Nurturing nature : testing the three-hit hypothesis of schizophrenia

Daskalakis, N.

Chapter 5

Testing the cumulative stress and mismatch hypotheses of psychopathology in a rat model of early-life adversity

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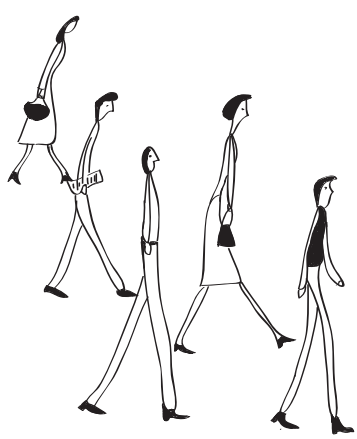

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Abstract

Background. In the present study, we tested both the *cumulative stress* and the *mismatch* hypothesis of psychopathology. For this purpose the combined effects of early-life adversity and later-life stress exposure on behavioral markers of psychosis susceptibility were studied in male Wistar rats.

Method. Experiment IA: rat pups divided on the basis of the levels of their maternal care experience in low, medium or high maternal care groups, were reared post-weaning in groups and tested at adulthood under basal conditions or after an acute corticosterone (CORT) administration. Maternal care levels were assessed by measuring the dam's licking and grooming (LG) the first postnatal week of life.

Experiment IB: another group of high, medium and low maternal care rat pups were reared post-weaning in social isolation and tested at adulthood under basal conditions.

Experiment II: rat pups exposed as neonates to daily sessions of 8 hours of maternal separation (MS) on postnatal days 3, 4 and 5 either altogether in their home cage (HOME SEP) or alone in a novel environment (NOVEL SEP), were reared post-weaning in groups and tested at adulthood under basal conditions.

Adult testing included behaviors marking psychosis susceptibility: apomorphine-induced gnawing (APO-gnawing), acoustic startle response and its modulation by a prepulse stimulus (PPI). The behavior of the Medium LG offspring was used as baseline reference for all the three experiments.

Results. Experiment IA: Low maternal LG history alone had limited effects on the behavior of Wistar offspring, although increased acoustic startle and increased PPI, at high prepulse intensity levels, were observed. An injection of CORT in the adult Low LG offspring, increased APO-gnawing and reduced PPI, whereas it was not effective in High LG offspring. These findings support the *cumulative stress* hypothesis.

Experiment IB: If Low maternal LG history was combined with post-weaning social isolation, APO-gnawing was decreased and PPI increased, compared to High LG and Med LG offspring. This reflects attenuated psychosis susceptibility. High LG offspring reared in isolation displayed, however, the highest APO-gnawing and the lowest PPI levels among rats reared in social isolation, which is indicative for increased psychosis susceptibility. These findings support the *mismatch* hypothesis.

Experiment II: MS increased psychosis susceptibility only NOVEL SEP rats that had experienced MS in the context of early social isolation. These individuals displayed increased adult APO-gnawing and reduced PPI, if reared post-weaning in a condition that did not *match* with their early life social environment (i.e. group housing). This finding supports the *mismatch* hypothesis.

Conclusion. The outcome of environmental manipulations on developmental programming of psychosis susceptibility depends on the interplay of early-life adversity and later-life stressors in a manner that can support either the *cumulative stress* or the *mismatch* hypothesis.

1. Introduction

Schizophrenia is a complex mental disorder often characterized by breakdown of

thoughts and a loss of contact with reality. Genetic risk-factors are clearly involved in the disease pathogenesis, and also the role of non-genetic/ environmental factors is established. The perinatal and pre-pubertal period are important time windows for the programming of psychosis susceptibility by the non-genetic factors [1]. This notion is reinforced by epidemiological studies showing an association of adversity of both early-rearing environment and later-life psychosocial stress with greater psychosis risk [2, 3].

Research on the role of these environmental factors in animal models for psychopathology has resulted in a wealth of data over the past 50 years [4, 5]. Although the data are not always consistent [6] the consensus is that increased adversity of the maternal environment is predictive of a vulnerable phenotype. Such phenotype has characteristics ranging from depressive-like behavior and enhanced stress reactivity to schizophrenia-like behavior and increased dopamine (DA) sensitivity [7, 8]. According to the "*cumulative stress*" hypothesis stressful experiences, acute or chronic, in later life will add to the already programmed vulnerability by early-life adversity [9, 10].

Using the postnatal variations in Licking and Grooming (LG) in rats as paradigm for the extent of early-life adversity, Champagne and colleagues found that, at adulthood, the Low LG offspring showed impaired hippocampal plasticity and poor memory performance. However, enhanced synaptic plasticity and better memory performance were observed in situations where the adult Low LG offspring experienced stressful or high CORT conditions. The opposite results were found when High LG offspring was exposed to stress or high CORT. These findings supported the "*mismatch*" hypothesis [11].

Also Ellenbroek & Cools found evidence for the *mismatch* hypothesis in schizophrenia endophenotypes. They showed decreased prepulse inhibition of acoustic startle (PPI) in rats that had been exposed as pups to 24 hours of maternal deprivation (24h-MD) on postnatal day (pnd) 9. However, if the maternally deprived rats were reared in social isolation, the 24h-MD did not lead to a decreased PPI [12]. Choy and van den Buuse also used the 24h-MD paradigm as an adverse early-life condition and, in an attempt to mimic chronic stress in later life, CORT was administered post puberty. They showed, at adulthood, in these rats less disruption of PPI by apomorphine or amphetamine, which suggests less psychosis susceptibility [13-15]. This programming of the PPI response is an example of the outcome of developmental phenotypic plasticity, which evolved to *match* an organism to its expected future environment. According to the "*mismatch*" hypothesis, as opposed to the *cumulative stress* hypothesis, a *mismatch* between the expected and the actual environment, predicts a maladaptive phenotype and enhanced risk for disease [16].

In this study, we tested both the *mismatch* and the *cumulative stress* hypotheses in the development of psychosis susceptibility induced by early-life adverse experiences. For this reason, we designed the following experiments: (i) In experiment IA, we combined early maternal environment with stress hormone exposure at adulthood.

We used the "naturally occurring variations of LG" paradigm that was previously shown to influence at adulthood the stress response and cognitive functions as well as psychosis-susceptibility [17-19]. Since previous research suggested a link of circulating corticosterone (CORT) levels with psychotic behavior [20, 21], the acute effect of the stress hormone on the phenotype of the different LG groups was investigated.

In experiment IB we measured, in the adult, the outcome of the exposure to maternal environment combined with post-weaning social isolation. Isolation rearing is a well characterized and validated post-weaning psychosocial stressor that precipitates behavioral disruptions comparable to the ones seen in schizophrenia, including PPI deficits, cognitive impairments and social dysfunction [22-25]. We hypothesized that early maternal environment interacts with later social environment: in case of a "*match*" (Low LG offspring in social isolation), psychosis susceptibility would decrease, and in case of a "*mismatch*" (High LG offspring in social isolation), it would increase.

In experiment II, we measured the influence of early social isolation in a novel context on the interaction between maternal separation (MS) and later social environment. We hypothesized that only if the pups are placed individually in a novel context during maternal absence, the enhanced experimentally-induced adversity will reveal a "*mismatch*" with a later group-housing condition, resulting in increased psychosis susceptibility. For this purpose, we used a modified version of the postnatal repeated MS-paradigm (8 hours/day on pnd 3,4,5), which we developed recently [26]. Rat pups stayed during maternal absence either in the home cage altogether with their siblings or they had the experience of being single away from peers in a socially isolated novel environment. Both separation conditions resulted in low levels of maternal LG [30].

2. Materials and Methods

2.1 Animals.

Wistar rats (originally obtained from Harlan, Horst, The Netherlands & Taconic Europe, Ejby, Denmark) were used in this study and housed in our animal facility under an 11:13 h light/dark cycle (lights on at 08.30 h, illumination inside the cage: 20-30 lux, temperature: 20 ± 1 °C, relative humidity: 60 ± 10%) and low volume background noise (40 dB). Food (RM3, Special Diet Services, Witham, Essex, UK) and water (containing 0.02% HCL) was ad libitum. Upon arrival males and females were housed in groups of 3-4 in macrolon-polycarbonate type IV cages (L60 x W38 x H20 cm) with wire lid. Each cage contained sawdust as bedding and tissue. These cages were also used for breeding following a one-week habituation period. From pnd 1-10 cages were not cleaned. From pnd 11, the cages were weekly changed.

Animal experiments were approved by the Local Committee for Animal Health, Ethics and Research of Leiden University and carried out in accordance with European Communities Council Directive 86/609/EEC.

2.2 Breeding.

Two or three females of the F1 generation were housed together for at least a week and then

mated with a male. After 10 days, the females were housed individually (macrolon-polycarbonate type III cages with wire lid; L42.5 x W26.6 x H18.5 cm; containing sawdust and two sheets of paper towels for nest material). We checked for litters daily at 19:30 h starting from 20 days after the start of breeding. If litters were present, the day of birth was defined as pnd 0 for that litter. On the day after parturition, pnd 1, each litter was culled to 8-10 healthy pups (males:females = 1:1).

2.3 Maternal care (pnd 1-7).

We assessed maternal behavior from pnd 1 to 7. The maternal behavior of each dam was observed and scored for five periods of 60min per as described previously [26-28]: at three periods during the light phase (10:00, 13:30, and 17:00 h) and two periods during the dark phase (07:30 and 19:30h; under 2x60 W red TLD-light). Note that the observation at 17.00 h at the day of maternal separation was the time of dam's re-union with the pups.

The behavior of each mother was scored every 3min (20 observations per period, 100 observations per day): pup retrieval, maternal contact, licking and grooming (LG), passive nursing posture, away from nest, nest building, burying, arched-back nursing [(passive) low arch/ blanket nursing, (active) low arch, middle arch, high arch]. Non-maternal care behaviors of the dam were also recorded: eating, drinking, chasing tail, self-grooming, digging, and sleeping. Litter conditions were also noticed: split litter, buried pups. We analyzed the percentage of observations in which the dam displayed each behavior.

2.4 Body weight.

Body weight pre-weaning (pnd 1-21) was measured with an electronic precision scale (MXX-2001, Denver Instrument, Göttingen Germany; readability 0.1 g, linearity 0.2 kg).

Body weight post-weaning was measured with an electronic precision scale (Access C 13 AB, Precia Molen, Breda, The Netherlands; readability 1 g, linearity 3 kg) during every weekly cage cleaning.

2.5 Eye-opening.

From pnd 11 onwards, any degree of eyelid separation in any of the pups of a litter was scored as a positive eye-opening for the litter. The observations took place at 11:00 hours. Pups were not removed from the nest during the observation so that the litters were not disturbed.

2.6 Chemicals.

Injections were prepared and preserved on low temperature (4oC). Injections were given subcutaneously (SC) in a volume of 1 ml/kg. Apomorphine (APO; APO-HCL: Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands) was dissolved in MilliQ (0.02% ascorbic acid). Corticosterone (CORT; 45mg Cort-HBC containing 66.7mg/g CORT; Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands) was dissolved in MilliQ (0.02% ascorbic acid). The dissolvent served as vehicle (VEH).

2.7 Experimental design.

2.7.1 Experiment IA – Assessing the impact of naturally occurring variations in the maternal environment on adult phenotypes tested in basal conditions and acute CORT conditions (Fig. 1A). In order to investigate the effects of variations in naturally occurring maternal environment on psychosis susceptibility under basal conditions and after CORT exposure at adulthood, two

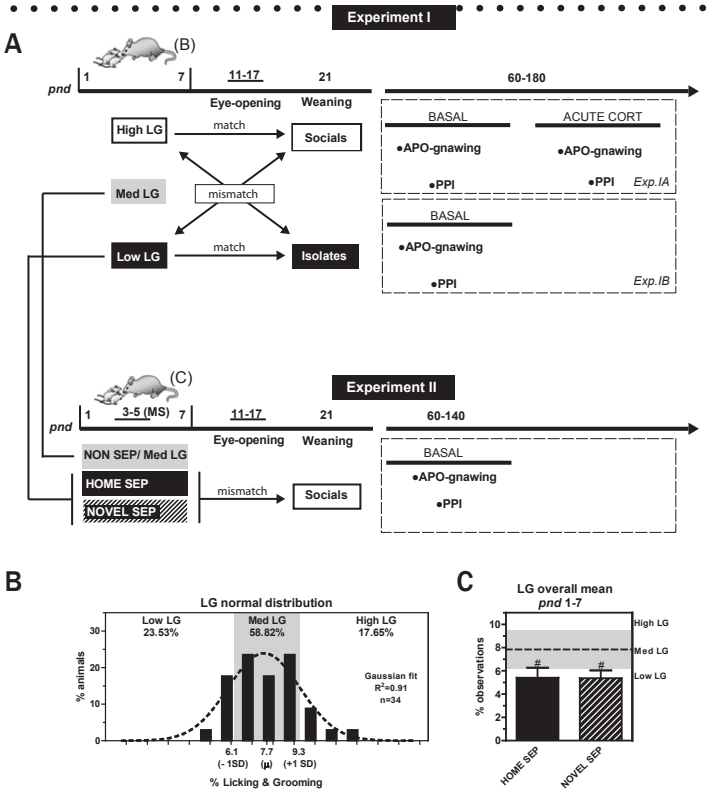

Figure 1. (A) Graphical representation of the experiments. Experiment IA & IB: Time line of longitudinal study. Maternal care was observed at postnatal days (pnd) 1-7. Litters were divided to three treatment groups: rats with maternal care history of High Licking & Grooming (High LG), Med LG or Low LG. On pnd 11-17, we observed the day of eye-opening. Weaning happened at pnd 21 and rats were housed either in groups (socials; Exp IA) or isolation (isolates; Exp IB). Note that High LG offspring were in a "*match*" condition when housed post-weaning in groups (Exp. IA) and in a "*mismatch*" condition when housed in isolation (Exp. IB). Similarly, Low LG offspring were in a "*mismatch*" when housed in groups (Exp. IA) and in a "*match*" condition when were housed post-weaning in isolation (Exp.IB). In the period of pnd 60-180 the behavioural testing was performed that included: (i) apomorphine-induced gnawing test (APO-gnawing) and prepulse inhibition of acoustic startle test (PPI) in basal condition & after acute CORT administration (Exp. IA), (ii) APO-gnawing and PPI in basal conditions (Exp. IB). Experiment II: Time line of longitudinal study. Litters were divided to three treatment groups: Treatment groups: non separated (NON SEP/Med LG) had no previous history of treatments, repeatedly separated pups were exposed to 8h-MS on pnd 3 and pnd 4 in a home (HOME SEP; home separated) or novel context (NOVEL SEP; novel separated). Maternal care was observed in the pre & post reunion periods (depicted with the design of the dam with the pups). On pnd 11-17, we observed the day of eye-opening. Weaning happened at pnd 21 and rats were housed in groups (socials). Note that HOME SEP and, especially, NOVEL SEP offspring were in a "*mismatch*" condition when housed post-weaning in groups. In the period of pnd 60-140 the behavioural testing was performed that included PPI test (in basal conditions) and apomorphine-induced gnawing test (in basal conditions).
(B) Frequency distribution of LG% of Experiment IA & B (Cohort 1: n=34). On top of the frequency distribution a normal distribution is superimposed. Those two distributions display a high Gaussian fit (R^2 =0.91). The mean of the population is indicated as well as the LG levels 1SD under and 1SD over the mean. These levels were used as cutoffs to divide the population in 3 groups: Low LG (23.53% of dams), Med LG (58.82% of dams) and High LG (17.65 % of dams).
(C) LG% average over pnd 1-7 of separated litters of Experiment II. Maternally separated litters had experienced 3 times 8h-MS on pnd 3, 4 & 5 in home (HOME SEP; n=6 dams) and novel context (NOVEL SEP; n=6 dams). The equivalent levels of High LG, Med LG and Low LG litters from Cohort 1 (Experiment I) are denoted. Data represented MEAN±SEM. Significance level was set at $p \leq 0.05$. # vs. High LG levels.

large cohorts of females were bred and after parturition were characterized for LG according to previously described methodology (see section 2.3 for maternal care scoring and Ref. [11, 27] for a detailed description of the LG paradigm). Dams were sorted into LG groups according to the following criterion (Fig. 1B): < one SD below the mean of the whole group (Low LG), between one SD below and one SD above the mean of the whole group (Medium LG, i.e. Med LG), and > one SD above the mean of the whole group (High LG) [11, 27].

Cohort 1 (n=34; LG: 7.71 ± 0.27 % observations): Using the LG data from pnd 1-7, we were able to obtain 6 High LG litters (17.65%) with a mean LG of 10.05 ± 0.36 (% observations), 20 Medium LG (58.82%) with a mean LG of 7.84 ± 0.18 (% observations), and 8 Low LG litters (23.53%) with a mean LG of 5.66 ± 0.09 (% observations). Cohort 2 (n=24; LG: 8.47 ± 0.39 % observations): Using the LG data from pnd 1-7, we were able to obtain 6 High LG litters (25.00%) with a mean LG of 9.97 ± 0.22 (% observations), 13 Medium LG (54.17%) with a mean LG of 8.11± 0.16 (% observations), and 5 Low LG litters (20.83%) with a mean LG of 5.82 ± 0.29 (% observations).

Weaning of the pups from the dams happened at pnd 21. From that day and until testing, three male rats of every litter were housed together (referred to as "socials") in Type IV cages. Note that High LG offspring were in a "*match*" condition when housed post-weaning in groups, whereas Low LG offspring were in a "*mismatch*" when housed post-weaning in groups. Adult rats were tested in basal conditions and acute CORT condition in apomorphine-induced gnawing and prepulse inhibition of acoustic startle (see section 2.10 for behavioral protocols).

2.7.2 Experiment IB – Assessing the lasting impact of naturally occurring maternal environment combined with post-weaning social isolation on adult phenotypes tested in basal conditions (Fig. 1A). In order to investigate the effects of the combination of High, Med and Low maternal LG history with post-weaning social isolation on psychosis susceptibility under basal conditions, the offspring of the various LG groups was used (rat cohorts as described in 2.7.1) housed post-weaning in social isolation. At the day of weaning (pnd 21), one or two males of each litter (characterized for maternal care) were isolated and placed individually (referred to as "isolates") in Type III cage until testing. This is the commonly used isolation rearing procedure [22]. Note that High LG offspring were in a "*mismatch*" condition when housed post-weaning in isolation, whereas Low LG offspring were in a "*match*" when housed post-weaning in isolation. Adult rats were tested in basal conditions in apomorphine-induced gnawing and prepulse inhibition of acoustic startle (see section 2.10 for behavioral protocols).

2.7.3 Experiment II – Assessing the lasting impact of experimentally-induced adverse maternal environment combined with early-postnatal social isolation on adult phenotypes tested in basal conditions (Fig. 1A). We investigated the effects of the combination of repeated-MS and isolation from peers on psychosis susceptibility in basal conditions. For this we used our recently developed repeated MS paradigm (i.e. HOME SEP & NOVEL SEP) [26]. MS occurred on pnd 3, 4 & 5; lasting 8h each. Litters were randomly distributed to the two experimental conditions.

Dams' transfer from the litter ("Dam out"). At 9:00h, dams selected for MS were removed from their cage ("home" cage), placed in a cage of the same type and transferred to an adjacent room ("dams" room). In the "dams" room, the environmental conditions were similar except the lighting intensity was higher (illumination inside the cage: 50-60 lux).

Separation procedure. After the dam was relocated to a new cage, litters were kept without any food or water available for 8h (9:00 to 17:00h). The home cage was placed on heating pads (33–38

°C; TM 22, Beurer, Ulm, Germany) to maintain the body temperature of the pups. To acquire the desired temperature, heating pads were turned on 30min prior use.
We used two following separation contexts:
"Home separation" (HOME SEP). The pups remained in their familiar environment (housing room, home cage) together with their littermates.
"Novel separation" (NOVEL SEP). The pups were moved to an adjacent unfamiliar room, with similar conditions as the housing room. Pups were additionally isolated from peers in new clean cages (Type II macrolon-polycarbonate, containing fresh sawdust bedding, divided in compartments of 18 x 20 x 14 cm) and placed on heating pads. The pups housed singly in this novel context, experienced the absence of their dam, the home cage environment and lacked contact with their littermates (isolation).
Reunion ("Dam back"). At 17:00h, the pups were returned to their home cage followed by their dams. Dams of separated pups in home and novel contexts were reunited with their litter at the same time.
Weaning of the pups from the dams happened at pnd 21. From that day and until testing, three male rats of every litter were housed together (referred to as "socials") in Type IV cages. Note that HOME SEP and, especially, NOVEL SEP offspring were in a "*mismatch*" condition when housed post-weaning in groups. Adult rats were tested in basal conditions in apomorphine-induced gnawing and prepulse inhibition of acoustic startle (see section 2.10 for behavioral protocols).

2.8 Common control group (Med LG).
Med LG offspring that were housed post-weaning in groups were used as controls in all experiments. Therefore their values were used as reference values for the other groups. Data are presented as % of controls. In Experiments IA and IB, the control group was named Med LG (in order to emphasize their Medium LG levels the first week of life) and was compared to High LG and Low LG. In Experiment II, the control group was named NON SEP/ Med LG (in order to emphasize that they don't have any maternal separation history) and was compared to HOME SEP and NOVEL SEP.

2.9 LG levels across experiments (Fig. 1C).
In Experiment II, HOME SEP and NOVEL SEP dams displayed the same levels of LG over the first week. We compared them with the average of High LG and Low LG dams of Experiments IA & IB. The mean LG of dams of Experiment II is in the range of the Low LG of Experiments IA & IB and statistically different only from the High LG dams (HOME SEP vs. High LG: $t10=4.98$, $p\leq0.001$, NOVEL SEP vs. High LG: $t12=6.16$, $p\leq0.001$).

2.10 Psychosis susceptibility behavioral parameters.
We defined as increased psychosis susceptibility increased stereotypic gnawing response to apomorphine and reduced sensorimotor gating.
2.10.1 Apomorphine-induced gnawing (APO-gnawing)
Rats given psychomimetic drugs often exhibit loco-motor hyperactivity, and at higher doses they might exhibit stereotyped/ perseverative behaviors [29-31]. In order to assess perseverative behavior in our rat populations, we measured the behavioral response to an injection of a large dose of APO using the so-called gnawing box [30, 32].

Apparatus. The gnawing box was slightly modified from the box originally described by Ljungberg and Ungerstedt [30]. It consisted of a Perspex hole-board (L69 x W69 x H25 cm) with a central cubicle (L25 x W25 x H25 cm). The board contained 32 holes (diameter approx. 3cm), each of which is surrounded by five concentric ridges. A microphone was placed underneath the central cubicle to allow registration of sounds. Stereotypic gnawing on the ridges surrounding the holes produced a characteristic sound that was detected by the microphone, fed into the computer and scored as a gnawing count [32, 33].

Procedure. The procedure followed for the APO-test was described before [32]. Briefly, after 60min of habituation period in the room of the gnawing box with food and water ad libitum (individually placed in type III cages), the rat was given a SC APO injection (dose: 1.5mg/kg). Immediately after injection the rat was put in the gnawing box (facing the front right corner) and measuring of gnawing lasted 45min. After the test, the rat was transferred back to its home cage.

Testing conditions.

Basal conditions (1st time – Experiment IA, IB & II): Rats were weighed in the housing room and transferred to the testing room containing the gnawing box.

Acute CORT condition (2nd time – Experiment IA): From our previous work we knew that rats APO-gnawing performance is stable across time [34]. Therefore, the rats were tested in exact the same way for a 2nd time at least one month after the 1st time, but the 2nd time they were pretreated with CORT (SC, dose: 3mg/kg) one hour prior the test in the beginning of the habituation period.

Measurements. The total number of gnawing counts per 45min was recorded for each rat. Data are presented either as the average gnawing counts of a given experimental group or as a population distribution of gnawing response into 3 sub-groups: no gnawing response (<10 counts/45min), a very intense gnawing response (>500/45min) and an intermediate response (11-500/45min) [32, 33, 35].

Note. Apomorphine susceptibility depends importantly on genetic background [32, 35, 36]. Therefore, in Experiment IA, we used the same rats both in the "1st time-no CORT pretreatment" condition and in the "2nd time-CORT pretreatment" condition in order to evaluate the effect of acute CORT, independent of the genetic background. In Experiment IB, we used rats coming from the same litters with the only difference the post-weaning environment in order to evaluate the effect of the combination isolation rearing with maternal care history independent of the genetic background. In Experiment II, since we used animals from different litters in the three conditions, we recognize that a percentage of the APO-gnawing variance reported could be due to a random genetic variation between the different experimental groups (NON SEP/ Med LG, HOME SEP and NOVEL SEP).

2.10.2 Sensorimotor gating

Apparatus. PPI measurements were performed in four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) consisting of a Plexiglas tube (diameter 8.7 cm, length 20.5 cm) attached on top of a piezoelectric accelerometer platform, which detected and transduced the movements of the rat. A speaker above the tube presented the acoustic pulses. Calibration of all of the four chambers was done with a calibration device and protocol provided by the manufacturer and adjustments of the chamber speakers were done daily with the help of decibel-meter (; dB[A] scaling was used).

PPI protocol. The PPI protocol was based on previously described rat protocols [37] and specifically on the protocol used before with apormorphine susceptible and unsusceptible rats [38]. Rats

were placed individually in the apparatus and the 17min testing protocol started. First, 5min of acclimatization were given with a background noise of 70 dB. Second, the PPI protocol started and consisted of 3 parts. The protocol started with a startle block of 6 pulse alone trials and ended with a startle block of 5 pulse alone trials. In pulse alone trials we used a pulse of 120 dB[A] for 40 ms. The main (middle) part of the protocol consisted of 39 trials: 10 pulse alone trials, 20 prepulse-pulse trials (5 trials for each of the four different prepulse intensities; prepulse intensities: 72, 74, 78, 86 dB[A] for 20 ms), 5 prepulse alone (86 dB[A] was only used) and 4 no-stimulus/background trials. These 39 trials were given in pseudorandom order; preventing two identical trials following each other and ensuring that the interval between two consecutive trials was different (mean intertrial interval duration 15 sec). The prepulse to pulse onset duration was stable at 100 ms.

Testing conditions. Basal conditions (Experiments IA, IB & II): Rats tested in baseline conditions were transferred in their home cage into the testing room with the startle chambers and allowed a 45min habituation period in the room. After PPI testing, they were weighted and then returned to their respective cages.

Acute CORT condition: Animals when tested after drug administrations (SC injections) were weighted before PPI testing, because injection volume was calculated using the body weight. Rats were weighted in the housing room and transferred individually in Type III cages to the testing room to habituate for 45min. Rats were SC injected with VEH, CORT (at dose 3 mg/kg) and APO (at dose 0.5 mg/kg). The three littermates were randomly subjected to one of the three injections. After injection, rats were put back in their cages for 5min, then into the startle chambers and the PPI protocol started. Note that injections happened 10min before PPI testing (counting additional 5min of acclimatization period as part of the PPI protocol). VEH and APO injections were the control conditions for the CORT injection since VEH was expected not to disrupt PPI (negative control) and APO was expected to disrupt it (positive control).

Measurements. The startle response after each trial is calculated by the software and the unit of measurement is Volts. The software was set to gather 1000 samples per sec for a sampling period of 100msec after the onset of the pulse. A Vmax is determined and Vaverage (Vavg) is calculated for the whole 100msec period. We used Vmax as more accurate since Vavg depends on the duration of the sampling period.

Startle reactivity and Startle Habituation: The initial startle response is considered too variable and was discarded according to previously described protocols [37]. The average of pulse alone trials No 2-6 was used as the startle reactivity. To access habituation of acoustic startle, we compared the startle responses in the initial part (1st startle block) of the testing protocol with the other two startle blocks (middle pulse alone trials, last five trials).

PPI: PPI % (for each prepulse intensity) was calculated as: 100% x (Avg. Middle 10 Pulse alone trials – Avg. PPIx trials)/ Middle 10 Pulse alone trials. The average of PPI of the four different prepulse intensities was used as a measure of overall PPI.

2.11 Statistical analysis.

Data are presented as mean ± SEM and were analyzed by one-way, one-way repeated measures or two-way analysis of variance (ANOVA) with the significance level set at $p < 0.05$. Where appropriate, simple and interaction main effects were investigated further with subsequent post-hoc comparisons (by Tukey test or student t-test). Wilcoxon Signed Ranks Test was used to compare APO-gnawing counts distribution of different groups of rats. The statistical analysis was

adjusted for non-equivalent groups when needed. When data from different generations were used together, we have performed separate analyses on each generation. If the different analyses showed the same main effects, the data was pooled.

3. Results

3.1 Experiment IA - Naturally occurring maternal environment (basal conditions & acute CORT condition).

3.1.1 Body weight (Table 1)

Low maternal LG history led to a significant reduction (vs. High LG or Med LG) in body weight only in two instances at adulthood (pnd 74 & 95).

3.1.2 Eye-opening (data not shown)

Maternal care history did not have a significant effect on eye-opening.

3.1.3 APO-gnawing - Basal conditions (Fig. 2A)

Maternal care history did not have a significant main effect on APO-gnawing.

3.1.4 Sensorimotor gating – Basal conditions (Fig. 2B,C)

Acoustic Startle (Fig.2B). There was a significant effect of startle block within the protocol on acoustic startle for all groups reflecting habituation ($F_{2,230}$=56.40; p<0.001/ p<0.001 for all groups). Maternal care history had also a significant effect ($F_{2,116}$=11.77; p<0.001). Overall, Low LG offspring startled more than the other LG groups (p<0.001 for both comparisons). They startled more than High LG and Med LG in all startle blocks (first: p=0.002 and p=0.001 respectively, middle: p=0.001 and p=0.002 respectively, last: p=0.003 and p=0.021 respectively).

PPI (Fig. 2C). There was a significant effect of the increasing prepulse intensity on PPI in all groups ($F_{3,345}$=166.59; p<0.001/ p<0.001 for all groups). The maternal care history and its interaction with prepulse intensity effect had also a significant effect ($F_{1,115}$=3.72; p=0.027). Surprisingly, Low LG, overall, displayed more PPI than the other LG groups (p=0.016 for both comparisons). In the highest prepulse intensity 16dB[A] over background and in average (Fig. 2D), Low LG offspring display more PPI than High LG (p=0.023 and p=0.026 respectively).

3.1.5 APO-gnawing – Acute CORT condition (Fig. 3)

Compared to the non CORT pretreated condition, CORT pretreated High LG offspring had more extreme responders to APO [both more rats with no gnawing response, more rats with intense gnawing response and less rats with intermediate responnse] in their gnawing distribution. Therefore, both the distribution and the average gnawing counts were not different between the two groups.

A Wilcoxon Signed Ranks Test showed a significant difference of the APO-gnawing distributions between CORT-pretreated and non CORT-preteated Low LG offspring (Z=-3.288; p<0.001). A right shift towards higher gnawing scores was actually found in CORT-pretreated rats (Fig. 3C). Additionally, Low LG offspring, displayed higher average APO-gnawing in the CORT pre-treatment condition than without CORT pretreatment (Fig. 3D; p=0.042).

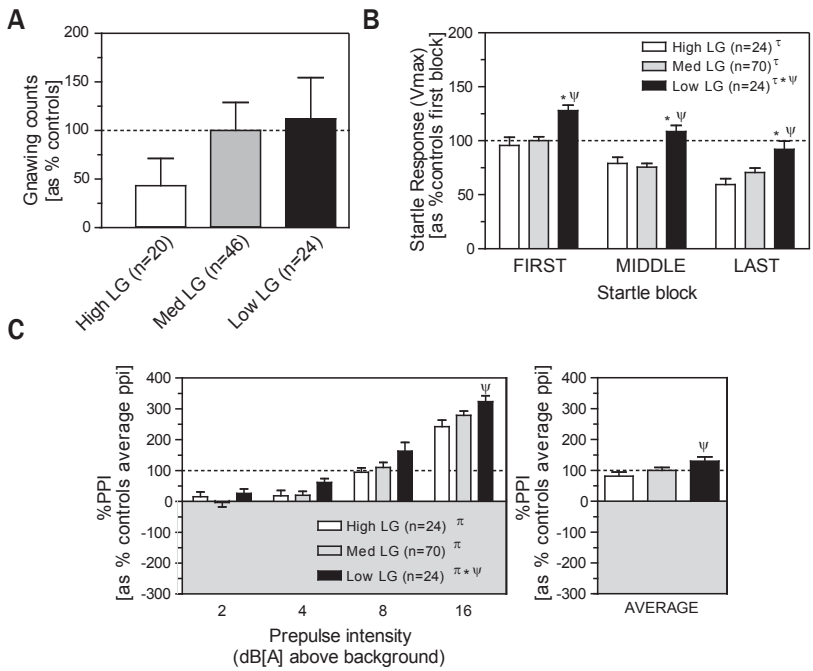

Figure 2. Apomorphine-induced gnawing (A), acoustic startle (B) and its prepulse inhibition (C) of socially reared rats with previous naturally occurring maternal environment. Treatment groups: rats with maternal care history of High Licking & Grooming (High LG), Med LG or Low LG. Post-weaning rats were socially reared (socials). Left panel C show PPI expressed per prepulse intensity level, whereas right panel C show PPI data expressed as average across all prepulse intensities. Data presented as MEAN ± SEM. τ denotes startle block effect, π denotes prepulse intensity effect, * vs. corresponding values of Med LG, Ψ vs. corresponding values of High LG. The exact number of rats used is indicated in the different panels.

3.1.6 Sensorimotor gating – Acute CORT condition (Fig. 4A-F)

High LG (Fig. 4A,B). Acoustic Startle: All the injected groups displayed a significant effect of startle block, indicating habituation (p≤0.001). CORT injected group, during the second startle block, startled less than the VEH injected group (p=0.043).

PPI: All the injected groups displayed a significant effect of prepulse intensity (VEH: p=0.001, CORT: p=0.001, 0.002). CORT injected High LG offspring socials displayed more PPI than VEH injected in 8 dB[A] prepulse intensity (p=0.046). There was a significant effect of APO injection ($F_{1,15}$=10.07; p=0.007) that interacts with the prepulse intensity effect ($F_{3,42}$=3.23; p=0.032). The APO injected rats displayed lower PPI than the VEH injected in all prepulse intensities, apart from 2 dB[A], (4: p=0.021, 8: p=0.006, 16:

Table 1

Body weight (Experiment IA & IB)

Early-life condition	Post-weaning	*Pnd*														
		1	11	21	46	53	60	74	81	88	95	102	109	116	130	165
High LG	socials	6.13 ± 0.20	22.62 ± 0.39	47.16 ± 0.71	201.75 ± 5.06	238.05 ± 4.61	270.33 ± 1.44	337.56 ± 6.17	353.00 ± 14.18	-	396.36 ± 6.04	410.44 ± 13.89	410.50 ± 16.14	431.00 ± 14.25	-	454.64 ± 6.48
Med LG	socials	5.86 ± 0.13	22.90 ± 0.26	47.65 ± 0.50	192.43 ± 4.17	239.06 ± 2.79	275.08 ± 6.60	342.30 ± 6.17	342.67 ± 4.54	379.70 ± 4.54	384.31 ± 7.52	397.41 ± 5.26	402.71 ± 5.21	418.33 ± 8.01	426.94 ± 15.96	-
Low LG	socials	5.69 ± 0.15	23.49 ± 0.29	48.68 ± 0.51	198.50 ± 3.15	241.00 ± 3.06	280.67 ± 7.55	301.00 ± 6.74$^{*\psi}$	357.13 ± 5.62	-	358.29 ± 14.30$^{\psi}$	399.89 ± 6.82	415.87 ± 8.08	430.80 ± 6.64	-	450.65 ± 19.21
High LG	isolates	-	-	49.24 ± 1.14	192.90 ± 4.83	239.60 ± 4.11	-	-	-	-	382.00 ± 7.79	398.33 ± 11.98	406.83 ± 9.67	425.40* ± 8.30	-	448.00 ± 8.36
Med LG	isolates	-	-	48.57 ± 0.80	192.33 ± 8.41	232.24 ± 3.86	-	295.80 ± 8.25$^{\delta}$	330.08 ± 8.80	370.33 ± 2.47	372.50 ± 8.07	386.43 ± 6.42$^{\delta}$	395.15 ± 7.47	400.54 ± 5.98	425.88 ± 13.51	-
Low LG	isolates	-	-	48.50 ± 0.79	195.17 ± 6.41	231.79 ± 7.04	-	-	-	-	372.57 ± 12.66	387.25 ± 6.26	411.14 ± 10.27	416.00 ± 11.28	-	448.81 ± 17.10

One-way ANOVA for the maternal care history effect on body weight revealed significant effects only for postnatal day (pnd) 74 ($F_{1,27}$=7.061; p=0.004) and for pnd 95 ($F_{1,33}$=4.112; p=0.026). One-way ANOVA for the post-weaning housing conditions revealed an effect on body weight on pnd 74 ($F_{1,17}$=10.618; p=0.005) and on pnd 102 ($F_{1,59}$=7.507; p=0.008). Data presented as MEAN ± SEM. * vs. corresponding values of Med LG, δ vs. corresponding values of Med LG socials, ψ vs. corresponding values of High LG.

Table 2

Body weight (Experiment II)

Early-life condition	Post-weaning	*Pnd*											
		1	11	21	28	32	39	46	53	67	88	95	137
NON SEP/ Med LG	socials	5.65 ± 0.07	23.93 ± 0.48	50.88 ± 0.63	82.63 ± 0.86	103.23 ± 2.20	148.43 ± 2.90	187.17 ± 1.91	237.53 ± 3.18	316.21 ± 2.67	394.00 ± 4.87	421.50 ± 5.21	455.61 ± 5.76
HOME SEP	socials	5.63 ± 0.08	22.09 ± 0.38	40.87 ± 0.79*	77.71 ± 1.28*	97.09 ± 2.32	145.50 ± 4.48	191.29 ± 6.78	219.60 ± 6.64*	282.31 ± 5.37*	364.11 ± 11.10*	385.50 ± 8.22*	439.75 ± 8.22
NOVEL SEP	socials	6.29 ± 0.10	24.86 ± 0.32$^{£}$	47.36 ± 0.44$^{£}$	85.86 ± 1.10$^{*£}$	108.35 ± 2.24$^{£}$	149.31 ± 4.58	221.19 ± 4.12$^{£}$	241.75 ± 1.87$^{£}$	323.00 ±5.45$^{£}$	382.50 ± 3.86	410.17 ± 4.80$^{£}$	471.93 ± 6.04$^{£}$

One-way ANOVA for the early-life adversity history effect on body weight revealed significant effects for postnatal day (pnd) 11 ($F_{1,56}$=10.499; p<0.001), pnd 21 ($F_{1,56}$=10.499; p<0.001), pnd 28 ($F_{1,54}$=13.173; p<0.001), pnd 32 ($F_{1,55}$=5.445; p=0.007), pnd 46 ($F_{1,55}$=17.359; p<0.001), pnd 53 ($F_{1,36}$=6.213; p=0.005), pnd 67 ($F_{1,40}$=24.796; p=0.005), pnd 88 ($F_{1,31}$=4.965; p=0.014), pnd 95 ($F_{1,27}$=8.217; p=0.002), pnd 137 ($F_{1,55}$=6.201; p=0.004). Data presented as MEAN ± SEM. τ denotes time effect, * vs. corresponding values of NON SEP/ Med LG, £ vs. corresponding values of HOME SEP.

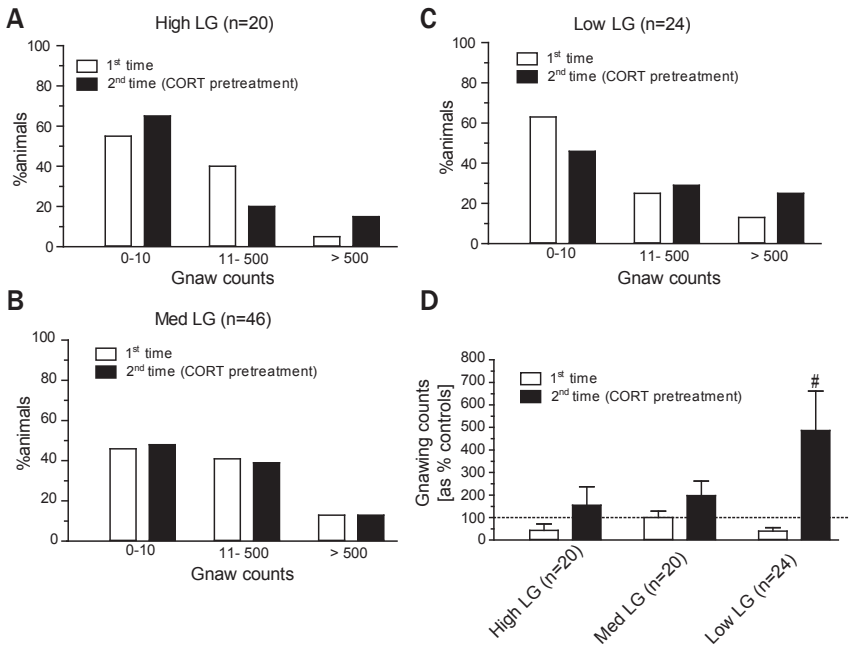

Figure 3. Apomorphine-induced gnawing distributions with/without CORT pre-treatment of socially reared rats with previous naturally occurring maternal environment. Treatment groups: rats with maternal care history of High Licking & Grooming (High LG: panel A), Med LG (panel B) or Low LG (panel C). In panel D the average gnawing counts of the different populations is presented. Post-weaning rats were socially reared (socials). Data presented as MEAN ± SEM. Z denotes significant shift of the gnawing distribution, # significant effect of CORT injection. The exact number of rats used is indicated in the different panels.

p=0.014) and in the overall average ($F_{1,15}$=10.07; p=0.007).

Med LG (Fig. 4C,D). Acoustic Startle: All the injected groups display a significant effect of startle block, indicating habituation (p≤0.001). There is a significant interaction effect of CORT injection and startle block ($F_{2,68}$=3.79; p=0.028). CORT injected Med LG socials startle more than the VEH injected in the first startle block (p=0.009).

PPI: All the injected groups display a significant effect of prepulse intensity (p≤0.001)

There is a significant effect of CORT injection ($F_{1,35}$=4.40; p=0.043) that interacts with the prepulse intensity effect ($F_{3,102}$=2.79; p=0.044). CORT injected Med LG socials display less PPI than VEH injected in 2 dB[A] prepulse intensity (p=0.001) and in the overall average ($F_{1,35}$=4.40; p=0.043). There is a significant effect of APO injection ($F_{1,35}$=26.59; p<0.001) that interacts with the prepulse intensity effect ($F_{3,102}$=4.47; p=0.005). The APO injected rats display lower PPI than the VEH injected in all prepulse intensities, apart from 2 dB[A], (4: p=0.021, 8: p<0.001, 16: p<0.001) and in the overall average ($F_{1,35}$=26.59; p<0.001).

Low LG (Fig. 4E,F). Acoustic Startle: CORT and APO injected groups display a significant effect of startle block, indicating habituation (p=0.004 for both).

PPI: All the injected groups displayed a significant effect of prepulse intensity (VEH: p<0.001, CORT: p=0.001, APO: p=0.003). There was also a significant effect of CORT injection ($F_{1,15}$=8.15; p=0.013). The CORT injected Low LG socials displayed lower PPI than the VEH injected in the lower prepulse intensities (2 dB[A]: p=0.030, 4 dB[A]: p=0.003) and in the overall average ($F_{1,15}$=8.15; p=0.013). There was a significant effect of APO injection ($F_{1,15}$=20.45; p<0.001). The APO injected rats displayed lower PPI than the VEH injected in all prepulse intensities, apart from 8 dB[A], (2: p=0.002, 4: 0.036, 16: p=0.011) and in overall average ($F_{1,15}$=40.94; p<0.001).

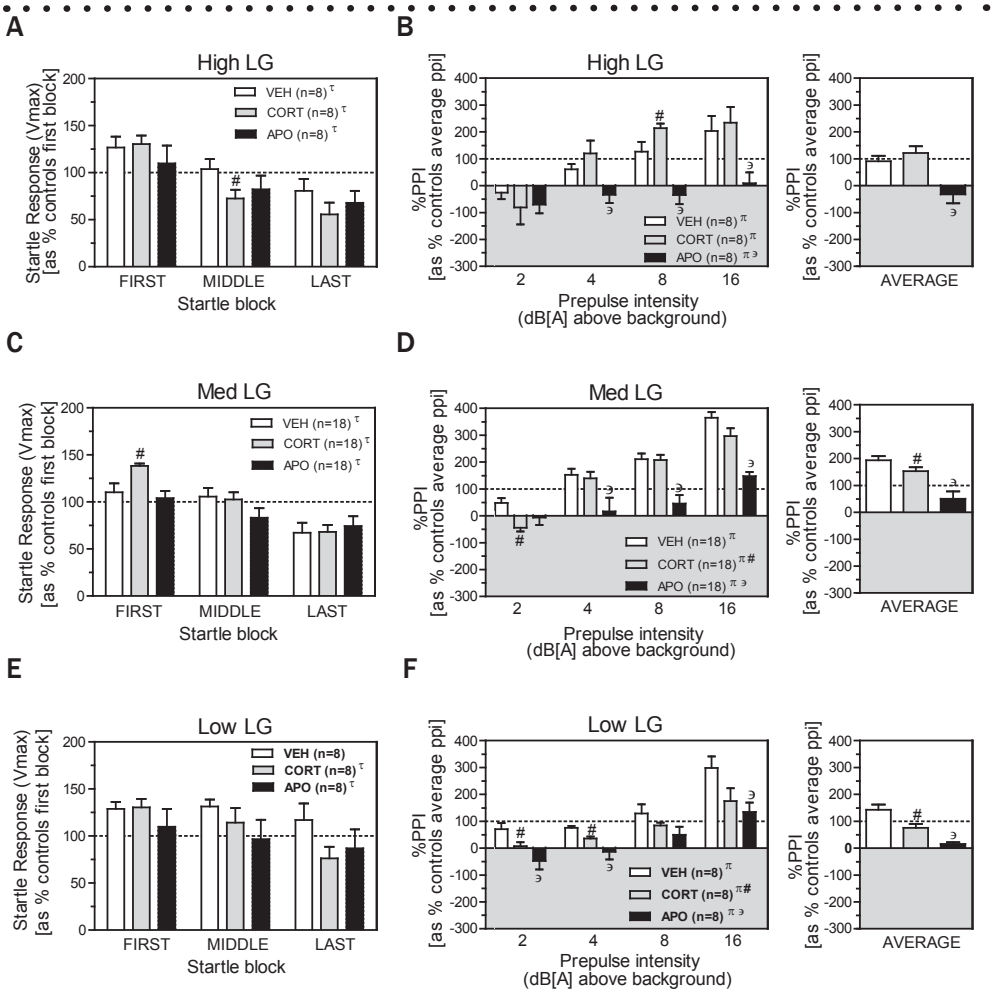

Figure 4. Acute effects of CORT on acoustic startle and its prepulse inhibition of socially reared rats with previous naturally occurring maternal environment. Treatment groups: rats with maternal care history of High Licking & Grooming (High LG: panel A, B), Med LG (panel C,D) or Low LG (panel E,F). Left panel at B, D and F show PPI expressed per prepulse intensity level, whereas right panel show PPI data expressed as average across all prepulse intensities. Post weaning rats were housed in groups (socials). Data presented as MEAN ± SEM. τ denotes startle block effect, π denotes prepulse intensity effect, * vs. corresponding values of Med LG, # significant effect of CORT injection, ə significant effect of APO injection. The exact number of rats used is indicated in the different panels.

3.1 Experiment IB - Naturally occurring maternal environment with post-weaning social isolation (basal conditions)

3.2.1 Body weight (Table 1)

Isolation rearing led to a reduction of body weight of Med LG rats in two instances at adulthood (pnd 74 & 102).

3.2.2 APO-gnawing - Basal conditions (Fig. 5A)

For rats reared in social isolation, there was a significant effect of maternal care history ($F_{2,63}$=5.61; p=0.006). Low LG isolates displayed lower gnaw count levels than both Med LG isolates (p=0.047) and High LG isolates (p=0.005).

3.2.3 Sensorimotor gating – Basal conditions (Fig. 5B,C)

Acoustic Startle (Fig. 5B). For rats reared in social isolation, there was a significant effect of startle block within the protocol on acoustic startle for all groups reflecting habituation ($F_{2,134}$=24.83; p<0.001/ High LG: p<0.001, Med LG: p<0.001, Low LG: p=0.006). Overall, High LG isolates startled less than the Med LG (p=0.035). In particular, they startled less during the second and third startle blocks (p=0.026 and p=0.021 respectively).

PPI (Fig. 5C). For rats reared in social isolation, there was a significant effect of the increasing prepulse intensity on PPI in all groups ($F_{3,201}$=190.22; p<0.001/ p<0.001 for all LG groups). The maternal care history and its interaction with prepulse intensity effect had also significant effects ($F_{2,69}$=8.63; p<0.001, $F_{6,201}$=15.54; p<0.001 respectively). Low LG isolates, overall, displayed more PPI than the other LG groups (vs. High LG: p<0.001, vs. Med LG: p=0.004). More in detail, Low LG isolates displayed higher PPI compared to Med LG (2 dB[A]: p=0.005, 16 dB[A]: p=0.002), which displayed higher PPI than the High LG (2 dB[A]: p<0.001). In almost all prepulse intensities, Low LG isolates displayed more PPI than High LG (2: p<0.001, 4: p=0.014, 16 p=0.034). However, at 8 dB[A] over background High LG displayed the highest PPI (vs. Med LG: p<0.001, vs. Low LG: p=0.012). In average PPI, Low LG displayed higher PPI levels than the other LG groups ($F_{2,69}$=34.69; p<0.001/ p<0.001 for both separate comparisons).

3.3 Experiment II - Experimentally-induced adverse maternal environment with early-postnatal social isolation (basal condition)

3.3.1 Body weight (table 2)

From pnd 11, HOME SEP rats were lighter than NON SEP/Med LG and NOVEL SEP. We have confirmed this difference in body weight, on a weekly basis, up to pnd 137.

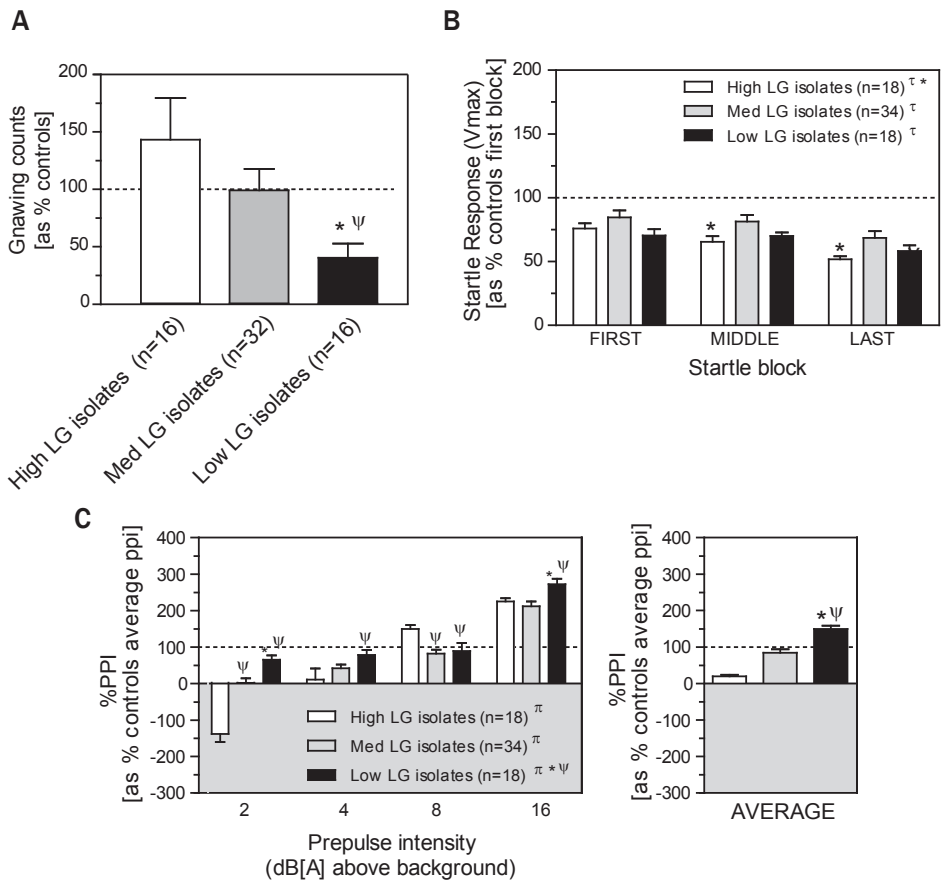

Figure 5. Apomorphine-induced gnawing (A), acoustic startle (B) and its prepulse inhibition (C) of rats reared in social isolation with previous naturally occurring maternal environment. Treatment groups: rats with with maternal care history of High Licking & Grooming (High LG), Med LG or Low LG. Post weaning rats were reared in social isolation (isolates). Left panel C show PPI expressed per prepulse intensity level, whereas right panel C show PPI data expressed as average across all prepulse intensities. Data presented as MEAN ± SEM. τ denotes startle block effect, π denotes prepulse intensity effect, * vs. corresponding values of Med LG, Ψ vs. corresponding values of High LG. The exact number of rats used is indicated in the different panels.

3.3.2 Eye-opening (data not shown)

NOVEL SEP pups eye-opening occurred approximately one day earlier compared to HOME SEP (p=0.038).

3.3.3 APO-gnawing - Basal conditions (Fig. 6A)

NOVEL SEP rats gnawed more than the HOME SEP (p=0.013).

3.3.4 Sensorimotor gating – Basal conditions (Fig. 6B,C)

Acoustic Startle (Fig. 6B). There was a significant effect of startle block within the protocol on acoustic startle for all groups reflecting habituation ($F_{2,96}$=95.508; p<0.001/ p<0.001 for all groups). The interaction of early-life adversity history and startle block had also significant effects ($F_{6,96}$=3.30; p=0.014 respectively). Overall, NOVEL SEP startled more than the controls NON SEP/Med LG (p=0.003). In the first startle block, NOVEL SEP more than NON SEP/Med LG (p=0.032) and the HOME SEP (p=0.028).

PPI (Fig. 6C). There was a significant effect of the increasing prepulse intensity on PPI in all groups ($F_{3,141}$=86.15; p<0.001/ p<0.001 for all groups). Overall and in average PPI, NOVEL SEP displayed less PPI than the HOME SEP (p=0.039).

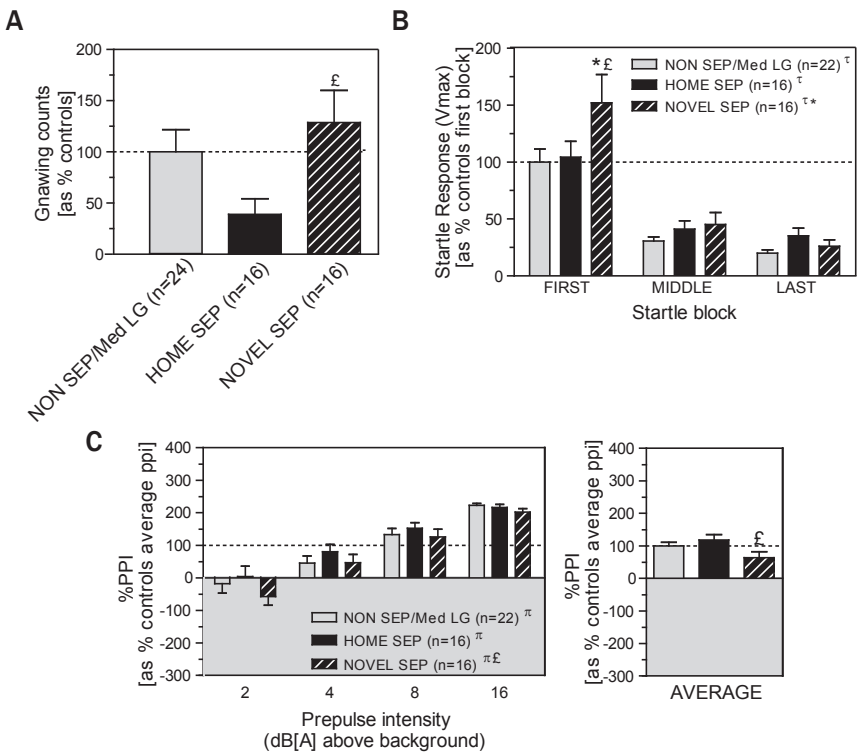

Figure 6. Apomorphine-induced gnawing (A), acoustic startle (B) and its prepulse inhibition (C) of rats exposed to experimentally-induced adverse maternal environment +/- postnatal social isolation. Treatment groups: non separated (NON SEP/Med LG) had no previous history of treatments, repeatedly separated pups were exposed to 8h-MS on pnd 3, 4 & 5 in a home (HOME SEP; home separated) or novel context (NOVEL SEP; novel separated). Post-weaning rats were socially reared. Left panel C show PPI expressed per prepulse intensity level, whereas right panel C show PPI data expressed as average across all prepulse intensities. Data presented as MEAN ± SEM. τ denotes startle block effect, π denotes prepulse intensity effect, £ vs. corresponding values of HOME SEP, * vs. corresponding values of NON SEP/Med LG. The exact number of rats used is indicated in the different panels.

4. Discussion

The goal of this study was to investigate the effect of the interaction of early-life adversity and later-life stress context on the programming of psychosis susceptibility. Our data of Experiment IA support the *cumulative stress* hypothesis since the adult Low LG offspring were more susceptible to the effects of acute CORT than Med LG or High LG offspring. However, the data of Experiments IB and II are in line with the predictions of the *mismatch* hypothesis since we demonstrated that if there is a "*mismatch*" between the early-life environment and the later social environment, the susceptibility for psychosis was increased. This was demonstrated in the case of the combination of High maternal LG history with social isolation at weaning and in the case of NOVEL SEP rats that matured post-weaning in social (group) housing conditions.

4.1 Naturally occurring variation in maternal environment (basal conditions)

In Long Evans rats, early-life adversity can predict altered social behavior, an enhanced psychoneuroendocrine response to stress and impaired cognitive functioning, but also alterations in working memory and social interaction, increased DA susceptibility and decreased sensorimotor gating [17-19, 39, 40]. A naturally occurring Low maternal LG in Wistar rats did not increase basal APO-gnawing, decrease basal PPI or reduce body weight. It increased only acoustic startle and, surprisingly, increased PPI at high pre-pulse intensity levels.

Low maternal care, alone, in Wistars did not yield the outcome expected from studies in Long Evans rats. This suggests that genetic background may influence the programming sensitivity of the pups to early-life adversity [41]. Our recent data indicate actually that genetic predisposition for DA susceptibility contributes to the sensitivity to naturally occurring variations of maternal care [33, 34, 42, 43]. Genotype dependent vulnerability (as seen in Long Evans rats) versus resilience (as seen in Wistar rats) towards adversity of the early-life environment has been demonstrated also in humans [44, 45].

4.2 Naturally occurring variation in maternal environment (acute CORT condition)

First, we observed that CORT-pretreatment, one hour prior testing, increased the average APO-gnawing of Low LG offspring due to a shift to the right of their gnawing distribution towards higher APO-gnawing counts. Acute stress and glucocorticoids can enhance DA synthesis and release [46, 47]. Studies with knock-out mice have demonstrated that this glucocorticoid action is mediated by GR expressed in the midbrain DA neurons of the reward circuitry and their targets (dopaminoceptive neurons of the NAc, caudate putamen, and PFC) [48]. The role of GR-mediated mechanisms in the observed phenotype of the Low LG offspring needs, however, further investigation.

Interestingly, our APO injections created a fast disruption, within 10min, of PPI in all groups. The high doses of APO, can be considered a control condition to validate the

maximum disruption of PPI possible in our experimental setup. All groups responded similarly to these injections indicating that the effect of APO we observed is indeed probably a "floor effect". Furthermore, CORT-injections also caused a fast disruption of PPI in Med LG and Low LG offspring, but not in High LG offspring. The effect was strong after a history of enhanced adversity of the maternal environment (Low LG). Previously in healthy human volunteers with no psychiatric history a similar fast reduction of PPI levels after infusion of CORT was observed [20]. The CORT effect is probably not mediated through a genomic pathway [49], but possibly via the recently identified membrane variant of the MR and/or GR [50, 51], as it was induced within 10min of administration.

Taken together, acute CORT administration could reveal the negative impact of enhanced adversity of the maternal environment on psychosis susceptibility (APO-gnawing and PPI), supporting the *cumulative stress* hypothesis. The difference of High and Low LG in the sensitivity to acute CORT calls for further investigation.

4.3 Naturally occurring variation in maternal environment with post-weaning social isolation

We investigated the interaction of early-life experience and post-weaning social environment. In previous studies using the combination of maternal deprivation and isolation rearing or chronic CORT administration it was discovered that the combination of two environmental stressors in development led to restoration of normal functions. One study showed that the basal PPI deficit caused by 24h-MD was restored after social isolation at adulthood [12]. Another two studies found that the DA (APO or amphetamine)-induced PPI deficit was reduced in maternally deprived rats chronically treated with CORT post puberty [14, 15]. These findings supported the *mismatch* hypothesis [4, 52], since when the later testing environment resembled (i.e. a "*match*") the early-postnatal social environment a low psychopathology susceptibility can be predicted, whereas if the early-postnatal and the later social environment were different (i.e. a "*mismatch*") there was increased susceptibility for psychopathology.

Interestingly, in our study when the (naturally occurring) Low LG offspring were housed in a post-weaning social isolation, psychosis susceptibility was reduced. Low LG displayed clearly an enhancement of PPI and their acoustic startle was no longer increased. In contrast, High LG individuals were sensitive to isolation rearing, which caused disruption of PPI. The Low LG offspring displayed better outcome than High LG offspring when reared in isolation in terms of DA susceptibility. Isolation rearing led to a reduced APO-gnawing in Low LG offspring, compared to High LG offspring. These findings demonstrate that maternal care history can modulate programming sensitivity to such an extent that the well groomed offspring becomes sensitive to social isolation, as an environmental factor precipitating psychosis susceptibility, while the reverse is observed in the offspring that received poor maternal care. Hence, this is a prime example of the "*mismatch*" hypothesis.

4.4 Experimentally-induced adverse maternal environment

MS imposed reduced levels of maternal care to the neonate Wistar pups and, as adults, they displayed reduced body weight, if they stayed as a litter in the home cage during maternal absence (HOME SEP). If during MS pups were in a novel environment isolated from their peers (NOVEL SEP), maternal care was also decreased, but body weight was not affected. However, this group compared to controls displayed increased DA-system sensitivity to APO and increased acoustic startle, while PPI was decreased.

An increase in perseverative behaviors, manifested here by APO-gnawing, and a reduction of sensorimotor gating induced by early-life stress was not unexpected. Maternally deprived rats (i.e. 24h-MD) also displayed higher APO-gnawing and disruption of basal sensorimotor gating in previous studies [36, 53-56]. In these studies, maternally deprived rats displayed deficits in PPI together with deficits in latent inhibition, that were both linked with altered NMDA receptor-dependent hippocampal plasticity [53-55].

Interestingly both MS groups (and especially NOVEL SEP) were in a *mismatch* condition when tested at adulthood: their adverse maternal environment did not *match* with later "normal" social rearing. The importance of our finding lies in the fact that we could distinguish the impact of early-life adversity per se from the effect of a non-shared early stressful experience because of the isolation in a novel environment. From our previous research using this model, we know that the NOVEL SEP pups' amygdala is activated prematurely early in life (pnd 5) and this predicted an adult fearful phenotype with deficits in social interaction [57]. Our studies imply that increased fear and stress reactivity and psychosis susceptibility coincide. This interesting co-precipitation of phenotypes, after early stressful experience that is not shared with peers, is supported by human data suggesting a link between HPA reactivity and psychotic symptoms [21]. Hence programming sensitivity can be modulated early in life not only by genetic background but also by the individual's experience.

4.5 Beyond maternal care levels: role of experience

HOME SEP/NOVEL SEP and Low LG groups shared reduced LG levels the first postnatal week, but yet their phenotypes in later life are different or even opposing. Obviously, experimentally-induced and naturally-occurring maternal care deficits do not represent the same degree or quality of early-life adversity and the maternal mediation hypothesis maybe not the sole mechanism programming adult phenotypic plasticity [58, 59]. It was proposed that environmental adversity contributes, on its own and together with the maternal repertoire, to lasting alterations on the offspring's HPA responses and behavior [60]. The notion that the pups' experience during maternal absence can influence the outcome [26, 57] is in line with recent pioneering studies on neonatal learning of preference or avoidance depending on dam's presence, the pup's CORT levels and amygdala maturation [61, 62].

5. Conclusion

This study was designed to investigate the lasting impact of the interaction between early-life adversity and later stress contexts on psychosis susceptibility in adult life. The CORT experiment revealed that possibly acute stress has an additive impact on psychosis susceptibility in individuals with increased early-life adversity, which is evidence in support of the *cumulative stress* hypothesis. We also found that the interaction of post-weaning social environment with the early-life experience supports the *mismatch* hypothesis for psychosis susceptibility. Finally, experience-related factors, like early isolation from peers, can influence the sensitivity to developmental programming.

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7. References

[1] van Os, J., Kenis, G., Rutten, B. P. The environment and schizophrenia. Nature. 2010,468:203-12.
[2] Brown, A. S. The environment and susceptibility to schizophrenia. Prog Neurobiol. 2011,93:23-58.
[3] Heins, M., Simons, C., Lataster, T., Pfeifer, S., Versmissen, D., Lardinois, M., et al. Childhood Trauma and Psychosis: A Case-Control and Case-Sibling Comparison Across Different Levels of Genetic Liability, Psychopathology, and Type of Trauma. The American journal of psychiatry. 2011.
[4] Champagne, D. L., Ronald de Kloet, E., Joels, M. Fundamental aspects of the impact of glucocorticoids on the (immature) brain. Seminars in fetal & neonatal medicine. 2009,14:136-42.
[5] Pryce, C. R., Ruedi-Bettschen, D., Dettling, A. C., Weston, A., Russig, H., Ferger, B., et al. Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. Neuroscience and biobehavioral reviews. 2005,29:649-74.
[6] Lehmann, J., Feldon, J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Reviews in the neurosciences. 2000,11:383-408.
[7] Schmidt, M. V. Molecular mechanisms of early life stress--lessons from mouse models. Neuroscience and biobehavioral reviews. 2010,34:845-52.
[8] Schmidt, M. V., Wang, X. D., Meijer, O. C. Early life stress paradigms in rodents: potential animal models of depression? Psychopharmacology. 2011,214:131-40.
[9] McEwen, B. S. Protective and damaging effects of stress mediators. N Engl J Med. 1998,338:171-9.
[10] Taylor, S. E. Mechanisms linking early life stress to adult health outcomes. Proceedings of the National Academy of Sciences of the United States of America. 2010,107:8507-12.
[11] Champagne, D. L., Bagot, R. C., van Hasselt, F., Ramakers, G., Meaney, M. J., de Kloet, E. R., et al. Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. J Neurosci. 2008,28:6037-45.
[12] Ellenbroek, B. A., Cools, A. R. Early maternal deprivation and prepulse inhibition: the role of the postdeprivation environment. Pharmacology, biochemistry, and behavior. 2002,73:177-84.

[13] Choy, K. H., de Visser, Y., Nichols, N. R., van den Buuse, M. Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce BDNF expression in hippocampus: effects on learning and memory. Hippocampus. 2008,18:655-67.
[14] Choy, K. H., de Visser, Y. P., van den Buuse, M. The effect of 'two hit' neonatal and young-adult stress on dopaminergic modulation of prepulse inhibition and dopamine receptor density. Br J Pharmacol. 2009,156:388-96.
[15] Choy, K. H., van den Buuse, M. Attenuated disruption of prepulse inhibition by dopaminergic stimulation after maternal deprivation and adolescent corticosterone treatment in rats. Eur Neuropsychopharmacol. 2008,18:1-13.
[16] Gluckman, P. D., Hanson, M. A., Buklijas, T., Low, F. M., Beedle, A. S. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. Nat Rev Endocrinol. 2009,5:401-8.
[17] Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science (New York, N.Y. 1997,277:1659-62.
[18] Liu, D., Diorio, J., Day, J. C., Francis, D. D., Meaney, M. J. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nature neuroscience. 2000,3:799-806.
[19] Zhang, T. Y., Chretien, P., Meaney, M. J., Gratton, A. Influence of naturally occurring variations in maternal care on prepulse inhibition of acoustic startle and the medial prefrontal cortical dopamine response to stress in adult rats. J Neurosci. 2005,25:1493-502.
[20] Richter, S., Schulz, A., Zech, C. M., Oitzl, M. S., Daskalakis, N. P., Blumenthal, T. D., et al. Cortisol rapidly disrupts prepulse inhibition in healthy men. Psychoneuroendocrinology. 2011,36:109-14.
[21] Walker, E., Mittal, V., Tessner, K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. Annu Rev Clin Psychol. 2008,4:189-216.
[22] Varty, G. B., Geyer, M. A. Effects of isolation rearing on startle reactivity, habituation, and prepulse inhibition in male Lewis, Sprague-Dawley, and Fischer F344 rats. Behavioral neuroscience. 1998,112:1450-7.
[23] Varty, G. B., Marsden, C. A., Higgins, G. A. Reduced synaptophysin immunoreactivity in the dentate gyrus of prepulse inhibition-impaired isolation-reared rats. Brain research. 1999,824:197-203.
[24] Varty, G. B., Braff, D. L., Geyer, M. A. Is there a critical developmental 'window' for isolation rearing-induced changes in prepulse inhibition of the acoustic startle response? Behavioural brain research. 1999,100:177-83.
[25] Varty, G. B., Paulus, M. P., Braff, D. L., Geyer, M. A. Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biological psychiatry. 2000,47:864-73.
[26] Daskalakis, N. P., Claessens, S. E., Laboyrie, J. J., Enthoven, L., Oitzl, M. S., Champagne, D. L., et al. The newborn rat's stress system readily habituates to repeated and prolonged maternal separation, while continuing to respond to stressors in context dependent fashion. Hormones and behavior. 2011,60:165-76.
[27] Champagne, F. A., Francis, D. D., Mar, A., Meaney, M. J. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiology & behavior. 2003,79:359-71.
[28] Myers, M. M., Brunelli, S. A., Squire, J. M., Shindeldecker, R. D., Hofer, M. A. Maternal behavior of SHR rats and its relationship to offspring blood pressures. Developmental psychobiology. 1989,22:29-53.
[29] Garner, J. P. Stereotypies and other abnormal repetitive behaviors: potential impact on validity, reliability, and replicability of scientific outcomes. ILAR J. 2005,46:106-17.
[30] Ljungberg, T., Ungerstedt, U. A method for simultaneous recording of eight behavioral parameters related to monoamine neurotransmission. Pharmacology, biochemistry, and behavior. 1978,8:483-9.
[31] Lazar, N. L., Neufeld, R. W., Cain, D. P. Contribution of nonprimate animal models in understanding the etiology of schizophrenia. J Psychiatry Neurosci. 2011,36:100054.
[32] Cools, A. R., Brachten, R., Heeren, D., Willemen, A., Ellenbroek, B. Search after neurobiological profile of individual-specific features of Wistar rats. Brain research bulletin. 1990,24:49-69.
[33] Ellenbroek, B. A., Sluyter, F., Cools, A. R. The role of genetic and early environmental factors in determining apomorphine susceptibility. Psychopharmacology. 2000,148:124-31.
[34] Daskalakis, N. P., Meelis, W., Fung, W. L. J., McNeilly, J., de Jong, I. E. M., McNeilly, A. S., et al. Testing the three-hit hypothesis of schizophrenia: ACTH hyper-reactivity and schizophrenia-like endophenotypes co-precipitate in genetically-susceptible rats following early-life adversity and post-weaning social isolation experiences. In preparation. 2011.
[35] Ellenbroek, B. A., Cools, A. R. Apomorphine susceptibility and animal models for psychopathology: genes and environment. Behavior genetics. 2002,32:349-61.
[36] Ellenbroek, B. A., Cools, A. R. The long-term effects of maternal deprivation depend on the genetic background. Neuropsychopharmacology. 2000,23:99-106.
[37] Geyer, M. A., Swerdlow, N. R. Measurement of startle response, prepulse inhibition, and habituation. Curr Protoc Neurosci. 2001,Chapter 8:Unit 8 7.
[38] Ellenbroek, B. A., Geyer, M. A., Cools, A. R. The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. J Neurosci. 1995,15:7604-11.
[39] Plotsky, P. M., Thrivikraman, K. V., Nemeroff, C.

B., Caldji, C., Sharma, S., Meaney, M. J. Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. Neuropsychopharmacology. 2005,30:2192-204.
[40] Parent, C. I., Meaney, M. J. The influence of natural variations in maternal care on play fighting in the rat. Developmental psychobiology. 2008,50:767-76.
[41] Claessens, S. E., Daskalakis, N. P., van der Veen, R., Oitzl, M. S., de Kloet, E. R., Champagne, D. L. Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. Psychopharmacology. 2011,214:141-54.
[42] Daskalakis, N. P., Fung, W. L., Schnorr, S., Meelis, W., Oitzl, M. S., De Kloet, E. R. The three hit hypothesis of schizophrenia tested in genetically selected rats exposed to early life experience and adolescent psycho-social stressor. International Journal of Developmental Neuroscience. 2010,28:698-.
[43] Daskalakis, N. P., Martens, G. J., Cools, A. R., de Kloet, E. R. Schizophrenia endophenotypes and stress hyper-reactivity co-precipitate following adverse life events in genetically susceptible rats. Eur Neuropsychopharmacol. 2011,21 S85-S6.
[44] Caspi, A., Moffitt, T. E. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nature reviews. 2006,7:583-90.
[45] Caspi, A., Hariri, A. R., Holmes, A., Uher, R., Moffitt, T. E. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. The American journal of psychiatry. 2010,167:509-27.
[46] Corcoran, C., Walker, E., Huot, R., Mittal, V., Tessner, K., Kestler, L., et al. The stress cascade and schizophrenia: etiology and onset. Schizophrenia bulletin. 2003,29:671-92.
[47] Marinelli, M., Piazza, P. V. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. The European journal of neuroscience. 2002,16:387-94.
[48] Ambroggi, F., Turiault, M., Milet, A., Deroche-Gamonet, V., Parnaudeau, S., Balado, E., et al. Stress and addiction: glucocorticoid receptor in dopaminoceptive neurons facilitates cocaine seeking. Nature neuroscience. 2009,12:247-9.
[49] Groenink, L., Dirks, A., Verdouw, P. M., de Graaff, M., Peeters, B. W., Millan, M. J., et al. CRF1 not glucocorticoid receptors mediate prepulse inhibition deficits in mice overexpressing CRF. Biological psychiatry. 2008,63:360-8.
[50] Karst, H., Berger, S., Turiault, M., Tronche, F., Schutz, G., Joels, M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. Proceedings of the National Academy of Sciences of the United States of America. 2005,102:19204-7.
[51] Karst, H., Berger, S., Erdmann, G., Schutz, G., Joels, M. Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proceedings of the National Academy of Sciences of the United States of America. 2010,107:14449-54.
[52] Gluckman, P. D., Hanson, M. A., Beedle, A. S. Early life events and their consequences for later disease: a life history and evolutionary perspective. Am J Hum Biol. 2007,19:1-19.
[53] Ellenbroek, B. A., van den Kroonenberg, P. T., Cools, A. R. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophrenia research. 1998,30:251-60.
[54] Ellenbroek, B. A., Cools, A. R. Maternal separation reduces latent inhibition in the conditioned taste aversion paradigm. Neuroscience Research Communications. 1995,vol. 17:27-33.
[55] Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B. A., Riva, M. A. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Molecular psychiatry. 2002,7:609-16.
[56] Rots, N. Y., de Jong, J., Workel, J. O., Levine, S., Cools, A. R., De Kloet, E. R. Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. Journal of neuroendocrinology. 1996,8:501-6.
[57] Daskalakis, N. P., Champagne, D. L., Diamantopoulou, A., Remmers, E., Tjälve, M., Van der Veen, R., et al. Beyond maternal absence: evidence for the role of peers and non-shared stressful experience in mediating the development of a fearful phenotype. Submitted. 2011.
[58] Macri, S., Mason, G. J., Wurbel, H. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. The European journal of neuroscience. 2004,20:1017-24.
[59] Tang, A. C., Akers, K. G., Reeb, B. C., Romeo, R. D., McEwen, B. S. Programming social, cognitive, and neuroendocrine development by early exposure to novelty. Proceedings of the National Academy of Sciences of the United States of America. 2006,103:15716-21.
[60] Macri, S., Wurbel, H. Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Hormones and behavior. 2006,50:667-80.
[61] Moriceau, S., Wilson, D. A., Levine, S., Sullivan, R. M. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. J Neurosci. 2006,26:6737-48.
[62] Sullivan, R. M., Holman, P. J. Transitions in sensitive period attachment learning in infancy: the role of corticosterone. Neuroscience and biobehavioral reviews. 2010,34:835-44.