$^{+/+}$ groups have more interest in the novel stimulus animal. The MS360 treatment group also seems to show more interest in the novel stimulus animal, because it has a significantly higher recognition index than 50% in all three of the genotypes.

A research where another social recognition test was performed with SERT knockout rats couldn't be found. But multiple researches indicated that SERT$^{-/-}$ rats show less social behaviour compared with the other genotypes (Kalueff et al. 2010).

Franklin et al. (2010) concluded in their research that unpredictability of maternal separation is essential to produce a lasting effect. This because, otherwise the mothers could adapt on the separation and could anticipate the absence of the pups by providing extra care before and after separation. This way any detrimental effect of the separation could be prevented. To find similar results in this research it would mean that there should be a lot of difference between the MSU(S)180 group and the MS180 or MS360 and CTR group. But no such differences were found.

There were multiple factors in this research that could be interesting for future research to change. In this research only females were investigated, it could be very interesting to look at males. Furthermore, it would be better if the groups were a little bigger, most of them were quite small, around the 8 rats. This could give clearer results. Also all the behavioural tests were executed in the same order for all of the rats. Maybe results would be different if the order of the tests would have been different per rat. Furthermore, this research was split up in two pilots, so half of the rats were born in a different time of the year than the other half. Which of course shouldn't have caused any differences in the results, but it could.

Conclusion

The results do not indicate an interaction between the SERT genotype and early life stress in the form of maternal separation. Therefore, the link Caspi et al. (2003) found couldn't be recreated. Furthermore, no real genotype effect can be found, because the behavioural tests do not show one particular result. The results that were found were often contradictory with the results found in literature. The same applies to the treatment effect, not one clear treatment effect could be found. It is difficult to find an explanation for the results that were found, especially because they are often contradictory with literature. One reason could be that MS is simply not a big enough stressor for the rats and bigger stressors should be used. Or is it like Dimetalis et al. (2015) say that female rats are resistant to developing a depressive phenotype induced by MS and that MS isn't the right way to induce a depressive-like phenotype. So finding a right way to recreate early life stress in rodents would be an important goal for future research.

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