

The

# GERMAN MOUSE CLINIC

## Report for NCAM

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# 1 Summary

## 1.1 Primary Screen

In a primary screen 72 NCAM mice have been analyzed in the German Mouse Clinic (GMC) in the screens Behavior, Dymorphology, Bone and Cartilage, Neurology, Eye, Clinical Chemistry, Immunology, Allergy, Nociception, Lung Function, Metabolism, Expression Profiling and a comprehensive Pathology.

**Behavior Screen:** We were able to confirm the previous finding of a general increase in exploratory locomotor activity in NCAM-deficient mice. In addition, under our mild testing conditions the increased exploratory drive phenotype in both sexes of NCAM-deficient mice was associated with reductions in anxiety-related behavior and social affinity, which extends the previous finding that NCAM mice do not seem to be generally more anxious as indicated by results in the Elevated Plus-maze test (Stork *et al.*, 1999), but rather seem to avoid high illumination intensities, as indicated by the finding of increased anxiety-related behavior sensitive to the treatment with anxiolytics in the light/dark avoidance test (Stork *et al.*, 1999).

**Dymorphology:** Differences in fat mass and fat content of NCAM mutants could be shown in three independent tests of our screen:

1. Some animals with less fat mass were already detected in the visual inspection of the animals.
2. Significant differences in fat mass and fat content between mutants and controls could be shown in males using the DEXA technique.
3. Quantitative analysis of the X-ray images detected differences in subcutaneous fat in both male and female data.

Also sBMD, Bone mineral content, Lean content, Body length, and Weight, were significantly changed in male NCAM mutants. In females alterations in the same direction could be observed, but the differences were less pronounced.

**Immunology Screen:** Minor differences in the level of IgG<sub>1</sub> between female mutants and their littermate controls were detected, although the observed values were not outside the expected values for this immunoglobulin.

**Metabolic Screen:** The reduced body weight in correlation with the high daily energy uptake indicates a disturbed body weight regulation which might be due to an elevated basal metabolic rate.

**Expression Profiling:** RNA expression profiling of brain tissue by means of a 21K cDNA microarrays revealed a very low number of genes differentially regulated between mutant and wild-type tissues in all experiments. These

genes may be of functional importance and of interest for the further analysis of the NCAM mutant mouse.

In the screens **Allergy, Nociception, Lung Function, Neurology, Clinical Chemistry** and **Pathology**, no genotype-specific differences could be found.

## 1.2 Secondary Screening

Secondary screening is suggested from the Neurology and Metabolic Screens. We would recommend analyzing:

**Neurology Screen:** Since homozygous NCAM knockout mice seem to have neuromuscular end plates that are 14% smaller as compared to controls, defects in muscular functioning might be possible. Therefore, we recommend secondary screening including a quantitative grip strength examination.

**Metabolic Screen:** The mutated gene in NCAM KO mice influences metabolic characteristics leading to lower body weight but higher assimilation capacity and probably basal metabolic rate. Hence, we recommend the determination of resting and basal metabolic rate under controlled conditions with a simultaneous monitoring of core body temperature and activity pattern.

Please contact Valérie Gailus-Durner to discuss further steps and details.

## 2 General Part

### 2.1 The Role of the Gene

Cell adhesion molecules (CAMs) of the immunoglobulin superfamily nucleate and maintain groups of cells at key sites during early development and in the adult (for a comprehensive review: Crossin and Krushel, 2000). The binding of neural CAM (NCAM) leads to a number of signaling events, some of which result in changes in gene expression. There is evidence that CAMs play critical functional roles in morphogenesis:

- Mediation of cell to cell interactions during development;
- Involved in axon pathfinding, fasciculation and synapse formation;
- Mediator of synaptic plasticity.

### 2.2 Previously Known Phenotypes

#### Fertility

- Reduced male fertility
- Female mice tend to kill their pups.

#### CNS

- Overall brain weight is reduced by 10%.
- Reduced size of olfactory bulb (app. 36%), accompanied by a dramatically reduced numbers of bulbar granule cells.
- Reduced LTP in the CA1 Region of the hippocampus.
- Reduced LTP in the CA3 region of the hippocampus.
- Fasciculation and laminar growth of hippocampal CA3 mossy fibers were strongly affected, leading to innervation of CA3 pyramidal cells by mossy fiber axons at ectopic sites.
- Severe hypoplasia of the corticospinal tract.
- Pathfinding errors of corticospinal axons: some corticospinal axons either stayed ventrally and extended laterally or axons turned dorsally, but instead of growing to the contralateral dorsal column, a significant fraction of axons projected ipsilaterally.
- Elevated levels of the G-protein coupled, inwardly rectifying potassium channel Kir3.

#### Behavior

- Impaired learning in the morris water maze
- Increased inter-male aggression
- Increased anxiety and anxiolytic sensitivity to serotonin 5-HT<sub>1A</sub> receptor agonists
- Impaired odor discrimination

## PNS

- Impaired maintenance of myelinated fibers: Degeneration of myelin and axons in MAG/NCAM double mutants, but not in one of the single mutants.
- Neuromuscular end plates were 14% smaller in NCAM-deficient mice, and formation of junctional folds was delayed.

## 2.3 Possible disease models

Schizophrenia

All further findings we consider as new.

## 2.4 Mice

### 2.4.1 Number and kind of mice

NCAM mice were mated at GSF. Due to the low fertility of NCAM mice, NCAM mice entered the GMC in three batches:

Date	Mutant Female	Mutant Male	Wild Type Female	Wild Type Male
02.06.2003	4	4	9	14
15.09.2003	9	5	18	10
08.12.2003	8	4	12	10
<b>Total Number</b>	<b>22</b>	<b>13</b>	<b>21(+9)</b>	<b>15(+19)</b>

Numbers in brackets indicate mice which were kept in reserve to replace dead animals. As described by the owner, the mice analyzed were generated by gene targeting and backcrossed to C57BL/6J.

### 2.4.2 Housing conditions

In the GMC mice are housed in type II polycarbonate cages in individually ventilated caging (IVC) systems (VentiRack Bioscreen TM, Biozone, Margate, UK) on wood fibre (Altromin, Lage, Germany). The IVCs operate with positive pressure. Mice are transferred in weekly intervals to new cages with forceps in Laminar Flow Class II changing stations. Mice are fed with irradiated standard rodent high energy breeding diet (Altromin 1314) and given semidemineralized filtered (0.2 µm) water *ad libitum*. Light is adjusted to a 12h/12h light/dark cycle; temperature and relative humidity are regulated to 22 ± 1°C and 55 ± 5%, respectively. In specified modules husbandry conditions are adjusted according to the experiment requirements (See corresponding sections). All people attending the facility completely change their garment (jackets and trousers autoclaved) and shoes and wear caps and masks before entering the GMC (Brielmeier *et al.*, 2004).

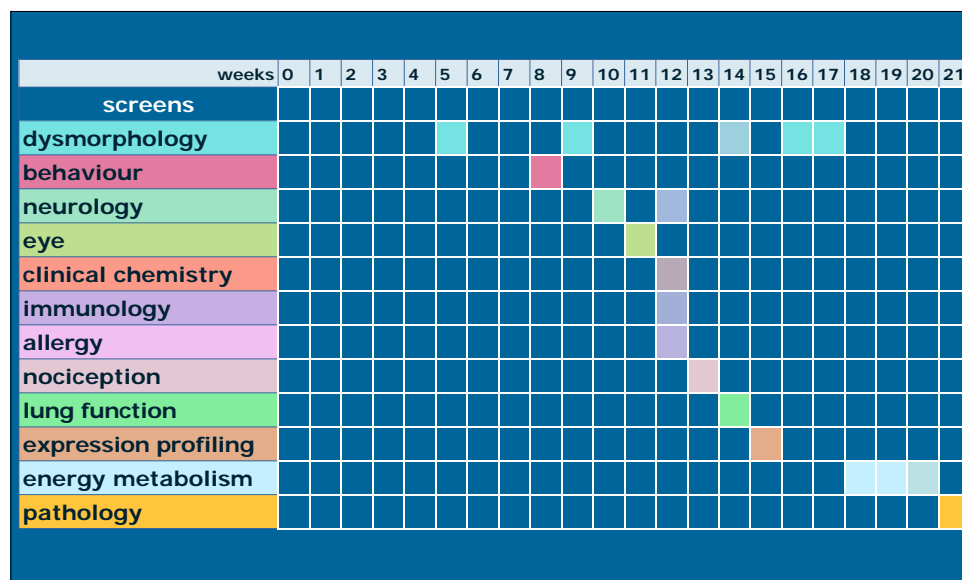
Outbred 8-week-old male SPF Swiss mice are used as sentinels and kept on a mixture of new bedding and aliquots of soiled bedding (50:50) from all cages of the IVC rack. In addition, the sentinels were also exposed to soiled air from all “upstream” cages of the IVC rack. Health monitoring is carried out by on-site examination of the sentinel mice by certified laboratories according to FELASA recommendations ([www.felasa.org](http://www.felasa.org)).

Mice are kept according to the German laws. Tests were carried out by authority of the Regierung von Oberbayern.


## 2.5 Workflow

### 2.5.1 Standardized workflow for the primary screen in the German Mouse Clinic

Mouse mutants entering the GMC are examined in a primary screen according to the following standard workflow (Fig. 1). Analyzed parameters are listed in Table. 1.



**Figure 1: Workflow of the primary screen**

Explanation below,  =Analysis of blood-based parameters.

After the mice arrive at the GMC, they are acclimatized in the new environment for one week. The males then start in the Behavior Screen. There they stay for three weeks. Directly after the Behavior Tests, the anatomical inspection of the Dysmorphology Screen is performed. In the next week, the Neurology Screen is applied. One week later the mice go through the tests of the Eye Screen. When the mice were 12 weeks old, blood is taken, and samples are distributed to the blood-based screens for Clinical Chemistry, Immunology, Allergy and the Lactate test. One week later, the animals are tested in the Nociceptive Screen. Two weeks after testing of the first blood sample, a

second sample is taken to confirm outliers, and to supply the Dymorpholgy Screen with material for determination of blood-based bone-related parameters. In parallel, 10 mutant animals (5 males / 5 females) and 10 controls (5 males / 5 females) leave the animal facility for the Lung Function Analysis, which for technical reasons is located elsewhere. These animals are, for hygienic reasons, not allowed to re-enter the German Mouse Clinic. The females go directly to Pathology. The males are used to freeze organs for future expression profiling on demand (remaining organs from those animals are analyzed by the Pathology). All other animals go through the bone and cartilage tests of the Dymorphology Screen, and then stay three weeks in the Metabolic Screen. After completion of the primary screen, all animals end up in the Pathology.

The screening of female animals starts one week later and follows the same workflow (with the exception of Expression Profiling sampling). Deviations from our Standard operation protocol (SOP) are listed below; please take the specific number of analyzed animals from the sections of the applied screen.

### **2.5.2 Applied screens**

The GMC standard workflow for the primary screen as described above was applied to analyze the NCAM mice. Due to the low fertility of NCAM mice, it was not possible to breed one large cohort of mice to enter the primary screen in one single batch. Thus the workflow was adapted to the available number of mice provided in three batches. Some parameters from the blood based screens could not be determined in all animals, as it was not possible to get the needed amount of blood from these animals. A few animals died during the primary screen and thus they could not be analyzed for all parameters.

### **2.5.3 Quality Management**

As a routine quality control, we take blood samples for serological tests of the sanitary status of all mice after they went through the GMC primary screen. When indicated, the serum is tested for MHV (BioDoc, Hannover). We chose MHV as a "sentinel" pathogen, as it is one of the most common viruses in mouse facilities worldwide and it is transmitted easily. To be open for collaboration for as many partners as possible, we allow MHV positive animals to enter our facility.

Microgranulomas in the liver are observed commonly in mice on a C57BL/6 genetic background. In those cases the results of the MHV tests are used to exclude MHV as one possible reason for these infiltrates (See chapter 3.12 Pathology Screen)

## **2.6 Statistical Analysis of Data**

If not otherwise stated, data of males and females was analyzed separately comparing mutant and control data using a Student's t-test. Sex differences within the mutant or the control group also were determined with a t-test. Tables summarizing the data will show mean  $\pm$  standard error of the mean. Sig-

nificant differences are indicated stepwise from 0.05, 0.02, 0.01, 0.001 to 0.0001.

## 2.7 References

Brielmeier M., H. Fuchs, G. Przemeck, V. Gailus-Durner, M. Hrabé de Angelis, J. Schmidt (2002) The GSF – Phenotype Analysis Center (German Mouse Clinic, GMC): A sentinel-based health-monitoring concept in a multi-user unit for standardized characterization of mouse mutants. In: J. Guenet and C. Herweg (Eds.) *Laboratory Animals Science - Basis and Strategy for Animal Experimentation* Vol. 11, Proceedings of the 8th FELASA Symposium, Laboratory Animals Ltd., Aachen, pp. 19-22.

Crossin, K. L. and L.A. Krushel (2000) Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. *Dev. Dynamics* 218: 260-279

### Abbreviations

NCAM	Neural cell adhesion molecule
GMC	German Mouse Clinic
IVC	individually ventilated cage
+/+	homozygous wild type
+/-	heterozygous mutant
-/-	homozygous mutant
KO	knockout
wt	wild type
B6	C57BL/6
FELASA	Federation of <b>E</b> uropean <b>L</b> aboratory <b>A</b> nimal <b>S</b> cience <b>A</b> ssociations, 25 Shaftesbury Avenue, London W1D 7EG, UK, <a href="http://www.felasa.org">www.felasa.org</a>

**Table 1: Primary Screen at GMC**

<b>Screens</b>	<b>Goal</b>	<b>Methods</b>
<b>Dysmorphology, Bone and Cartilage</b>	morphological analysis of body, skeleton, bone and cartilage	morphological observation, bone densitometry, X-ray, AVL analyzer, micro-computer tomography
<b>Behavior</b>	locomotor, exploratory, emotional and social behavior, object recognition memory	modified hole board
<b>Neurology</b>	assessment of muscle, spinocerebellar, sensory, and autonomic function	modified SHIRPA protocol
<b>Eye</b>	assessment of morphological and functional alterations of the eye	electroretinography, slit lamp biomicroscopy
<b>Clinical Chemistry</b>	determination of clinical-chemical and hematological parameters in blood	blood autoanalyzer, ABC-animal blood counter
<b>Immunology</b>	analysis of peripheral blood samples for immunological parameters	flow cytometry, ELISA
<b>Allergy</b>	analysis of total plasma IgE	ELISA
<b>Nociception</b>	detection of altered pain response	hot plate assay
<b>Lung function</b>	assessment of alterations in breathing patterns	whole body plethysmography (Buxco®)
<b>Expression Profiling</b>	RNA expression profiling	DNA-chip technology
<b>Energy Metabolism</b>	measurement of altered body weight regulation, body temperature and energy balance	bomb calorimetry
<b>Pathology</b>	microscopic and macroscopic histological examination	histology, immunochemistry

## 3 Specific part

### 3.1 Behavior Screen

#### 3.1.1 Summary

The modified hole board test is used as primary screen in the behavioral phenotyping module of the GMC, because it allows the comprehensive analysis of a range of behavioral parameters known to be indicative of behavioral dimensions such as locomotor activity, exploratory behavior, arousal, emotionality, memory and social affinity in a single short test (see Ohl *et al.*, 2001).

With this test, which is performed under testing conditions which are relatively stress-free for the animals (medium illumination level, cage mates are present in adjacent group compartment during the test), we could confirm the previous finding of a general increase in exploratory locomotor activity in NCAM-deficient mice (Stork *et al.*, 1999). In addition, under our mild testing conditions the increased exploratory drive phenotype in both sexes of NCAM-deficient mice was associated with reductions in anxiety-related behavior and social affinity, which extends the previous finding that NCAM mice do not seem to be generally more anxious as indicated by results in the Elevated Plus-maze test (Stork *et al.*, 1999), but rather seem to avoid high illumination intensities, as indicated by the finding of increased anxiety-related behavior sensitive to the treatment with anxiolytics in the light/dark avoidance test (Stork *et al.*, 1999).

#### 3.1.2 Mice

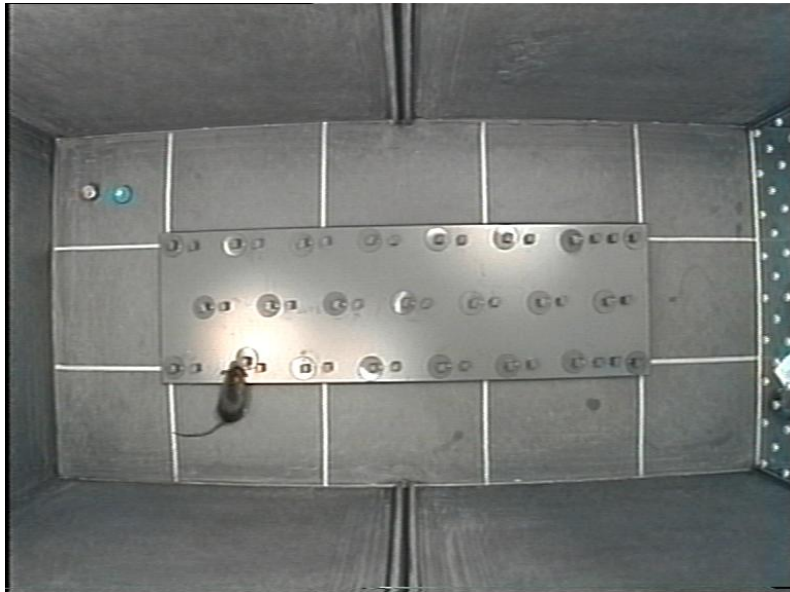
Mice were housed with food and water *ad libitum* under standard laboratory conditions. Animals were separated based on sex, but not genotype. They entered the laboratory at the age of six weeks, were given two weeks for acclimatization and were tested at the age of eight weeks. Three days before testing, an object (metal cube) was placed into the home cage and removed one day before testing.

In this screen, 40 female mice (19 wt, 21 mutants) and 25 male mice (14 wt, 11 mutants) were available for analysis.

#### 3.1.3 Material and Methods

The modified hole board test was carried out according to the procedures described by Ohl *et al.*, 2001. The test apparatus consisted of a test arena (100 x 50 cm), in the middle of which a board (60 x 20 x 2 cm) with 23 holes (1.5 x 0.5 cm) staggered in 3 lines with all holes covered by movable lids was placed, thus representing the central area of the test arena as an open field. The area around the board was divided into 12 similarly sized quadrants by lines taped onto the floor of the box (See Ohl *et al.*, 2001). Both box and board were made of dark grey PVC. All lids were closed before the start of a trial. For each trial, an unfamiliar object (a blue plastic tube lid, similar in size to the metal cube) and the familiar object (metal cube) were placed into the

test arena with a distance of 2 cm between them. The illumination levels were set at approximately 150 lux in the corners and 200 lux in the middle of the test arena.



**Figure 2: Test Arena for modified hole board test.**

For testing, each animal was placed individually into the test arena and allowed to explore it freely for 5 min. The animals were always placed into the test arena in the same corner next to the partition, facing the board diagonally. The two objects were placed in the corner quadrant diametrical to the starting point. During the 5 min trial, the animal's behavior was recorded by a trained observer with a hand-held computer. Data were analyzed by using the Observer 4.1 Software (Noldus, Wageningen). Additionally, a camera was mounted 1.20 m above the center of the test arena, and the animal's track was videotaped and its locomotor path analyzed with a video-tracking system (Ethovision 2.3, Noldus, Wageningen). After each trial, the test arena was cleaned carefully with a disinfectant.

Data were statistically analyzed using SPSS software (SPSS Inc, Chicago, USA). The chosen level of significance was  $p < 0.05$ .

### 3.1.4 Parameters

<b>Manually recorded behavior</b>
Line crossings, rearings, board entries, hole explorations, hole visits, stretched attends (risk assessment), partition (group contact), grooming, defecation, unfamiliar and familiar object exploration
<b>Video-track analysis</b>
Total distance moved, mean velocity, maximum velocity

### 3.1.5 Results

Analysis of both the behavioral observation data (Table 2) and the video-track data (Table 3) revealed increased locomotor and exploratory activity in NCAM-deficient mice in both sexes. The increase in locomotor activity was reflected in the number of line crossings and total distance moved, as well as in increased mean and maximal speed of movement. The increased exploratory activity was reflected by increases in rearings and hole explorations, as well as an increased exploration of the board. Group contacts were reduced in NCAM-deficient mice, most likely as a consequence of locomotor hyperactivity.

### 3.1.6 Discussion

We found a locomotor hyperactivity and increased exploratory drive phenotype in both sexes of NCAM-deficient mice, with associated **reductions in anxiety-related and social affinity parameters**, which are most likely non-specific consequences of the increased exploratory drive. The whole behavioral repertoire was shifted towards exploration, at the expense of other behaviors. These results do not contradict, but confirm and extend previous findings obtained in other anxiety tests with these mice, e.g. Light/dark avoidance and Elevated Plus-maze (Stork *et al.*, 1999). What was consistent in all of these tests was a clear increase in exploratory locomotor activity, but NCAM mice do not seem to be generally more anxious under any testing condition, as indicated by our results and the findings in the Elevated Plus-maze test (Stork *et al.*, 1999). They may rather avoid high illumination intensities, as indicated by the finding of increased anxiety-related behavior sensitive to the treatment with anxiolytics in the light/dark avoidance test (Stork *et al.*, 1999).

### 3.1.7 Reference

- Ohl, F., Sillaber, I., Binder, E., Keck, M.E. & Holsboer, F. (2001) Differential analysis of behavior and diazepam-induced alterations in C57BL/6N and BALB/c mice using the modified hole board test. *J. Psychiatr. Res.* 35: 147-154.
- Stork, O., Welzl, H., Wotjak, C.T., Hoyer, D., Delling, M., Cremer, H. and M. Schachner (1999) Anxiety and increased 5-HT<sub>1A</sub> receptor response in NCAM null mutant mice. *Neurobiol.* 40:343-355.

**Table 2: Results of Behavioral Observation in the Modified Hole Board Test**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Wild Type (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=19)	<i>p - value</i>	(n=11)	(n=21)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
Line crossing [frequency]	141.71 $\pm$ 9.3	131.53 $\pm$ 3.82	N.A.	203.64 $\pm$ 16.06	160.1 $\pm$ 9.07	N.A.	p<0.05	p<0.05
Line crossing [latency]	0.77 $\pm$ 0.13	0.95 $\pm$ 0.24	N.A.	1.03 $\pm$ 0.22	2.22 $\pm$ 0.66	N.A.	n.s.	n.s.
Rearings in box [frequency]	33.93 $\pm$ 4.1	29.68 $\pm$ 1.77	N.A.	31.45 $\pm$ 1.85	29.19 $\pm$ 1.71	N.A.	n.s.	n.s.
Rearings in box [latency]	27.55 $\pm$ 4.55	26.23 $\pm$ 3.19	N.A.	31.95 $\pm$ 4.59	37.61 $\pm$ 4.23	N.A.	n.s.	p<0.05
Hole exploration [frequency]	24.64 $\pm$ 2.97	1.51 $\pm$ 1.51	N.A.	35.73 $\pm$ 3.71	32.9 $\pm$ 2.68	N.A.	p<0.05	p<0.05
Hole exploration [latency]	29.46 $\pm$ 5.58	31.9 $\pm$ 5.13	N.A.	15.38 $\pm$ 4.41	16.74 $\pm$ 4.37	N.A.	n.s.	p<0.05
Hole visit [frequency]	0 $\pm$ 0	0 $\pm$ 0	N.A.	0 $\pm$ 0	0 $\pm$ 0	N.A.	N.A.	N.A.
Hole visit [latency]	300 $\pm$ 0	300 $\pm$ 0	N.A.	300 $\pm$ 0	300 $\pm$ 0	N.A.	N.A.	N.A.
Board entry [frequency]	8.29 $\pm$ 0.83	7.37 $\pm$ 0.63	N.A.	17.09 $\pm$ 2.65	14.48 $\pm$ 1.48	N.A.	p<0.05	p<0.001
Board entry [latency]	62.79 $\pm$ 9	60.78 $\pm$ 9.19	N.A.	33.81 $\pm$ 3.64	41.16 $\pm$ 6.84	N.A.	p<0.05	n.s.
Board entry [total duration %]	8.99 $\pm$ 1.02	7.22 $\pm$ 0.8	N.A.	13.53 $\pm$ 1.81	13.22 $\pm$ 1.16	N.A.	n.s.	p<0.001
Rearing on board [frequency]	0.29 $\pm$ 0.16	0.16 $\pm$ 0.09	N.A.	0 $\pm$ 0	0.86 $\pm$ 0.27	N.A.	n.s.	p<0.05
Rearing on board [latency]	284.2 $\pm$ 8.94	287.05 $\pm$ 7.65	N.A.	300 $\pm$ 0	235.32 $\pm$ 18.91	N.A.	n.s.	n.s.
Risk assessment [frequency]	0 $\pm$ 0	0.05 $\pm$ 0.05	N.A.	0 $\pm$ 0	0 $\pm$ 0	N.A.	n.s.	n.s.

Parameter	Wild Type (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
Group contact [frequency]	12,86 ± 0,88	11 ± 0,39	N.A.	11,18 ± 1,51	9,24 ± 0,62	N.A.	n.s.	p<0.05
Group contact [latency]	21,49 ± 3,45	13,73 ± 1,63	N.A.	31,6 ± 7,3	33,87 ± 5,03	N.A.	n.s.	p<0.05
Group contact [total duration %]	24,75 ± 2,81	19,54 ± 1,14	N.A.	12,79 ± 2,11	10,89 ± 1,36	N.A.	p<0.05	p<0.001
Grooming [frequency]	0.07 ± 0.07	0.42 ± 0.12	N.A.	0.27 ± 0.19	0.14 ± 0.08	N.A.	n.s.	n.s.
Grooming [latency]	299.97 ± 0.03	276.01 ± 9.75	N.A.	264.69 ± 0	296.17 ± 2.43	N.A.	n.s.	n.s.
Grooming [total duration %]	0,01 ± 0,01	0.37 ± 0.14	N.A.	0.4 ± 0.27	0.31 ± 0.18	N.A.	n.s.	n.s.
Defecation [frequency]	0.07 ± 0.07	0 ± 0	N.A.	0 ± 0	0 ± 0	N.A.	n.s.	n.s.
Defecation [latency]	288.3 ± 11.7	300 ± 0	N.A.	300 ± 0	300 ± 0	N.A.	n.s.	n.s.
Unfamiliar object exploration [frequency]	6.29 ± 0.62	7.26 ± 0.53	N.A.	7.27 ± 0.7	8.43 ± 0.62	N.A.	n.s.	n.s.
Familiar object exploration [frequency]	7.36 ± 0.61	8.32 ± 0.48	N.A.	8.27 ± 0.74	9.52 ± 0.7	N.A.	n.s.	n.s.
Unfamiliar object exploration [latency]	16.56 ± 3.17	23.16 ± 3.75	N.A.	13.47 ± 2.47	34.67 ± 6.08	N.A.	n.s.	n.s.
Familiar object exploration [latency]	23.59 ± 4.74	33.47 ± 8.55	N.A.	30.68 ± 7.58	25.9 ± 5.39	N.A.	n.s.	n.s.
Unfamiliar object exploration [total duration %]	1.91 ± 0.2	2.6 ± 0.28	N.A.	1.21 ± 0.12	1.6 ± 0.11	N.A.	n.s.	n.s.
Familiar object exploration [total duration %]	2.04 ± 0.34	2.63 ± 0.14	N.A.	1.43 ± 0.14	1.87 ± 0.15	N.A.	n.s.	n.s.
Object Index	0.17 ± 0.05	0.19 ± 0.04	N.A.	0.13 ± 0.06	0.18 ± 0.04	N.A.	n.s.	n.s.

<b>Table 3: Video-Tracking Results Regarding Locomotor Behavior</b>								
Data are presented as mean ± standard error of mean.								
Parameter	Wild Type (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=14)	(n=19)	<i>p - value</i>	(n=11)	(n=21)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
<b>Total distance moved [cm]</b>	3617.46 ± 191.58	3393.85 ± 82.02	<b>N.A.</b>	5295.48 ± 351.77	4238.75 ± 213	<b>N.A.</b>	<b>p&lt;0.001</b>	<b>p&lt;0.05</b>
<b>Mean velocity [cm/sec]</b>	20.93 ± 0.92	20.27 ± 0.39	<b>N.A.</b>	26.31 ± 1.27	22.04 ± 0.77	<b>N.A.</b>	<b>p&lt;0.05</b>	<b>p&lt;0.05</b>
<b>Maximum velocity [cm/sec]</b>	62.84 ± 3.3	58.73 ± 1.6	<b>N.A.</b>	79.51 ± 3.74	67.49 ± 2.55	<b>N.A.</b>	<b>p&lt;0.05</b>	<b>p&lt;0.05</b>
<b>Turns [Frequency]</b>	1831.86 ± 39.96	1785.68 ± 26.32	<b>N.A.</b>	2253.64 ± 67.58	2065.33 ± 54.54	<b>N.A.</b>	<b>p&lt;0.001</b>	<b>p&lt;0.001</b>
<b>Board entry [max. duration, sec.]</b>	7.78 ± 0.74	6.87 ± 0.74	<b>N.A.</b>	7.78 ± 0.81	8.6 ± 0.8	<b>N.A.</b>	n.s.	n.s.
<b>Mean distance to wall [cm]</b>	7.53 ± 0.3	6.81 ± 0.16	<b>N.A.</b>	8.93 ± 0.39	8.91 ± 0.22	<b>N.A.</b>	<b>p&lt;0.05</b>	<b>p&lt;0.001</b>
<b>Mean distance to board [cm]</b>	8.29 ± 0.25	8.73 ± 0.11	<b>N.A.</b>	7.15 ± 0.26	7.19 ± 0.15	<b>N.A.</b>	<b>p&lt;0.05</b>	<b>p&lt;0.001</b>

## 3.2 Dymorphology, Bone and Cartilage

### 3.2.1 Summary

In the Dymorphology, Bone and Cartilage Screen of the German Mouse Clinic mice are analyzed for morphological abnormalities in different organ systems with special focus on bone and cartilage development and homeostasis. We adapted the successful dymorphology screening protocol from the Munich ENU-Mutagenesis Screen (Hrabé de Angelis *et al.* 2000) for the use in the German Mouse Clinic. The nomenclature of the parameters was adapted according to the “Phenoslim” wording (<http://www.informatics.jax.org/userdocs/phenoslim.shtml>). Further tests for defects in bone development and homeostasis were taken over from human diagnosis, and were adapted for the use in mice analysis: X-ray analysis, bone densitometry and, in a limited number of animals, micro-computer tomography.

A total of 70 animals of NCAM mice have been analyzed in the Dymorphology, Bone and Cartilage module of the German Mouse Clinic. We detected reduced fat mass in NCAM mutants. sBMD, Bone mineral content, Fat mass, Fat content, Lean content, Body length, Weight, and Subcutaneous fat were significantly changed in male NCAM mutants. In females alterations in the same direction could be observed, but the differences were less pronounced.

In the morphological investigation via visual inspection and X-ray analysis some additional phenotypes were observed in single individuals, however no correlation of any phenotype with a certain genotype was detected.

### 3.2.2 Mice

Twenty-eight male (15 +/+, 13 -/-) and 42 female (21 +/+, 21 -/-) NCAM-mice were analyzed by morphological inspection at the age of nine weeks. Blood was taken at the age of 14 weeks for determination of ionic calcium from 20 Mutants and 26 control animals. Sixteen-week-old NCAM-mutants (17 animals) and controls (21 animals) entered the bone density tests and X-ray analysis.

### 3.2.3 Material and Methods

The Dymorphology, Bone and Cartilage module of the German Mouse Clinic analyzed the mice in different phases:

1. At the age of 5 weeks, when the mice entered the facility, the general physical condition and health were checked;
2. At the age of 9 weeks, a morphological whole-body checkup was carried out;
3. The ionized fraction of calcium in blood was analyzed in 14-week-old mice, and
4. At the age of 16 to 17 weeks, X-ray analysis and bone densitometry were performed.

### **Morphological test**

The animals were screened using the protocol for morphological analysis from Fuchs *et al.* (2000) as adapted for the German Mouse Clinic.

### **Ionized calcium Analysis**

*Equipment:* AVL 9180 Electrolyte Analyzer (distributed by Roche Diagnostics GmbH, Mannheim, Germany)

cleaning solution and conditioning solution (Roche),

ISEtrol Quality Control Solutions (Roche),

lithium-heparin polypropylen tubes,

glass capillary (0.8 mm diameter, 32 mm length, without heparin; special product of Laborteam K+K, Munich).

*Quality control:* Calibration of the system and quality control were performed at intervals recommended and with solutions provided by the manufacturer. The results from the quality control were recorded by the system. Before blood measurement, daily cleaning, conditioning and calibration of the analyzer were performed.

*Procedure:* Blood (100 µl) was collected from anesthetized mice in lithium heparin tubes and transferred directly to the analyzer. Values were transferred directly to the database.

### **X-ray images**

*Equipment:* Faxitron X-ray Model MX-20 (Specimen Radiography System, Illinois, USA),

NTB Digital X-ray Scanner EZ 40 (NTB GmbH, Diepholz, Germany),

*Quality control:* Calibration of the system is done in monthly intervals,

*Settings:* Voltage 25 kV, integration time 40 ms,

*Procedure:* The anesthetized mouse was fixed on an X-ray-permeable plate and placed in the machine. Using iX-Pect software supplied by the manufacturer of the X-ray scanner, the image was taken and analyzed. Analysis was done qualitatively by visual inspection of the images as well as quantitatively by using the ruler tool of iX-Pect software.

### **Bone density analysis**

*Equipment:* pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems. Inc., Basingstoke, Hampshire, UK; distributed by Stratec Medizintechnik GmbH, Pforzheim, Germany),

*Quality control:* Calibration of the system was done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. Results from the quality control were recorded by the system.

*Settings:* Scan speed 20 mm/s, Resolution 0.5 mm x 1.0 mm, HAW 0.020

*Procedure:* After anesthesia, the weight and length of the mouse were recorded, and the mouse was placed in the analyzer. After a scout scan, the area of interest was optimized and the measure scan started.

*Data-analysis:* For analysis of the data, regions have to be defined. The standard analysis comprises a whole body analysis as well as a whole body analysis excluding the skull.

### Statistical analysis of data

Analysis of quantitative data sets was carried out using StatView software package (SAS corporation).

### 3.2.4 Parameters

<b>Morphological inspection</b>
<i>Growth/weight/body size:</i> abnormality <i>Eye:</i> dysmorphology, corneal or lens defect, <i>Coat:</i> hair growth defects, hair texture defects, color anomalies, hair follicle, structure/orientation anomalies <i>Skin:</i> pigmentation anomalies, texture/condition, anomalies <i>Vibrissae:</i> dysmorphology, <i>Extremities:</i> limb dysmorphology, digit dysmorphology, tail dysmorphology <i>Teeth:</i> tooth dysmorphology <i>Ears:</i> auditory defects/deafness, dysmorphology <i>Musculature:</i> muscle dysmorphology, <i>Skeletal:</i> osteogenesis/developmental anomalies, axial defects, extremities defects, craniofacial defects <i>Neurological / behavioral:</i> seizures/epilepsy, motor capabilities / coordination / movement anomalies, feeding / drinking anomalies <i>Respiratory system:</i> dysmorphology <i>Reproductive system:</i> dysmorphology <i>Other aberrant phenotype</i>
<b>X-ray analysis</b>
Skull shape, mandibles, maxilla, teeth, orbit, number of vertebrae (cervical, thoracic, lumbar, pelvic, sacral), vertebrae shape, number of ribs, rib shape, scapulas, clavicle, pelvis, femur diameter, femur shape, tibia, fibula, humerus, ulna, radius, number of digits, completeness of digits, subcutaneous fat, joints
<b>Dual energy X-ray absorption</b>
Bone mineral density (BMD), partial bone mineral density (pBMD, whole body excluding skull), specific bone mineral density (sBMD), bone mineral content (BMC), lean mass, fat mass, bone content, lean content, fat content
<b>AVL analyzer</b>
Free ionic calcium
<b>Computer tomography</b>
3D-visualization of whole skeleton, 2D-examination of inner organs and soft tissue, high-resolution analysis of regions of interest

### 3.2.5 Results and Discussion

In the morphological inspection of the mice and in the X-ray analysis, a few abnormalities were observed, however they were found in mutants and controls (Tabs. 4 and 5).

In the DEXA analysis **differences between the genotypes** in males could be found in the parameters specific BMD, Bone mineral content, Fat mass, Fat content, Lean content, Body length, Weight, and Subcutaneous fat. In females alterations in the same direction could be observed, but only in Weight and Subcutaneous fat (Tab. 7) the differences were significant in the t-test.

Two bone-related parameters sBMD and BMC are changed in mutant males. However, if the BMC data is related to the weight of the animals (bone content), the differences are less pronounced. The different values in sBMD between mutants and controls can be caused by the differences in weight of the animals (sBMD=BMD/Weight). Summarizing up, there is no hint from our data to a defect in bone metabolism in the NCAM mutants.

Differences in fat mass and fat content of NCAM mutants could be shown in three independent tests of our screen: Some animals with less fat were already detected in the visual inspection of the animals. Significant differences in fat mass and fat content between mutants and controls could be shown in males using the DEXA technique. Quantitative analysis of the X-ray images detected differences in subcutaneous fat in both male and female data. Additional information is given in chapter 3.11.5 (Metabolic Screen).

The data of the wild-type animals corresponded perfectly with the data from C57BL/6J animals obtained in our baseline experiment.

Raw data will be available on demand.

### 3.2.6 References

Fuchs H, Schughart K, Wolf E, Balling R, Hrabé de Angelis M. (2000): Screening for dysmorphological abnormalities - a powerful tool to isolate new mouse mutants. *Mammalian Genome* 11: 528-30.

Hrabé de Angelis, M., H. Flaswinkel, H. Fuchs, B. Rathkolb, D. Soewarto, S. Marschall, S. Heffner, W. Pargent, K. Wuensch, M. Jung, A. Reis, T. Richter, F. Alessandrini, T. Jakob, E. Fuchs, H. Kolb, E. Kremmer, K. Schaeble, B. Rollinski, A. Roscher, C. Peters, T. Meitinger, T. Strom, T. Steckler, F. Holsboer, T. Klopstock, F. Gekeler, C. Schindewolf, T. Jung, K. Avraham, H. Behrendt, J. Ring, A. Zimmer, K. Schughart, K. Pfeffer, E. Wolf and R. Balling (2000): Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nature Genetics* 25: 444 – 447

#### Abbreviations

KO	knockout
BMC	bone mineral content
BMD	bone mineral density
pBMD	partial bone mineral density
sBMD	specific bone mineral density

<b>Table 4: Results from the Morphological Inspection</b>				
<b>Phenotype</b>	<b>Male</b>		<b>Female</b>	
	<b>KO</b>	<b>wt</b>	<b>KO</b>	<b>wt</b>
Growth / Weight / Body size abnormality	2	-	4	-
Skin pigmentation anomaly	1	-	5	1
Craniofacial dysmorphology	-	-	5	-
Tooth dysmorphology	-	-	1	-
Eye dysmorphology	1	-	8	1
<b>Animals analyzed</b>	<b>13</b>	<b>15</b>	<b>21</b>	<b>21</b>

<b>Table 5: Results from the X-Ray Analysis</b>				
<b>Phenotype</b>	<b>Male</b>		<b>Female</b>	
	<b>KO</b>	<b>wt</b>	<b>KO</b>	<b>wt</b>
Craniofacial dysmorphology	1	-	1	-
Mandibles/Maxilla abnormality	3	-	-	-
<b>Animals analyzed</b>	<b>8</b>	<b>10</b>	<b>9</b>	<b>11</b>

**Table 6: Bone-Related Quantitative Parameters**Data are presented as mean  $\pm$  standard error of mean.

	Wild Type (A)			Mutant (B)			Male A~B	Female A~B
Parameter	Male (n=10)	Female (n=11)	p-value	Male (n=8)	Female (n=9)	p-value	p-value	p-value
BMD [mg/ cm <sup>2</sup> ]	61 $\pm 1$	65 $\pm 2$	n.s.	60 $\pm 2$	60 $\pm 2$	n.s.	n.s.	n.s.
pBMD [mg/ cm <sup>2</sup> ]	51 $\pm 1$	49 $\pm 1$	n.s.	48 $\pm 2$	46 $\pm 2$	n.s.	n.s.	n.s.
sBMD [10 <sup>-3</sup> x cm <sup>-2</sup> ]	1.79 $\pm 0.04$	2.86 $\pm 0.15$	< 0.001	2.20 $\pm 0.11$	3.02 $\pm 0.16$	< 0.001	< 0.01	n.s.
BMC [mg]	789 $\pm 46$	465 $\pm 34$	< 0.001	534 $\pm 59$	368 $\pm 33$	< 0.05	< 0.01	n.s.
Lean mass [g]	21.55 $\pm 0.83$	17.90 $\pm 0.45$	< 0.001	21.78 $\pm 0.97$	16.74 $\pm 0.72$	< 0.001	n.s.	n.s.
Fat mass [g]	8.83 $\pm 1.50$	2.72 $\pm 0.51$	< 0.01	3.84 $\pm 1.25$	1.71 $\pm 0.70$	n.s.	< 0.05	n.s.
Body Length [cm]	8.85 $\pm 0.11$	8.32 $\pm 0.08$	< 0.001	8.50 $\pm 0.09$	8.11 $\pm 0.07$	< 0.01	< 0.05	n.s.
Weight [g]	34.26 $\pm 1.33$	23.01 $\pm 0.65$	< 0.001	27.39 $\pm 0.61$	20.11 $\pm 0.59$	< 0.001	< 0.001	< 0.01
Bone Content [%]	2.29 $\pm 0.08$	2.01 $\pm 0.12$	n.s.	1.93 $\pm 0.18$	1.82 $\pm 0.14$	n.s.	n.s.	n.s.
Lean Content [%]	64.07 $\pm 4.08$	78.50 $\pm 3.14$	< 0.02	79.98 $\pm 4.36$	83.33 $\pm 3.00$	n.s.	< 0.02	n.s.
Fat Content [%]	24.83 $\pm 3.46$	10.40 $\pm 2.11$	< 0.01	10.10 $\pm 3.68$	4.70 $\pm 2.30$	n.s.	< 0.02	n.s.
Subcutaneous fat span [mm]	4.3 $\pm 0.3$	3.7 $\pm 0.1$	n.s.	3.3 $\pm 0.3$	3.1 $\pm 0.1$	n.s.	< 0.05	< 0.01
	Male (n=15)	Female (n=11)	p - value	Male (n=11)	Female (n=9)	p - value	p - value	p - value
Ionized Calcium [mM]	1.18 $\pm 0.02$	1.18 $\pm 0.02$	n.s.	1.15 $\pm 0.02$	1.17 $\pm 0.02$	n.s.	n.s.	n.s.

## 3.3 Neurology Screen

### 3.3.1 Summary

In the primary neurological screen, 35 NCAM knockout mice (13 males/21 females) and 37 control mice (16 males/21 females) were screened. Animals were analyzed according to our modified SHIRPA protocol where a battery of behavioral tests is carried out. This primary observation screen is a modification of the Irwin procedure (Irwin, 1968) and was proposed as a rapid, comprehensive and semi-quantitative screening method for qualitative analysis of abnormal phenotypes in a mouse strain (Rogers *et al.*, 1994).

We carried out 37 of 40 designed test parameters (See web page: [http://www.mgu.har.mrc.ac.uk/mutabase/shirpa\\_summary.html](http://www.mgu.har.mrc.ac.uk/mutabase/shirpa_summary.html)) to detect phenotypic differences between knockout and control mice. Each test parameter contributes to an overall assessment in muscle, lower motor neuron, spinocerebellar, sensory and autonomic function. The primary neurological screen is focused on investigating neurological reflexes to determine the neurological functioning of a mouse. We also examine lactate levels in the blood of mice to draw conclusions about energy metabolism.

The comparison of NCAM knockout to control mice revealed no obvious neurological phenotype. We found significant differences in the body weight between NCAM knockout and control mice only.

### 3.3.2 Mice

As mentioned in Chapter 2.4.1, thirteen 10-week-old male NCAM-KO and sixteen 10-week-old male NCAM control mice entered the neurological screen at three time points, female mice (21 NCAM-knockouts and 21 NCAM controls) one week later respectively. All animals were fed *ad libitum* for a period of one week during their stay in the neurological screen.

### 3.3.3 Material and Methods

At the age of 10 weeks assessment of each animal started with observation of undisturbed behavior (*Viewing Jar Behavior*) in a glass cylinder (11 cm in diameter). The mice were then transferred to an arena consisting of a clear Perspex box (420 x 260 x 180 mm) in which a Perspex sheet on the floor is marked with 15 squares. In this arena, locomotor activity and motor behavior was observed (*Behavior recorded in the Arena*). This was followed by a sequence of manipulations testing reflexes, grip strength, toe pinch and wire maneuver (*Behavior recorded on or above the arena*). For the wire maneuver test, a rigid horizontal wire (3 mm in diameter) is secured across the rear right corner of the arena. For grip strength testing, a grid (270 x 275 mm) is secured across the width of the arena. In the last part of the observation (*Behavior recorded during Supine Restraint*), the animals were restrained in a supine position to record autonomic responses such as salivation. Measurements were completed with the recording of limb tone, provoked biting, and body length. The last part of the primary screen also involves the analysis of righting reflex, negative geotaxis and contact righting reflex. A glass cylinder

(35 mm diameter, 135 mm length) is used for testing the contact-righting reflex. Throughout the entire procedure, abnormal behavior, irritability, fear, aggression and vocalization were recorded. Between testing of each mouse, faecal pellets and urination were removed from the viewing jar and arena. All experimental equipment is thoroughly cleaned with Pursept-A and dried prior to testing.

Values for body length, body weight and locomotor activity are presented as means  $\pm$  SEM. Kruskal-Wallis-test (S-PLUS, Insightful) was used to test for effects of genotype and gender factors on these parameters. The Chi-Squared test was applied for all other parameters.

### 3.3.4 Parameters

<b>Muscle/lower motor neuron function</b>
Body position, gait, Positional passivity, wire maneuver, tail elevation, limb tone, body tone, abdominal tone, grip strength, urination, defecation
<b>Spinocerebellar function</b>
Body position, gait, righting reflex, tail elevation, visual placing, limb tone, body tone, abdominal tone, grip strength
<b>Sensory function</b>
Transfer arousal, touch escape, gait, visual placing, toe pinch, pinna reflex, righting reflex
<b>Autonomic function</b>
Palpebral closure, urination, salivation, respiration rate, defecation
<b>Neurological reflexes</b>
Righting reflex (pons), contact righting reflex, visual placing, toe pinch/flexion reflex (cerebellar/spinal cord), negative geotaxis, corneal reflex (medulla), pinna reflex (hearing test)
<b>Physiological parameters</b>
Body weight, body length
<b>General appearance</b>
Body weight, body length, body position, transfer arousal, fear, touch escape, irritability, vocalization, positional passivity, aggression, spontaneous activity, locomotor activity, skin color

### 3.3.5 Results

All **SHIRPA test parameters** were without significant pathological findings. **Blood lactate** screening showed that both male and female NCAM knockout mice had no significant changes in their blood lactate level as compared to control mice. The only obvious parameter we found was a significantly reduced body weight of NCAM knockout mice compared to control mice (Tab. 7).

Raw data for each individual are available on demand in Excel sheets.

### 3.3.6 Discussion

In the Neurological Screen, male and female NCAM knockout mice did not show any significant SHIRPA parameter in comparison to controls with the exception of reduced body weight. Since homozygous NCAM knockout mice seem to have neuromuscular end plates that are 14% smaller as compared to controls, defects in muscular functioning might be possible. Therefore, we recommend **secondary screening** including a quantitative grip strength examination.

### 3.3.7 References

Irwin S. (1968) Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia*. 13(3): 222-257.

Rogers D. C., E.M. Fisher, S.D. Brown, J. Peters, A.J. Hunter, J.E. Martin (1997) Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome*. 8(10): 711-713.

### Abbreviations

SHIRPA **S**mithKline Beecham Pharmazeuticals; **H**arwell, MRC Mouse Genome Centre and Mammalian Genetics Unit; **I**mperial Col-  
legeschool of Medicine at St. Mary`s; **R**oyal London Hospital, **P**he-  
notype Assessment  
[http://www.mgu.har.mrc.ac.uk/mutabase/shirpa\\_summary.html](http://www.mgu.har.mrc.ac.uk/mutabase/shirpa_summary.html)

s.a. Sub-maxillary area

**Table 7: Recording of Body Length and Body Weight**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Male			Female		
	Wild Type (n=16)	Mutant (n=13)	<i>p-value</i>	Wild Type (n=21)	Mutant (n=21)	<i>p-value</i>
<b>Body Length [g]</b>	8.29 $\pm$ 0.03	8.25 $\pm$ 0.04	<i>n.s.</i>	7.6 $\pm$ 0.02	7.4 $\pm$ 0.03	<i>n.s.</i>
<b>Body Weight [g]</b>	28.4 $\pm$ 0.17	23.03 $\pm$ 0.2	<b>0.001</b>	20.5 $\pm$ 0.1	18.2 $\pm$ 0.1	<b>0.003</b>

**Table 8: Behavior Recorded in Viewing Jar**Data shown represents the results of test parameters from major tests where a behavioral response was observed. Test parameters which did not elicit any response were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0.05$ 

Parameter	Male			Female		
	Wild Type (n=16)	Mutant (n=13)	<i>p-value</i>	Wild Type (n=21)	Mutant (n=21)	<i>p-value</i>
<b>Body Position</b>						
Sitting or standing	9	8	<i>n.s.</i>	14	11	<i>n.s.</i>
Rearing on hind legs	7	5		7	9	
<b>Spontaneous Behaviour</b>						
Slow movement	0	0	<i>n.s.</i>	0	0	<i>n.s.</i>
Moderate movement	10	6		12	11	
Vigorous	6	7		9	10	
<b>Tremor</b>						
None	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
Mild	0	0		0	0	

**Table 9: Recording of Locomotor Activity and Behavior in the Arena**

Locomotor activity data are shown as mean ( $\pm$  SEM). Data from behavior recorded in the Arena represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0,05$

Parameter	Male			Female		
	Wild Type (n=16)	Mutant (n=13)	<i>p-value</i>	Wild Type (n=21)	Mutant (n=21)	<i>p-value</i>
<b>Locomotor Activity</b>	21.69 $\pm$ 0.5	22.9 $\pm$ 0.5	<i>n.s.</i>	19.38 $\pm$ 0.5	23.9 $\pm$ 0.3	<i>n.s.</i>
<b>Transfer arousal</b>						
Brief freeze	9	3	<i>n.s.</i>	10	12	<i>n.s.</i>
Momentary freeze	1	1		0	0	
No freeze	6	9		11	9	
<b>Palpebral Closure</b>						
Eyes wide open	16	12	<i>n.s.</i>	21	21	<i>n.s.</i>
Eyes 1/2 closed	0	1		0	0	
<b>Piloerection</b>						
None	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Gait</b>						
Normal	16	13	<i>n.s.</i>	20	20	<i>n.s.</i>
Fluid but abnormal	0	0		1	0	
<b>Pelvic Elevation</b>						
Markedly flattened	0	0	<i>n.s.</i>	1	1	<i>n.s.</i>
Barely touches	2	1		0	0	
Normal	13	9		16	11	
Elevated	1	3		4	9	
<b>Tail Elevation</b>						
Horizontally extended	16	12	<i>n.s.</i>	19	20	<i>n.s.</i>
Elevated/Straub tail	0	1		2	1	
<b>Touch Escape</b>						
Mild	0	0	<i>n.s.</i>	1	0	<i>n.s.</i>
Moderate	8	7		10	9	
Vigorous	8	6		10	12	
<b>Positional Passivity</b>						
Struggles when held by tail	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>

**Table 10: Behavior Recorded in or above the Arena**

Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0,05$

Parameter	Male			Female		
	Wild Type (n=16)	Mutant (n=13)	<i>p-value</i>	Wild Type (n=21)	Mutant (n=21)	<i>p-value</i>
<b>Trunk Curl</b>						
Absent	5	2		7	8	
Present	11	11	<i>n.s.</i>	14	13	<i>n.s.</i>
<b>Limb Grasping</b>						
Absent	16	13		20	20	
Present	0	0	<i>n.s.</i>	1	1	<i>n.s.</i>
<b>Visual Placing</b>						
Upon vibrassee contact	10	10		14	15	
Before vibrassee contact	5	3		6	6	
Early vigorous extension	1	0	<i>n.s.</i>	1	0	<i>n.s.</i>
<b>Grip strength</b>						
Moderate	12	7		7	8	
Active grip	4	6	<i>n.s.</i>	14	13	<i>n.s.</i>
<b>Body Tone</b>						
Slight resistance	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Pinna reflex</b>						
Active retraction	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Corneal Reflex</b>						
Active single eye blink	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Toe Pinch</b>						
None	1	0		0	0	
Moderate withdrawal	6	4	<i>n.s.</i>	7	8	<i>n.s.</i>
Brisk	9	9		14	13	
<b>Wire manoeuver</b>						
Active grip	1	2		1	4	
Difficulty to grasp	13	9		15	11	
Unable to grasp	1	1		5	4	
Falls within seconds	1	1	<i>n.s.</i>	0	2	<i>n.s.</i>

**Table 11: Behavior during Supine Restraint**

Data shown represent the results of test parameters from major tests where a behavioral response was observed. Test parameters, which did not elicit any response, were excluded from this data. Statistical analysis: chi-squared test; significance  $p < 0,05$ .

Parameter	Male			Female		
	Wild Type (n=16)	Mutant (n=13)	<i>p-value</i>	Wild Type (n=21)	Mutant (n=21)	<i>p-value</i>
<b>Skin Color</b>						
Pink	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Limb Tone</b>						
No resistance	11	9		14	13	
Slight resistance	5	4	<i>n.s.</i>	7	8	<i>n.s.</i>
<b>Abdominal Tone</b>						
Slight resistance	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Lacrimation</b>						
None	15	13		21	21	
Present	1	0	<i>n.s.</i>	0	0	<i>n.s.</i>
<b>Salivation</b>						
None	0	2		0	0	
Slight margin of s.a	14	6		16	19	
Wet zone entire of s.a.	2	5	<i>n.s.</i>	5	2	<i>n.s.</i>
<b>Provoked biting</b>						
Absent	8	9		13	16	
Present	8	4	<i>n.s.</i>	8	5	<i>n.s.</i>
<b>Righting reflex</b>						
No impairment	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Contact righting reflex</b>						
Present	16	13	<i>n.s.</i>	21	21	<i>n.s.</i>
<b>Negative Geotaxis</b>						
Turns and climbs the grid	14	11		18	18	
Turns but then freezes	1	0		0	0	
Moves, but fails to turn	1	2	<i>n.s.</i>	3	3	<i>n.s.</i>
<b>Irritability</b>						
None						
Struggles during supine restraint	6 10	6 7	<i>n.s.</i>	12 9	15 6	<i>n.s.</i>
<b>Aggression</b>						
None	12	9		16	20	
Provoked biting or attack	4	4	<i>n.s.</i>	5	1	<i>n.s.</i>
<b>Vocalization</b>						
None	0	5		3	3	
Provoked during handling	16	8	<i>n.s.</i>	18	18	<i>n.s.</i>

<b>Table 12: Lactate Levels</b>						
Data shown represent the results of the mean blood lactate concentrations, value ( $\pm$ SEM)						
	<b>Male</b>			<b>Female</b>		
	<b>Wild Type (n=16)</b>	<b>Mutant (n=13)</b>	<b><i>p-value</i></b>	<b>Wild Type (n=21)</b>	<b>Mutant (n=21)</b>	<b><i>p-value</i></b>
<b>Lactate (mmo/l)</b>	5.3 $\pm$ 0.56	5.3 $\pm$ 0.4	<b><i>n.s.</i></b>	4 $\pm$ 0.6	4 $\pm$ 0.1	<b><i>n.s.</i></b>

## 3.4 Eye Screen

### 3.4.1 Summary

In the Eye Screen, a high throughput electroretinography method (ERG) was employed to examine mice for retinal impairment (Dalke *et al.*, 2004). Furthermore, mice were examined for anterior segment abnormalities by slitlamp biomicroscopy (Favor, 1983).

In humans blindness several different ocular diseases cause. Among these, the cataracts are responsible for half of all cases (Johnson and Foster, 2003). The retinal disorders cover a broad variety of clinical symptoms and many different genes are involved in the corresponding pathological conditions in humans. The two most important groups are retinitis pigmentosa (RP) and age-related-macular-degeneration (ARMD; for recent reviews, see Rivolta *et al.*, 2002 and Stone *et al.*, 2001). Mouse models are appropriate tools to understand the genetic and biochemical mechanisms of ocular disorders. There is a rapid increasing number of mouse mutants available suffering from various types of eye diseases (for a recent review see Graw, 2003).

No genotype-specific differences between wild-type and mutant NCAM mice were detected.

### 3.4.2 Mice

Thirty-six NCAM *+/+* (15 male, 21 female) and 34 NCAM *-/-* mice (13 male, 21 female) entered the Eye Screen at the age of 11 weeks. Mice were first examined by slit lamp biomicroscopy and on the following day, an ERG was performed. Mice were kept under standard laboratory conditions with food and water *ad libitum*.

### 3.4.3 Materials and Methods

**Electroretinography (ERG)** was used to examine the retinal function as described (Dalke *et al.*, 2004). Mice were dark-adapted for at least 12 hours and anaesthetized with 137 mg Ketamine and 6.6 mg Xylazine per kg body weight. After pupil dilation (1 drop Atropine 1%), individual mice were fixed on a sled with Velcro straps. Gold wires (as active electrodes) were placed on the cornea; care was taken not to obstruct the pupillary opening. The ground electrode was a subcutaneous needle in the tail; a reference electrode was placed subcutaneously between the eyes. The mice were introduced into an ESPION ColorBurst Handheld Ganzfeld LED stimulator (Diagnosys LLC, Littleton, MA, USA) on a rail to guide the sled (High-Throughput Mouse-ERG, STZ for Biomedical Optics and Function Testing, Tübingen, Germany). To minimize temperature influences on the ERG, body temperature was kept at 37°C using a warming plate. 10 ms light pulses were delivered at a frequency of 0.48 Hz in two steps at 500 and 12,500 cd/m<sup>2</sup>. Bandpass filter was set ranging from 0.15 to 1000 Hz. Responses were recorded simultaneously from both eyes with an ESPION Console (Diagnosys LLC, Littleton, MA, USA) and stored for offline analysis after averaging 10-40 individual measurements at each step.

**Slit Lamp Biomicroscopy:** Mice were examined biomicroscopically for eye abnormalities as previously described (Favor, 1983). Briefly, pupils were dilated with a 1% atropine solution applied to the eyes at least 10 min prior to examination. Both eyes of the mice were examined by slit lamp biomicroscopy (Zeiss SLM30) at 48x magnification with a narrow beam slit lamp illumination at 25-30° angle from the direction of observation. Observed phenotypic variants of the eyes were carefully documented.

**Statistical Analysis:** ERG data were statistically analyzed using MS-Excel. Differences between mouse groups were evaluated with the Student's t-test. Statistical significance was set at  $p < 0.05$ . Data are presented as mean values  $\pm$  standard error of the mean (SEM).

### 3.4.4 Parameters

<b>Electroretinography (ERG)</b>
a/b-wave, left/right eye at 500/12.50 cd/m <sup>2</sup>
<b>Slit lamp biomicroscopy</b>
(qualitative) abnormalities of lens and cornea like opacity and development disorders
<b>Histology</b>
(qualitative) retinal lamination and morphology of cell layers and lens
<b>Morphology</b>
(qualitative) like size and degree of closure

### 3.4.5 Results and Discussion

**ERG responses** were recorded from the groups of NCAM (wild type – mutant) mice with light pulses at two different light intensities. These two luminance levels were chosen because at 500 cd/m<sup>2</sup> a well discernable b-wave amplitude (nearly no a-wave) mainly stemming from the rod system is induced, while light pulses at 12,500 cd/m<sup>2</sup> induce a maximally developed b-wave response and an a-wave, coming presumably from rods and cones (Dalke et al., 2004).

At first, a comparison of the left and right eyes for each group was performed on the amplitudes of a- and b-wave for both luminance intensities (data not shown). Since no differences were observed between the left and right eye, ERG amplitudes of both eyes were averaged for further evaluation. The mean value and standard error was calculated for each group of mice, male and female, wild type and mutant (Tab. 13). The comparison of a- and b-wave amplitudes of males and females revealed no significant differences. Between the groups of mutant and wild-type NCAM mice no consistent differences were found, neither in the male nor in the female group.

A total of 70 mice were examined ophthalmologically by **slit lamp biomicroscopy**. No eye phenotype was shown to be associated with the NCAM mutation (Tab. 14).

### 3.4.6 References

- Dalke C., J. Löster, H. Fuchs, V. Gailus-Durner, D. Soewarto, J. Favor, A. Neuhäuser-Klaus, W. Pretsch, F. Gekeler, K. Shinoda, E. Zrenner, T. Meitinger, M. Hrabé de Angelis and J. Graw (2004): Electroretinography as a screening method for mutations causing retinal dysfunction in mice. *IOVS* 45: 601-609.
- Favor, J. (1983): A comparison of the dominant cataract and recessive specific-locus mutation rates induced by treatment of male mice with ethylnitrosourea. *Mutation Research* 110: 367-382.
- Graw J. (2003): The genetic and molecular basis of congenital eye defects. *Nat. Rev. Genet.* 4: 876-888.
- Johnson G.J. and A. Foster (2003): Prevalence, incidence and distribution of visual impairment. In: G.J. Johnson, D.C. Minassian, R.A. Weale, S.K. West (eds.): *The epidemiology of the eye disease*. Arnold, London, UK, 2003, 3-28.
- Rivolta C., D. Sharon, M. Hrabé de Angelis and T.P. Dryja (2002): Retinitis pigmentosa and allied diseases: numerous diseases, genes, and inheritance patterns. *Hum. Mol. Genet.* 11: 1219-1227.
- Stone E.M., V.C. Sheffield and G.S. Hageman (2001): Molecular genetics of age-related macular degeneration. *Hum. Mol. Genet.* 10: 2285-2292.

**Table 13: Comparison of ERG-Responses at Illumination Levels of 500 and 12,500 cd/m<sup>2</sup>.**  
Mean ± standard error is calculated for a- and b-wave amplitudes.

Parameter	Wild Type (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=15)	(n=21)	<i>p</i> -value	(n=13)	(n=21)	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
<b>a-wave</b> 500 cd/m <sup>2</sup>	-9 ± 1.5	-10 ± 1.5	n.s.	-11 ± 1.2	-10 ± 1.4	n.s.	n.s.	n.s.
<b>b-wave</b> 500 cd/m <sup>2</sup>	158 ± 6.8	166 ± 6.5	n.s.	142 ± 7.0	150 ± 4.4	n.s.	n.s.	n.s.
<b>a-wave</b> 12,500 cd/m <sup>2</sup>	-41 ± 3.2	-37 ± 2.5	n.s.	-42 ± 4.2	-45 ± 2.8	n.s.	n.s.	<0.05
<b>b-wave</b> 12,500 cd/m <sup>2</sup>	193 ± 11.7	194 ± 7.4	n.s.	182 ± 10.0	184 ± 6.8	n.s.	n.s.	n.s.

**Table 14: Results from Slit Lamp Biomicroscopy**

Genotype	Normal	Nuclear flecks	Corneal erosions	Microphthalmia
<b>NCAM -/-</b>	11	14	5	4
<b>NCAM +/+</b>	8	19	6	3

**Abbreviations**

cd/m<sup>2</sup>      candela per square meter  
 ERG        electroretinography  
 Hz         hertz

## 3.5 Clinical-Chemical Screen

### 3.5.1 Summary

The aim of the Clinical-Chemical Screen is the detection of hematological changes, defects of various organ systems, and changes in metabolic pathways and electrolyte homeostasis by means of suitable laboratory diagnostic tools. Since most inherited metabolic disorders are known to lead directly or indirectly, via altered organ function, to changes in the parameters investigated, this screening process provides a comprehensive investigation of clinical phenotypes with counterparts in humans and animal species (Rathkolb *et al.*, 2001). The methods used are routine procedures, allowing the appropriate screen of large numbers of mice for a broad spectrum of clinical-chemical and hematological parameters (Chamby *et al.*, 2004; Hough *et al.*, 2002).

In the primary clinical chemical screen, 36 (15 males/ 21 females) control mice and 32 (13 males/ 19 females) NCAM mice were analyzed. Nineteen different clinical-chemical parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes. Additionally, we measured 8 basic hematological parameters. All parameters of both mutants and control mice were within normal ranges.

### 3.5.2 Mice

The NCAM mice were delivered in three groups:

- The first group (9 males/9 females) entered the Clinical-Chemical Screen at the beginning of the 28<sup>th</sup> calendar week in 2003.
- The second batch (10 males/18 females) started at the beginning of the 43<sup>rd</sup> calendar week 2003, and
- The last group (9 males/15 females) started in 2004 at the beginning of the 3<sup>rd</sup> calendar week (Chapter 2.4.1; Table 15).

Group Name	Date	Number	Sex	Genotype	
				Mutant	Wild Type
NCAM1	08.07.2003	18	9 males	4	5
			9 females	4	5
NCAM2	21.10.2003	28	10 males	5	5
			18 females	9	9
NCAM3	15.01.2004	24	9 males	4	5
			15 females	8	7

### 3.5.3 Materials and Methods

#### Blood Withdrawal and Storage

The Clinical-chemical Screen of the German Mouse Clinic routinely analyzed 12-week-old mice. A blood sample was taken from an ether-anesthetized

mouse by puncturing the retro-orbital sinus with a non-heparinized capillary (0.8 mm in diameter; Laborteam K&K; Munich, Germany; Art.No. 1.28.13.1.2). The time for sample taking was recorded in a work list. A total of 600µl blood was collected in a heparinized tube (Li-heparin, KABE; Nümbrecht, Germany; Art. No. 078028). In addition, 50 µl blood was collected (using the same capillary) in EDTA-coated tubes (KABE, Art. No 078035). The tube was immediately inverted five times to achieve a homogeneous distribution of the anticoagulant.

The Li-heparin-coated tubes were stored in a rack at room temperature for two hours. Afterwards, cells and plasma were separated by a centrifugation step (10 min, 4656 x g; Biofuge, Heraeus; Hanau, Germany). Plasma (130 µl) was transferred into an Eppendorf tube and diluted 1:2 with aqua dest. The solution was mixed for a few seconds (Vortex genie, Scientific Industries, New York, America) to prevent clotting and then centrifuged again for 10 min at 4656 x g. Blood samples were distributed to Clinical Chemical, Immunology and Neurology (Lactate) Screens, whereas the Immunology, Allergy, and Clinical Chemical Screens received plasma samples .

### **Clinical Chemistry**

The high-throughput of the screen was insured by the use of an Olympus AU 400 autoanalyzer and adapted reagents from Olympus (Hamburg, Germany) and Roche (Mannheim, Germany). In the primary screen, 20 different parameters were measured including various enzyme activities, as well as plasma concentrations of specific substrates and electrolytes.

### **Hematology**

A volume of 50 µl EDTA-blood was used to measure basic hematological parameters with a blood analyzer, which has been carefully validated for the analysis of mouse blood (ABC-Blutbild-Analyzer, Scil Animal Care Company GmbH, Viernheim). Red blood cells, white blood cells, and platelets are measured by electrical impedance, and hemoglobin by spectrophotometry. Mean corpuscular volume (MCV) is calculated directly from the cell volume measurements, the hematocrit (HCT) from  $MCV \times \text{red blood cell count}$ . Mean corpuscular hemoglobin (MCH) and mean concentration of corpuscular hemoglobin (MCHC) are calculated from hemoglobin/red blood cells count (MCH) and hemoglobin/hematocrit (MCHC).

### **Analysis of Data**

Data were statistically analyzed using Excel and Sigma Stat 2.0 with the level of significance set at  $p < 0.05$ .

### 3.5.4 Parameters

<b>Plasma enzyme activities</b>
Alkaline phosphatase (EC 3.1.3.1), $\alpha$ -Amylase (EC 3.2.1.1), Creatine kinase (EC 2.7.3.2), Aspartate-aminotransferase (AST/GOT; EC 2.6.1.1), Alanine-aminotransferase (ALT/GPT; EC 2.6.1.2), Ferritin, Transferrin, Lipase (EC 3.1.1.3)
<b>Plasma concentrations of specific substrates</b>
Glucose, Cholesterol, Triglycerides, Total protein, Uric acid, Urea, Creatinine
<b>Plasma concentrations of electrolytes</b>
Potassium, Sodium, Chloride, Calcium, Inorganic phosphate
<b>Basic hematology</b>
White blood cell count (WBC), Red blood cell count (RBC) Hematocrit (HCT), Hemoglobin (HGB), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Platelet count (PLT)

### 3.5.5 Results

#### Clinical Chemistry

Most values obtained for the clinical chemical parameters were within the normal ranges usually found in C57BL/6 mice at the age of three months and were supported by previously published data (Tab. 16; Suckow *et al*, 2001; Quimby, 1999 and publications cited therein). In the control animals, sex differences were observed in inorganic phosphorus, urea, triglyceride, alanine-aminotransferase, alkaline phosphatase, and amylase concentrations. In the NCAM mice, we found sex differences in sodium, inorganic phosphorus, urea, triglyceride, and amylase concentrations.

Sodium and chloride concentrations differed significantly between mutants and wild-type animals only in males (Tab. 16).

#### Hematology

In the primary screen, all hematological parameters of both wild type and mutants were in normal ranges (Tab. 17). Significant differences between mutants and controls were detected in white blood cell count (males) and platelet count (females) only.

Raw data for each individual are available on demand in Excel sheets.

### 3.5.6 Discussion

All clinical chemical and haematological parameters were within the normal ranges published for mice of that age. We did not find any profound differences in the clinical chemical and hematological parameters between the two genotypes. Although sodium and chloride values as well as white blood cell and platelet count differed significantly between mutants and wild type, devia-

tions in these two parameters only do not allow to draw any firm conclusion on an altered metabolism of a specific organ system, especially taking into consideration that the values measured were within normal ranges.

### **3.5.7 References**

Champy, M.-F., M. Selloum, L. Piard, V. Zeitler, C. Caradec, P. Chambon and J. Auwerx (2004): Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mammalian Genome* 15: 768-783

Hough T.A., P. Nolan, V. Tsipouri, A.. Toye, I. Gray, M. Goldsworthy, L. Moir, R. Cox, S. Clements, P. Glenister, J. Wood, R. Selley, M. Strivens, L. Vizor, S. McCormack, J. Peters, E. Fisher, N. Spurr, S. Rastan, J. Martin, S. Brown and A. Hunter (2002): Novel phenotypes identified by plasma biochemical screening in the mouse. *Mammalian Genome* 13: 595-602

Quimby, F. (1999) The Mouse. In: *The clinical chemistry of laboratory animals*, ed. by W. F. Loeb and F. W. Quimby. Taylor and Francis, New York, pp. 3-31

Rathkolb B, Decker T, Fuchs E, Soewarto D, Fella C, Heffner S, Pargent W, Wanke R, Balling R, Hrabe de Angelis M, Kolb HJ and E. Wolf (2000): The clinical-chemical screen in the Munich ENU Mouse Mutagenesis Project: screening for clinically relevant phenotypes. *Mammalian Genome* 11: 543-546

Suckow, M.A., P. Dannemann and C. Brayton (2001) *The laboratory mouse* CRC Press, Boca Raton, London, New York, Washington

**Table 16: Clinical-Chemical Parameters.**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutant (A)			Wild Type (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=13)	(n=19)	<i>p</i> -value	(n=15)	(n=21)	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
Sodium [mmol/l]	160 $\pm 0.73$	157 $\pm 0.69$	<0.01	158 $\pm 0.60$	156 $\pm 1.08$	n.s.	<0.05	n.s.
Potassium [mmol/l]	4.0 $\pm 0.10$	3.7 $\pm 0.06$	<0.02	3.9 $\pm 0.09$	3.8 $\pm 0.07$	n.s.	n.s.	n.s.
Calcium [mmol/l]	2.1 $\pm 0.03$	2.0 $\pm 0.01$	n.s.	2.0 $\pm 0.02$	2.0 $\pm 0.03$	n.s.	n.s.	n.s.
Chloride [mmol/l]	113.9 $\pm 0.45$	113.6 $\pm 0.70$	n.s.	111.6 $\pm 0.40$	113.0 $\pm 0.79$	n.s.	<0.001	n.s.
Inorganic Phosphate [mmol/l]	2.2 $\pm 0.10$	1.7 $\pm 0.08$	<0.01	2.0 $\pm 0.07$	1.7 $\pm 0.06$	<0.01	n.s.	n.s.
Total Protein [g/dl]	5.2 $\pm 0.10$	4.9 $\pm 0.06$	<0.05	5.1 $\pm 0.10$	5.1 $\pm 0.09$	n.s.	n.s.	n.s.
Creatinine [mg/dl]	0.362 $\pm 0.01$	0.353 $\pm 0.01$	n.s.	0.358 $\pm 0.01$	0.361 $\pm 0.01$	n.s.	n.s.	n.s.
Urea [mg/dl]	69.8 $\pm 3.43$	60.1 $\pm 2.78$	<0.05	70.3 $\pm 2.2$	62.3 $\pm 2.2$	<0.02	n.s.	n.s.
Uric acid [mg/dl]	2.7 $\pm 0.33$	2.8 $\pm 0.24$	n.s.	2.8 $\pm 0.4$	2.7 $\pm 0.2$	n.s.	n.s.	n.s.
Cholesterol [mg/dl]	98.4 $\pm 3.64$	82.7 $\pm 2.92$	<0.01	130.7 $\pm 26.6$	76.9 $\pm 2.5$	n.s.	n.s.	n.s.
Triglyceride [mg/dl]	147.8 $\pm 12.86$	99.6 $\pm 8.08$	<0.01	145.8 $\pm 15.0$	82.4 $\pm 6.4$	<0.001	n.s.	n.s.
Creatine Kinase [U/l]	121 $\pm 22.78$	107 $\pm 31.10$	n.s.	108 $\pm 28.00$	72 $\pm 15.00$	n.s.	n.s.	n.s.
Alanine-Amino-transferase (ALAT,GPT) [U/l]	24 $\pm 1.58$	18 $\pm 2.54$	n.s.	35 $\pm 7.00$	16 $\pm 2.00$	<0.02	n.s.	n.s.
Aspartate-Amino-transferase (AST,GOT) [U/l]	36 $\pm 2.71$	36 $\pm 3.20$	n.s.	38 $\pm 5.00$	30 $\pm 2.00$	n.s.	n.s.	n.s.
Alkaline Phosphatase [U/l]	166 $\pm 14.25$	193 $\pm 15.66$	n.s.	133 $\pm 9.00$	162 $\pm 9.00$	<0.05	n.s.	n.s.
$\alpha$ -Amylase [U/l]	2352 $\pm 83.70$	2029 $\pm 66.12$	<0.01	2451 $\pm 78.00$	1944 $\pm 42.00$	<0.001	n.s.	n.s.
Glucose [mg/dl]	188.7 $\pm 9.78$	180.2 $\pm 10.70$	n.s.	180.6 $\pm 8.6$	168.9 $\pm 8.4$	n.s.	n.s.	n.s.
Ferritin [ng/ml]	29.1 $\pm 1.84$	30.3 $\pm 1.55$	n.s.	30.2 $\pm 2.1$	31.3 $\pm 1.9$	n.s.	n.s.	n.s.
Transferrin [mg/dl]	143.1 $\pm 2.98$	147.2 $\pm 2.07$	n.s.	143.8 $\pm 2.6$	145.8 $\pm 2.7$	n.s.	n.s.	n.s.
Lipase [U/l]	66.2 $\pm 17.9$ 4	63.7 $\pm 16.$ 00	n.s.	55.4 $\pm 6.9$	48.0 $\pm 3.9$	n.s.	n.s.	n.s.

**Table 17: Hematological Parameters.**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutant (A)			Wild Type (B)			A~B	A~B
	Male	Female		Male	Female		Male	Female
	(n=13)	(n=19)	<i>p</i> - value	(n=15)	(n=21)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
White blood cell count [10 <sup>3</sup> /μl]	6.29± 0.48	6.67± 0.52	n.s.	7.96± 0.61	6.40± 0.41	<0.05	<0.05	n.s
Red blood cell count [10 <sup>3</sup> /μl]	10.30± 0.12	10.25± 0.18	n.s.	10.00± 0.14	10.17± 0.13	n.s	n.s	n.s
Hemoglobin [g/dl]	15.50± 0.19	15.68± 0.23	n.s.	15.26± 0.22	15.64± 0.16	n.s	n.s	n.s
Hematocrit [%]	47± 0.61	47± 0.73	n.s.	46± 0.65	47± 0.44	n.s	n.s	n.s
Mean corpuscular volume [fl]	46.00± 0.16	45.80± 0.17	n.s.	46.07± 0.28	45.90± 0.30	n.s	n.s	n.s
Mean corpuscular hemoglobin [pg]	15.06± 0.14	15.32± 0.15	n.s.	15.26± 0.14	15.40± 0.15	n.s	n.s	n.s
Mean corpuscular hemoglobin concentration [g/dl]	32.74± 0.32	33.49± 0.26	n.s.	33.12± 0.26	33.51± 0.24	n.s	n.s	n.s
Platelet count [10 <sup>3</sup> /μl]	791± 24.45	674± 26.69	<0.01	871± 30.88	746± 13.80	<0.01	n.s.	<0.05

## 3.6 Immunology Screen

### 3.6.1 Summary

Mouse models have been a primary source of information for understanding the intricate mechanisms of the immune system (Blüethmann and Ohashi, 1994; Mak *et al.*, 2001; Fischer 2002; Rogner and Avner, 2003). The Immunology Screen at the GMC was set up to conduct a broad immunological phenotyping of mouse mutant lines with the intention of identifying distinct gene functions, which play key roles in the immune defenses of the organism through a complex network of cellular and soluble components (Janeway *et al.*, 2004).

According to the data summary presented to the GMC, no immunological phenotype was known in NCAM mutant mice. Their analysis in the Immunology Screen revealed minor differences in the level of IgG<sub>1</sub> between female mutants and their littermate controls.

### 3.6.2 Mice

We analyzed 33 mutant animals (20 females and 13 males) and 35 age- and sex-matched littermate controls (20 females and 15 males).

### 3.6.3 Material and Methods

Peripheral blood leukocytes (PBLs) were isolated from 500  $\mu$ l blood by erythrocyte lysis with NH<sub>4</sub>Cl (0.17M) - Tris buffer (pH 7.45) directly in 96-well microtiter plates. After subsequent washing with FACS staining buffer (PBS, 0.5% BSA, 0.02% sodium azide, pH 7.45), PBLs were incubated for 20 min with 1  $\mu$ M ethidium monazide bromide (EMA, Molecular Probes, The Netherlands) and Fc block (clone 2.4G2, PharMingen, San Diego, USA). EMA bound to the DNA of dead cells was photocrosslinked by brief light exposure. Cells were then stained with fluorescence-conjugated monoclonal antibodies (PharMingen).

The following main cell populations were analyzed: B cells (CD19<sup>+</sup> clone 1D3), B1 B cells (CD19<sup>+</sup>CD5<sup>+</sup>, clone 53-7.3), B2 B cells (CD19<sup>+</sup>CD5<sup>-</sup>), T cells (CD3<sup>+</sup>, clone 145-2C11), CD4<sup>+</sup> T cells (clone RM4-5), CD8<sup>+</sup> T cells (CD8 $\alpha$ , clone 53-6.7; CD8 $\beta$ , clone H35-17.2),  $\gamma/\delta$ T cells (clone GL3), granulocytes (Gr-1<sup>+</sup>, clone RB6-8C5), and NK cells (CD49b<sup>+</sup>, clone DX5). We also analyzed additional subpopulations based on the following surface antigens: IgD (clone 11-26c.2a), B220 (clone RA3-6B2), CD11b (clone M1/70), CD103 (clone 2E7), CD25 (clone PC61), CD62L (clone MEL-14), CD45RA (clone 14.8), Ly-6C (clone AL-21), and CD44 (clone IM7). Data were acquired on a FACS Calibur (Becton Dickinson, San Diego, USA) and were analyzed using FlowJo software (TreeStar Inc, USA). All samples were acquired until a total number of 25,000 cells was reached.

The plasma levels of IgM, IgG<sub>1</sub>, IgG<sub>2a</sub>, IgG<sub>2b</sub>, IgG<sub>3</sub>, and IgA were determined by standard sandwich ELISAs using goat anti-mouse immunoglobulin antibodies and alkaline phosphatase (AP) conjugates (SouthernBiotech, Birmingham, USA). The presence of rheumatoid factor and anti-DNA antibodies was evaluated by indirect ELISA with rabbit IgG (Sigma-Aldrich, Steinheim, Ger-

many) and calf thymus DNA (Sigma-Aldrich), respectively, as antigens and AP-conjugated goat anti-mouse secondary antibody (Sigma-Aldrich). Serum samples from MRL/MpJ-Tnfrsf6<sup>lpr</sup> mice (Jackson Laboratory, Bar Harbor, USA) were used as positive controls in the autoantibody assays.

### 3.6.4 Parameters

<b>Flow cytometry</b>
B cells (CD19 <sup>+</sup> ), B1 B cells (CD19 <sup>+</sup> CD5 <sup>+</sup> ), B2 B cells (CD19 <sup>+</sup> CD5 <sup>-</sup> ), T cells (CD3 <sup>+</sup> ), CD4 <sup>+</sup> T cells, CD8 <sup>+</sup> T cells, $\gamma/\delta$ T cells, granulocytes (Gr-1 <sup>+</sup> ), and NK cells (CD49b <sup>+</sup> ). Furthermore, all potential subpopulations which can be identified by co-staining for other surface markers (IgD, B220, CD11b, MHC II, I-A <sup>k</sup> , CD25, CD8 $\beta$ , CD62L, CD45RA, Ly-6C, CD44) using 6 parameter/5 color flow cytometry were analyzed.
<b>ELISA</b>
IgM, IgG <sub>1</sub> , IgG <sub>2a</sub> , IgG <sub>2b</sub> , IgG <sub>3</sub> , IgA; anti-DNA antibodies, rheumatoid factor

### 3.6.5 Results

The analysis of standard immunological parameters measured in the primary screen (Tab. 18) revealed significantly lower IgG<sub>1</sub> levels in female NCAM mutant mice as compared to their littermate controls.

### 3.6.6 Discussion

Under standard screening conditions all immunological parameters measured in NCAM mice were within normal ranges. Although we established significantly lower IgG<sub>1</sub> levels in mutant females, the observed values were not outside the expected values for this immunoglobulin. This finding is most likely due to natural variation, or differences between the tested groups of animals (the NCAM line was screened in three independent experiments due to breeding problems). Without any evidence for further dysregulation of immune function in NCAM mice, no additional immunological investigations are recommended.

### 3.6.7 References

Bluethmann, H., and P. S. Ohashi (Eds.) (1994): Transgenesis and targeted mutagenesis in immunology. Academic Press, San Diego.

Fischer, A. (2002): Natural mutants of the immune system: a lot to learn! Eur J Immunol 32: 1519-1523.

Janeway C, Travers P, Walport M, Shlomchik M and M.J. Shlomchik (2004)  
Immunobiology: The Immune System in Health and Disease. 6th edition,  
Garland Publishing, London.

Mak, T. W., J. M. Penninger and P. S. Ohashi (2001): Knockout mice: a para-  
digm shift in modern immunology. *Nat Rev Immunol* 1: 11-19.

Rogner, U. C., and P. Avner (2003): Congenic mice: cutting tools for complex  
immune disorders. *Nat Rev Immunol* 3: 243-252.

**Table 18: Basic Parameters Analyzed in the Immunology Screen.**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Mutants (A)			Wild Type (B)			A - B	
	Male	Female	<i>p</i> - value	Male	Female	<i>p</i> - value	Male	Female
	(n=13)	(n=20)		(n=15)	(n=20)		<i>p</i> - value	<i>p</i> - value
CD19 <sup>+</sup> [%]	44.3 $\pm$ 1.8	44.0 $\pm$ 1.8	n.s.	49.5 $\pm$ 2.1	49.9 $\pm$ 1.1	n.s.	n.s.	n.s.
CD19 <sup>+</sup> CD5 <sup>-</sup> [%]	96.9 $\pm$ 0.3	95.3 $\pm$ 0.5	<0.05	96.8 $\pm$ 0.4	95.5 $\pm$ 0.6	n.s.	n.s.	n.s.
CD19 <sup>+</sup> CD5 <sup>+</sup> [%]	3.1 $\pm$ 0.3	4.6 $\pm$ 0.5	<0.05	3.1 $\pm$ 0.4	4.4 $\pm$ 0.6	n.s.	n.s.	n.s.
CD3 <sup>+</sup> [%]	19.8 $\pm$ 1.4	26.2 $\pm$ 1.6	<0.02	25.1 $\pm$ 2.5	30.3 $\pm$ 1.8	n.s.	n.s.	n.s.
$\gamma/\delta$ TCR <sup>+</sup> [%]	0.3 $\pm$ 0.02	0.4 $\pm$ 0.05	n.s.	0.3 $\pm$ 0.04	0.3 $\pm$ 0.04	n.s.	n.s.	n.s.
Gr-1 <sup>+</sup> [%]	24.3 $\pm$ 1.8	18.5 $\pm$ 1.7	<0.05	23.2 $\pm$ 2.8	17.0 $\pm$ 1.0	<0.05	n.s.	n.s.
CD49b <sup>+</sup> [%]	30.9 $\pm$ 3.1	25.3 $\pm$ 2.4	n.s.	26.0 $\pm$ 2.9	23.1 $\pm$ 3.1	n.s.	n.s.	n.s.
CD4 <sup>+</sup> [%]	13.4 $\pm$ 0.4	22.6 $\pm$ 2.7	<0.05	12.3 $\pm$ 0.4	15.2 $\pm$ 0.5	<0.001	n.s.	n.s.
CD8 $\beta$ <sup>+</sup> [%]	8.5 $\pm$ 0.2	9.9 $\pm$ 0.4	<0.05	8.1 $\pm$ 0.2	10.1 $\pm$ 0.3	<0.001	n.s.	n.s.
IgG <sub>1</sub> [ $\mu$ g/ml]	247.3 $\pm$ 33.5	254.8 $\pm$ 31.6	n.s.	332.6 $\pm$ 55.2	448.0 $\pm$ 38.0	n.s.	n.s.	<0.001
IgG <sub>2a</sub> [ $\mu$ g/ml]	451.2 $\pm$ 88.9	406.1 $\pm$ 68.2	n.s.	422.5 $\pm$ 65.3	484.4 $\pm$ 70.5	n.s.	n.s.	n.s.
IgG <sub>2b</sub> [ $\mu$ g/ml]	287.7 $\pm$ 123.2	182.5 $\pm$ 84.0	n.s.	311.6 $\pm$ 115.2	198.2 $\pm$ 50.8	n.s.	n.s.	n.s.
IgG <sub>3</sub> [ $\mu$ g/ml]	287.8 $\pm$ 123.2	182.5 $\pm$ 54.0	n.s.	311.6 $\pm$ 115.2	198.2 $\pm$ 50.8	n.s.	n.s.	n.s.
IgM [ $\mu$ g/ml]	202.6 $\pm$ 41.0	101.6 $\pm$ 18.6	<0.05	137.2 $\pm$ 28.7	121.3 $\pm$ 21.7	n.s.	n.s.	n.s.
IgA [ $\mu$ g/ml]	85.8 $\pm$ 24.5	65.6 $\pm$ 19.6	n.s.	119.8 $\pm$ 28.2	74.0 $\pm$ 11.6	n.s.	n.s.	n.s.
Anti-DNA Ab [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.
Rheumatoid factor [%]	0	0	n.s.	0	0	n.s.	n.s.	n.s.

Raw data will be available on demand.

## 3.7 Allergy Screen

### 3.7.1 Summary

The goal of the Allergy screen within the German Mouse Clinic (GMC) is to search for IgE mutants in order to establish mouse models for allergic diseases and to find new strategies for antiallergic therapy. The increased production of IgE in response to common environmental antigens is the hallmark of atopic diseases in man (Hamelmann *et al.* 1999). Mouse mutants with phenotypic alterations in IgE production represent a valuable tool to study and characterize the molecular mechanisms of IgE-mediated allergic hypersensitivity (Zhang *et al.* 1997).

In the primary Allergy screen 31 NCAM-deficient mice 32 wild-type animals were screened. The analysis of NCAM mice in Allergy screen did not reveal any profound differences between knockout and wild-type mice.

### 3.7.2 Mice

An age- and sex-matched group of 32 wild-type (17 females, 15 males) and 31 knockout (18 females, 13 males) mice aged 12 weeks was analyzed in Allergy screen.

### 3.7.3 Material and Methods

Twelve-week-old male and female mice were screened for alterations in plasma total IgE concentrations. Blood samples were taken from animals by puncturing the retroorbital plexus under ether anesthesia. Plasma IgE concentrations were measured by isotype-specific sandwich ELISA technique with a lower detection limit of 1 ng/ml. briefly, microtiter plates were coated with the IgG fraction of sheep anti-mouse IgE in sodium bicarbonate buffer (pH 9.6). After incubation, plates were washed with Tris buffer (pH 7.4) and blocked with 3% (w/v) bovine serum albumin at room temperature. Diluted plasma samples and standard were added to the plates. After overnight incubation biotinylated rat anti-mouse IgE was added and plates were incubated at room temperature for 2 h. Then plates were incubated in the presence of peroxidase-labeled streptavidin. After washing, tetramethylbenzidine (TMB) substrate solution was added and after an appropriate incubation time the stop solution (sulphuric acid, 2M) was added. The plates were read in a standard microplate reader at a wavelength of 450 nm. Total murine IgE data are reported in ng/ml, based on a standard curve of purified murine IgE. (Alessandrini *et al.*, 2001)

### 3.7.4 Results

The analysis of total IgE levels in plasma (mean  $\pm$  SE) of NCAM-KO mice and their sex- and age-matched wild-type littermates revealed higher mean IgE concentrations of females KO. and wild-type animals. This sex-difference was statistically not significant (Tab. 19).

**Table 19: Total plasma IgE in NCAM mice**Data are presented as mean  $\pm$  standard error of mean.

	Mutant (A)			Wild Type (B)			A~B	A~B
	Female	Male		Female	Male		Female	Male
	(n=18)	(n=13)	<i>p - value</i>	(n=17)	(n=15)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
<b>Total IgE [ng/ml]</b>	46 $\pm$ 7.8	43.2 $\pm$ 10	n.s.	56 $\pm$ 7.9	38.5 $\pm$ 7.1	n.s.	n.s.	n.s.

Raw data will be available on demand.

### 3.7.5 Discussion

No statistically significant difference between NCAM-KO and wild-type mice was found. In both NCAM-KO and wild-type animals, the mean concentration of total IgE in females was higher than in males, which was not significant.

The mean concentration of total plasma IgE of both NCAM-KO and wild-type females and males was close to the normal range for total IgE in C57BL/6 mice established in our laboratory (females 87.6  $\pm$  20.12 ng/ml vs. males 30.3  $\pm$  4.41 ng/ml).

Taken together, under standard screening conditions for primary Allergy screen, NCAM-KO mice did not show changes in total plasma IgE levels that would reveal a major allergy phenotype.

### 3.7.6 Reference

Alessandrini, F., Jakob, T., Wolf, A., Wolf, E., Balling, R., Hrabé de Angelis, M., Ring, J., Behrendt, H. (2001): ENU mouse mutagenesis: Generation of mouse mutants with aberrant plasma IgE levels. *Int Arch Allergy Immunol.* 124: 25-28

Hamelmann, E., K. Takeda, A. Oshiba and E.W. Gelfand (1999): Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness – a murine model. *Allergy* 54: 297-305

Zhang, Y., W.J.E. Lamm, R.K. Albert, E.Y. Chi, W.R.Henderson and D.B. Lewis (1997) Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am J Respir Crit Care Med.* 155: 661-669

## 3.8 Nociceptive Screen

### 3.8.1 Summary

Pain is the perception of an aversive or unpleasant sensation that originates from a specific region of the body. The highly subjective nature of pain is one of the factors that make it difficult to define and to treat clinically. Pain is more than a conspicuous sensory experience that warns of danger.

Nociceptors are activated by tissue injury but also by mechanical, thermal, or chemical stimuli. Harmful stimuli applied to the skin or to subcutaneous tissue, activate nociceptors, the peripheral endings of primary sensory neurons whose cell bodies are located in the dorsal root or in the trigeminal ganglia.

A noxious stimulus activates the nociceptor by depolarising the membrane of the sensory ending. When peripheral tissues are damaged, the sensation of pain in response to subsequent stimuli is enhanced. This phenomenon termed hyperalgesia, may involve a lowering of threshold of the nociceptors or an increase in the magnitude of pain evoked by suprathreshold stimuli. Hyperalgesia can occur both at the site of tissue damage (primary hyperalgesia) and in the surrounding undamaged areas (secondary hyperalgesia; Wall and Melzak, 1984). By means of different inbred mouse strains it could be demonstrated that rodents display large and heritable differences in both nociceptive and analgesic sensitivity (Mogil, 1999; Mogil *et al.*, 1999)

In the primary screen we tested the responsiveness of the intact somatosensory system to thermal pain of the NCAM mice by means of the hot plate test. We could not find any significant differences in pain reactivity between the two genotypes. Additionally, there were no sex-specific differences. Therefore we do not suggest continuing pain related studies in NCAM mice.

### 3.8.2 Mice

Twenty-seven NCAM knockout mice (11 male, 16 female), and 34 control animals (15 male, 19 female) were tested in our first screen.

### 3.8.3 Material and Methods

#### Hot plate test

The mice were placed on a metal surface maintained at  $52 \pm 0.2^\circ\text{C}$  (Hot plate system was made by TSE GMBH, Germany; Eddy and Leimbach, 1953). Locomotion of the mouse on the hot plate was constrained by 20 cm high plexiglas wall to a circular area with a diameter of 28 cm. Mice remained on the plate until they performed one of three behaviors regarded as indicative of nociception: hind paw lick (h.p. licking), hind paw shake/flutter (h.p. shaking) or jumping.

We evaluated only hind paw but not the front paw responses, because fore paw licking and lifting are components of normal grooming behavior. Each mouse was tested only once since repeated testing leads to profound changes in response latencies. The latency was recorded to the nearest 0.1 s.

To avoid tissue injury 60 s cut-off time was used. The data values are given in seconds.

### Statistical analysis

Statistical analysis was performed using a statistical package Statgraphics® (Statistical Graphics Corporation, Rockville, MD). The differences between the groups were compared with ANOVA, LSD test was used as *post hoc*. Statistical significance was assumed at  $p < 0.05$ .

### 3.8.4 Parameters

<b>Hind paw licking</b>
Reaction with licking of hind paw to the thermal pain
<b>Hind paw shaking</b>
Reaction with shaking of hind paw to the thermal pain
<b>Jumping</b>
Jumping reaction to the thermal pain

### 3.8.5 Results and Discussion

Typically, the first nociceptive response observed in these mice was *hind paw shaking*. Both genotypes showed *hind paw licking*, another typical nociceptive response. The third examined response was the *jumping* of animals.

We could not find any differences in the pain response neither between the genotypes nor between the sexes (Tab. 20). Therefore we do not suggest performing any additional pain related studies on NCAM mice.

<b>Table 20: Nociceptive Screen</b>									
Data are presented as mean $\pm$ standard error of mean.									
Parameter	Mutant (A)			Wild Type (B)			A~B	A~B	AN-NOVA
	Female	Male		Male	Male		Female	Male	
	(n=16)	(n=11)	<i>p</i> -value	<i>p</i> -value	(n=15)	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value	<i>p</i> -value
<b>H.p. licking</b>	13,9 $\pm$ 1,2	16,4 $\pm$ 1,4	n.s.	n.s.	14,4 $\pm$ 1,2	n.s.	n.s.	n.s.	0,27025
<b>H.p. shaking</b>	11,5 $\pm$ 0,79	8,9 $\pm$ 0,9	n.s.	n.s.	9,9 $\pm$ 0,8	n.s.	n.s.	n.s.	0,36757
<b>Jumping</b>	53,9 $\pm$ 2,4	51,9 $\pm$ 2,9	n.s.	n.s.	54,9 $\pm$ 2,5	n.s.	n.s.	n.s.	0,73722

### **3.8.6 Reference**

Eddy, N.B. Leimbach, D. (1953): Synthetic analgesics II. Diethienylbutenyl – and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* 107: 385-393

Mogil J.S. (1999): The genetic mediation of individual differences in sensitivity to pain and its inhibition. *Proc. Nat. Acad. Sci.* 96: 7744-7751

Mogil J.S., S.G. Wilson, K. Bon, S.E. Lee, K. Chung, P. Raber, J.O. Pieper, H.S. Hain, J.K. Belknap, L. Hubert, G.I. Elmerl, J.M. Chung and M. Devor (1999): Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain.* 80:67-82.

Wall P.D. and R. Melzack (Eds.) *Textbook of Pain*, Churchill Livingstone, London, 1984

### **Abbreviations**

h.p. hind paw

## 3.9 Lung Function Screen

### 3.9.1 Summary

Neural and mechanical processes that control breathing frequency have been investigated in man for a long time (Mead, 1960; Otis *et al.*, 1959), but only with the availability of mouse inbred strains the contribution of genetic determinants to differential baseline breathing patterns could be elucidated (Tankersley *et al.*, 1997; Tankersley, 1999). By use of genetically engineered mice, candidate genes for human developmental disorders of breathing have been identified (Katz, 2003).

In the Primary screen, spontaneous breathing patterns during rest and activity were studied in 15-week-old male and female NCAM-deficient and wild-type mice. For the absolute values, neither sex specific differences nor differences between wild-type and mutant mice were found. Thus, in general breathing patterns were similar. The significantly higher values for specific tidal volume and specific minute ventilation in the mutant mice compared to the wild types are most likely related to the significant differences in body weight.

### 3.9.2 Mice

The workflow of the screen provides male and female mice with a mean age of 15 weeks. Body weights differed significantly between wild-type mice and mutants. Four wild-type males (bw = 28.1 g  $\pm$  0.2) were compared to three mutant males (bw = 23.8 g  $\pm$  0.7). Further, eight wild-type (bw = 21.1  $\pm$  0.6) and seven mutant females (bw = 17.6  $\pm$  0.6), respectively, were analyzed. (Tab. 21).

### 3.9.3 Material and Methods

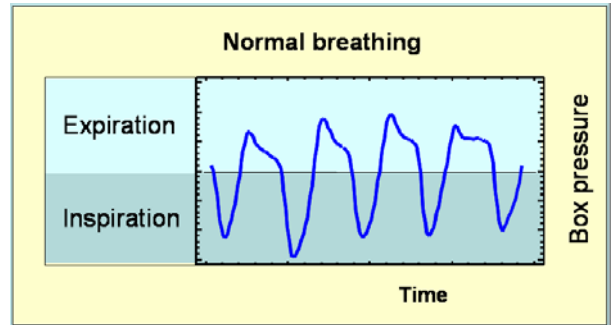
#### Whole Body Plethysmography

A commercially available system from Buxco<sup>®</sup> Electronics (Sharon, Connecticut) was used to assess breathing patterns in unrestrained animals according to the principle described by Drorbaugh and Fenn (1955). It measures the pressure changes which arise from inspiratory and expiratory temperature and humidity fluctuations during breathing (Fig. 3 and 4).

Calibration of the system allows to transform these pressure swings into flow and volume signals so that automated data analysis provides tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF). These data were stored online as mean values at 10 s intervals.



**Figure 3: System used at GMC to assess breathing patterns.**



**Figure 4: Recorded data used to calculate the breathing parameters.**

Measurements were always performed between 8 a.m. and 11 a.m. to account for potential diurnal variations in breathing. The system was set up in a quiet room where temperature and humidity were kept constant throughout the measurements. Before each measurement, the system was calibrated and the actual barometric pressure, temperature, and humidity were supplied to warrant adequate calculations of flow rates and volumes. After placing the animals into the chamber, data recording was immediately started and was continued for 40 min. Mice underwent typical phases during the measuring period. Primarily, the animals were stressed so that the respiratory rate was highest at the beginning. Usually after 5 min. the animals became calmer, they slightly reduced their respiratory rate, and began to explore the chamber and start cleaning themselves – *phase of activity*. Later activity was more and more interrupted by phases of rest or even short periods of snoozing – *resting phase*. Some of the animals even went to *phases of sleep*, which resulted in a further marked decrease in respiratory rate. The frequency histogram of the respiratory rates was determined for each individual, and breathing was analyzed for the above mentioned parameters during the phases of activity and rest. In addition to the directly recorded parameters, mean inspiratory and expiratory flow rates (MEF, MIF) were calculated offline from the ratio of tidal volume and the respective time interval. The relative duration of inspiration ( $T_i/TT$ ) was determined from the ratio of inspiratory time to total time required for the breathing cycle. Specific tidal volumes and minute ventilations (sTV, sMV) were calculated by relating the absolute values to the body weight of the

animal. Furthermore, the mean of all breathing frequencies (mean\_f) measured during the 40-minute-period was calculated as a rough and ready parameter to assess whether the duration of rest and activity was similar in all mouse strains.

### Statistical Analysis of Data

Statistical analyses were performed using a commercially available statistics package (Statgraphics®, Statistical Graphics Corporation, Rockville, MD). Differences between strains were evaluated by Students t-test. Statistical significance was assumed at  $p < 0.05$ . Data are presented as mean values  $\pm$  standard error of the mean (SEM).

### 3.9.4 Parameters

<b>Directly recorded data</b>
Tidal volumes (TV), respiratory rates (f), minute ventilation (MV), inspiratory and expiratory times (Ti, Te), as well as peak inspiratory and peak expiratory flow rates (PIF, PEF).
<b>Calculated data</b>
mean inspiratory flow rates (MEF), expiratory flow rates (MIF), relative duration of inspiration (Ti/TT), specific tidal volumes (sTV), minute ventilations (sMV), mean of all breathing frequencies (mean_f)

### 3.9.5 Results and Discussion

Table 22 summarizes the results obtained for spontaneous breathing under resting and active conditions.

For the absolute values tidal volume and minute ventilation, neither sex specific differences nor differences between wild-type and mutant mice were found meaning that in general, breathing patterns were comparable. During both rest and activity female mutant mice exhibited significantly lower breathing rates resulting in lower minute ventilation, higher expiratory timing, and lower mean expiratory flow compared to male mutant mice. We have to date no explanation for these sex-related differences, but since only 3 male mutant mice were available, data has to be interpreted carefully and should be verified in a higher number of animals.

Concerning differences between wild-type and mutant mice, mutants showed significantly higher specific tidal volumes and specific minute ventilation. This is most likely related to the significant differences in body weight between wild-type and mutant mice.

In conclusion, no evidence was found that the mutation affects the breathing pattern. Studies in the Metabolic Screen might reveal insights in the causation of the differences in body weight.

Raw data are available on demand.

### 3.9.6 References

- Drorbaugh J.E. and W.O. Fenn (1955): A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16: 81-87
- Katz D.M. (2003): Neuronal growth factors and development of respiratory control. *Respir. Physiol. Neurobiol.* 135: 155-165
- Mead, J. (1960): Control of respiratory frequency. *J. Appl. Physiol.* 15: 325-336
- Otis, A.B., W.O. Fenn and H. Rahn (1950): Mechanics of breathing in man. *J. Appl. Physiol.* 2: 592-607
- Tankersley, C.G. (1999): Genetic control of ventilation: What are we learning from murine models? *Current Opinion in Pulmonary Medicine* 5: 344-348
- Tankersley, C.G., Fitzgerald R.S., Levitt R.C., Mitzner W.A., Ewart S.L. and S.R. Kleeberger (1997): Genetic control of differential baseline breathing pattern. *J. Appl. Physiol.* 82: 874-81

## Abbreviations

bw	body weight (g)
mean_f	mean of all respiratory rates (1/min)
f	respiratory rate (1/min)
TV	tidal volume (ml)
sTV	specific tidal volume ( $\mu\text{l/g}$ )
MV	minute ventilation (ml/min)
sMV	specific ventilation (ml/min/g)
Ti	inspiratory time (ms)
Te	expiratory time (ms)
Ti/TT	relative duration of inspiration
PIF	peak inspiratory flow rate (ml/s)
PEF	peak expiratory flow rate (ml/s)
MIF	mean inspiratory flow rate (ml/s)
MEF	mean expiratory flow rate (ml/s).

**Table 21: Characterization of the Studied Mice**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Wild Type (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=4)	(n=8)	<i>p - value</i>	(n=3)	(n=7)	<i>p - value</i>	<i>p - value</i>	<i>p - value</i>
<b>bw</b>	28.1 $\pm$ 0.2	21.1 $\pm$ 0.6	< 0.001	23.8 $\pm$ 0.7	17.6 $\pm$ 0.6	< 0.001	< 0.01	< 0.01
<b>mean_f</b>	452.7 $\pm$ 17.9	427.5 $\pm$ 15.9	n.s.	470.3 $\pm$ 13.3	400.8 $\pm$ 7.5	< 0.01	n.s.	n.s.

**Table 22: Spontaneous Breathing Pattern during Rest and Activity**Data are presented as mean  $\pm$  standard error of mean.

Parameter	Wildtype (A)			Mutant (B)			A-B	A-B
	Male	Female		Male	Female		Male	Female
	(n=4)	(n=8)	<i>p</i> - value	(n=3)	(n=7)	<i>p</i> - value	<i>p</i> - value	<i>p</i> - value
<b>Rest</b>								
<b>f</b>	385.6 $\pm$ 17.4	368.6 $\pm$ 14.1	n.s.	397.9 $\pm$ 10.2	349.1 $\pm$ 5.9	< 0.05	n.s.	n.s.
<b>TV</b>	0.26 $\pm$ 0.02	0.24 $\pm$ 0.01	n.s.	0.27 $\pm$ 0.02	0.25 $\pm$ 0.01	n.s.	n.s.	n.s.
<b>sTV</b>	9.4 $\pm$ 0.6	11.3 $\pm$ 0.9	n.s.	11.2 $\pm$ 0.6	14.3 $\pm$ 0.7	n.s.	0.07545	< 0.05
<b>MV</b>	99.8 $\pm$ 6.2	84.4 $\pm$ 4.4	n.s.	103.6 $\pm$ 5.2	85.2 $\pm$ 4.2	n.s.	n.s.	n.s.
<b>sMV</b>	3.5 $\pm$ 0.2	4.0 $\pm$ 0.2	n.s.	4.4 $\pm$ 0.1	4.9 $\pm$ 0.2	n.s.	< 0.05	< 0.02
<b>Ti</b>	52.3 $\pm$ 2.0	49.2 $\pm$ 1.9	n.s.	51.7 $\pm$ 1.8	49.3 $\pm$ 1.0	n.s.	n.s.	n.s.
<b>Te</b>	104.3 $\pm$ 5.8	115.2 $\pm$ 4.5	n.s.	99.3 $\pm$ 2.2	122.9 $\pm$ 3.0	< 0.01	n.s.	n.s.
<b>Ti/TT</b>	0.33 $\pm$ 0.01	0.30 $\pm$ 0.006	< 0.02	0.34 $\pm$ 0.003	0.29 $\pm$ 0.007	< 0.01	n.s.	n.s.
<b>PIF</b>	8.9 $\pm$ 0.6	8.5 $\pm$ 0.5	n.s.	8.8 $\pm$ 0.4	8.6 $\pm$ 0.5	n.s.	n.s.	n.s.
<b>PEF</b>	5.7 $\pm$ 0.4	5.0 $\pm$ 0.3	n.s.	6.5 $\pm$ 0.6	5.4 $\pm$ 0.3	n.s.	n.s.	n.s.
<b>MIF</b>	5.1 $\pm$ 0.3	4.8 $\pm$ 0.3	n.s.	5.2 $\pm$ 0.2	5.1 $\pm$ 0.3	n.s.	n.s.	n.s.
<b>MEF</b>	2.5 $\pm$ 0.2	2.0 $\pm$ 0.1	< 0.05	2.7 $\pm$ 0.2	2.0 $\pm$ 0.09	< 0.01	n.s.	n.s.
<b>Activity</b>								
<b>f</b>	510.2 $\pm$ 12.7	487.9 $\pm$ 12.6	n.s.	522.8 $\pm$ 4.4	465.7 $\pm$ 7.6	< 0.01	n.s.	n.s.
<b>TV</b>	0.26 $\pm$ 0.01	0.26 $\pm$ 0.01	n.s.	0.29 $\pm$ 0.008	0.26 $\pm$ 0.01	n.s.	n.s.	n.s.
<b>sTV</b>	9.3 $\pm$ 0.4	12.2 $\pm$ 0.8	< 0.05	12.2 $\pm$ 0.3	15.0 $\pm$ 0.7	n.s.	< 0.01	< 0.05
<b>MV</b>	131.9 $\pm$ 7.7	122.6 $\pm$ 5.0	n.s.	149.1 $\pm$ 3.3	120.4 $\pm$ 5.3	< 0.02	n.s.	n.s.
<b>sMV</b>	4.7 $\pm$ 0.3	5.8 $\pm$ 0.3	< 0.02	6.3 $\pm$ 0.2	6.7 $\pm$ 0.3	n.s.	< 0.01	< 0.05
<b>Ti</b>	41.9 $\pm$ 1.0	40.8 $\pm$ 0.7	n.s.	42.2 $\pm$ 0.5	43.5 $\pm$ 0.5	n.s.	0.8132	< 0.01
<b>Te</b>	75.9 $\pm$ 2.6	82.8 $\pm$ 2.6	n.s.	72.6 $\pm$ 0.6	85.5 $\pm$ 2.0	< 0.01	n.s.	n.s.
<b>Ti/TT</b>	0.36 $\pm$ 0.01	0.33 $\pm$ 0.006	< 0.05	0.37 $\pm$ 0.003	0.34 $\pm$ 0.005	< 0.01	n.s.	n.s.
<b>PIF</b>	10.8 $\pm$ 0.7	10.7 $\pm$ 0.6	n.s.	11.4 $\pm$ 0.4	10.1 $\pm$ 0.6	n.s.	n.s.	n.s.
<b>PEF</b>	7.5 $\pm$ 0.5	7.2 $\pm$ 0.4	n.s.	9.2 $\pm$ 0.5	7.3 $\pm$ 0.5	n.s.	n.s.	n.s.
<b>MIF</b>	6.3 $\pm$ 0.4	6.3 $\pm$ 0.3	n.s.	6.8 $\pm$ 0.2	6.0 $\pm$ 0.3	n.s.	n.s.	n.s.
<b>MEF</b>	3.5 $\pm$ 0.2	3.1 $\pm$ 0.1	n.s.	4.0 $\pm$ 0.1	3.1 $\pm$ 0.1	< 0.01	n.s.	n.s.

## 3.10 Expression Profiling

### 3.10.1 Summary

In this report, we describe the results of using close to genome-wide 21K cDNA microarrays for the RNA expression profiling of brain of 3 animals of the NCAM<sup>-/-</sup> mutant mouse line. The data analysis and various statistical methods detected a very low number of genes differentially regulated between mutant and wild-type tissues in all experiments. These genes may be of functional importance and of interest for the further analysis of the NCAM mutant mouse.

### 3.10.2 Mice

The molecular phenotyping screen archives organs of mutant mice for subsequent DNA-chip expression profiling analysis. Seven male mice of the NCAM strain were provided to the molecular phenotyping screen (Tab. 23).

Organs were collected at the age of 105-110 days. To minimize the influence of circadian rhythm on gene expression, mice were killed between 9 a.m. and 12 a.m. by carbon dioxide asphyxiation. The following 17 organs were collected and archived in liquid nitrogen following our established standard operating protocols: bulbourethral gland, spleen, kidney, seminal vesicles, testis, white fat, liver, heart, lung, thymus, skin/cartilage (outer ear), bone (femur), skeletal muscle, salivary gland, brain, brown fat, and eye.

**Table 23: NCAM-deficient and Wild-type Mice Stored for Expression Profiling.**

Mouse ID	Strain	Sex	Date of Birth	Genotype	Date of Collection
30014194	NCAM	m	17.10.2003	-/-	04.02.2004
30014195	NCAM	m	17.10.2003	-/-	04.02.2004
30014220	NCAM	m	17.10.2003	-/-	04.02.2004
30014103	NCAM	m	16.10.2003	+/+	04.02.2004
30014105	NCAM	m	16.10.2003	+/+	04.02.2004
30014177	NCAM	m	17.10.2003	+/+	04.02.2004
30014112	NCAM	m	16.10.2003	+/+	04.02.2004

### 3.10.3 Material and Methods

#### Isolation of Total RNA

Total RNA was isolated just before processing for expression profiling. For preparation of total RNA individual organs were thawed in buffer containing chaotropic salt (RLT buffer, Qiagen) and homogenised using a Polytron homogeniser. Total RNA from individual samples was obtained according to manufacturer's protocols using RNeasy Midi kits (Qiagen). 2µg RNA aliquots were run on a formaldehyde agarose gel to check for RNA integrity and the concentration was calculated from OD<sub>260/280</sub> measurement. The RNA was stored at -80°C in RNase free water (Qiagen).

#### Chip Design

We use a glass-surface DNA-chip containing ≈ 21,000 probes. About 20,200 of these probes are from the commercial Lion mouse array-TAG clone set, which is mostly derived from 3'UTRs. All Lion probes have been sequenced. The remaining probes were isolated in a subtractive screen for differentially expressed genes in the mesoderm of Delta/Notch pathway deficient mouse embryos. Mouse array-TAG clones have the general ID MG-VW-XYZ and the Delta/Notch specific probes are named rda-X.

#### DNA Microarrays

PCR products with 5'-aminogroup were amplified from the mouse arrayTAG library from Lion Bioscience comprising approximately 20.200 clones (Heidelberg, Germany). PCR products were dissolved in 3-fold SSC buffer and spotted on aldehyde-coated slides (Telechem, USA) using a Microgrid TAS II spotter (Biorobotics) with 48 Stealth™ SMP3 pins (Telechem). Spotted slides were rehydrated overnight in a humid chamber containing 50-70% aqueous solution of glycerol. Rehydrated slides were immersed in blocking solution (0,1 M sodium borohydride in 0.75fold PBS with 25% ethanol) for 5 minutes, oiled in water for 2 minutes, briefly immersed in 100% ethanol and air-dried. Slides were pre-hybridised for 1 hour in pre-hybridisation buffer (6-fold SSC, 1%BSA, 0.5%SDS) rinsed in water, dried and hybridised the same day. (Seltmann *et al*, in press)

#### Reverse Transcription and Fluorescent Labelling

For labelling 20µg of total RNA were used for reverse transcription and indirectly labelled with Cy3 or Cy5 fluorescent dye according the TIGR protocol ([http://pga.tigr.org/sop/M004\\_1a.pdf](http://pga.tigr.org/sop/M004_1a.pdf)). Labelled cDNA was dissolved in 30µl hybridisation buffer (6x SSC, 0,5% SDS 5fold Denhardt's solution and 50% formamide) and mixed with 30 µl of reference cDNA solution (pool from 5 wt animals) labelled with the second dye. This hybridisation mixture was placed on a pre-hybridised microarray, under a cover slip, placed into a hybridisation chamber (Genetix) and immersed in a thermostatic bath at 42°C for at least 16 hours. After hybridisation slides were washed in 40 ml of 3x SSC, 40 ml of 1x SSC and 40 l of 0,25x SSC at room temperature. For drying slides were placed in an empty 50 ml Falcon tube (Becton Dickinson, USA) and centrifuged at 4000 m/s<sup>2</sup>. Dried slides were scanned with a GenePix 4000A microarray scanner and the images were analysed using the GenePix Pro3.0 image processing software (Axon Instruments, USA). All data were normal-

ised by adjusting the median of log-ratios of Cy5 to Cy3 intensities to 0. For data analysis, in-house produced LabView based software was used (Drobyshev *et al.*, in press).

### 3.10.4 Results

#### Selected Organs and Isolated RNA

Brain was selected as organ for expression profiling analysis based on data from other GMC-screens. We isolated total RNA of these organs of three NCAM-mutant mice and four wild-type individuals (Table 24).

<b>Table 24: Amount of Total RNA [<math>\mu</math>g] Isolated from Brain of Each Individual.</b>	
<b>Mouse ID</b>	<b><math>\mu</math>g total RNA</b>
30014194	272
30014195	334
30014220	360
30014103	343
30014105	275
30014177	288
30014112	346

#### Chip Hybridisation

Four chip hybridisations were performed with RNA from brain of each 3 individual mutant mice. Each chip hybridisation was performed against the identical pool of wt RNAs (reference RNA pool). For each individual the chip experiments included 2 colour-flip experiments. All 12 chip hybridisations were used for data analysis.

#### Expression of the NCAM

In all experiments, signals on the NCAM probe were below detection thresholds.

#### Data analysis: Genes that may be of interest for the NCAM mutant line

The selected genes are reproducibly up-regulated in all 12 experiments; genes were ranked according to the minimum of (12) ratios (minimum of maximum). Only signals with intensity above 200 were selected.

### 1. Down-regulated Genes

No down-regulated genes found in brain of NCAM mutant mice.

### 2. Up-regulated Genes

Table 25: Up-regulated Genes				
Gene Name	UniGene ID	Fold Induction	p-value	Function
Myt1	Mm.86639	2,01 ± 0,21	< 0,01	Myelin transcription factor 1

The selected genes below were ranked according the lowest of 12 ratios of reproducibility in those chip-hybridisations (“minimum of maximum”). In this ranking all genes were either consistently up- or down-regulated. For the different selection of genes the estimated minimal number of false positive, non-differentially expressed (NDE) genes is given for the probabilities  $p < 0,05$  und  $p < 0,1$  (Table 26).

Table 26: Pattern analysis of gene expression data				
Number of genes	Number non-reproducible	NDE		Range of fold inductions (Minimum in 12 repetitions)
		$p < 0,05$	$p < 0,1$	
5	3	4	3	1,79 – 1,12
10	8	9	9	1,12 – 1,11
25	20	21	21	1,11 – 1,08
50	41	42	42	1,08 – 1,06
100	88	89	89	1,06 – 1,05

Among the top 10 genes 8 genes are not reproducibly expressed and for the probability  $p < 0,1$  nine genes are false positive (NDE).

Inspection of expression data from **individual mice** revealed a stronger correlation of up- and down-regulated genes in samples 30014194 and 30014220. Expression pattern of sample 30014195 shows anti-correlation to the other two samples. May be genes expressed in individual 30014194 and 30014220 are of interest for the NCAM mutant line (Table 27):

Table 27: Up-regulated genes				
Gene Name	UniGene ID	Fold Induction	P-Value	Function
Myt1	Mm.86639	2,1 ± 0,21	< 0,01	Myelin transcription factor 1: regulatory function in neuronal differentiation
Waspip	Mm.24110 Mm.20031	1,5 ± 0,27	< 0,01	Wiskott-Aldrich syndrome protein interacting protein: regulates synaptic growth in Drosophila; Modulation of synaptic transmission in hippocampal CA1 neurons

Table 28: Pattern analysis of gene expression data				
Number of genes	Number non-reproducible	NDE p<0,05	NDE p<0,1	Range of fold inductions (min in 8 repetitions)
10	3	4	4	1,79 – 1,14
25	14	16	16	1,14 – 1,12
50	35	37	37	1,12 – 1,10
100	78	82	81	1,10 – 1,07

### 2.10.5 Discussion

Inspection of expression data from individual mice revealed stronger correlation of differentially expressed genes in animals 194 and 220. An anti-correlation in expression pattern for some genes of animal 195 was found (data not shown). Maybe biological variability in gene expression oscillating in a circadian rhythm and stress-responsive genes are the reason for anti-correlation in the expression patterns between single individuals. Also, several recent publications have provided evidence for biological variability of expression levels for particular genes (Oishi *et al.*, 2003; Pritchard *et al.* 2001; Drobyshev *et al.*, 2003a; Drobyshev *et al.*, 2003b; Churchill *et al.* 2002).

Excluding animal 195 in data analysis we detected two significantly differentially expressed genes in brain of NCAM mutant mice:

- **Myt1** (myelin transcription factor1) has regulatory functions in neuronal differentiation. Maybe the up-regulation of this gene compensates the loss of NCAM that is responsible for cell to cell interaction during development.
- **Waspip** (Wiskott-Aldrich syndrome protein interacting protein) plays a role in modulation of synaptic transmission in hippocampal CA1 neurons. The overexpression of Waspip could be a compensation of the

missing NCAM gene product that also acts as a mediator of synaptic plasticity.

Using the selection criteria described above, we could identify two genes (shown above) that are differentially expressed in brain of NCAM mutant mice. It is suggested evaluating the relevance of these genes in terms of the studied allele. This may be done by a detailed inspection of the functional annotations for both genes, as initiated here in the discussion. Since the number of significantly expressed genes is very limited we also suggest in a next step to analyse the *in situ* expression patterns in mutant and wt tissues. We would be grateful for any feedback on this and would be glad to support you in this process. Please, contact us if you have questions concerning this analysis.

### 3.10.5 References

- Churchill G.A. (2002): Fundamentals of experimental design for cDNA microarrays. *Nat Genet* 32 Suppl.490-495
- Drobyshev A., M. Hrabé de Angelis and J. Beckers (2003a): Artefacts and reliability of DNA microarrays expression profiling data. *Current Genomics* 4: 615-621
- Drobyshev A., C. Machka, M. Horsch, M. Seltmann, V. Liebscher, M. Hrabé de Angelis and J. Beckers (2003b): Specificity assessment from fractionalization experiments (SAFE): a novel method to evaluate microarray probe specificity base on hybridisation stringencies. *Nucleic Acids Res.* 31(2):E1-1.
- Oishi K., K. Miyazaki, K. Kadota, R. Kikuno, T. Nagase, G. Atsumi, N. Ohkura, T. Azama, M. Mesaki, S. Yukimasa, H. Kobayashi, C. Iitaka, T. Ume-hara, M. Horikoshi, T. Kudo, Y. Shimizu, M. Yano, M. Monden, K. Machida, J. Matsuda, S. Horie, T. Todo and N. Ishida (2003): Genome wide expression analysis of mouse liver reveals CLOCK regulated circadian output genes. *J Biol Chem* 278: 41519-41527
- Pritchard C.C., L. Hsu, J. Delrow and P.S. Nelson (2001): Project normal: defining normal variance in mouse gene expression. *Proc Natl Acad Sci USA* 98: 13266-13271
- Seltmann M., M. Horsch, A. Drobyshev, Y. Chen, M. Hrabé de Angelis and J., Beckers (2004): Assessment of a Systematic Expression Profiling. Approach In *ENU-Induced Mouse Mutant Lines. Mam Gene*; in press

## 3.11 Metabolic Screen

### 3.11.1 Summary

The metabolic screening provides a comparative analysis of bioenergetic parameters in mice. Mechanisms which lead to disturbances in body weight regulation and energy metabolism are determined. Hence, the basal energetic demands are monitored during *ad libitum* feeding and under food restricted conditions. In humans unbalanced energy uptake and energy expenditure cause the development of obesity (Spiegelman and Flier, 2001) or anorexia nervosa with severe weight loss (Hebebrand *et al.*, 2003). Some rodent and other species tend to increase activity upon food restriction leading to weight loss when given access to an activity wheel (Exner *et al.*, 2000). Several studies described that fasting in mice results in transient depression of metabolic rate, heart rate, body temperature and locomotor activity (Duffy *et al.*, 1990; Williams *et al.*, 2002). Therefore the primary Metabolic Screening focussed on the determination of food and energy uptake under *ad libitum* conditions and metabolic adaptations during food restriction and serves as the origin for further investigations in the Secondary and Tertiary screening which go into details of energy expenditure and energy storage.

Nineteen (8 male / 11 female) wild-type mice and fourteen (7 male / 7 female) NCAM-KO mice were analyzed. They were first fed under *ad libitum* conditions for two weeks, followed by one week of food restriction to 60% of *ad libitum*.

KO mice had lower body weights but consumed more food and energy related to their body weight. Simultaneously to higher metabolic demands under *ad libitum* conditions, NCAM-KO mice increased food assimilation efficiency during food restriction.

The reduced body weight in correlation with elevated energetic demands indicated a disturbed body weight regulation and might reflect an **elevated basal metabolic rate**. The increase of food assimilation during food restriction supports the theory of elevated basal metabolic demands, because the limited food supply lead to a decrease of metabolic rate, which causes the decrease of body temperature.

### 3.11.2 Mice

Three batches of control and NCAM ko mice entered the metabolic screen, in total, eight adult control and seven adult KO males as well as seven wild-type and seven KO females. The mice were single caged on grid panels (0.5 cm grid hole diameter). They were fed *ad libitum* for a period of 14 days, followed by a period of food restriction to 60% of *ad libitum* for seven days to analyze adaptive responses of metabolism.

### 3.11.3 Material and Methods

#### Recorded Data

During the different feeding regimes body weight, food consumption ( $F_{con}$ ), rectal temperature ( $T_{re}$ ), daily feces production (Fec), energy uptake ( $E_{up}$ ), energy content of the feces ( $E_{fec}$ ), metabolizable energy ( $E_{met}$ ) and the food assimilation coefficient ( $F_{ass}$ ) were recorded.

#### Analysis of Feces

The separation of mice in single cages allowed collection of feces in three day intervals. Samples of lab chow and feces (~1 g) were dried at 60°C for two days, homogenized in a coffee grinder and squeezed to a pill for determination of energy content in a bomb calorimeter (IKA Calorimeter C7000) based on dry measurement principle. Energy uptake is determined as the product of food consumed and the caloric value of the food. To obtain metabolizable energy ( $E_{met}$ ) the energy content of feces and urine (2% of  $E_{up}$ ; Drozd 1975) were subtracted from energy uptake.

#### Statistical Analysis

All values are presented as means  $\pm$  SEM. Two-way-ANOVA (SigmaStat, Jandel Scientific) was used to test for effects of the factors genotype and gender. The Tukey test was applied for *post hoc* multiple comparisons. The Mann-Whitney-Test for paired samples was used to analyze the effect of nutritional status on parameters of energy metabolism.

### 3.11.4 Parameters

Recorded Data during the different feeding regimes
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body weight, food consumption ( $F_{con}$ ), rectal temperature ( $T_{re}$ ), daily feces production (Fec), energy uptake ( $E_{up}$ ), energy content of the feces ( $E_{fec}$ ), metabolizable energy ( $E_{met}$ ), food assimilation coefficient ( $F_{ass}$ )
--

### 3.11.5 Results

Wild-type mice displayed higher body weights than KO mice, which also reduced body temperature during food restriction. This could be caused by a decreased basal metabolic rate as an indication for metabolic adaptation. Food consumption or energy uptake was not different between wt and KO mice, but calculating energy uptake or ratio of metabolized energy per unit body weight KO mice show higher values. The assimilation efficiency of energy was not different during *ad libitum* conditions but increased in KO mice when exposed to food restriction. Especially KO females extract significantly more energy from food chow as a reaction on food restriction.

Raw data for each individual are available on demand in Excel sheets.

### 3.11.6 Discussion

No information about metabolic parameters were available prior the metabolic screening of NCAM KO mice. The metabolic phenotyping revealed several characteristics for NCAM KO mice. Based on the difference in body weight between both strains, the body weight related parameters like **energy uptake** and **ratio of metabolised energy** are significantly higher in KO mice. This indicates **higher metabolic demands** of KO compared to wt mice.

To test whether KO mice are able to adapt to food challenges, food was reduced to 60% of *ad libitum* amount and food assimilation coefficient was determined in comparison to the *ad libitum* period. While KO mice produced less feces per day and females even have lower values in energy content of feces samples, the **extraction rate of energy** out of food chow was higher in KO mice. This was even more pronounced when KO mice are food restricted. The mutated gene in NCAM KO mice influences metabolic characteristics leading to lower body weight but higher assimilation capacity and probably basal metabolic rate. It is suggested determining and validating the basal metabolic rate in the **secondary screening**.

### 3.11.7 References

- Drozdz M. (1975) A Food habits and food assimilation in mammals. In: Methods for Ecological Bioenergetics, edited by W. Grodzinski, R.Z. Klekowski and A Duncan. Oxford, UK: Blackwell, p: 23-47
- Duffy, P.H., R. J. Feuers and R. W. Hart (1990): Effect of chronic caloric restriction on the circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. *Chronobiol Int* 7: 291-303
- Exner, C., J. Hebebrand, H. Remschmidt, C. Wewetzer, A. Ziegler, S. Herpertz, U. Schweiger, W. F. Blum, G. Preibisch, G. Heldmaier and M. Klingenspor (2000): Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. *Mol Psychiatry* 5: 476-481.
- Hebebrand J., C. Exner, K. Hebebrand, C. Holtcamp, R.C. Casper, H. Remschmidt, B. Herpertz-Dahlmann, M. Klingenspor (2003): Hyperactivity in patients with anorexia nervosa and in semistarved rats: Evidence for a pivotal role of hypoleptinemia. *Physiology and Behavior* 79: 25-37
- Spiegelman B.M. and J.S. Flier (2001): Obesity and the regulation of energy balance. *Cell* 104: 531-543
- Williams T. D., J.B. Chambers, R.P. Henderson, M.E. Rashotte and J.M. Overton (2002): Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. *Am J Physiol Regul Integr Comp Physiol* 282: R1459-67

## Abbreviations

$F_{\text{con}}$	Food consumption
$T_{\text{re}}$	rectal temperature
$F_{\text{fec}}$	daily feces production
$E_{\text{up}}$	energy uptake
$E_{\text{fec}}$	energy content of the feces
$E_{\text{met}}$	metabolizable energy
$F_{\text{ass}}$	food assimilation coefficient

**Table 29: Metabolic Parameters Recorded in the Primary Screen**

Data are presented as mean  $\pm$  standard error of mean.

Parameter	Wild Type (A)					Mutant (B)					A~B	
	<i>ad libitum</i>		<i>p</i> -value	food reduction, 7 days to 60%		<i>ad libitum</i>		<i>p</i> -value	food reduction, 7 days to 60%		<i>p</i> -value	<i>p</i> -value
	Male	Female		Male	Female	Male	Female		Male	Female		
(n=8)	(n=11)	(n=8)	(n=11)	(n=7)	(n=7)	(n=7)	(n=7)	(n=7)	(n=7)			
<b>Body weight [g]</b>	33.75 $\pm$ 1.54	23.41 $\pm$ 0.39	<0.001	29.0 $\pm$ 1.48	20.1 $\pm$ 0.32	28.3 $\pm$ 0.96	21.6 $\pm$ 0.45	<0.001	23.7 $\pm$ 1.13	17.6 $\pm$ 0.56	<0.01	0.01
<b>Rectal body temperature [°C]</b>	36.6 $\pm$ 0.18	37.3 $\pm$ 0.12	< 0.01	35.3 $\pm$ 0.3	35.6 $\pm$ 0.26	36.7 $\pm$ 0.19	37.2 $\pm$ 0.26	n.s.	34.6 $\pm$ 0.37	34.8 $\pm$ 0.24	n.s.	n.s.
<b>Food consumption [g day<sup>-1</sup>]</b>	3.94 $\pm$ 0.21	3.52 $\pm$ 0.1	<0.05	60% of <i>ad libitum</i>		3.86 $\pm$ 0.09	3.54 $\pm$ 0.12	n.s.	60% of <i>ad libitum</i>		n.s.	n.s.
<b>Energy uptake [kJ day<sup>-1</sup>]</b>	72.99 $\pm$ 3.91	65.2 $\pm$ 1.79	<0.05	43.79 $\pm$ 2.35	39.12 $\pm$ 1.07	71.5 $\pm$ 1.75	65.47 $\pm$ 2.27	n.s.	42.9 $\pm$ 1.05	39.28 $\pm$ 1.36	n.s.	n.s.
<b>Energy uptake BW<sup>-1</sup> [kJ g<sup>-1</sup> day<sup>-1</sup>]</b>	2.17 $\pm$ 0.1	2.78 $\pm$ 0.08	<0.001	1.51 $\pm$ 0.07	1.95 $\pm$ 0.05	2.53 $\pm$ 0.05	3.02 $\pm$ 0.09	<0.001	1.82 $\pm$ 0.05	2.23 $\pm$ 0.08	<0.02	n.s.
<b>Feces production [g day<sup>-1</sup>]</b>	0.69 $\pm$ 0.03	0.6 $\pm$ 0.01	<0.05	0.41 $\pm$ 0.03	0.39 $\pm$ 0.01	0.64 $\pm$ 0.02	0.56 $\pm$ 0.02	<0.02	0.33 $\pm$ 0.03	0.32 $\pm$ 0.02	n.s.	n.s.
<b>Energy content feces [kJ g<sup>-1</sup>]</b>	16.16 $\pm$ 0.1	16.26 $\pm$ 0.1	n.s.	16.42 $\pm$ 0.08	16.2 $\pm$ 0.08	16.19 $\pm$ 0.11	15.93 $\pm$ 0.06	n.s.	16.11 $\pm$ 0.13	16.12 $\pm$ 0.05	n.s.	<0.05
<b>Metabolized energy [kJ day<sup>-1</sup>]</b>	62.0 $\pm$ 3.46	55.7 $\pm$ 1.7	<0.05	37.2 $\pm$ 1.98	32.9 $\pm$ 0.88	61.3 $\pm$ 1.55	56.6 $\pm$ 2.05	n.s.	37.6 $\pm$ 0.94	34.3 $\pm$ 1.07	n.s.	n.s.
<b>Metabolized energy [kJ g<sup>-1</sup> day<sup>-1</sup>]</b>	1.85 $\pm$ 0.09	2.38 $\pm$ 0.08	<0.001	1.28 $\pm$ 0.05	1.64 $\pm$ 0.04	2.17 $\pm$ 0.06	2.62 $\pm$ 0.08	<0.001	1.6 $\pm$ 0.06	1.95 $\pm$ 0.07	<0.01	n.s.
<b>Food assimilation coefficient [%]</b>	84.9 $\pm$ 0.04	85.3 $\pm$ 0.37	n.s.	85.0 $\pm$ 0.34	84.3 $\pm$ 0.37	85.7 $\pm$ 0.32	86.5 $\pm$ 0.32	n.s.	87.8 $\pm$ 1.19	87.3 $\pm$ 0.44	n.s.	<0.05

## 3.12 Pathology Screen

### 3.12.1 Summary

The Pathology screen performed a complete morphological analysis with standard stains.

We did not find any genotype-specific morphological differences between the mutant and the knockout mice. Therefore, we can conclude that NCAM mice do not show a specific morphological phenotype.

### 3.12.2 Mice

A total of 54 mice, 28 knockout mice (9 males, 19 females) and 26 control animals (10 males, 16 females) were analyzed. Due to the workflow in the GMC, mice of different ages were received from different screens (Table 30). The term “other screens” is used when a mouse was received from any other screen not listed in Table 30.

<b>Table 30: NCAM-deficient mice and their control littermates analyzed.</b>						
	<b>Wild Type</b>		<b>Mutant</b>		<b>Number of Animals</b>	<b>Age [weeks]</b>
	<b>Males</b>	<b>Females</b>	<b>Males</b>	<b>Females</b>		
<b>Lung Screen</b>		8		7	15	16
<b>Dysmorphology Screen</b>	2		1	1	4	19 - 22
<b>Metabolic Screen</b>	8	7	7	6	28	21 - 22
<b>Other Screens</b>		1	1	5	7	11 - 14
<b>Total Number of Animals</b>	<b>10</b>	<b>16</b>	<b>9</b>	<b>19</b>	<b>54</b>	

### 3.12.3 Materials and Methods

Mice received in the laboratory of pathology were sacrificed with CO<sub>2</sub>. The animals were analyzed macroscopically and weighed ([www.eulep.org/Necropsy\\_of\\_the\\_Mouse/index\\_2004.php](http://www.eulep.org/Necropsy_of_the_Mouse/index_2004.php)). The thymus and left lobe of the liver were measured. Blood samples were taken, centrifuged and the serum was saved at -20°C. Tails were preserved at -70°C for further genetic analysis. Following a complete dissection, an x-ray of the complete bone structure was taken, when indicated (Hewlett Packard, Cabinet X-Ray System Faxitron Series). All organs were fixed in 4% buffered formalin and embedded in paraffin for histological examination. Two-μm-thick sections from skin, heart, muscle, lung, brain, cerebellum, thymus, spleen, cervical lymph nodes, thyroid, parathyroid, adrenal gland, stomach, intestine, liver, pancreas, kid-

ney, reproductive organs, and urinary bladder were cut and stained with haematoxylin and eosin (H&E). Prussian's Blue staining was performed when indicated.

### 3.12.4 Results

In both genotypes, NCAM-KO mice and their littermate controls, only non-specific inflammatory changes in different organs were observed (Tab. 31).

<b>Table 31: NCAM-mice. Genotype-specific Morphological Alterations.</b>					
<b>Organ</b>	<b>Skin</b>	<b>Musculoskeletal System</b>	<b>Eyes</b>	<b>Brain</b>	<b>Cerebellum</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Heart</b>	<b>Trachea</b>	<b>Lung</b>	<b>Teeth</b>	<b>Salivary glands</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Esophagus</b>	<b>Stomach</b>	<b>Small Intestine</b>	<b>Large Intestine</b>	<b>Liver</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Pancreas</b>	<b>Cervical Lymph Nodes</b>	<b>Thymus</b>	<b>Spleen</b>	<b>Thyroid</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Parathyroid</b>	<b>Adrenal Gland</b>	<b>Kidneys</b>	<b>Urinary Bladder</b>	<b>Testes</b>
<b>Alteration</b>	no	no	no	no	no
<b>Organ</b>	<b>Epididymis</b>	<b>Funiculus spermaticus</b>	<b>Ovaries</b>	<b>Uterus</b>	<b>Vagina</b>
<b>Alteration</b>	no	no	no	no	no

**Liver:** In 30 of 54 animals (14 wild type, and 16 knockouts), non-specific infiltrates (composed of tree to eight lymphocytes), and one to five microgranulomas were observed. In addition, seven animals (six wild type and one knockout) developed a micro- and macrovesicular steatosis of the liver.

**Urinary Tract:** In five mice (2 wild type, and 3 knockouts) hydronephrosis of the kidney was observed. In addition, one knockout male mouse showed a mild dilatation of the renal pelvis. In one knockout female mouse, an inflammation of the ureter, in combination with hydronephrosis, was found. Two wild-type mice also had a dilatation of the urinary bladder. The term hydronephrosis used here signifies the combination of obstructive pelvic dilatation and obstructive renal disease. The glomeruli are well preserved, but the tubuli are partially atrophic.

**Thymus:** Eighteen of 54 animals (11 knockouts and 7 wild types) received from the metabolic screen revealed cortical thymus atrophy, as a reaction to food restriction.

### **3.12.5 Discussion**

The infiltration of the liver with non-specific lymphocytes and the finding of microgranulomas confirm earlier results of C57BL/6J screening. Mice of this background seems to be more susceptible to liver alterations. Concerning the steatosis in the liver, we have observed this change in different strains and lines. Therefore we conclude that this finding is more related to the type and amount of food that mice ingest than to some pathological feature.

A susceptibility to “obstructive nephropathy” was identified in this screen, including hydronephrosis with tubular atrophy and urinary bladder dilatation. However, the renal alterations were present in both wild-type and knockout mice. This means that the renal phenotype found here is strain- and not genotype-specific. Interestingly, such renal alterations were never observed in the analysis of about 100 non-manipulated C57BL/6J mice. To our knowledge, the renal alterations observed here, have not been recognized as part of the normal phenotype in wild-type C57BL/6J. In humans, segmental atrophy, as well as dysplasia of renal tubules, has been associated with urinary reflux most probably secondary to obstruction, even though, the association cannot be confirmed in all the cases. Furthermore, in the NCAM line, dilatation of the urinary bladder was not necessarily linked to the hydronephrosis, suggesting alterations at different levels of the urinary tract.

Thymic atrophy is a frequent finding in mice coming from the metabolic screen. We do believe that the thymic atrophy is secondary to food restriction and/or the stress induced during this analysis (unpublished observation). The atrophy usually correlates with the amount of fat loss during the food restriction period. The complete morphological analysis indicates that NCAM mice do not show a specific phenotype when compared to their littermates.

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