We have shown previously that while the separation context is not affecting the pup’s adaptation to maternal absence, it has profound consequences for other aspects of the stress response. If the neonates are housed individually in a novel cage during maternal separation (MS), the outcome is strikingly different than when the pups remain housed as a group in the home cage (Daskalakis, Claessens et al. 2011). In this study we wanted to extend these findings by further investigating the impact of the MS-context on juvenile social interactions.

METHODS
Wistar rats were used in this study and housed under an 11:13 h light/dark cycle. After parturition, litters were randomly assigned to the experimental conditions. Non-separated litters remained undisturbed with their dams in the housing room until the time of testing. The procedure of repeated MS in home context (HOME SEP) or novel context (NOVEL SEP) has been described before (Daskalakis, Claessens et al. 2011): (i) “home-separation” (i.e. HOME SEP); during daily 8h absence of the dam, starting postnatal day (pnd) 3 the pups remained together in the home cage; (ii) “novel-separation” (i.e. NOVEL SEP); the same separation procedure, but now the pups were individually housed in a novel environment. From pnd 1-10 cages were not cleaned. From pnd 11, the cages were weekly changed. On pnd 21, the pups were weaned from their dams; males and females were separated. The males of the same litters were housed together in groups. We measured social play in a novel setting at pnd 30.

Apparatus
The cameras were placed above the test arenas connected to an automated video-tracking system EthoVision 3.1 (Noldus Information Technology, Wageningen, Netherlands). EthoVision was used for video capturing (Fig.1) and to live measure rats’ proximity to each other and duration of interaction. We later used the video files to determine whether patterns of social interaction between individuals were differently influenced by the MS contexts using The Observer XT (Noldus Information Technology, Wageningen, Netherlands).

Procedure
The social interaction experiment was performed on pnd 30 in a novel environment setting. 4H of single housing was followed by 20min observation of social interaction in dyads of rats. Two days before the testing, rats were habituated to a dim-lighted (5-10 lux) room. The single housing of the juvenile rat increases the amount of social play (Niesink and Van Ree 1989). During the first 2h, rats...
had access to a plastic tube (open in one end) from which they could dig out light-weighted stones (from a total of 300 gr). This was to assess the general behavioral function and muscular strength of the rats in an ecological relevant way (Deacon 2006). Thereafter, the tube was removed and the quantity of stones (gr) retrieved by the rat was calculated. The bedding was refreshed with new bedding (sawdust) and the rat remained in this setting for another 2h.

To observe social interactions, two rats of the same age that were unfamiliar to each other but with the same early-life history were put together for 20 min in a novel testing cage. Behavior was videotaped and analyzed at a later time point. The 20min experiments took place during either a morning session at 9:00h or an afternoon session at 13:30h. Rats within and between experimental groups were counterbalanced between morning and afternoon session. In order to distinguish between rats during the analysis of the tapes, one rat was painted with odorless mark the previous day while the other was equally handled but not labeled. Note that there was only a marginal difference in weight (up to maximum of 10 gr) between rats tested in pair.

**Measurements**

The scoring of the behaviors was done using The Observer XT program. The first 5 min of each video recording were analyzed. Behaviors were scored according to a protocol previously described by Vanderschuren and colleagues (Vanderschuren, Niesink et al. 1995; Veenema and Neumann 2009): (i) Playful activities, (ii) Non-playful, but social activities (iii) Non-playful, but non-social. An additional analysis of rats’ proximity was performed with the use of EthoVision 3.1 program (Noldus, Wageningen, The Netherlands). In order to get an overall picture from results of the individual behaviors, we added the frequencies of behaviors indicating initiation of social play sequelae.

**Results**

In the first 2h of the habituation phase to the novel setting, all groups burrowed to the same extent, indicating that all rats had similar muscular strength. During this period they also displayed the same number of visits to the plastic tube (EthoVision). In the second 2h of the habituation phase, we monitored the locomotion of the rats in order to evaluate if the rats displayed comparable activity levels and they did (EthoVision). During the 20-min social interaction, rats of all treatment groups explored the cage to the same extent (EthoVision & The Observer XT). NOVEL SEP rats initiate less frequently social play compared to the other two groups (The Observer XT).

**Discussion**

That the NOVEL SEP individuals initiated less social play, than the other groups, can be interpreted as a precursor of the later fearful phenotype. This phenotype could be more related to a difference in basal or stress-induced CORT levels between NOVEL SEP and the other groups (Veenema and Neumann 2009).

**References**

1. Daskalakis, N. P.; Claessens, S. E. et al. (2011). 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, 60* (2), 165-176.

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