AUSWIRKUNGEN EINES DEFIZITS DES NEURONALEN ZELLADHÄSIONSMOLEKÜLS (*NCAM*) IM TELENCEPHALON AUF LERNEN, GEDÄCHTNIS UND INDIVIDUALITÄT BEI EINER GENTECHNISCH VERÄNDERTEN LABORMAUS

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Dissertation an der Universität Hamburg, Deutschland, 2003

CONSEQUENCES OF A DEFICIT OF THE NEURONAL CELL ADHESION MOLECULE (NCAM) IN THE TELENCEPHALON FOR LEARNING, MEMORY AND INDIVIDUALITY IN A GENETICALLY MANIPULATED LABORATORY-MOUSE

Dissertation at the department of Biology of the University of Hamburg, Germany, 2003
German with English summary

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Verlag: **Litis Press Publishers**, München 2003
1. Auflage 10/2003
246 Seiten, 70 s/w Abbildungen, 99 Tabellen
Email: **info@litispress.com**

ISBN 0-9751285-1-5

Elektronische Version

zum Download vorhanden / to be downloaded for free
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Die **gedruckte und gebundene Ausgabe** mit der **ISBN 0-9751285-0-7** (inhaltlich identisch mit der elektronischen Version) kann im Buchhandel oder direkt bei **Litis Press Publishers** vertrieb@LitisPress.com bestellt werden.

The **printed version** may be ordered through bookstores or directly at **Litis Press Publishers** vertrieb@LitisPress.com with **ISBN 0-9751285-0-7**

Bibliografische Angaben: Fentrop, N. (2003), Auswirkungen eines Defizits des Neuronalen Zelladhäsionsmoleküls (*NCAM*) im Telencephalon auf Lernen, Gedächtnis und Individualität bei einer gentechnisch veränderten Labormaus, *Litis Press Publishers*, München.

English summary

This study could not confirm the hypothesis that NCAM-deficient mice have a profound deficit in spatial long-term memory in the Morris water maze - besides some subtle shortfalls in the short- and long-term search precision. However, with a unique approach of a behavioural analysis to genetically manipulated animals it could be shown that NCAM knock-out mice possess a longer-term individual behavioural consistency. This is the first study to show a group of "normal" animals (wild-type) to change their individual behaviour over time while the knock-out littermates persistently keep their individual behavioural traits.

Based on the hypothesis that synaptic plasticity underlies learning and memory it has been hypothesised that the neuronal cell adhesion molecule (NCAM) could be involved in long-term memory consolidation, in particular spatial memory. Sixteen conditional knock-out male mice (ko), and 15 male wild-type litter mates (wt) were tested in various behavioural paradigms. In the ko-mice a 90 % reduction of NCAM expression was previously demonstrated in the hippocampus and to a lesser extend in the neocortex from post-natal week 3 onwards.

Mice were singly housed under an inverse light cycle and tested in the dark phase. All animals completed the following tests successively over 80 days. (I) On day 1 an **open-field test** for exploratory activity; (II) on day 3 a **light-dark avoidance test** for light-induced, anxiogenic behaviour; (III) on day 21 to 54 a **Morris water-maze test** series (mice must search for a platform hidden under the water surface using distal optical landmarks); (IV) on day 76 an **open-field test** identical to day 1; (V) on day 80 an **open-field test** in a circular instead of a square arena.

No significant differences between ko- and wt-mice were found in **general parameters** (e.g. body weight, frequency of defecation or urin-patches) reflecting their ability to perform tests of learning and memory or to use coping strategies. Furthermore, there was no significant difference in their preference of the dark quadrant in the light-dark avoidance test, nor in the distribution of activity in illuminated versus dark zones, which would indicate differences in **anxiogenic behaviour**.

Like previous findings in hippocampus-deficient rodents, the **locomotor activity** was significantly greater in the ko-mice than in the wt-mice. In all three open field tests and in the light-dark avoidance test, the NCAM-deficient mice covered 36-80 % more distance and visited the different zones of the arena more frequently.

An analysis of **exploratory behaviour** in the circular arena showed that mice of both genotypes made disproportional use of the arena zones. Thus, during the first five minutes they covered greater distances in an area within 10 cm of the wall than in the central areas, an effect, however, which was not evident in the entire test time of 20 minutes. Furthermore, half of the time mice spent moving they did so within the third of the arena adjacent to the wall.

In all open-field tests, significant differences between genotypes were found in the relative distribution of activity in arena zones. In general, ko-mice moved further from the **walls**, **visited** the zones closer to the centre more often, **stayed** longer in the intermediate zone, and **covered** a greater relative distance (proportion of distance moved in zone to total distance moved) in the intermediate zone. Furthermore, ko-mice became active earlier towards the centre or remained there active for longer periods of time. In the contrary, all mice were slowest in the zone adjacent to the wall and increased their speed of locomotion gradually towards the centre by about 50%.

Learning and memory performance of mice was studied using three measures: (1) **habituation** (2) **individual consistency** in behaviour as an expression of a general ability to encode experience, and (3) memory of **spatial configurations** in the Morris water-maze test.

Mice of both genotypes exhibited significant **short-** (intra-test) and **long-term** (inter-test) **habituation** for the distance travelled. For travel speed, on the other hand, only short-term habituation was significant. However, for none of these variables a significant difference between ko- and wt-mice was detected.

Individual consistency of behaviour was measured by the individual's stability of responding to repeated stimuli, in terms of the rank of the individual's responding within its own genotype group. For each variable, the values of all individuals of the same genotype were correlated between two corresponding tests (Pearson correlation). In addition, the individual habituation curves were analysed for consistency.

Short-term consistency: Medium ($r \geq 0.50$) to high ($r \geq 0.80$) correlations were found within the variables "distance moved", "speed", and "frequency of visits" both for ko- and wt-mice if tests were separated by a few

days (open-field tests and light-dark avoidance test: "dry tests") or up to three weeks (Morris water-maze: "wet test"). The correlations were independent of the type of test (open-field tests or light-dark avoidance test), independent of the kind of arena (circular or square), and independent of the novelty of the test. However, there was hardly any correlation between dry and wet tests.

Long-term consistency: Medium to high correlations were obtained for ko-mice, but not for wt-mice if tests were separated by ten weeks. The comparison of individual habituation curves for the two identical open field tests (separated by 79 days) revealed more shared characteristics within animals for ko-mice than for wt-mice. Medium correlations of characteristics could be detected for ko- but not for wt-mice.

In the Morris water-maze test for spatial memory ko-mice did not differ significantly from wt-mice in the learning curve of latency to locate the target platform. However, during the learning period, ko-mice were delayed by a few days in swimming close to the platform compared to wt-mice (approaching significance, $p < 0.100$). In addition, ko-mice took longer than wt-mice to reach a smaller platform (diameter 15 instead of 20 cm) on day 3 of testing. Nevertheless, when searching ko- and wt-mice did not differ significantly in their exclusive proximity to the former platform position ("exclusive": compared to three additional, virtual platform positions) in the probe trials (no platform present) which took place 1 hr and 65 hrs after the last learning trial.

In two further probe trials, 22 days after the last learning trial and a series of learning trials with a new platform location, ko-mice did not show any significant spatial preference for the target area whereas wt-mice at least preferred the virtual target position compared to a position opposite.

There was no difference between ko- and wt-mice in (1) **navigation strategy** or (2) the **signals used** for orientation while searching. Mice of both genotypes did not swim to the switched target position when the distal optical landmarks where moved 180° to point to the opposite side of the water maze. Presumably, the moved landmarks in combination with further uncontrolled landmarks present in the room where not utilisable for orientation since they were contradictory. Only when distal optical landmarks were removed mice of both genotypes swam in exclusive proximity of the target platform, apparently using uncontrolled landmarks. Mice of both genotypes did not swim in exclusive proximity to the target platform whether in continuous darkness or when the light was switched off within four seconds from the start. Latency to reach the platform did not improve between three learning trials under initial light and subsequent darkness. It appears that mice depended on visual landmarks for navigation.

There were no significant differences detected in **motivational status**, **sensomotor abilities** or **coping strategies** between ko- and wt-mice (e.g. floating, thigmotaxis or latency to a visible platform or distance covered while searching).

In conclusion, this study could not confirm that NCAM-deficient mice have a profound deficit in long-term memory, especially in the spatial domain. However, compared to wt-mice, ko-mice showed (1) subtle shortfalls in the short- and long-term search precision in the Morris water maze, (2) altered exploratory behaviour directed more towards the centre of the arena, and (3) longer-term individual behavioural consistency.

The finding of short-term behavioural consistency for all mice when tested in identical or similar, but not in rather different situations supports the "interactionistic model" of individuality (Magnusson & Endler 1977, p. 4). Since wt-mice showed a time-restricted individual behavioural consistency, one can assume a time-dependent natural loss of individual consistency. Given that this loss was smaller in the ko-mice, NCAM seems to be involved in the development of behavioural individuality in mice for reactive variables (distance travelled and speed). This effect could be due to the dependence of behavioural individuality on learning and memory processes.

Regarding the role of NCAM, it is possible that ko-mice having a NCAM deficit also have reduced synaptic plasticity resulting in a limited ability for behavioural adaptation (behavioural inflexibility). However, due to the likelihood of mechanisms compensating for the NCAM deficit, active on various organismic, integrative levels, a direct relationship between NCAM and the observed behavioural deviations compared to wt-mice is unlikely. In the future cellular mechanisms of long-lasting behavioural consistency or behavioural inflexibility could be investigated using conditional NCAM mice.

Keywords: NCAM, mouse, behaviour, learning, memory, consistency, individuality, Morris water maze, test battery