EFFECT OF EARLY LIFE STRESS

Measuring learning and memory in rats with EthoVision

By Charlotte A. Oomen - In humans, traumatic experiences during childhood increase the risk for developing stress-related pathologies such as depression in adulthood[1]. Findings from animal studies have shown that experiences early in life can exert long term changes in brain structure[2], especially in the hippocampus, a brain region involved in learning and memory. In order to study whether learning and memory is altered after early stress, we subjected male rats to maternal deprivation (MD) and tested their spatial learning ability and emotional memory in adulthood.

MATERIAL AND METHODS

Animals and maternal deprivation
Rats were separated from their mother during 24 hours on day 3 after birth[3]. Ten control and ten MD-males were used for behavioral analysis (aged 11 weeks).

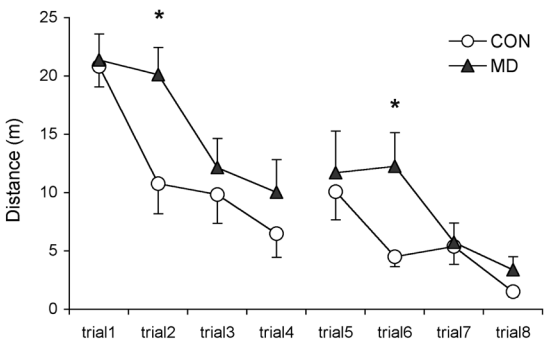
Anxiety behavior
To study changes in exploration and anxiety, rats were tested on an elevated plus maze; shaped like a cross with two opposite open arms (10 x 40 cm) and two opposite closed arms (10 x 40 cm, 25 cm high walls) connected to an open center. Animals were put on the center and were allowed to explore for 5 minutes. Exploration was recorded and analyzed for total time, frequency, and latency to first appearance in all compartments using EthoVision (Noldus, Wageningen, The Netherlands).

Spatial learning and memory
To asses spatial learning, animals were trained in a water maze[3] during 2 days, 4 trials/ day with an inter-trial interval of 15 minutes. The maze (diameter: 150 cm) was filled with opaque water (22 ºC). In one quadrant, a platform (diameter: 12 cm) was hidden under the surface. Trials started -randomized- in one of the three quadrants without a platform and were analyzed for latency, swim distance and time in platform quadrant using EthoVision.

Emotional memory
To assess hippocampus-dependent emotional memory, rats were subjected to contextual fear conditioning[4]. Rats learned to associate a fearful stimulus (footshock) with a context (conditioning box) and the strength of fear-memory was determined by scoring the amount of freezing behavior manually. Animals were allowed to explore the conditioning box freely for three minutes after which a footshock (2 seconds, 0.4 mA) was administered. After 24 hours, contextual memory was measured by measuring the amount of freezing in the same box.

MAIN RESULTS

Anxiety
There were no significant differences in latency to first appearance (CON 51.7 ± 21.0 sec; MD 32.2 ± 10.7 sec, p=0.33), visiting frequency (CON 7.8 ± 1.5 times; MD 7.5 ± 1.7 times, p=0.89) and / or percentage of time spent in the open arms of the maze (CON 40.1 ± 6.3%; MD 45.3 ± 6.5%; p=0.55) between control and stressed animals.

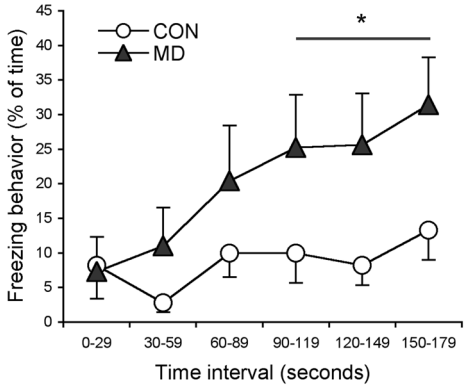


Spatial learning

Spatial acquisition of the water maze test was significantly impaired by maternal deprivation (figure 1). MD animals required more time (repeated measures ANOVA, $F_{1,18}= 5.83$; $p=0.03$) and travel distance ($F_{1,18}= 4.45$; $p<0.05$) to reach the platform especially on the second trial of both days ($p=0.02$).

Emotional memory

MD animals showed more freezing behavior 24 hours after learning the context-footshock association (figure 2, group x interval; $F_{5,125}= 2.3$; $p<0.05$), especially during the last three intervals ($p=0.02$).



DISCUSSION

We show that severe early life stress results in impaired spatial learning, yet leads to improved fear memory formation, without affecting basal anxiety levels. This demonstrates that even a severely adverse early life event in rats does not impair overall hippocampal functionality in adulthood.

Stress in early life might prepare animals to respond optimally under conditions associated with high stress levels during adulthood, i.e. when the adult and early life conditions closely match[5]. Ultimately, interaction between the individual genetic profile and the early environment may amplify individual responsiveness in animals and can be involved in adaptive programming.

REFERENCES

1. Heim, C.; Nemeroff, C.B. (2001.) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry,* **49(12)**, 1023-1039.
2. Mirescu, C.; Peters, J.D.; Gould, E. (2004.) Early life experience alters response of adult neurogenesis to stress. *Nature Neuroscience,* **7**, 841-846.
3. Morris, R. (1984.) Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods,* **11(1)**, 47-60.
4. Phillips, R.G.; LeDoux, J.E. (1992.) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioural Neuroscience,* **106(2)**, 274-285.
5. Champagne, D.L.; De Kloet, E.R.; Joëls M. (2009.) Fundamental aspects of the impact of glucocorticoids on the (immature) brain. *Seminars in Fetal and Neonatal Medicine,* **(14)3**, 136-142.

CONTACT INFORMATION

Charlotte Oomen, PhD Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, The Netherlands.

Current address: Department of Experimental Psychology, University of Cambridge, United Kingdom (cao34@cam.ac.uk).

This report is based on the following publication:
Oomen, C.A.; Soeters, H.; Audureau, N.; Vermunt, L.; Van Hasselt, F.N.; Manders, E.M.M.; Joëls, M.; Lucassen, P.J.; Krugers, H. (2010.) Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *The Journal of Neuroscience*, **30(19)**, 6635-6645.