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

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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 I: 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 (Exp. IA) or in social isolation (Exp. IB) 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 II: rat pups exposed as neonates to daily sessions of 8 h 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 I: 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. When low maternal LG history was combined with post-weaning social isolation, basal 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. For demonstration of the cumulative stress hypothesis an injection of CORT in the adult Low LG offspring was required that increased APO-gnawing and reduced PPI. This CORT-induced PPI disruption was greatly enhanced after additional isolation rearing. The High LG group, either socially housed or reared in isolation, was resistant to the acute effects of CORT at adulthood.
Experiment II: MS increased psychosis susceptibility only in 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 does 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 supports the mismatch hypothesis. However, evidence for the cumulative stress hypothesis arises if vulnerable individuals are exposed in later life additionally to excess of the stress hormone CORT.


## 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 periods are important time windows for

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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 a 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 brains of the adult Low LG offspring experienced stressful or high CORT conditions. The opposite results were found when High LG offspring or its hippocampus was exposed to stress or high CORT, respectively. These findings supported the so-called "mismatch" hypothesis which states that experience early in life programs the developing brain in preparation of later life environment [4,11,12].

Ellenbroek and 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 h of maternal deprivation (24 h-MD) on postnatal day (*pnd*) 9. However, if the maternally deprived rats were reared in social isolation, the 24 h-MD did not lead to a decreased PPI [13]. Choy and van den Buuse also used the 24 h-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 [14–16]. 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 physical and mental disease as reviewed by Gluckman et al. and Schmidt et al. [12,17].

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 [18–20]. Since previous research suggested a link of circulating corticosterone (CORT) levels with psychotic behavior [21,22], 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 [23–26]. 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 h/day on *pnd* 3,4,5), which we developed recently [27]. 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.

## 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 lx, 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) were ad libitum. Upon arrival males and females were housed in groups of 3–4 in macrolon-polycarbonate type IV cages (L60 × W38 × 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 × W26.6 × 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 60 min per as described previously [27–29]: 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 19:30 h; under 2 × 60 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 3 min (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.