Neuropeptides' manipulations in the nucleus accumbens shell impair maternal behavior in lactating rats

Subjects and husbandry

- Lactating Sprague-Dawley rats (230g – 250g, Charles River)
- Standard laboratory conditions (12/12 h light/dark cycle, lights on at 7:00, 22 °C, 55 % humidity) with water and food *ad libitum*

Stereotaxic surgery

On pregnancy day 18 (PD18, Exp. 1) or lactation day 2 (LD2, Exp. 2) animals were bilaterally implanted with guide cannulas (23G; 12mm length; stainless steel) 2 mm above the NAcSh (AP 1mm; L 3mm; D 5.3mm; angle 17.5°) under isoflurane anesthesia.
On LD2 (Exp. 3), rats were unilaterally implanted with a microdialysis probe (molecular cut-off: 18 kDa; Hemophan, Gambro Dialysatoren, Hechingen, Germany) targeting the NAc (AP 1mm; L 3mm; D 7.3mm; angle 17.5°).

Maternal care observation

Maternal care (MC) was monitored for 10s every 2nd minute in 30-min blocks, following an established protocol (Bosch and Neumann, 2008). Under undisturbed conditions (Exp. 1), MC was observed under basal conditions as well as immediately after treatment infusion for 120 min, followed by a 60-min observation in the afternoon to assess for any long-lasting effects of drug treatment. In Exp. 2, MC was monitored under basal conditions as well as after the maternal defense test (MDT, a psychosocial stressor).
The occurrence of the following nursing parameters was scored: arched back nursing (ABN), blanket nursing posture, other nursing posture (together, these parameters account for "total nursing"). Other maternal as well as non-maternal behaviors (e.g., self-grooming) were monitored.

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Pup retrieval test

Maternal motivation was assessed in the pup retrieval test (PRT). Behavior was recorded for 15 min and the latency to retrieve the first pup, the time to retrieve all the pups, and the total number of pups retrieved were scored.

Anxiety-related behavior

Anxiety-related behavior was evaluated with the light-dark box test (LDB). The behavior was monitored in lactating and virgin female rats, as well as male rats, to identify any differences due to the different reproductive state or sex. The percentage of time in the light zone was the parameter indicating anxiety-like behavior.

Maternal defense test

Maternal aggression was monitored in the MDT by measuring the number of aggressive behaviors within 10 min. To define an "aggression score" and enhance the accuracy of behavioral phenotyping (Guillox et al., 2011), z-score was calculated as follows:

$$z - aggression =$$

$$\frac{\left(\frac{x-\mu}{\sigma}\right)attack + \left(\frac{x-\mu}{\sigma}\right)keep\ down + \left(\frac{x-\mu}{\sigma}\right)aggr.grooming + \left(\frac{x-\mu}{\sigma}\right)lateral\ threat + \left(\frac{x-\mu}{\sigma}\right)aggr.sniffing + \left(\frac{x-\mu}{\sigma}\right)off.upright}{number\ of\ parameters}$$

X: individual value

μ: mean of control group

σ: standard deviation control group

Retrodialysis and oxytocin measurement

On LD 4, the inflow adapter of the microdialysis probe was connected via PE-20 tubing to a syringe mounted onto a microinfusion pump; the outflow adapter was attached to a 1.5 ml collection tube. Ringer's solution was flushed to collect basal samples (-60, -30), then the syringes

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were switched to those containing CRF or Ucn3 to allow drug infusion. After 30 min, the syringes were switched back to vehicle. Samples were collected in 30-min intervals and immediately frozen on dry-ice. Oxytocin content was measured with radioimmunoassay by an external company (RIAgnosis).

Immunohistofluorescence (IHF)

On LD 7, animals were exposed to the MDT 90 min before collection of the brains. To assess for any soothing effect of pup presence, a group underwent pup separation right at the end of the MDT.

Groups:

1. No test, with pups (control)
2. No test, pups removed
3. MDT, with pups after test
4. MDT, pups removed after test

All rats were anesthetized and transcardially perfused with PBS 1X and brains were flash-frozen. For IHF, 16 µm-thick coronal sections were collected on slide and post-fixed in 4% paraformaldehyde for 20 min. After 3 washings of 10 min each, slides were incubated in blocking solution for 1h (3% BSA in PBS 1X + 0.3% Triton-X-100), and afterwards incubated O/N at 4°C with primary antibody (AbI) prepared in the same blocking solution (AbI: Abcam cFos 1:2000, Santa Cruz CRF 1:1000). The following day, slides were washed 3x10 min in PBS 1X and incubated 1h at room temperature with AbII (Alexa Fluor 488 αRabbit 1:800; Alexa Fluor 546 αGoat 1:500, 546). Slides were covered with mounting medium containing DAPI. Images were acquired at 20x magnification.

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