Behavioral investigation of mice deficient for the extracellular matrix protein tenascin-R

and

Investigation of the influence of maternal separation on the behavior of mice deficient for the cell recognition molecule CHL1

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften

der Fakultät für Biologie

an der Universität Bielefeld

vorgelegt von

Sandra Freitag

Bielefeld 2009

TABLE OF CONTENTS

PART B: INVESTIGATION OF THE INFLUENCE OF MATERNAL SEPARATION ON THE BEHAVIOR OF MICE DEFICIENT FOR THE CELL RECOGNITION MOLECULE CHL1

Abstract

The function of the central nervous system depends on the interaction and communication between neurons. Extracellular matrix proteins in the extracellular space and cell recognition molecules on the cellular membrane are important mediators of this interaction. In the present study the effects of the ablation of the extracellular matrix glycoprotein tenascin-R (TN-R) and of the cell recognition molecule CHL1 were investigated at the behavioral level in mice.

TN-R is an important component of the extracellular matrix of the brain and is attributed to fulfill a broad spectrum of different functions. In this study the behavior of mice deficient for the extracellular matrix glycoprotein TN-R in comparison to their wild-type littermates was investigated. Tests for exploration and anxiety, motor coordination and cognition were carried out. Mice were tested at different ages and under different housing conditions. TN-R deficient mice displayed decreased motivation to explore and an increased anxiety profile in the open field, free choice open field and elevated plus maze tests. Also, the anxiety level of TN-R deficient mice was more strongly influenced by environmental factors as compared to wild-type littermates. TN-R deficient mice showed motor coordination impairments in the wire hanging, Rotarod and pole tests. Thus TN-R ablation leads to an altered behavioral phenotype in mice that may negatively affect their fitness under natural conditions.

Mice deficient for the cell recognition molecule CHL1 display an altered behavior that is reminiscent of some symptoms found in schizophrenic patients. The development of schizophrenia is supposed to depend on the combination of genetic predisposition and negative experiences during early development. Thus, we wondered whether CHL1 deficient mice would be more vulnerable towards environmental postnatal insults and investigated the impact of daily maternal separation on the behavior of CHL1 deficient mice and their wild-type littermates. Male and female mice were tested as adults in a longitudinal study including tests for exploration and anxiety, social interaction, motor coordination and cognition. Maternal separation induced hyperactivity in males of both genotypes and a more impulsive or disinhibited behavior in females of both genotypes. Thus, maternal separation is a paradigm that can alter the behavioral responses expressed by male and female mice later in adulthood. For most of the investigated parameters maternal separation had a similar effect on both genotypes, although some evidence for impairment in working memory, a core symptom of schizophrenia, could be found specifically in maternally separated CHL1 deficient male mice.

.

Zusammenfassung

Die Funktionen des zentralen Nervensystems werden maßgeblich durch die Interaktion und Kommunikation zwischen den Neuronen gesteuert. Proteine der extrazellulären Matrix und Zellerkennungsmoleküle auf der Zellmembran sind wichtige Bestandteile dieser Interaktion. In der vorliegenden Arbeit wurden die möglichen Auswirkungen des Fehlens des extrazellulären Matrix Proteins Tenascin-R (TN-R) und des Zellerkennungsmoleküls CHL1 auf das Verhalten von Mäusen untersucht.

TN-R ist ein wichtiger Bestandteil der extrazellulären Matrix des Gehirns. In dieser Studie wurde das Verhalten TN-R defizienter Mäuse im Vergleich zu ihren Wildtyp Geschwistern untersucht. Eine Langzeitstudie, welche Tests zum Explorationsverhalten und Angstverhalten, sowie zur Motorik und Kognition beinhaltete, wurde durchgeführt. Die Mäuse wurden in unterschiedlichen Altersstufen und unter unterschiedlichen Haltungsbedingungen getestet. TN-R defiziente Mäuse zeigten eine verminderte Motivation zu Erkunden und erhöhte Ängstlichkeit im offenen Feld, freiwilligen offenen Feld und erhöhtem Plus Labyrinth. Des Weiteren zeigte sich, daß das Angstverhalten der TN-R defizienten Mäusen stärker von Umweltfaktoren beeinflußt wurde als das der Wildtyp Mäuse. TN-R defiziente Mäuse wiesen zudem Beeinträchtigungen in der motorischen Koordination beim Seil-Hängen, Rotarod und Stab Test auf. Demzufolge führt das Fehlen von Tenascin-R bei Mäusen zu einem veränderten Verhaltensprofil, welches sich unter natürlichen Bedingungen nachteilig auf das Überleben auswirken könnte.

Mäuse, denen das Zellerkennungsmolekül CHL1 fehlt, zeigen Verhaltensänderungen, welche einigen Symptomen der Schizophrenie ähneln. Die Entstehung von Schizophrenie wird auf das Zusammentreffen von genetischer Prädisposition und von negativen Erfahrungen in der frühen Entwicklung zurückgeführt. Somit stellte sich die Frage, ob CHL1 defiziente Mäuse anfälliger für postnatale Störungen sein könnten als Wildtyp Mäuse. Wir untersuchten, welchen Einfluß eine tägliche Trennung vom Muttertier auf das Verhalten von CHL1 defizienten Mäusen und deren Wildtyp Geschwistern hat. Männchen und Weibchen wurden als Erwachsene in einer Langzeitstudie mit Tests zum Explorationsverhalten und Angstverhalten, zur sozialen Interaktion, Motorik und Kognition getestet. Die maternale Separation führte bei Männchen beider Genotypen zu Hyperaktivität und bei Weibchen beider Genotypen zu einem impulsiveren oder weniger gehemmten Verhalten. Somit kann maternale Separation das Verhalten männlicher und weiblicher Mäuse langfristig beeinflussen. Die maternale Separation zeigte bei den meisten Parametern einen ähnlichen Effekt bei beiden

Genotypen, jedoch konnten Hinweise auf eine mögliche Verminderung des Arbeitsgedächtnisses (ein Hauptsymptom der Schizophrenie) spezifisch bei männlichen CHL1 defizienten Mäusen gefunden werden.

General Introduction

Expression of behavior is regulated by the central nervous system (CNS) which in turn depends on the proper contact and interaction between cells (i.e., neuron-neuron, neuron-glia and glia-glia interactions) and their surrounding environment, the extracellular matrix (ECM). These interactions are mediated by cell recognition molecules and ECM proteins. Several functional *in vitro* and *in vivo* studies have shown that indeed ECM proteins and cell recognition molecules regulate not only important processes during the development of the CNS (Maness and Schachner, 2007) but also the function of the adult brain (Schachner, 1997; Dityatev and Schachner, 2003). In the present study, the role of one ECM glycoprotein and of one cell recognition molecule in regulating behavioral responses was investigated with the help of genetically engineered mice in which the expression of the protein in question was constitutively ablated. In the first part the behavioral phenotype of a mouse deficient for tenascin-R was investigated. The multifunction of tenascin-R *in vitro*, did not allow for a strong hypothesis, therefore the experiment was designed to address a spectrum of different behavioral traits under different aspects. In the second part the impact of maternal separation on the behavior of a mouse deficient for CHL1 was investigated. Here the emphasis was put on the interaction of adverse environmental factors with the lack of CHL1 and the consequences for the outcome of behavior.

The development of gene targeting in embryonic stem cells and the possibility to generate mice of theoretically any desired genotype by the end of the 1980s (Capecchi, 2005), offered new options for the study of the influence of a determined protein on the behavior of the mouse. So far, several mice lines lacking a defined protein (knockout mice) have been generated and their behavior has been investigated. In some cases, a direct link between a mutated gene and a distinct behavioral deficit could be defined, as for example the specific impairment of spatial learning observed in α–Calcium-Calmodulin Kinase II mutant mice (Silva et al., 1992). However, a complex behavior cannot be attributed to a single protein, but relies on a whole set of different genes that act in concert. Moreover, it is obviously not only the genotype that defines behavioral traits. Environmental and epigenetic factors play an important role in the shaping of behavior as well. Crabbe et al. (1999) reported remarkable differences in the anxiety-like behavior of mice that were tested in three different laboratories under otherwise identical conditions. Also test order and training history have been shown to affect the outcome of some behavioral tests (McIlwain et al., 2001). The beneficial influences of an enriched environment are described to be manifold including enhanced learning and

memory performance and compensation of neurodegenerative impairments (overview in: Rampon and Tsien, 2000; Laviola et al., 2008). Shaping of behavior through environmental factors has a main impact during early development and particularly the maternal factors play a key role. Francis et al. (2003) used cross-fostering methods to demonstrate the important effect of the maternal care on the behavior displayed in adulthood. Furthermore the sex ratio and genotype ratio within a litter (Crews, 2008), as well as the intrauterine position (Ryan and Vandenbergh, 2002), have been shown to influence adult sexual and social behavior. Despite the paramount importance of environmental factors in shaping behavior of the mouse, they are often underestimated when the behavior of mutant mice is analyzed. In this context, the two projects described in the present thesis aim to analyze two mutant mice taking into consideration also the possible effects of the environment and experience on the expression of behavioral responses.

A

Behavioral investigation of mice deficient for the extracellular matrix protein tenascin-R

Introduction

The Tenascin family of Glycoproteins

The development and function of multicellular organisms depends on the communication between cells and between cells and their surrounding environment, the extracellular matrix (ECM). The ECM of the nervous system is a specialized composition of macromolecules including members of the tenascin family of glycoproteins. The tenascins fulfill a broad spectrum of important functions in cell differentiation, cell migration, neurite outgrowth and cell communication.

The tenascins comprise at present four closely related members: tenascin-C (TN-C) (Chiquet and Fambrough, 1984; Grumet et al., 1985; Kruse et al., 1985), tenascin-R (TN-R) (Pesheva et al., 1989; Rathjen et al., 1991; Nörenberg et al., 1992), tenascin-X (TN-X) (Bristow et al., 1993) and tenascin-W (TN-W) (Weber et al., 1998). Since a tenascin gene has been found in the urochordate *Ciona intestinalis*, but not in any invertebrate phyla it is likely, that tenascins are exclusively expressed by chordates (Tucker et al., 2006). All tenascins display a common modular structure existing of an amino terminal cystein-rich segment which is unique to the family, followed by diverse numbers of epidermal growth factor-like (EGFL) domains, fibronectin type III (FN III) domains and a fibrinogen-like (FBG) knob. The name tenascin was derived from a combination of the Latin words *tenere* (to hold) and *nasci* (to be born) (Chiquet-Ehrismann et al., 1986).

Tenascin-R

TN-R has been discovered together with TN-C by Kruse et al. in 1985 as HNK-1 carbohydrate carrying protein with a molecular mass of 220, 200, 180 and 160 kD. The yet unknown protein got the name J1. Later it was possible to recognize J1-200/220 and J1-160/180 as two strongly related but different molecules and to identify J1-200/220 as TN-C (Grumet et al., 1985). Due to its dual function *in vitro*, where it has been shown to be either repulsive or adhesive, the J1-160/180 protein was named janusin after the two headed roman god Janus (Pesheva et al., 1993). Rathjen et al. (1991) described a protein in chicken with a distinct spatio-temporal expression pattern in the CNS and therefore named it restrictin. Restrictin was shown to be the avian homologue of janusin and a member of the tenascin family resulting in the final name tenascin-restrictin (TN-R).

Structure of TN-R TN-R is composed of an amino-terminal cystein-rich domain, followed by 4.5 epidermal growth factor like repeats, 9 fibronectin type III domains (the 6th

domain can be alternatively spliced), and a fibrinogen knob (Nörenberg et al., 1992) (Fig. 1). TN-R occurs in two isoforms of 160 and 180 kD that are capable of building dimers and trimers, respectively (Pesheva et al., 1989). As a glycoprotein TN-R is a carrier of the HNK-1 carbohydrate (Kruse et al., 1985) and diverse chondroitin sulfate proteoglycans (Zamze et al., 1999; Probstmeier et al., 2000). TN-R is strongly conserved during evolution, thus TN-R of rat, chicken and human shows a high degree of sequence homology, ranging from 75 to 93 % (Erickson, 1993; Fuss et al., 1993; Erickson, 1994).

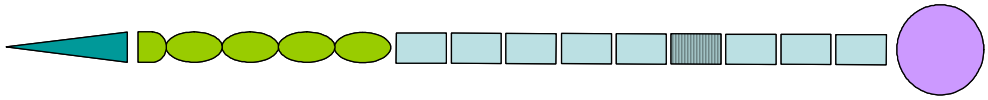

Fig. 1 Schematic presentation of the tenascin-R protein.
Proceeding in a carboxyl terminal direction, the domains are as follows: cystein rich stretch (turquoise triangle), 4,5 EGFL repeats (green ovals), 9 Fibronectin type III domains (blue rectangles) with the 6th domain alternatively spliced (striped) and a fibrinogen globe (purple circle).

Expression and localization of TN-R. Apart from a possible expression by Schwann-cells during embryogenesis (Probstmeier et al., 2001), TN-R is restricted to the CNS. Studies in rats and mice revealed that TN-R is mainly expressed by oligodendrocytes during the period of active myelination. Neuronal cells expressing TN-R have been identified as a small subset of neurons in the hippocampus and neurons in the olfactory bulb, motorneurons in the spinal cord and stellate and basket cells in the cerbellum (Fuss et al., 1993; Wintergerst et al., 1993; Saghatelyan et al., 2004). In the myelinated part of the murine optic nerve TN-R expression peaks around 2-3 weeks postnatal. In the retina expression of TN-R by horizontal cells remains stable up to adulthood. TN-R is found on unmyelinated and myelinated axons, on oligodendrocytes and on the outer aspects of myelin sheaths (Bartsch et al., 1993), at axon astrocyte contact areas and between the somata of pyramidal neurons (Schuster et al., 2001). TN-R has been found to be particularly accumulated at the nodes of Ranvier (ffrench-Constant et al., 1986; Bartsch et al., 1993). Another very important feature is the involvement of TN-R in the building of perineuronal nets (PN) (Celio and Rathjen, 1993; Wintergerst et al., 1996). First described by Camillo Golgi (Golgi, 1893) the PNs have nowadays been identified as lattice or honeycomb like accumulation of extracellular matrix that surround the soma, the proximal parts of dendrites and the axon initial segments of several neuronal cell types in various parts of the brain. The PNs consist mainly of three classes of substances, namely: hyaluronan, proteoglycans and glycoproteins like TN-C (Celio and Chiquet-

Ehrismann, 1993) and TN-R (Celio and Rathjen, 1993). A common feature of cells with well developed nets is their extensive coverage of synaptic contacts and the diameter of holes within the nets agrees roughly to the size of axonal boutons (Celio and Blümke, 1994). Since PNs are mainly found around neurons with fast firing properties, they may serve as a polyanionic microenvironment, sub serving fast recruitment phases of fast firing neurons (Brückner et al., 1993). On the other hand PNs may stabilize existing synapses and prevent the formation of new contacts. Underpinning this hypothesis PNs form late in postnatal life and contain substances that are repellent for neurons and their processes like chondroitin sulfate proteoglycans, TN-C and TN-R (Celio and Blümcke, 1994). Other theories see PN as possible storage rooms for growth factors around neurons, or a linkage of ECM with the cytoskeleton (Wintergerst et al., 1996). TN-R bearing PNs are found predominantly around interneurons in the cortex, hippocampus, cerebellum, brainstem and spinal cord (Hagihara et al., 1999; Brückner et al., 2000).

Function of TN-R. Several *in vitro* studies have been carried out to investigate the functional attributes of TN-R. One of the most striking features is the dualism of properties. TN-R has been found to be either adhesive or repulsive, neurite outgrowth promoting or inhibiting, depending on the time course, cell type and developmental stage of the cell, the presentation of the TN-R protein and the interaction with different ligands and receptors. TN-R is a non permissive substrate for the attachment of cerebellar neurons, astrocytes and fibroblasts while neurons first attach and then detach from the substrate (Pesheva et al., 1989; Morganti et al., 1990). Interestingly TN-R promotes or retards growth cone advance depending on the spatial expression pattern and the neuronal cell type. If TN-R is presented as a sharp boundary, it is non permissive for dorsal root ganglia and retinal ganglion cell neurites, whereas, if presented as a uniform substrate, TN-R enhances the outgrowth of dorsal root ganglia, but abolishes the outgrowth of retinal ganglion cells completely (Taylor et al., 1993). Studies on TN-R fragments have provided information on the binding abilities and functional properties of single domains (Taylor et al., 1993; Nörenberg et al., 1995; Xiao et al., 1996; Aspberg et al., 1997; Xiao et al., 1997). The interaction of TN-R and the cell adhesion molecule F3/F11/Contactin mediates repulsion of murine neurons (Pesheva et al., 1993) and attachment and neurite outgrowth of chicken neurons (Rathjen et al., 1991; Nörenberg et al., 1995). Zacharias and Rauch (2006) found the promoting or inhibiting effect of TN-R on the attachment and outgrowth of chicken tectal cells to be dependent on the interaction of TN-R with contactin 1 and diverse chondroitin sulphate proteoglycans. The peak in TN-R expression during the period of myelination suggests relevance for CNS

myelination. Accordingly it has been shown that substrate bound TN-R supports the adhesion of oligodendrocyte progenitors and influences oligodendrocyte differentiation (Pesheva et al., 1997). The strong attachment of TN-R to oligodendrocytes led to the filing of a patent in 2005, using TN-R fragments as a molecular fishing rod to purify oligodendrocytes from other cells. Furthermore, the myelin associated glycoprotein (MAG) has been identified as a binding partner. MAG shows an overlapping expression pattern with TN-R and is part of the signaling pathway of TN-R for cell repulsion (Yang et al., 1999). TN-R binds to and is able to modulate the beta-2 subunit of voltage gated sodium channels, suggesting a role of TN-R in localizing sodium channels at axon initial segments and at nodes of Ranvier (Srinivasan et al., 1998; Xiao et al., 1999). Taken together, *in vitro* studies revealed a broad spectrum of different attributes, depicting TN-R as a multifunctional, modular protein with a great variety of (sometimes ambivalent) functions.

The anti-adhesive properties of TN-R led to the hypothesis that TN-R may play a key role in preventing CNS regeneration in higher vertebrates. Thus several *in vivo* studies have been carried out. Lesion experiments in rodents showed that TN-R is upregulated in regions of neuronal degeneration and reactive astrogliosis (Wintergerst et al., 1997; Probstmeier et al., 2000). TN-R inhibits the outgrowth of retinal ganglion cell axons of both mice and salamander but remains expressed after lesion of the optic nerve in mice and is strongly down regulated after lesion of the optic nerve of the salamander. This correlates with the finding that, in contrast to mammals, amphibians are able to regenerate axons after optic nerve crush (Becker et al., 1999; Becker et al., 2000). In zebrafish TN-R borders the optic tract and is repellent for developing and newly growing and regenerating optic axons, thus TN-R may have a guiding function for the proper building of the optic tract (Becker et al., 2003; Becker et al., 2004). Other studies found that fish TN-R is not repellent for fish neurons, but mammalian TN-R is repellent for mammalian neurons and postulate a development from adhesive to antiadehesive functions of TN-R during vertebrate evolution (Pesheva et al., 2006). Congruently it has been shown, that TN-R is upregulated in the optic tract of lizards and does not prevent the outgrowth of optic axons (Lang et al., 2008).

Electrophysiological studies revealed the involvement of TN-R in synaptic plasticity of the hippocampus. In the murine CNS TN-R and its HNK-1 carbohydrate modulate perisomatic inhibition and LTP in the CA1 region (Saghatelyan et al., 2000). Furthermore TN-R is involved in the recruitment of neuroblasts in the adult mouse brain, as has been shown for the olfactory bulb (Saghatelyan et al., 2004). Further information about the involvement of TN-R in the living system has been gained from a mouse mutant deficient for TN-R.

The tenascin-R knockout mutant

Weber et al. (1999) generated a murine null mutant for TN-R. TN-R deficient mice are viable and fertile and TN-R expressing brain areas are apparently normal. Ultrastructural investigations revealed a normal building, appearance and density of myelin, and a normal morphology of the nodes of Ranvier with no obvious changes in the expression and distribution of voltage gated sodium channels. However, immunostaining for phosphacan, a binding partner of TN-R (Xiao et a., 1997; Milev et al., 1998), is weak and diffuse in the mutant, especially at nodes of Ranvier, suggesting an altered distribution of this ECM component in the mutant. Furthermore the conduction velocity in the optic nerve is decreased to about half that in WT animals, thus underpinning the finding that TN-R is a functional modulator of sodium channels (Xiao et al., 1999). A prominent feature of TN-R deficient mice is a disturbed morphology of perineuronal nets (PN). The PNs of the mutant are found in similar numbers and show the same distribution and development as in the WT. However, the appearance of the nets is altered. The staining for chondroitin sulfate proteoglycans is diminished or absent in the PNs of TN-R deficient mice, the nets are less regularly shaped and less extensive in their covering of somata and dendrites. In comparison to the evenly honeycomb-like meshes of the WT, the TN-R deficient nets show a more granular configuration, thus revealing an important role of TN-R for the composition and maintenance of the matrix components in PNs. (Weber et al., 1999; Brückner et al., 2000; Haunso et al., 2000). The finding of the relevance of TN-R for perisomatic inhibition (Saghatelyan et al., 2000) led to extensive studies of the electrophysiological properties of TN-R deficient mice. The mutants display a disturbed balance between excitation and inhibition in the hippocampus. They show an increase of excitatory synaptic transmission and a reduced expression of LTP in CA1, and display a two-fold decrease in theta-burst stimulation induced LTP (Bukalo et al., 2001; Saghatelyan et al., 2001; Gurevicius et al., 2004). It was found that the deficiency of TN-R increases the threshold for induction of LTP at CA3-CA1 synapses, due to hippocampal metaplasticity (Bukalo et al., 2007). TN-R deficient mice display increased hippocampal excitability of CA1 pyramidal cells, resulting from a deficit in GABAergic inhibition, but not an increased susceptibility to seizures (Brenneke et al., 2004). Correlating with the abnormal inhibition in CA1, the spatial arrangement of neuronal cell bodies in the pyramidal cell layer is more diffuse in the mutant and the coverage of pyramidal cell bodies by active zones of symmetric synapses is strongly reduced (Nikonenko et al., 2003). Furthermore, the extracellular space in the brain of TN-R deficient mice is reduced,

which may also contribute to a disturbed diffusion of neurotransmitters, trophic factors and ions (Sykova et al., 2005). The hypothesis of TN-R as an inhibitor of regeneration in mammals (see above) led also to lesion studies in TN-R deficient mice. After transection of the facial nerve, no alteration of motoneuron reinnervation was found, but a better recovery of vibrissal whisking in TN-R deficient mice in comparison to WT mice. This suggests that TN-R impedes recovery after nerve lesion (Guntinas-Lichius et al., 2005). Also after compression of the thoracic spinal cord, open field locomotion in TN-R deficient mice recovered better than in WT mice, affirming TN-R as an inhibitory protein for CNS regeneration (Apostolova et al., 2006).

In summary the results obtained from investigation of TN-R deficient mutants add further insights into the multiple functional properties of the protein *in vivo.* The studies revealed that TN-R is involved in building a proper ECM environment around neurons, in synaptic plasticity of the hippocampus and in regenerative processes of the CNS.

Aim of the Study

The extracellular matrix glycoprotein TN-R is a phylogenetically conserved protein suggesting that it is important for the expression of an adaptive phenotype. Morphological and electrophysiological investigation of a mouse mutant deficient for TN-R revealed several alterations in the mutant, but the behavioral phenotype has never been described. Behavior is the end-point of integrated systems and even subtle alterations in any of the components are likely to be reflected in a disrupted or modified behavior. The aim of this study was to perform a longitudinal behavioral study on TN-R deficient mice in order to test the influence of the lack of TN-R on the behavior of the mouse.

Experimental design

In order to gain comprehensive information about the behavior of TN-R deficient mice several behavioral tests were performed. For investigation of exploration and reaction towards novel stimuli, the open field and free choice open field were included. The elevated plus maze was accomplished as a classical test for anxiety related behavior in rodents. For evaluation of social behavior the observation of spontaneous social interactions between siblings, the resident intruder and the Reeperbahn tests were used. Hippocampus-dependent cognitive functions were tested with the Morris water-maze (for spatial learning and memory) and the step through passive avoidance (for one-trial learning and memory). Motor functions and motor learning were examined with the pole test, ROTAROD and wire hanging. The aim of this study was not just to screen the mice systematically for any possible difference between KO and WT. Furthermore it ought to be tested whether environmental changes did differentially affect the development of behavior in WT and TN-R deficient mice. Therefore the free choice open field, open field and elevated plus maze where performed repeatedly before and after isolation and at different developmental ages. Although it is clear, that investigation of inbred mice under laboratory conditions does not meet the criteria of classical ethology, the experiments were performed and analyzed taking in consideration the biology of the mouse and the adaptive functions of determined behavioral responses. The order was designed starting with tests that are assumed to be the least invasive ones.

Materials and Methods

Husbandry and general procedures

Mice were transferred from the breeding facility into a *vivarium* with an inverted 12:12 light: dark cycle (light on at 7:00 am) and maintained under standard housing conditions (21 ± 1°C, 40-50% humidity, food and water *ad libitum*). All behavioral tests were performed during the dark cycle of the animals in a room next to the *vivarium* that was illuminated with dim red light. Tests were started and ended at least 2 hours after light offset and 2 hours before light onset, respectively. After a mouse finished a test the fecal boli and urine-drops were counted and the experimental material was cleaned with soap, water, and ethanol (70%) successively.

Animals

16 *tenascin-R* +/+ (WT), 34 *tenascin-R* +/- (HET) and 18 *tenascin-R* -/- (KO) male littermates from heterozygous breeding pairs (mixed C57Bl/6J x 129Ola genetic background, 2 backcrosses into C57Bl/6J) were separated from their mothers at postnatal day (PD) 21 and transferred into the animal room and, after one week, housed in pairs composed by one HET mouse and either one WT or one KO littermate in Macrolon type II-long cages (15 x 20 x 30 cm). To avoid a litter effect, no more than 2 males per genotype were used from the same litter. Body weight was recorded daily up to PD 35, and then weekly up to the age of 18 weeks. Starting at PD 29-33 WT and KO mice were tested in several behavioral paradigms in a longitudinal study (Table 1).

Table 1

Day	Age	Experiment
1	21 d	Weaning
6	27 d	Housing in twos
8	29 - 33 d	Free choice openfield (FCOF) 1
12	33 - 37 d	Open field 1
52 -57	11 - 15 w	Home cage spontaneous behavior
66	13 - 16 w	Open field 2
69	14 - 17 w	Elevated Plus maze 1
77	14 - 17 w	Isolation
80	14 - 17 w	Resident/intruder test
82	14 - 17 w	Open field 3
84	14 - 18 w	Elevated plus maze 2
87 - 89	15 - 18 w	FCOF 2 and 3, Reeperbahn test
90	16 - 18 w	Pole test
96 - 104	17 - 20 w	Water-maze
116	20 - 22 w	ROTAROD
136	23 - 25 w	Wire hanging
265	9 - 10 m	Circadian activity
280	11 m	Step through passive avoidance

Aggressive interaction between siblings

The observation of aggressive interactions between WT or KO mice and their HET brothers were performed to assess possible major differences in social hierarchy between caged-together littermates. Aggressive interactions were scored for 60 min after changing the

cage during the second half of the dark cycle. The new cage contained new sawdust and new nesting material with the addition of part of the old nesting material and sawdust. The HET mice had been marked on the tail with non-toxic dye on the previous days. Observations were performed in red dim-light on PD 40-44, PD 54-58, PD 68-72, and PD 94-98. Observation of aggressive interactions was also performed in the first half of the dark cycle without disturbing the mice on PD 40-44, as well as during the observation of home cage spontaneous behavior (see below). In addition, aggressive interactions were scored for 30 min after mice were returned into the home cage after the three open field tests and after the elevated plus maze test (as the WT or KO mouse was in the open field or elevated plus-maze the HET mouse was placed in a new cage without bedding). During all observations latency, frequency and direction (i.e. HET towards WT) of attacks, counterattacks and mounting events were scored. For each attack it was scored whether the mouse being attacked was counterattacking. The following method was then used to determine social hierarchy within each cage (Bartolomucci et al., 2001; Grant et al., 1963; Terranova et al., 1998): a mouse was ranked as submissive when it showed, in the last two aggressive interactions, submissive behavior (submissive upright position, squeaking, crouched posture) when attacked; a mouse was considered dominant when it was initiating attacks, with the partner showing defensive or submissive behavior in the last two aggressive interactions.

Free choice open field and Reeperbahn test

Mice were housed for 2 days in a Macrolon type II-long cage equipped with a lockable hole (4 cm in diameter) at the bottom of one of the smaller walls. The cage (with the hole closed) was placed next to an arena (75 x 90 cm). For the test performed on periadolescent mice, the arena was enclosed by a 45 cm high wall on one 90 cm and one 75 cm side and an 85 cm high cliff on the other two sides. For the test performed on adult mice, the whole arena was surrounded by 45 cm high walls. One short wall contained a gap where the small side of the cage fitted in perfectly allowing direct access of the mouse from its home cage into the arena through the hole. After 5 min the hole was opened and the mouse had 10 min to recognize the opening. After the mouse recognized the open door, it was given a maximum of 10 min to enter the arena with four paws (Fig. 2). The test lasted 10 min after the first entrance into the arena. During this time the mouse could freely move between the arena and the home cage. Mice were caged in pairs when tested at PD 29-33. To distinguish the focal animal (either WT or KO) from the HET littermate, the tail of the HET mouse was marked 30 min before the test with a non-toxic white dye. This marking stimulated toilet behavior in the

HET mice, so that only in three cases the HET mouse entered the arena before the focal mouse. The arena was illuminated by 25 Lux and 5 Lux for the young and adult mice, respectively (the lower light density for the adult mice was used since previous data showed low exit rates with higher illumination). The young mice were tested once, whereas the adult mice were tested twice on two consecutive days and, 24 hrs later, they underwent a modified FCOF where a new social stimulus (a cage containing adult females) was introduced (for brevity named Reeperbahn test after the red light district of Hamburg). For the Reeperbahn test all conditions were as for the free choice open fields (FCOFs), besides that a Plexiglas cage (14 x 20 x 26 cm) containing three adult virgin females was placed in the arena opposite to the entrance. All females were previously caged together and had never been exposed either to males or male's urine since weaning, conditions that cause prolonged diestrous intervals (named anestrous or pseudopregnancy) in mice (Lee-Boot effect) (Lee and Boot, 1955; Lee and Boot, 1956; Whitten, 1959). A new group of three females was used every four trials (two WT mice and two KO mice). The females' cage had several holes (11 mm diameter) to allow diffusion of odor and limited tactile contacts between male and females. The two FCOFs on adult mice were performed to observe long-term habituation to the FCOF before testing the mice in the same apparatus containing the cage with the females. The new social stimulus should induce an altered behavioral response to the arena as compared to the FCOFs performed on the previous days, possibly increasing the motivation of the male mice to enter the arena and to explore the cage containing the females.

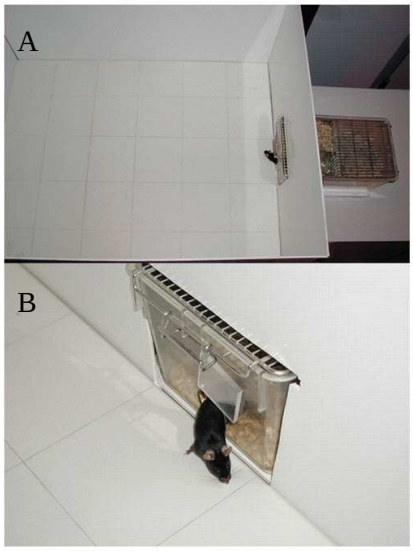

Fig. 2 Free choice open field. *(A) Setup. (B) Mouse entering the arena.*

The behavior of the mice in their home cage and in the arena was analyzed with the software The Observer (Noldus, Wageningen, The Netherlands). Following parameters were scored: time spent investigating the door, latency to enter the arena, transitions home cage/arena, time spent in different zones of the arena, locomotion (number of 15 x 15 cm squares crossed in the arena), rearing in the arena and time spent at females' cage (Reeperbahn test only).

Open field

The open field (OF) consisted of a 50 x 50 cm arena enclosed by 40 cm high walls, illuminated with 25 Lux (Fig. 3A). The mouse was placed in a Plexiglas cylinder located in one corner of the arena. As the cylinder was lifted, the mouse could freely move in the arena for 15 min. Tracks were produced with the software EthoVision (Noldus). The following behavioral parameters were analyzed with The Observer for the first 5 min of the test: stretch attend posture (calculated when the mouse stretched forward and then retracted to the original position without forward locomotion; Rodgers and Johnson, 1995), rearing on wall (vertical exploration by standing on the back paws with one or two forepaws touching the wall, Fig. 3B), rearing off wall (Fig. 3C) and self-grooming.

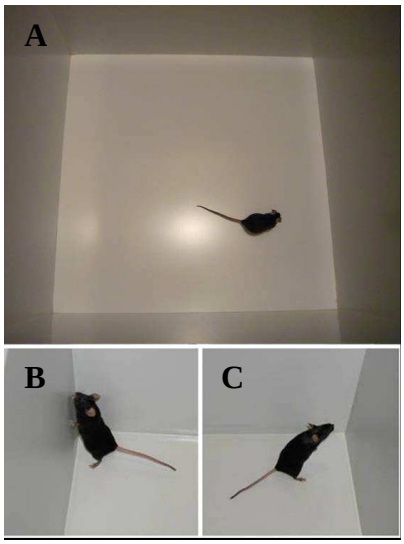

Fig. 3 Open field. *(A) Open field box. (B) Rearing on wall. (C) Rearing off wall.*

Elevated plus maze

The arena of the plus maze had the shape of a plus with four 30 cm long and 5 cm wide arms, connected by a 5 x 5 cm center. Two opposing arms were bordered by 15 cm high walls (closed arms), whereas the other two arms (open arms) were bordered by a 2 mm rim. The plus was elevated 75 cm from the floor and illuminated with 5 Lux. The mouse was placed in the center facing an open arm and observed for 5 min (Fig 4). The following parameters were analyzed with The Observer: latency to enter the open arms, latency to reach the edge of an open arm, open and closed arm entries (calculated when all the four paws were on an arm), number of entries in the edges of the open arms (calculated when the mouse reached with its snout the edge of an open arm), stretch attend posture (SAP, calculated when the mouse stretched forward and retracted to the original position without forward locomotion (Rodgers and Johnson, 1995), rearing on wall, self-grooming, and head dips (exploratory head movement over the side of an open arm with the snout pointing downwards).

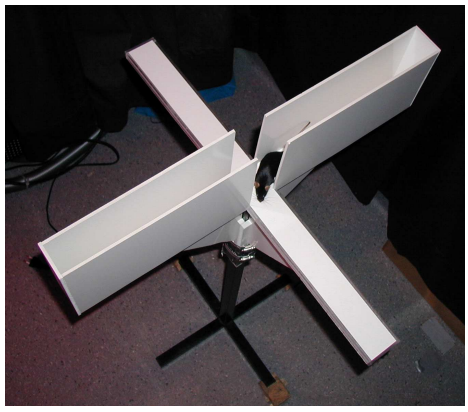

Fig. 4 Elevated Plus Maze.

Resident/intruder test

Mice underwent the resident/intruder test three days after being single housed in a Macrolon type II cage (15 x 20 x 30 cm). The home cage of the mouse was gently taken from the *vivarium* and placed in the experimental room under a video camera. A Plexiglas panel provided with holes for ventilation substituted the top of the cage. After 5 min, a C57Bl/6J age- and body-weight-matched unfamiliar male (intruder) was introduced in the cage of the focal animal (resident). The test lasted 10 min from the first resident-intruder contact. Possible aggressive interactions between resident and intruder were analyzed.

Home cage spontaneous behavior

The spontaneous behavior of 11 to 15 week-old mice in their home cage was observed 2 and 7 days after the mice were placed in a fresh cage. In order to distinguish the focal animal from the HET partner, the tale of the HET animal was painted with animal marking color. The behavior of the mice was observed by instantaneous sampling for 1 hour with an interval of 3 min (20 samples / hour) at three different time points: at the beginning of the dark phase (08:30), in the middle of the dark phase (14:00) and at the end of the dark phase (17:30). The behavioral parameter shown by the mouse at the moment the experimenter looked in the cage was immediately recorded, namely: resting, eating, drinking, being active, climbing at the grid of the cage, self-grooming, allo-grooming (passive and active), social investigation (passive and active), biting (passive and active), fighting and urination/defecation. The frequency of expression of the several behavioral parameters (measured as number of time the mouse was involved in a behavioral parameter over the maximum of 20) was analyzed.

Circadian activity

The circadian activity of a single housed mouse was monitored by using an infrared sensor connected to a recording and data storing system of the size of a cigarette pocket (Mouse-E-Motion by Infra-e-motion, Henstedt-Ulzburg, Germany, see technical descriptions at http://www.infra-e-motion.de). The mice were placed into a standard cage (15 x 20 x 23 cm) two days before starting monitoring the circadian activity. A Mouse-E-Motion was placed 10 cm above the top of each cage so that the mouse could be detected in any position inside the cage. The Mouse-E-Motion sampled every second whether the mouse was moving or not over a period of 4 days. The sensor could detect body movement of the mouse of at least 1.5 cm from one sample point to the successive one. The activity of the mouse is expressed as percentage of samples showing a movement over the total amount of samples for a certain

time interval. For example, if, over 5 min, 50 samples showed that the mouse was moving, the mouse was scored as active for 16.7% over this time (50 * 100 / 5 min * 60 s). The data measured by each Mouse-E-Motion were downloaded and processed with Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA).

Pole test

The animals were placed on top of a vertical 48.5 cm long rod made of rough wood with a diameter of 0.8 cm. To motivate the mouse to climb down, nesting material of the animals' home cage was placed at the bottom of the pole. The mouse was placed grasping the rod with four paws and the head pointing upwards. The time required by the mouse to reach the floor (maximum duration of each trial was 80 sec) was recorded. Each mouse had to perform 3 consecutive trials with an inter-trial interval (ITI) of 30 sec. The ability of a mouse to turn 180° and climb down with the head pointing downwards was evaluated. In case the mouse turned, it was recorded whether it turned at the top (level 1, above 32 cm), at the middle (level 2, between 32 and 16 cm) or at the bottom of the rod (level 3, below 16 cm).

Wire hanging test

Mice were placed with their forepaws gripping the middle of a 50 cm long horizontal metallic wire (1.5 mm in diameter) that was suspended between two rods 30 cm above a foam mattress. The mice had to perform 3 trials with an ITI of 45 min (maximum duration of each trial was 10 min). The latency to fall down and the ability to grip the wire with 2, 3 or 4 paws was scored.

Rotarod test

Mice had to walk on a turning, corrugated rod (3.2 cm in diameter) (Accelerating Rotarod for mice, Jones & Roberts, TSE systems, Bad Homburg, Germany). The rod was started to rotate 5 sec after the mice were placed onto it (Fig. 5). Mice underwent 5 trials with an ITI of 45 min. Trials 1 and 2 were performed at slow, constant speed (4 rpm) for a maximum duration of 3 min. Trials 3-5 were performed with the accelerating rod, starting with 4 rpm up to 40 rpm within 4 min, with a maximum duration of 10 min. On the following day, a sixth trial with the accelerating rod was carried out. The performance of the mice was evaluated by scoring the latency to fall down.

Fig. 5 Rotarod

Water maze

Mice were trained in a 155 cm diameter water maze filled with water at 20 ± 1°C, made opaque by a non-toxic white paint. A platform (14.5 cm diameter) was placed 1 cm below the water surface 40 cm from the white wall (20 cm above the water surface) (Fig. 6). The maximal trial duration was 90 s. The maze was placed in the center of a room (3.5 x 3.5 m) provided with several visual cues at the walls and illuminated by 100 Lux. During the experiment, mice were kept in an adjacent room illuminated by dim red light. Mice were started from six symmetrical positions in a pseudo-randomized order. After staying on the platform for 15 s, the mice were given the opportunity to climb onto a wire-mesh grid and then returned to their home cage placed under infrared light. The training was started with a visible platform so that the mice could learn to associate the platform with the escape from the pool. For the visible platform protocol (days 1 to 2, 4 trials per day, ITI of 1 hr), visual cues were occluded by a curtain and the platform was tagged by a black flag (6 x 7 x 15 cm) and located pseudo-randomly in different locations across trials. For the spatial learning protocol the platform was hidden and the cues at the walls were visible. Animals were trained over 6 days (days 3-5, six trials per day, days 6-8, 4 trials per day, ITI 45 min). On day 9 a transfer trial was performed (the platform was removed and mice swam for 80 s). Time spent in the four imaginary quadrants was used to test the preference of the mice for the former platform location. The swimming behavior of the mice was analyzed by measuring the time they spent without regular coordinated forward swimming when released into the pool. Uncoordinated swimming was defined as contractions of the body from one side to the other, circling in narrow circles or struggling with the forepaws in the water or at the wall of the pool.

Fig. 6 Morris water maze pool

Flinch-jump threshold test and step-through passive avoidance task

Before performing the passive avoidance test, the sensibility of WT and KO mice to a foot-shock was analyzed: 2 month-old naïve female mice (n_{wt} = 6; n_{ko} = 7) were used for the flinch/jump test. Mice were placed in a 24 x 24 x 48 cm box with a grid-floor (0.6 cm space between bars ∅ 1.1 mm) that allowed releasing an electric shock. 20 s after the mouse was

placed in the box, a train of consecutive 0.5 s long foot-shocks was administered (30 s interval between foot-shocks) stepwise from 13 to maximal 101 µA in steps of 8 µA. Behavioral responses (flinch and jump) were recorded at each shock intensity. The lowest shock intensity eliciting flinch and/or jump was taken as threshold values.

9 WT mice and 10 KO mice were used for the step-through passive avoidance test. A two-chamber-box equipped with a grid-floor (0.6 cm between bars, ∅ 1.1 mm) was used. The box was made of white plastic with a sliding door (5 x 5 cm) connecting the two chambers. One smaller chamber (13 x 21 x 30 cm) was illuminated (50 Lux) while the other (25 x 21 x 30 cm) remained dark (0.5 Lux). On day 1 mice were familiarized with the set-up by placing them in the light chamber without opening the door. After 5 min mice were returned to their home cages. On the second day mice were placed again in the light chamber. After 1 min the sliding door was raised. After the mouse encountered the open door for the first time the latency to enter the dark chamber was taken. When the mouse entered the dark compartment with 4 paws the door was closed and a foot-shock (1 s, 0.25 mA) was delivered. After the foot-shock mice were immediately taken back to their home cages. Retention was tested 24 hours later on day 2 by repeating the whole procedure without foot shock.

Analysis of behavioral parameters

With exception of the Rotarod, all tests were video-recorded. Tracks representing the position of the mice were created and analyzed with the software Ethovision for the open field and water maze tests (sampling rate of 5 samples per second). For the analysis of the tracks the minimal distance moved was set at 1.6 cm, except for the parameter “minimal distance to a zone”, which was analyzed with a minimal distance moved of 0 cm. The following parameters were obtained: distance moved, mean velocity and maximal velocity. Defined zones were designed within the arena of the open field and of the water maze to calculate the following parameters: time spent and distance moved in different zones, latency to the first entrance and number of entries in the zones and minimal distance from different zones. Three zones were defined for the open field: “border”, a rim of 5 cm at the walls of the open field; “center”, a 25 x 25 cm square in the center of the open field; “wall”, a rim of 2 cm at the walls which was used to calculate the minimal distance the mice kept from the walls of the open field (being 0 the value given by a mouse touching the walls with its body). For the water maze, four quadrants containing one circle with the diameter of 14.5 cm at their center were designed. One quadrant contained the platform at its center and was therefore called target

quadrant. A circular rim of 2 cm was designed at the border of the wall to calculate the minimal distance from the wall of the pool.

The behavior of the mice was analyzed blind to the genotype with the software The Observer. Observation was trained until at least 85% of consistency could repeatedly be scored between two analyzes performed at different times on the same mice, as calculated with the Reliability Test provided by the software The Observer (having 2 s as maximal time discrepancy).

Statistics

To compare the WT and KO group, data were analyzed with the non-parametric Mann-Whitney U test. For multi-factorial analysis of paired values obtained at different time points (different time intervals in the open field; different testing days for the open field and for the elevated plus maze; different hours and days for the analysis of home cage spontaneous behavior and circadian activity; different trials and days for the water maze; different trials for pole, wire hanging, and ROTAROD tests) the ANOVA for repeated measures was performed (having Genotype as between factor), followed by post-hoc analysis (Newmann-Keuls) when appropriate. Since the three 5 min intervals of the open field test were arbitrary, values calculated for the total 15 min duration of the test were analyzed with the Mann-Whitney U test. For brevity the results of this analysis are presented only if in discordance with the results obtained from the ANOVA for repeated measures. Comparison between KO and WT mice in the proportion of mice showing a particular performance was tested with Fisher's exact probability test. All tests were performed two-tailed.

Results

General appearance

No obvious difference in the appearance of TN-R deficient mice (KO) in comparison to their wild-type (WT) littermates was observed when mice were examined starting from postnatal day (PD) 21. No difference was found in body weight as regularly recorded from weaning up to 4 months of age (Fig. 7). At the age of 11 months KO mice were slightly but significantly heavier than WT mice (WT: 34.6 ± 1.3 g; KO: 38.6 ± 1.1 g, $P < 0.05$).

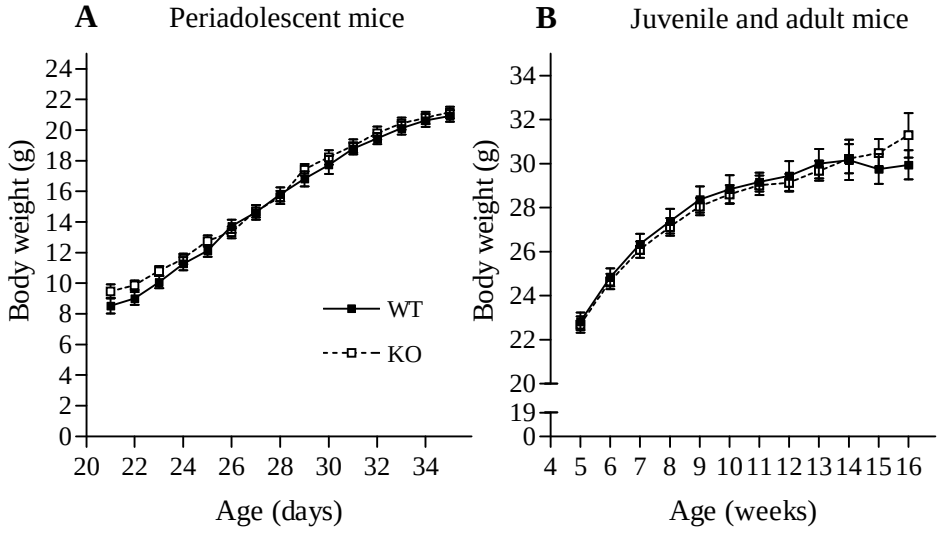

Fig. 7 No difference in body weight between KO mice and WT littermates. *Body weight of WT mice (n=16) and KO littermates (n=18). Data are expressed as mean ± SEM. (A) Daily bodyweight of periadolescent mice (PD 21–35). (B) Weekly body weight of juvenile and adult mice (5–16 weeks).*

Aggressive interactions with littermates

The observation of aggressive interactions performed until mice were isolated at the age of 3 months did not show any significant difference between KO mice and WT littermates. WT mice and KO mice started aggressive interactions with their HET littermate at the age of approximately 6-8 weeks. WT mice and KO mice did not differ in their social rank. 11 WT mice and 10 KO mice were involved in aggressive interactions with their HET partner without showing a clear hierarchy (both siblings initiated attacks towards the brother that regularly counterattacked and no of the two siblings showed submissive behavior); 2 WT mice and 4 KO mice appeared to be submissive whereas 2 WT mice and none of the KO mice were dominant; 1 WT mouse and 4 KO mice were never seen in aggressive interactions with their HET littermate.

Free choice open field and Reeperbahn test

When tested at periadolescence, most WT and KO mice freely entered the arena and no difference between genotypes was detected. On the contrary, when re-exposed as adults after isolation, KO mice showed to be extremely anxious towards the arena as compared to their WT littermates by avoiding entering and exploring the arena both during the free choice open field (FCOF) and Reeperbahn tests.

FCOF 1. Periadolescent mice caged with a heterozygous littermate

As shown in Table 2, no difference between periadolescent WT mice and KO littermates was found in their behavioral response to the arena. 9/16 WT mice and 13/18 KO mice freely entered the arena with no difference between genotypes in the latency to enter it. Both genotypes spent a similar amount of time in the arena and crossed comparable numbers of squares. KO and WT mice showed similar patterns of movement within the arena, namely staying most of the time in an area close to the cage and spending little time in the rest of the arena. There was no significant difference in time spent at the door while being in the cage and amount of rearing in the arena.

FCOF 2 and 3 and Reeperbahn test. Adult mice after isolation

Few KO mice entered the arena during the FCOF when adult (FCOF 2: 5/18; FCOF 3: 5/18; Reeperbahn test: 3/18), while most of the adult WT mice entered the arena (FCOF 2: 14/16; FCOF 3: 14/16; Reeperbahn test: 15/16). The difference between genotypes was significant for all three trials (Fisher's exact $P< 0.001$). Due to the low number of KO mice, it was not possible to test the effect of Genotype on the behavior shown by the mice after they had entered the open field. Nevertheless, as compared to WT littermates, KO mice that had entered the open field showed lower mean values of time spent in the arena, of amount of rearing and of locomotion (number of squares crossed) (Table 2).

Table 2. Behavioral analysis of the FCOF tests and Reeeperbahn test.

	FCOF 1		FCOF 2		FCOF 3		Reeperbahn	
	WT (n=8) Median;P_{25}/P_{75}	KO (n=13) Median;P_{25}/P_{75}	WT (n=14) Median	KO (n=5) Median	WT (n=14) Median	KO (n=5) Median	WT (n=15) Median	KO (n=3) Median
Latency to enter the arena (s) +	500; 118/600	167.5; 42/600	114.5	600	30.5	600	40.5	600
Home cage-arena transitions	13.5; 7/14.3	7; 6/11	15.5	8	11	10	15	16
Squares crossed	33; 10/56	8; 0/52	174.5	6	183.5	153	281	196
Time in arena (%)	31.5; 14.3/44	18.4; 7.1/28.9	69	8.7	81.7	74.5	86.6	76.5
Time in center (%)	0; 0/3.1	0; 0/3.1	3.8	0	3.4	3.3	6.2	7.1
Time in square at the home cage (%)	23.7; 9.1/26	15.6; 6.0/18	22.7	8.7	68.4	87.4	10.1	7.8
Time in squares at the cliff (%)	3.8; 1.6/4.9	1.1; 0/2.5	-	-	-	-	-	-
Time in squares at the wall (%)	4.2; 0.6/6.6	0; 0/5.3	-	-	-	-	-	-
Rearing	1; 0/5	0; 0/1	19.5	0	38	4	11	1.5
Time at the door (%)	19.1; 10.5/27	24.2; 17.3/28.7	8.9	38.2	15.2	16.6	4.3	7.7

+: WT (n=16); KO (n=18); P_{25} and P_{75} are 25^{th} and 75^{th} percentiles, respectively.

Open field

In all three open fields (OFs) performed KO mice moved and explored less as compared to WT littermates. KO mice avoided staying in the center and spent more time close to the walls. Both genotypes changed their behavior over the successive OF tests (Fig. 8 and Table 3).

OF 1. Periadolescent mice caged with a heterozygous littermate

To test possible differences between genotypes in short-term habituation all parameters were analyzed for the three consecutive 5 min intervals. As shown in Table 3, the 2-way ANOVA for repeated measures (having Genotype as between factor and Interval as within factor) showed an effect of Genotype on distance moved, mean velocity, time in the border, time in the center, and mean distance to the wall (MDW). KO mice moved less and at lower velocity, spent more time in the border and less time in the center and had a lower MDW as compared to WT mice (Fig. 8A-E). Since the lower percentage of time spent in the center (and more time in the border) could have been caused by a lower locomotor activity of KO mice, the distance moved in the border and in the center was analyzed as percentages of the total distance moved in the arena. KO mice showed a higher percentage of distance moved in the border and lower percentage of distance moved in the center as compared to WT mice (Fig. 9F and G). Therefore, the higher “preference” for the border and “avoidance” of the center shown by KO mice as compared to WT mice indicates an enhanced anxiety towards the center in KO mice. There was an effect of Interval on distance moved, mean velocity, time in the border (all these parameters decreased from the first to the last 5 min interval) and on MDW, which increased from the first to the last 5 min interval. Therefore, as the mice spent more time in the open field, they moved less and showed less thigmotaxis, as expected from mice familiar with the arena. There was no effect of the interaction Genotype x Interval on any of the parameters observed, suggesting that WT and KO mice had a similar short term habituation to the open field. The behavioral response of mice during the first minutes after exposure to the open field can be indicative of the novelty induced reactivity of the mice. Therefore an ethological analysis of the first 5 min of the test was performed. KO mice did less rearing on wall and less rearing off wall as compared to their WT littermates (Fig. 9H and I). There was no difference in the number of stretch attend posture (SAP) between genotypes probably due to the fact that this behavior was rarely done by the two genotypes. Both genotypes spent a similar amount of time self-grooming.

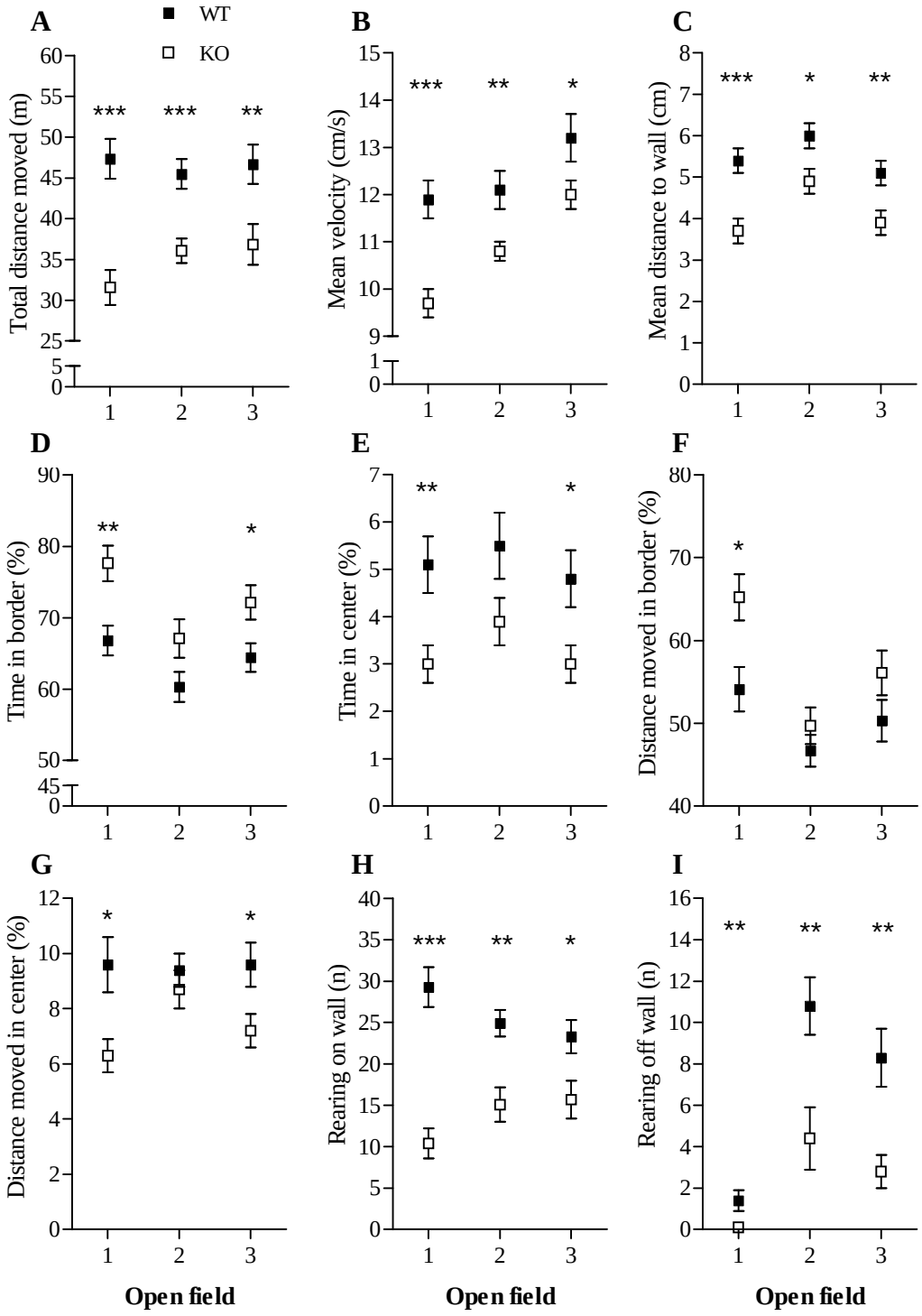

Fig 8. Decreased exploration and increased anxiety-like behavior of KO mice in the open field test. *Performance of WT mice (n=16) and KO littermates (n=18) in three open field tests of the duration of 15 min each performed as mice were periadolescent (Open field 1), adult (Open field 2) and 7 days after adults were single housed (Open field 3). Data are expressed as mean ± SEM. (A) Distance moved. (B) Mean velocity of locomotion. (C) Mean distance to the wall. (D) Percentage of time spent in the border (an imaginary outer rim 5 cm wide). (E) Percentage of time spent in the center (an imaginary 20 x 20 cm square in the middle of the open field. (F) Distance moved in the border expressed as percentage of total distance moved. (G) Distance moved in the center expressed as percentage of total distance moved. (H) Number of rearing on wall as measured for the first 5 min of the test. (I) Number of rearing off wall as measured for the first 5 min of the test. For each test, a 2-way ANOVA for repeated measures was performed having Genotype as between factor and Interval (three consecutive 5 min intervals) as within factor. *, **, *** P < 0.05, 0.01, 0.001, respectively (effect of Genotype).*

Table 3. 2-way ANOVA for repeated measures on open fields 1-3

	Open field 1						Open field 2						Open field 3					
	Genotype		Interval		Gen. x Int.		Genotype		Interval		Gen. x Int.		Genotype		Interval		Gen. x Int.	
	$F_{1,32}$	p	$F_{2,64}$	p	$F_{2,64}$	p	$F_{1,32}$	p	$F_{2,64}$	p	$F_{2,64}$	p	$F_{1,32}$	p	$F_{2,64}$	p	$F_{2,64}$	p
Distance moved (DM)	24.1	0.00	21.0	0.00	2.3	0.11	16.2	0.00	5.0	0.01	1.5	0.23	8.0	0.01	23.4	0.00	1.9	0.16
Mean velocity	18.7	0.00	4.3	0.02	0.5	0.60	12.2	0.00	1.2	0.30	0.1	0.92	4.6	0.04	23.2	0.00	0.0	0.96
Time in border	10.4	0.00	6.3	0.00	0.6	0.53	3.8	0.06	2.7	0.07	1.2	0.30	5.8	0.02	31.2	0.00	4.4	0.02
Time in center	8.2	0.01	1.9	0.15	1.3	0.26	3.8	0.06	1.0	0.37	0.7	0.49	6.0	0.02	7.1	0.00	0.0	0.94
MDW	17.6	0.00	4.3	0.02	0.4	0.66	7.3	0.01	2.8	0.07	0.3	0.77	7.6	0.01	33.9	0.00	3.4	0.04
DM border/arena	8.8	0.01	14.0	0.00	0.2	0.83	1.2	0.28	16.2	0.00	0.8	0.44	3.1	0.09	34.8	0.00	2.4	0.10
DM center/arena	8.2	0.01	6.7	0.00	0.2	0.78	0.6	0.45	5.10	0.01	0.1	0.94	5.8	0.02	19.7	0.00	0.8	0.46

DM: distance moved; MDW: mean distance to the wall

OF 2. Adult mice caged with a heterozygous littermate

When tested the second time in the OF at the age of 13-16 weeks, KO mice showed again reduced locomotor activity (lower distance moved and lower mean velocity) and higher thigmotaxis (lower MDW) as compared to WT littermates, as shown by the significant effect of Genotype on these parameters (Table 3, Fig. 8A-C). There was no effect of Genotype on time in the border, time in the center, percentage of distance moved in the border and percentage of distance moved in the center, although KO mice tended to spend more time in the border and less time in the center as compared to WT mice ($p < 0.1$) (Fig. 8D-F). The 2-way ANOVA showed an effect of Interval on distance moved and mean velocity (both parameters decreased from the first to the last 5 min interval). There was no effect of the interaction Genotype x Interval on any parameter observed. The ethological observation during the first 5 min of the open field revealed a significant difference in the amount of rearing on and off wall (KO mice reared less than WT mice, see Fig. 8H,I). No difference was seen in SAP and in self-grooming.

OF 3. Adult mice after isolation

As in OF 1, KO mice moved less and with lower mean velocity, spent more time in the border and less time in the center and stayed closer to the wall (decreased MDW) as compared to WT littermates (there was a significant effect of Genotype on these parameters, see Table 3 and Fig. 8A-C). There was an effect of Interval on distance moved, mean velocity, time in the border (all these parameters decreased from the first to the last 5 min interval), time in the center and on MDW, which increased from the first to the last 5 min interval. There was an effect of the interaction Genotype x Interval on time in the border and MDW (Table 3). The post-hoc analysis showed that KO mice had lower values of time in the border and MDW as compared to WT mice in the first and second 5 min interval (during the first 10 min) but not in the third 5 min interval. Moreover, although both genotypes reduced the time in the border and increased the MDW during the test, WT mice drastically reduced their time in the border already at the second 5 min interval as compared to the first one,

whereas KO mice did not reduce their time until the third 5 min interval. Behavioral analysis of the first 5 min of the test revealed that KO mice performed less rearing on wall and off wall than their WT littermates (Fig. 8H,I) and self-groomed more than WT mice (WT = 1.6 ± 0.4; KO = 3.9 ± 0.5; P < 0.001), while there was no difference between genotypes in SAP.

Meta-analysis of the open field tests

It is known that the behavioral response of a mouse to the OF also depends on the age of the animal and on previous experience. Therefore a statistical analysis to test whether the two genotypes differentially changed their behavior over the successive OF tests was performed.

In concordance to the statistical analyses performed on single OF tests, the 2-way ANOVA (being Genotype the between factor and Age the within factor) showed an effect of Genotype on distance moved, velocity, time in border, time in center, MDW, percentage of distance moved in the border, rearing on wall, rearing off wall and self grooming (Table 4). There was an effect of Age on velocity (it increases from OF 1 to OF 3), time in border, MDW, percentage of distance moved in the border and rearing off wall. The post-hoc analysis showed that both genotypes were less thigmotactic (less time in the border and percentage of distance moved in the border and higher MDW) in OF 2, as compared to OF 1 and OF 3. Both genotypes did more rearing off wall in OF 2 and 3 as compared to OF 1. There was an effect of the interaction Genotype x Age on the parameter rearing on wall. While WT mice tended to do less rearing on wall when adult (OF 2 and 3), KO mice tended to increase the number of rearing on wall when adult, although KO mice always had lower values as compared to WT littermates.

Table 4. Meta-analysis with 2-way ANOVA for repeated measures for open fields 1-3

	Genotype		Age		Gen. x Age	
	$F_{1,32}$	P	$F_{2,64}$	P	$F_{2,64}$	P
Distance moved (DM)	25.8	0.00	0.9	0.42	2.1	0.13
Mean velocity	15.7	0.00	28.2	0.00	2.8	0.06
Time in border	9.7	0.00	12.3	0.00	0.7	0.48
Time in center	13.1	0.00	1.4	0.25	0.1	0.89
MDW	18.2	0.00	10.3	0.00	0.9	0.41
DM border/arena	5.1	0.03	23.8	0.00	3.0	0.05
DM center/arena	8.8	0.01	1.6	0.21	2.2	0.12
Rearing on wall	32.9	0.00	0.0	0.96	5.7	0.00
Rearing off wall	25.2	0.00	22.4	0.00	3.2	0.04
Selg grooming	8.3	0.01	0.0	0.98	1.4	0.25

MDW: mean distance to the wall

In conclusion, the meta-analysis confirmed that KO mice moved less and explored less in the OF and spent more time close to the walls and less time in the center as compared to their WT littermates, regardless of their age or previous exposure to the arena. Moreover, the meta-analysis showed that both genotypes similarly changed their response to the OF as they were re-exposed to it.

Elevated plus maze

In the elevated plus maze (EPM) tests KO mice stayed less time on the open arms as compared to WT littermates, indicating higher anxiety. The differences between genotypes were more pronounced as mice were re-tested one week after isolation (Fig. 9).

EPM 1. Adult mice caged with a heterozygous littermate

When tested for the first time on the elevated plus maze 11/16 WT and 8/18 KO mice entered at least one of the open arms. KO mice stayed less time on the open arms and did less head dipping and less rearing as compared to WT mice (Fig. 10). No other significant difference was detected between the two genotypes, although KO mice tended to have a lower percentage of entries into the open arms and into the open arm edges and tended to stay less time at the edges of the open arms as compared to WT littermates ($P < 0.1$) (Fig. 9).

EPM 2. Adult mice after isolation

When tested for the second time on the EPM after social isolation, a lower proportion of KO mice entered the open arms as compared to WT mice (13/16 WT and 7/18 KO mice, Fisher's exact $P = 0.017$). KO mice differed from WT littermates in all parameters analyzed (Fig. 3). KO mice entered the open arms with higher latencies and showed a lower percentage of time and entries into the open arms as compared to WT mice. Concordantly, KO mice reached less frequently the edge of the open arms and stayed less time there. KO mice were also less active as compared to WT littermates as shown by the lower values of total transitions, closed arms entries and rearing. KO mice did less SAP and head dipping as compared to WT mice and spent less time in the center, a position where the mouse usually stays when assessing the risk of the open arms by remaining in a more protected area. KO mice spent more time sitting in the corners of the closed arms and did more self-grooming as compared to WT mice, additionally indicating lower exploratory activity. Moreover KO mice spent more time self-grooming (6.1 ± 1.3) as compared to WT littermates (2.3 ± 0.5) ($P < 0.01$).

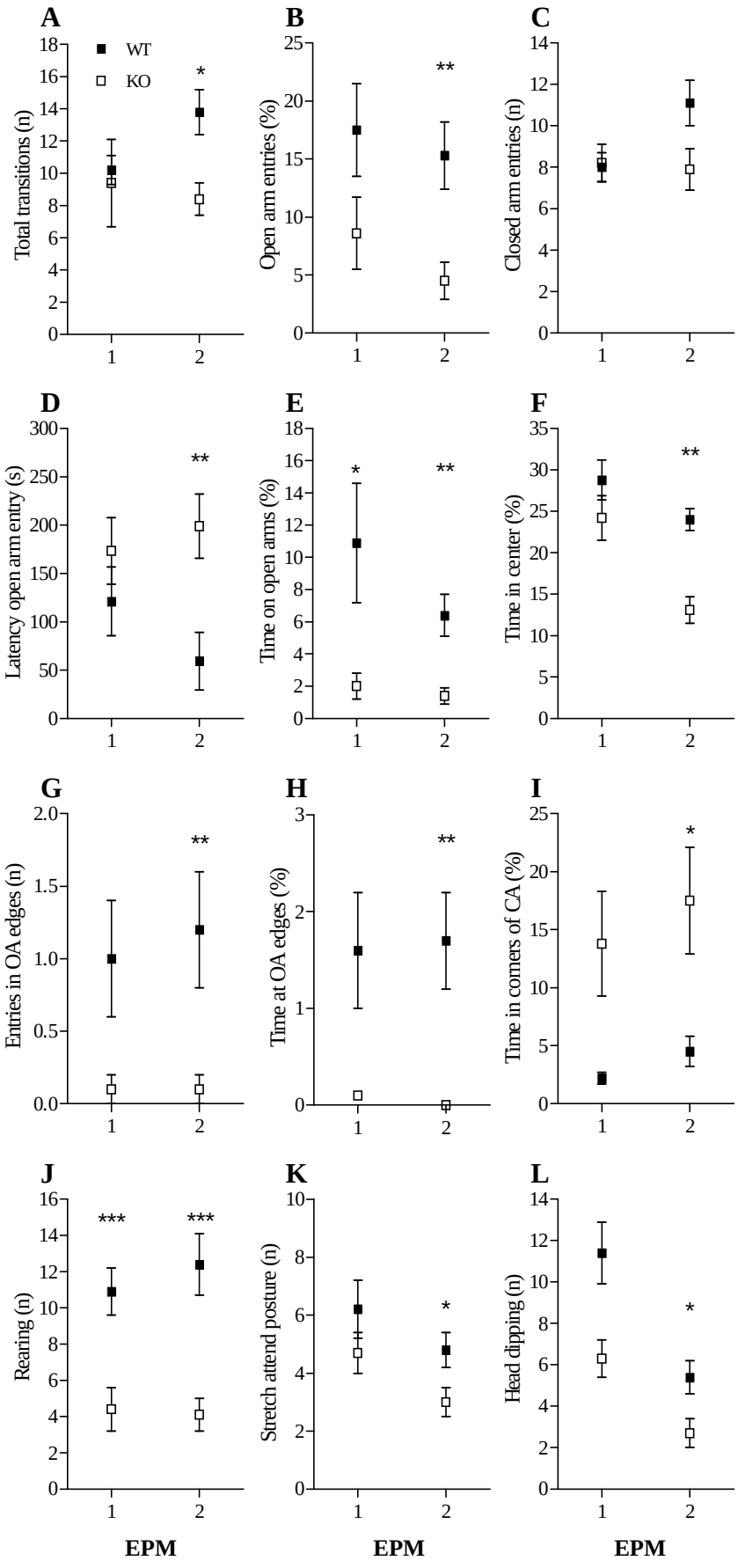

Fig 9. Enhanced anxiety-like behavior of KO mice in the elevated-plus maze.
*Performance of WT mice (n=16) and KO littermates (n=18) in the elevated plus maze. Two tests of 5 min were performed before (EPM 1) and after (EPM 2) mice were single housed. Data expressed as mean ± SEM. (A) Number of total transitions (open arms + closed arms transitions). (B) Open arm entries expressed as percentage of total transitions. (C) Number of closed arm entries. (D) Latency to enter one open arm. (E) Time spent on the open arms as percentage of total time. (F) Time spent in the center as percentage of total time. (G) Number of entries on the open arm edges. (H) Time spent at the open arm edges as percentage of time spent on the open arms. (I) Time spent in the corners of the closed arms as percentage of time spent in the closed arms. (J) Number of rearing in the closed arms. (K) Number of stretch attend postures. (L) Number of head dipping. *, **, *** P < 0.05, 0.01, 0.001, respectively, as analyzed with the Mann-Whitney U-test.*

Meta-analysis of the EPM tests

A 2-way ANOVA (having Genotype as between factor and Day as within factor) was performed to test whether the two genotypes differentially changed their response to the EPM from the first to the second exposure to the apparatus (Table 5). There was an effect of Genotype on all parameters analyzed. There was an effect of Day on head dipping, SAP and time spent in the center (they all decreased in EPM 2 as compared to EPM 1). There was an effect of the interaction Genotype x Day on two parameters for locomotor activity, total transitions and closed arm entries. While WT mice increased the total transitions and closed arm entries in EPM 2 as compared to EPM 1, KO mice maintained the same locomotor activity.

Table 5. Meta-analysis with 2-way ANOVA for repeated measures for elevated plus-mazes 1 and 2

	Genotype	Day	Gen. x Day
	$F_{1,32}$; P	$F_{1,32}$; P	$F_{1,32}$; P
Latency to enter OA (s)	8.03; 0.01	0:30; 0.59	1.74; 0.20
Total transitions (n)	5.22; 0.03	2.62; 0.11	7.77; 0.01
CA entries (n)	1.71; 0.20	8.18; 0.05	8.18; 0.02
OA enttries (%)	8.36; 0.01	1.72; 0.20	0.15; 0.70
Time in OA (%)	8.84; 0.00	3.54; 0.07	2.00; 0.17
Time in center (%)	7.54; 0.01	18.53; 0.00	2.83; 0.10
Time in corners of CA (%)	7.58; 0.01	2.17; 0.15	0.12; 0.73
Time in OA edges (%)	9.35; 0.00	0.05; 0.81	0.39; 0.53
Entries in OA edges (n)	8.86; 0.00	0.50; 0.48	1.71; 0.20
Rearing (n)	21.95; 0.00	0.38; 0.54	0.99; 0.33
SAP (n)	4.20; 0.04	5.60; 0.02	0.04; 0.83
Head dipping (n)	11.7; 0.00	34.00; 0.00	2.31; 0.14
Time self grooming (%)	5.30; 0.03	0.01; 0.93	3.02; 0.09

OA: open arms; CA: closed arms; SAP stretch attend posture

In conclusion, in the EPM performed after isolation both genotypes decreased not only the exploration of the open arms, but also the frequency of behaviors as head dipping, SAP and time in the center that are indicative of the risk assessment the mouse performs when in conflict between exploring the open arms (the anxiogenic stimulus) and staying in the protected part of the maze. In WT mice the decreased exploration of the open arms was coupled to an enhanced exploration of the closed arms (more transitions), while this was not true for KO mice that did not change the locomotor activity between the two EPM tests.

Resident/Intruder test

There was no difference between KO mice and WT littermates in their behavioral response towards an unfamiliar male placed into their home cage for 10 min. Only very few WT (3/16) and KO (2/18) attacked the intruder.

Home cage spontaneous behavior and circadian activity

Mice home cage spontaneous behavior was scored by instantaneous sampling observations of 60 min performed at three time points at two different days. Data were analyzed by 3-way ANOVA (having Genotype as between factor and Day and Hour as within factors). There was a significant effect of Genotype on resting ($F_{1,32} = 6.3$; $P = 0.02$) and eating/drinking ($F_{1,32} =$

6.7; P = 0.01). KO mice spent more time resting and less time eating/drinking. These parameters were also affected by the day of observation (resting: $F_{1,32}$ = 7.2; P = 0.02; eating/drinking: $F_{1,32}$ = 5.0; P = 0.03; active: $F_{1,32}$ = 33.2; P = 0.000). Both genotypes decreased the time spent resting and eating/drinking, and increased their active time 7 days after being placed in the new cage as compared to the second day (Fig. 10A).

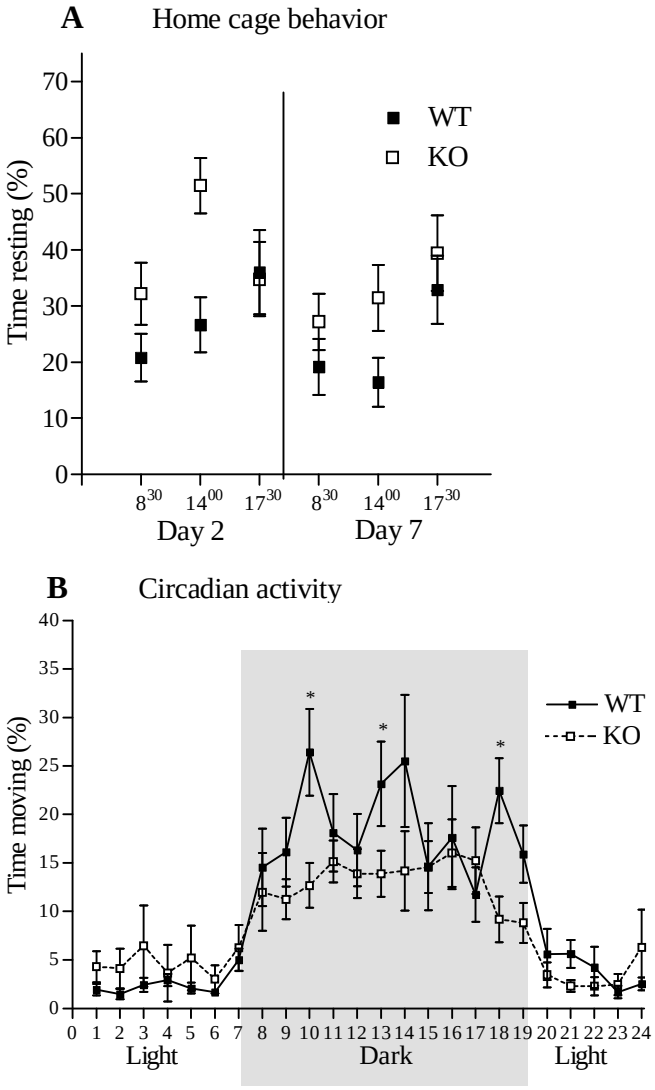

Fig 10. Home cage spontaneous behavior and circadian activity. *(A) Percentage of time WT mice (n=16) and KO mice (n=18) spent resting during one hour of observation three times a day, 2 and 7 days after changing of the cage. Data are expressed as mean ± SEM. KO mice spent significantly more time resting as shown by 3-way ANOVA. (B) Percentage of time WT mice (n=9) and KO mice (n=9) spent moving in their home cage during the light and dark cycle. Data points are given per hour and represent the mean of four consecutive days ± SEM. * P < 0.05 (comparison between genotypes at a determined hour by Newmann-Keuls post-hoc after 4 way ANOVA).*

Since the data pointed into the direction of KO mice being less active as compared to WT littermates, a more detailed analysis via automatic recording of the circadian activity was performed. The recording was performed as mice were 10 months old and revealed some subtle differences between WT and KO mice. Since both genotypes did not change their circadian activity during the 4 recording days, the circadian activity of WT and KO mice is presented as a mean of the four days with single values for each hour (Fig. 10B). Data were analyzed with 4-way-ANOVA for repeated measures having Genotype as between factor and Day (4 levels), Dark/Light (2 levels) and Hours (12 levels) as within factors. No difference was observed between KO and WT mice during the dark and light periods. Both genotypes were more active during the dark period as compared to the light period. No effect of the interaction Genotype x Dark/Light was found, whereas there was a significant interaction of Genotype x Dark/Light x Hour ($F_{11,187}$ = 2.04 and P = 0.027). Post-hoc analyses showed a significantly

decreased activity of KO mice as compared to WT littermates 3, 6 and 11 hrs after light offset (Fig. 4B). Indeed, by observation of the graphical representation it appears that WT mice showed a cyclic activity during the dark period with activity peaks at around 3, 6 and 11 hrs after light offset, whereas KO mice maintained a relatively constant activity throughout the dark period.

Pole test, Rotarod and wire hanging test

When tested in the pole, Rotarod and wire hanging tests KO mice showed a clear impairment in motor-coordination as compared to WT mice. In the Pole test, the 2-way ANOVA (having Genotype as between factor and Trial as within factor) showed an effect of Genotype on the latency to climb down the pole, being KO mice slower than WT littermates ($F_{1,31}$ = 7.82; P < 0.01) (Fig. 11A). There was also an effect of Trials. The post-hoc analysis showed that both genotypes decreased the latency to climb down from the first to the third trial. There was no effect of the interaction Genotype x Trial. However, the latency to climb down did not always correspond to the motor performance since mice that were slipping or falling also scored low latencies. Therefore more emphasis was put on the strategy the animals

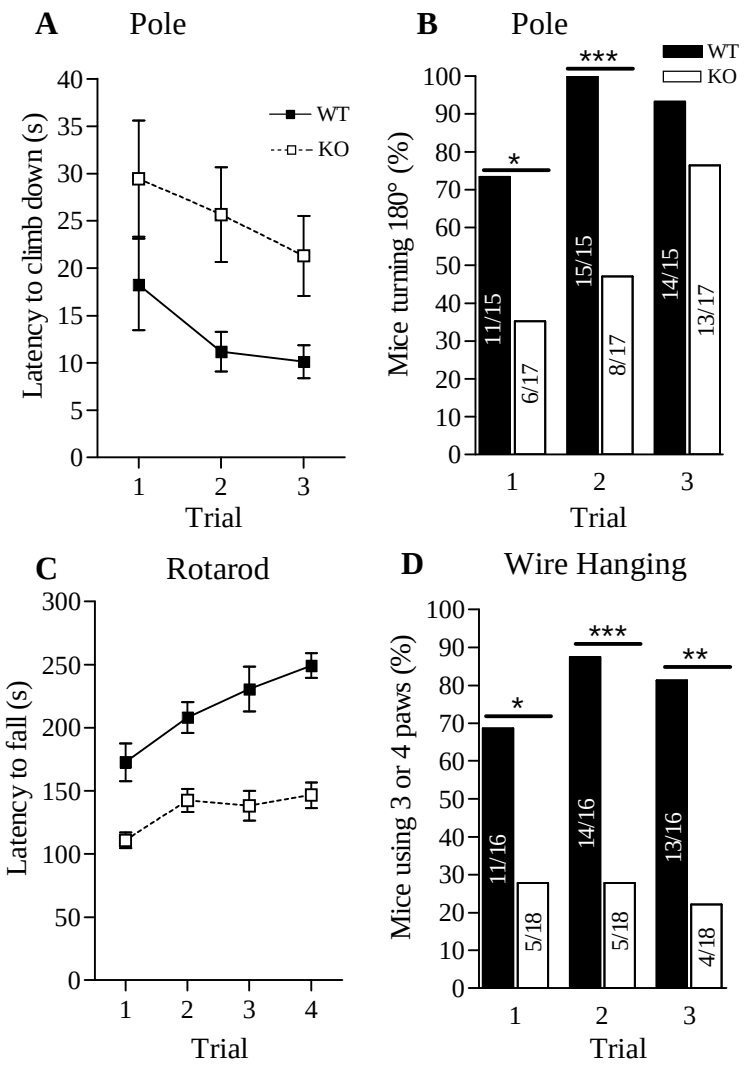

Fig 11. Impaired motor coordination of KO mice. *Motor coordination abilities of WT mice (n=15 pole, n = 16 wire hanging and Rotarod tests) and KO littermates (n=17 pole, n= 18 wire hanging and Rotarod tests). Data are expressed in (A) and (C) as mean ± SEM, in (B) and (D) as percentages. (A) Time mice needed to climb down the vertical pole on 3 consecutive trials. (B) Percentage of mice turning 180° and climbing down the pole with head foremost. (C) Latency to fall from the accelerating Rotarod on 3 consecutive trials and on a 4th trial performed 24 hrs later. (D) Percentage of mice performing an uplift and using 3 or 4 paws instead of only 2 while hanging on the wire in 3 consecutive trials. *, **, *** P < 0.05, 0.01, 0.001, respectively, as analyzed with Fisher's exact probability test.*

used to climb down. While almost all WT mice first turned 180° and then climbed down the pole with the head pointing downwards (Fig. 12A-C), a lower proportion of KO mice were able to turn 180° during the first and second trials. Most KO mice failed to turn and instead kept their body in a position horizontal to the pole and climbed down often in a corkscrew-like manner (Fig. 12D-F and Fig. 11B).

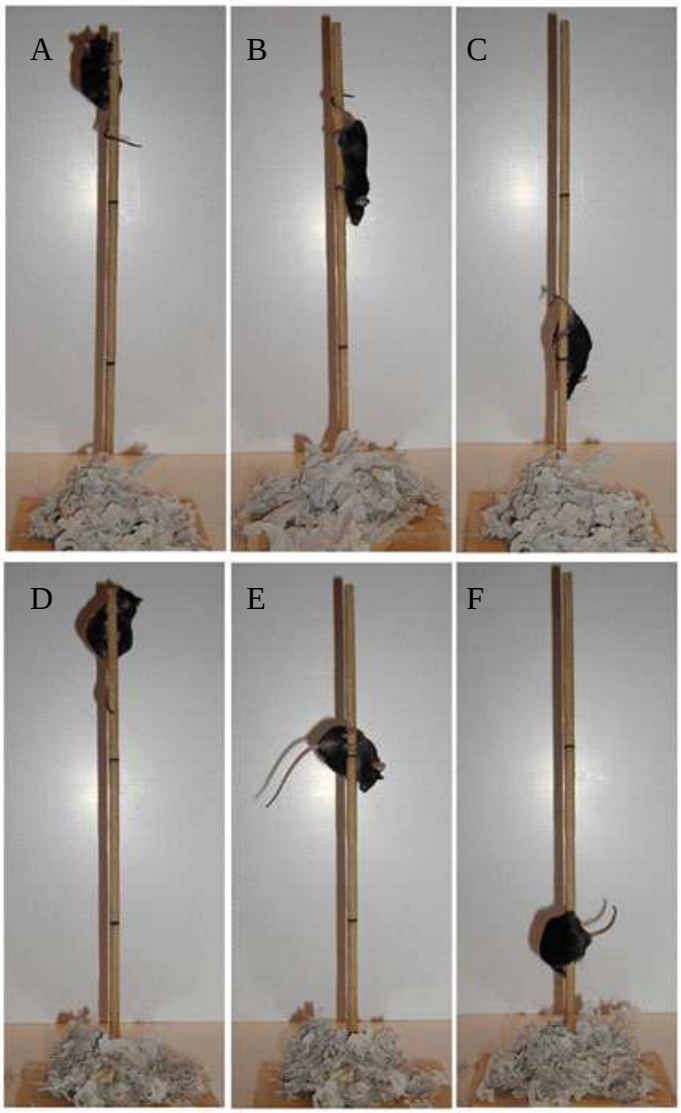

Fig 6. Altered motor coordination of KO mice in the pole test
Two possibilities of climbing down the pole. (A), (B), and (C) example of a mouse that turns 180°. (D), (E), and (F) example of a mouse that climbs down sideways. (A.) Starting position. (B) The mouse turned 180° already at the top of the rod, the head is pointing downwards. (C) The mouse climbed the rod to the end in a turned position. (D) Starting position. (E) The mouse did not turn 180°, but kept the body in a horizontal position. (F) The mouse climbed down the rod laterally.

In the Rotarod test both genotypes were able to walk on the Rotarod for a given maximal time of 180 s when the rod was rotating at a constant speed of 4 rpm, whereas KO mice showed impairment as compared to WT mice when the speed of the rod was accelerating. The 2-way ANOVA (having Genotype as between factor and Trial as within factor) showed an effect of Genotype on the latency to fall down the accelerating rod ($F_{1,32} = 51.59$, $P < 0.001$). Indeed, KO mice fell down the accelerating Rotarod at lower latencies as compared to WT mice during all accelerating trials performed. Both genotypes improved their performance on

successive trials (significant effect of Trial, $F_{3,96} = 11.7$, $P < 0.001$), while there was no effect of the interaction Genotype x Trial, suggesting that both KO mice and WT littermates were able to improve their performance with training (Fig. 11C).

In the wire hanging test there was no effect of Genotype, Trial or interaction of Genotype x Trial on the latency to fall from the wire. Nevertheless, while most of WT mice were able to lift their body up allowing them to grip the wire also with the hind limbs, a lower proportion of KO mice, although they seemed to try, was able to do so during all three trials (Fisher's exact $P < 0.01$) (Fig. 11D and Fig. 13).

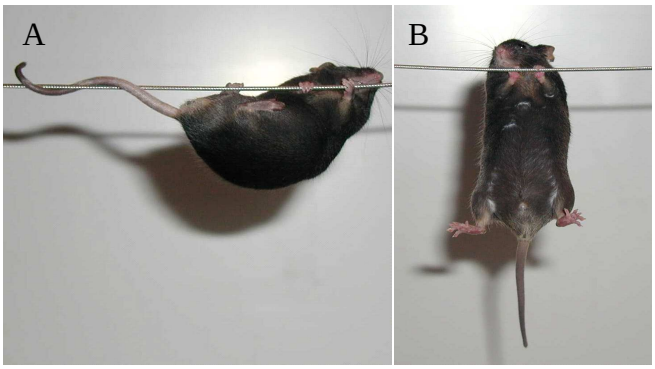

Fig 13 Two possibilities of hanging at the wire.
(A) Example typical for WT mice: the mouse performed an uplift and uses 3 or 4 paws and the tail for gripping. (B) Example typical for KO mice: the mouse uses only the forepaws for gripping.

Water maze test

Before being tested for spatial learning and memory, mice were first trained for two days with a cued platform. At the end of this training all mice learnt to associate the cue with the escape from the maze, and both genotypes reached, on the second day, a mean escape latency of less than 10 s. No mice showed floating during the training with a cued platform. The 2-way ANOVA did not show an effect of Genotype on escape latency, but there was a significant effect of the interaction Genotype x Trial ($F_{7,140} = 370.6$; $P < 0.005$). Post-hoc analyses showed that KO mice needed more time to successfully find and climb the platform as compared to their WT littermates only during the first trial (Fig. 14A). When observed for their ability to perform coordinated swimming behavior in the first four visible trials, KO mice spent a longer time with uncoordinated movements before starting with regular swimming behavior as compared to their WT littermates on the first trial, going in parallel with the increased escape latency of KO mice in this trial (Fig. 14B). No differences in swimming behavior were observed in the first trial of the hidden platform. When mice were trained for spatial learning and memory with a hidden platform, no difference was found between the two genotypes. The 2-way ANOVA did not show any difference between KO mice and WT littermates in any of the parameters observed. KO mice and WT littermates swam normally and climbed successfully onto the escape platform in the pool. The 2-way

ANOVA for repeated measure did not show any effect of Genotype and of the interaction between Genotype and Trials on escape latency, distance moved, velocity and MDW. There was an effect of Trial on escape latency ($F_{1,20} = 7.48$; $P < 0.000$), distance moved ($F_{1,20} = 8.17$; $P < 0.000$), mean velocity ($F_{1,20} = 7.94$; $P < 0.000$), and MDW ($F_{1,20} = 5.63$; $P = 0.001$). Both genotypes decreased the escape latency, distance moved and mean velocity and increased the MDW over trials (Fig. 14C). When a transfer trial was performed 24 hrs after the last training, both KO mice and WT littermates did not preferentially search in the target quadrant (SE) (Fig. 14D).

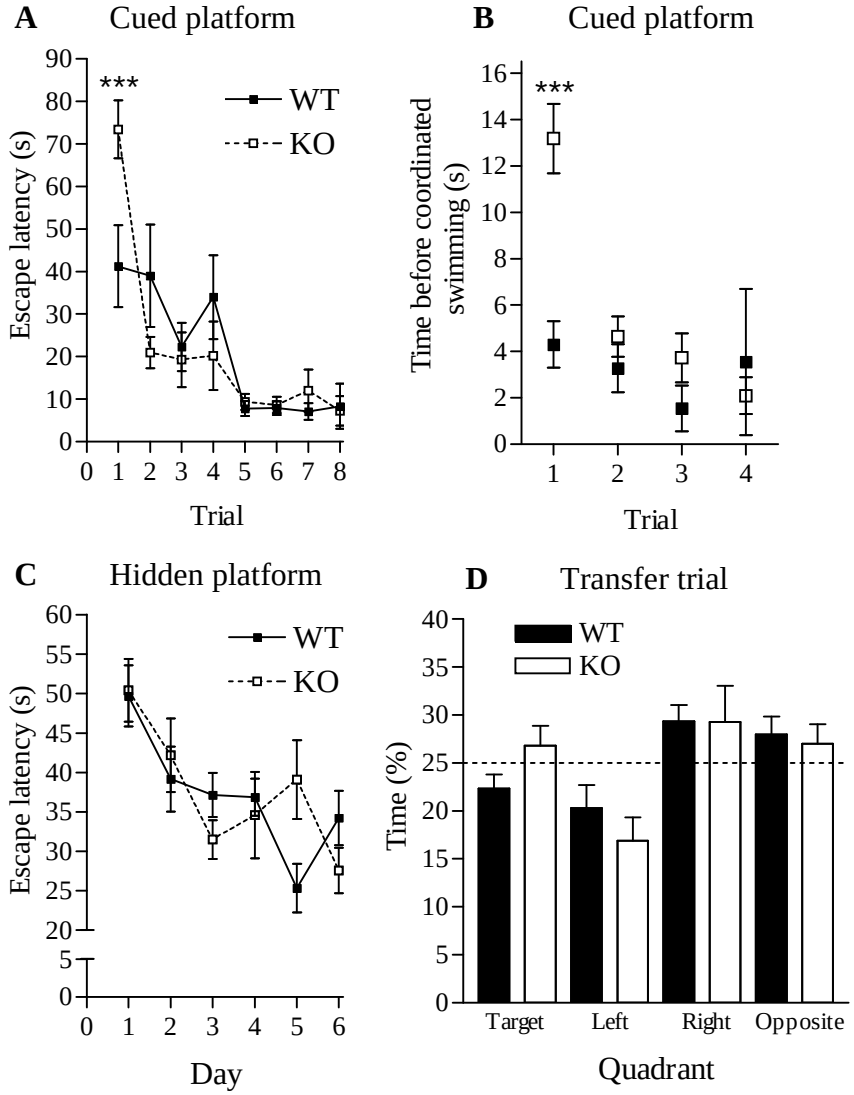

Fig 14. Morris water maze. *Performance of WT (n=11) and KO (n=11) in the water maze. Data are expressed as mean ± SEM. (A) Latency to reach the cued platform in 8 trials (B) Time spent performing uncoordinated movements before starting coordinated swimming directly after placement in the pool on the first four visible trials. (C) Latency to reach the hidden platform on 6 consecutive days. (D) Time spent in imaginary quadrants of the pool during the transfer trial (no platform) as percentage of the total 90 s trial duration. The transfer trial was performed 1 day after the last trial with the hidden platform. The dotted line indicates the chance level of 25 %. *** = P < 0.001 as analyzed by post-hoc analysis (Newman-Keuls test) after 2-way ANOVA for repeated measures (having Genotype as between factor and Trial as within factor) that showed an effect of the interaction of Genotype x Trial.*

Flinch-jump threshold test and step-through passive avoidance task

There was no difference in foot-shock sensitivity between WT and KO female mice as tested in the flinch-jump threshold test. The flinch threshold was 28.2 ± 2.1 and 31.6 ± 7 µA for the WT mice and KO mice, respectively; the jump threshold was 50.5 ± 5.9 and 53.0 ± 3.9 µA for the WT mice and KO mice, respectively.

When trained in the step-through passive avoidance task, KO mice entered the dark chamber (where the foot-shock was given) with higher latencies as compared to WT littermates (WT: 12.2 ± 2.2 s; KO: 126.5 ± 32.8 s, 1 KO mouse did not enter the dark chamber). The difference between genotypes was significant ($P < 0.01$) revealing a strong discrepancy already in the baseline between the two genotypes. Since performing a passive avoidance task emanating from already very different baseline levels between WT mice and KO mice would not lead to interpretable results, the test was discontinued.

Discussion

Weber et al. (1999) generated a mouse deficient for the extracellular matrix glycoprotein TN-R to investigate the molecule's functions *in vivo*. Since then, TN-R deficient mice have been studied extensively under morphological and electrophysiological aspects and considerable abnormalities were found (Weber et al., 1999; Brückner et al., 2000; Haunso et al., 2000; Bukalo et al., 2001; Saghatelyan et al., 2001; Nikonenko et al., 2003). However, the behavioral phenotype of this mutant has not yet been described. Therefore tests for different behavioral parameters, including motor coordination, novelty-induced behavior, intra-sexual aggressive behavior, circadian activity and cognitive function, were performed. There was no obvious difference in the general appearance, health and body weight of WT and KO mice during the experiments and handling of animals. Instantaneous sampling did not reveal major changes in the spontaneous behavior. Therefore it is unlikely that the observed behavioral alterations are due to differences in general health or body conditions. However it is interesting to note that at the age of 11 month the KO animals have been significantly heavier, although the underlying causes remain to be elucidated.

Exploratory and anxiety related behavior

Novel stimuli, such as a new environment generate an approach/avoidance conflict in the mouse. The reaction of a mouse is composed of the drive to explore the novelty to gain information and of anxiety related or cautious behavior to protect the animal from possible danger or harm (Bardo et al., 1996). Several environmental and intrinsic factors influence the behavior of mice in tests for exploratory behavior and/or anxiety, such as: laboratory environment, social isolation, age, rearing conditions, social stress and previous exposure to the same or different tests (for review see: Holmes, 2001). Therefore the anxiety and exploratory behavior of KO mice and WT littermates was investigated by using paradigms known to diversely elicit behavioral responses of the mouse (FCOF, OF and EPM), additionally mice were tested at different ontogenetic stages and under different housing conditions.

Anxiety behavior can be divided into state and trait anxiety, with state anxiety being anxiety that occurs temporarily as a reaction to an anxiogenic stimulus, whereas the trait anxiety is intrinsic for each individual (Lister, 1990). The open field (OF) was performed as a classical test for state anxiety and for exploration by means of novelty (Belzung and Griebel, 2001). A first OF was run on periadolescent mice and additional OFs were conducted as mice

were adult, one before and one after social isolation. In all OFs KO mice explored less as compared to WT littermates, as shown by the shorter distance moved, lower velocity and, in particular, by the decreased amount of rearing, an ethological marker for exploratory behavior (Crusio, 2001). Furthermore, KO mice entered the center less often and spent more time in the border and showed more thigmotaxis, revealing an increased state anxiety. Also a change in the behavior of both genotypes was observed in the different OFs. WT and KO mice showed less anxiety when paired housed in the adulthood as compared to periadolescence and after isolation. In summary, KO mice clearly showed alterations in their behavior in the OFs that remained constant under various conditions.

A similar increase in anxiety was seen as mice were tested in the elevated plus maze (EPM), one of the most common tests for state anxiety and exploration in rodents (Belzung and Griebel, 2001). Avoiding the open arms and performing less risk assessment are interpreted as anxious behavior (Rodgers and Dalvi, 1997). Two EPMs were performed as mice were adult, one before and one after isolation. As observed in the OF, both genotypes changed their behavioral response between the two EPMs, showing a more anxious profile in the second EPM as compared to the first one. Interestingly, while both genotypes decreased exploration of the open arms, WT mice increased exploration of the closed arms whereas KO mice did not change their behavior in the closed arms. This indicates that KO mice and WT littermates had a different reaction to re-exposure to the apparatus and/or to social isolation. Indeed, in the first EPM KO mice only tended to show increased anxiety and decreased exploration in comparison to WT littermates, whereas there was a clear difference between genotypes in the second EPM, indicating that the increase in anxiety shown by both genotypes in the latter EPM was more pronounced in KO mice as compared to WT littermates.

Particularly interesting are the results from the free choice open field (FCOF). Since mice decide themselves if, when and how often they enter the arena and since they are not forcibly exposed to an anxiogenic stimulus this test is thought to assess trait anxiety (Griebel et al., 1993). No difference was found between KO mice and WT littermates when they were periadolescent, indicating that there was no difference in trait anxiety or in novelty seeking behavior between genotypes at this age. Strikingly, there was a dramatic change in the behavior of KO mice in the FCOF when mice were adult. The number of adult KO mice entering the arena was strongly decreased in contrast to the first FCOF on periadolescent mice, although the arena in the second FCOF was supposed to be less anxiogenic (only closed

sides). However, the number of WT mice entering the arena increased when they were adult and they also increased the exploration of the arena.

The results from the EPM and FCOF experiments indicate that KO mice displayed increased state and trait anxiety and explored less under certain conditions, possibly due to re-exposure to the apparatus, to age or to social isolation. Also the social and competitive behavior of both genotypes was monitored as they were caged with a heterozygous brother and after they were single housed. No difference was found between genotypes. Therefore it seems unlikely, that the traits observed in the KO mice were due to different social rank or competitive behavior.

The results from the instantaneous sampling and automatic measurement of the circadian activity indicate that KO mice were less active when WT mice showed characteristic peaks of activity. Therefore, it is important to validate that our results from the anxiety and exploratory tests were not biased by possible differences in activity. For the OF the distance moved in the border and in the center was normalized by the total distance moved. The results indicate that KO mice displayed more thigmotaxis regardless of the lower distance moved, clearly pointing to an increase in anxiety. The same holds true for the percentage of time and entries in the open arms of the EPM. Moreover, in the first EPM KO mice showed no difference in total transitions and closed arms entries, two parameters for general activity (Rodgers and Johnson, 1995), but spent less time than WT mice on the open arms. Nevertheless it will be important to perform more focused experiments on the circadian rhythm of KO mice, in particular on their ability to reset the biological clock after a shift in the light dark cycle, as well as their ability to maintain circadian rhythm activity under constant darkness.

Mice explore a novel environment to gain information (Bardo et al., 1996). It can be assumed that TN-R KO mutants will have disadvantages being confronted with a novel environment, since their increased anxiety and reduced exploration will decrease the amount of information they may acquire. This is particularly clear when considering the results from the FCOF that seems to be the most valuable test in terms of ethological interpretation of novelty seeking behavior.

Motor coordination

The pole, wire hanging and Rotarod tests revealed an impairment of motor coordination in KO mice. In the pole test a lower proportion of KO mice were able to turn 180° on the rod and hence failed to climb down head foremost. Watching the performance of KO mice it seemed that they unsuccessfully tried to turn 180° and therefore climbed down sideward.

However, KO mice were able to improve their performance during three consecutive trials. An impairment of KO mice to coordinate in space was also observed in the wire hanging test, where KO mice were not able to lift their body and thus grip the wire with their hind paws. Similar to the pole test KO mice seemed to try to turn upwards but failed. The Rotarod is used to examine motor coordination in rodents and is often included in test batteries for mouse mutants (Brandon et al., 1998; Tarantino et al., 2000). In concordance with the data from pole and wire hanging tests, KO mice displayed problems by staying shorter times on the accelerating rod. The results clearly reveal a distinct defect in motor coordination in KO mice that occurs only under aggravated conditions, since KO mice did not display any obvious motor problems such as ataxia, tremor or disabilities in walking.

One may argue that the observed impairments were caused by lower muscle strength, but the specific expression of TN-R in the CNS rules out this hypothesis. Alternatively, the observed impairment might be a sequel of increased anxiety and not a primary motor problem as it has been reported that stress can impair the performance of rats in different motor tasks (Metz et al., 2001). However, KO mice did not show disabilities in the non-accelerating Rotarod, indicating that KO mice have no motor problems under mild demands, even if the test is new and unfamiliar and therefore anxiogenic and stressful. However, it is conceivable that anxiety has an impact on motor performance.

With respect to the morphological phenotype of the mutant, it is interesting to note that TN-R bearing perineuronal nets are found in the deep cerebellar nuclei, around motor neurons in the spinal cord and in cortical areas (Celio and Blümcke, 1994; Hagihara et al., 1999; Brückner et al., 2000). Thus one may speculate that an irregular appearance of perineuronal nets as it is seen in the TN-R KO mutant (Weber et al., 1999; Brückner et al., 2000; Haunso et al., 2000) could be the cause of impairments in motor coordination. On the other hand, TN-R is also accumulated at the nodes of Ranvier (ffrench-Constant et al., 1986; Bartsch et al., 1993) where its function has not been defined. A disturbed molecular composition of the node of Ranvier, especially in the spinal cord, may lead to a decreased conduction velocity in the TN-R KO mutant as it has already been observed for the optic nerve (Weber et al., 1999), thus resulting in impaired motor coordination. It is noteworthy in this respect that TN-R binds to voltage-activated sodium channels and activates them (Srinivasan et al., 1998; Xiao et al., 1999). In conclusion, ablation of TN-R causes impairments in finely coordinated movements, possibly relating to the function of perineuronal nets or of nodes of Ranvier in the motor cortex, cerebellum and spinal cord.

Cognitive behavior

Cognitive abilities were tested in a spatial learning task (water maze) and in a passive avoidance task (step-through). Unfortunately, the results do not permit a plausible interpretation regarding learning and memory in KO mice. In the water maze both genotypes performed poorly, thus compromising evaluations of possible cognitive impairments in KO mice. In the step-through passive avoidance, KO mice differed from WT littermates already in their baseline, showing a “passive” behavior that made this paradigm inappropriate for testing learning and memory in the mutant.

However, the behavioral profile shown by KO mice in the spatial learning and passive avoidance tasks confirmed the conclusions drawn from the experiments related to exploratory behavior and motor coordination. In the first visible trial of the water maze KO mice spent significantly more time with uncoordinated movements until they started to swim regularly. The lack of coordination in the first seconds in the pool emphasizes again the impairment in motor coordination and may also suggest increased anxiety displayed by the mutant when confronted with a novel situation. As observed in the OF, FCOF and EPM, KO mice were less active during the conditioning trial of the step-through passive avoidance test, indicating that they had a lower motivation to explore and displayed increased anxiety in comparison to WT littermates.

Although the use of null mutants has been recognized as a powerful tool in understanding the function of a targeted gene product, one cannot rule out possible compensatory effects during development and/or an influence of the genetic background on the alterations of a mutant (Gerlai, 1996). Generating transgenic animals is often performed by using embryonic stem cells from the 129/Ola strain and chimeras bred with C56Bl/6J mice, this also holds true for the tenascin-R KO mice used in this study. However, the results shown here replicate the results obtained on two former experiments where KO mice from F1 homozygous breeding have been compared to a strain generated from 129/Ola x C56Bl/6J F1 mice. Therefore it is most likely that the observed behavioral alterations are not linked to the genetic background, but caused by the ablation of TN-R.

Concluding remarks

In this study was shown that deficiency in TN-R leads to increased anxiety, decreased exploration and impaired motor coordination. Taken together, these behavioral alterations may be a disadvantage for mice living under natural conditions where natural selection works. Due to the decreased drive to explore and increased anxiety when confronted with a new

stimulus, KO mice may not be able to acquire new resources in the environment, with a potentially negative impact on their fitness. In addition, the observed motor impairment may be a grave handicap when mice have to coordinate in three dimensions and are forced to be quick and precise in their movements, for instance when fleeing from a predator or when interacting with the environment. Therefore, TN-R appears to be important for the expression of an adaptive behavioral response and, by consequence, for the fitness of an individual, holding a first functional explanation to the highly conserved structure of TN-R during evolution.

B

Investigation of the influence of maternal separation on the behavior of mice deficient for the cell recognition molecule CHL1

Introduction

Cell recognition molecules in the nervous System

The nervous System, as one of the most complex organs, depends crucially on a proper organization mediated by cell-cell contacts and cell-cell interactions. Molecules of the Immunoglobulin superfamily of neural cell recognition molecules (formerly named cell adhesion molecules) have been found to play important roles for the development, maintenance and function of the nervous system. The functional properties of recognition molecules are manifold and include regulation of cell differentiation and migration, neurite outgrowth, synapse formation, regeneration and synaptic plasticity (Rutishauer, 1993; Maness and Schachner, 2007).

The L1 family of recognition molecules

Within the nervous system three classes of recognition molecules have been described, the cadherins (Shapiro et al., 1998), integrins (Albeda et al., 1990) and the immunoglobulin superfamily (Salzer and Colman 1998). Proteins of the immunoglobulin superfamily are characterized by their immunoglobulin-domain which is responsible for the high specific recognition and binding. Proteins of the immune system display similar immunoglobulin modules as found in the superfamily, suggesting an evolutionary connection (Edelman, 1987). The immunoglobulin superfamily comprises several subfamilies (overview in Edelman and Crossin, 1991 and Crossin and Krushel, 2000) including the L1 subfamily with members found in invertebrates and vertebrates. The most prominent members within the vertebrates are: L1 (Rathjen and Schachner, 1984), CHL1 (close homologue of L1, Holm et al., 1996) neurofascin (Volkmer et al., 1992) and Nr-CAM (neuron-glia CAM related cell adhesion molecule, Grumet et al., 1991). Proteins of the L1 subfamily are characterized by a modular structure composed of six immunoglobulin-like domains, three to five fibronectin type III domains, a transmembrane domain and a short, highly conserved cytoplasmic tail (Hortsch, 2000). Members of the L1 subfamily are expressed predominately by neuronal cells, some also by glial cells. They act through homophillic or heterophillic binding and have the ability to mediate different aspects of neuronal and glial interactions, including myelination and morphogenesis. The early onset of expression with high levels along major axonal pathways highlights the important role for neurite guidance and promotion (Hortsch, 1996).

L1, the eponym of the subfamily, was one of the first described cell recognition molecules (Rathjen and Schachner, 1984). During development L1 plays a role in cell migration,

outgrowth and fasciculation of axons and myelination (reviewed in: Maness and Schachner, 2007). In the established nervous system L1 is involved in memory formation and synaptic plasticity (Lüthi et al., 1996; Venero et al., 2004) and triggers neuronal survival and axonal regeneration (Nishimune et al., 2005; Chen et al., 2007; Loers and Schachner, 2007).

CHL1

When the attempt was made to search for new cDNA clones of L1, one of the isolated clones was found to encode for a protein that is closely related, but distinct from L1 (Tacke et al., 1987). Due to its high similarity with L1 it was termed: close homologue of L1 (CHL1). CHL1 comprises an N-terminal signal sequence, six immunoglobulin-like domains, 4.5 fibronectin type III -like repeats, a transmembrane domain and a C-terminal domain (Holm et al., 1996) (Fig 16).

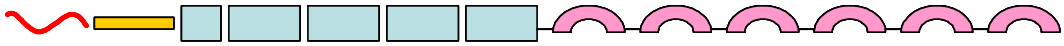

Fig. 16 schematic presentation of the CHL1 protein. *Proceeding in a carboxyl terminal direction, the domains are as follows: cytoplasmic tail (red line), transmembrane domain (orange rectangle), 4,5 Fibronectin type III domains (blue rectangles) and 6 immunglobulin like domains (pink bows).*

CHL1 expression is restricted to the nervous system. In mice CHL1 is first expressed around embryonic day 13 at times of neurite outgrowth, and is detectable in subpopulations of neurons, astrocytes, oligodendrocyte precursors and Schwann cells. CHL1 expression decreases from PD 7 to adulthood, but remains significant in the adult (Holm et al., 1996; Hillenbrand et al., 1999). CHL1 is a strong promoter of neurite outgrowth *in vitro* (Hillenbrand et al., 1999). Furthermore CHL1 promotes neuronal survival (Chen et al., 1999; Nishimune et al., 2005) and regulates neuronal migration (Buhusi et al., 2003).

The importance of CHL1 for the development of the nervous system has also been shown in a mouse mutant deficient for CHL1. At the morphological level, these mice show alterations of hippocampal mossy fiber organization and of olfactory axon projections, suggesting participation of CHL1 in the establishment of neuronal networks (Montag-Sallaz et al., 2002). Furthermore CHL1 deficient mice show reduced migration and positioning of pyramidal cells in the visual cortex (Demyanenko et al., 2004). Behavioral investigations of the mutants revealed no differences in life span, viability, general behavior, reflexes or in motoric and sensory functions, but detected an altered explorative behavior, when mice are confronted with a novel environment (Montag-Sallaz et al., 2002). The finding, that CHL1 mice show impaired prepulse inhibition of the acoustic startle response and therefore display a disturbance of sensorimotor gating (Irintschev et al., 2004) led to a detailed analysis of the

behavior and of synaptic activity in the hippocampus of CHL1 deficient mice. This study revealed a delayed response to novel environmental stimuli and to social stimuli, suggesting, that CHL1 deficient mice are impaired in extracting relevant information from the environment. The electrophysiological investigation did not reveal alterations in synaptic plasticity of major excitatory connections of the hippocampus, but an enhanced basal excitatory synaptic transmission in perforant path projections to the dentate gyrus in CHL1 deficient mice, providing a possible correlate to the delayed behavioral responses (Morellini et al., 2007).

CHL1 and mental diseases in humans

Interestingly, the above described behavioral alterations found in CHL1 mice are among the typical symptoms found in several neuropsychological disorders in humans. A disturbed prepulse inhibition, as described for the CHL 1 deficient mouse (Irintchev et al., 2004), is a typical symptom found in schizophrenic patients (Braff et al., 2001). The observed impaired reaction towards novel stimuli, indicative of reduced attention, as well as disturbances in social behavior of CHL1 deficient mice (Morellini et al., 2007) are also core features of schizophrenia (Nuechterlein et al., 2006; Burns and Patrick, 2007). CHL1 in humans (also referred to as CALL) localizes to chromosome 3p and its loss or mutation may contribute to mental impairment associated with the “3p-syndrome” (Angeloni et al., 1999) and to mental retardation (Frints et al., 2003). Furthermore a correlation between mutations in the human CHL1 gene and the occurrence of schizophrenia has been reported (Sakurai et al., 2002; Chen et al., 2005). Schizophrenia is a complex mental disorder and as with many other neuropsychological dysfunctions, the underlying mechanisms and causes are not yet well understood. Nevertheless it is widely accepted that the occurrence of schizophrenia is associated with both, genetic and environmental factors (Tandon et al., 2008). Most of the possible environmental disturbances that may have an impact on the development of schizophrenia take place either prenatal, in form of obstetric complications (Mittal et al., 2008), or during early childhood in form of trauma, stress or abnormal hormonal homeostasis (Morgan and Fischer 2007).

Maternal separation in rodents as an ecological model of postnatal stress

A mechanism to induce early life stress in mammals is the separation of dam and pups, at stages when the pups are still depending on the care of the mother in form of nursing, maintenance of body temperature and physical contact. The laboratory rat has been one of the first animals studied in maternal separation (MS) (Levine, 1957; Hofer, 1975) and ever since

many studies have contributed to the understanding of the impact of MS on the behavior of the rat. Depending on the method and strain used, the alterations found are manifold. Reported changes induced by MS in rats are increase in anxiety- and depression-like behavior, hyperactivity, changes in social/defensive behavior, increase of voluntary drug-intake and hyperactivity of the hypothalamic-pituitary-adrenal (HPA)-axis (overview in: Champagne and Meaney, 2001; Cameron et al., 2005; Holmes et al., 2005; Moffet et al., 2007). MS has also been applied in mice, but the results are not as extensive and consistent as in the rat. An increase in anxiety–like behavior after MS has been reported for C57BL/6J male, but not female mice (Romeo et al., 2003), whereas other studies report no increase in anxiety-like behavior in this mouse strain after MS (Parfitt et al., 2004; Parfitt el al., 2007). A mild increase in depression-like behavior (MacQueen et al., 2003), or increased exploration (Venerosi et al., 2003) are also alterations found after MS. In the most extensive study the influence of MS on the anxiety- and depression-like behavior of eight different mouse strains has been investigated systematically and the authors come to the conclusion that MS is not a robust model of early life stress affecting anxiety- and depression-related behaviors in mice (Millstein and Holmes 2007). Taken together, mice seem to be more robust against the adverse qualities of MS in comparison to rats (Millstein and Holmes 2007). Furthermore the outcome seems very much dependent on the gender and on the mouse strain used, indicating that the combination of genetic predisposition and the treatment determines the character and magnitude of the effect of MS in mice.

Aim of the Study

Mice deficient for the neural cell recognition molecule CHL1 display behavioral alterations in their reaction towards novel stimuli that are reminiscent of alterations observed in neuropsychological disorders like schizophrenia. The etiology of schizophrenia is thought to be a combination of genetic predisposition and adverse environmental factors. The aim of the study was to perform a maternal separation paradigm on CHL1 deficient mice and their WT littermates and to compare the behavioral alterations evoked by this early life stress. The question was if CHL1 deficient mice are, due to their genetic predisposition, more vulnerable to the effects of maternal separation, and thus display alterations that are not, or only to a minor extend, observed in WT mice. Furthermore the choice of several tests covering a broad spectrum of different behaviors and the investigation of male and female mice ought to add information on the influence of maternal separation on mice in general.

Experimental design

CHL1 KO mice have already been investigated in order to compile a behavioral phenotype. In this present study the focus was put on the impact of the maternal separation on the behavior of the mouse. It was not intended to apply a standardized test-battery to search for general differences between genotypes. Therefore the type, procedure and order of the behavioral tests was designed considering on the one hand results obtained from the behavioral analysis of the CHL1 KO mouse (Montag-Sallaz et al., 2002; Pratte et al., 2003; Frints et al., 2003; Morellini et al., 2007), and on the other hand the possible influence of maternal separation on the behavior as reported in the literature (Romeo et al., 2003; Parfitt et al., 2004; Millstein et al., 2006; Millstein and Holmes 2007). The study from Morellini et al. (2007) showed that CHL1 KO mice display alterations in their reaction to novel environmental stimuli and furthermore exhibit reduced social behavior. To gain information on a possible influence of MS, the open field test in which the mice have to react with a new environment and the novel object test where the reaction towards new stimuli is observed were performed to test novelty-induced behavior. The social preference, urine marking and resident intruder tests were chosen to assess social behavior. Since anxiety-like behavior seems to be affected by MS in mice (Romeo et al., 2003), the elevated plus maze (EPM) as one of the most established tests for state anxiety in rodents was performed in addition to the anxiety-related parameters that can be extrapolated from the open field and novel object tests. Another emphasis was placed on a possible interaction between CHL1 deficiency and MS on

the memory performance of mice. It is known that psychotic illnesses like schizophrenia also influence cognitive abilities and in particular working memory (Lewis and Liebermann, 2000; Manoach, 2003), thus tests for working memory (spontaneous alternation) and long term memory (step through passive avoidance) have been accomplished. No differences in motor performance between CHL1 KO and WT have been found and no influence of MS on the motor behavior of mice has been reported so far. Therefore two tests of motor behavior (pole test and Rotarod) have been included to serve as an internal control and to scrutinize a possible influence of the combination of CHL1 KO and MS on the motor function. Finally, the effect of MS on the depression like behavior of mice as described by MacQueen et al. (2003) was meant to be assessed by the tail suspension test. While planning the schedule the invasiveness of the tests was taken into account, thus beginning with tests considered to be the least stressful for the mice.

Material and Methods

Husbandry and general procedures

Husbandry and general procedures were performed as described in part A.

Animals

Heterozygous CHL1 mice with a 129Ola x C57Bl/6J background and 6 backcrosses to C57Bl/6J were used for breeding. Sixteen breeding cages were composed of 1 CHL1 +/- male and 2 CHL1 +/- females, 2 cages contained 1 male and 1 female only. After 13 days, females with a weight gain of at least 10% were considered to be bearing and single housed in Macrolon cages (15 x 20 x 23 cm). Cages were checked for litters daily starting 19 days after breeding onset. The inspection was performed carefully without opening the cage to prevent disturbance of dam and pups. When a litter was found, the pups were designated postnatal day (PD) 1 and the normal food was exchanged with food for nursing mice (ssniff, Soest, Germany).

Long Maternal separation

The whole procedure was performed wearing gloves. On PD 2 pups were counted and checked for gender by evaluation of the anogenital distance. Each litter was either assigned to the control group (CON), or long maternal separation (MS) group allowing for a balanced litter composition. The CON group was left undisturbed. During MS, the dam was transferred in a new cage to avoid the destruction of the nest by the dam in search of the pups and brought into the *vivarium*. In the meantime, the pups were kept in an adjacent room to avoid ultrasound communication between pups and dam. Each pup was put in a single compartment (4 x 4 cm) of a cardboard box covered with tissue. Red lights were arranged around the boxes to obtain a temperature of 32 ± 2° Celsius (Fig. 17). The separation lasted 180 min and took place daily from PD 2 to PD 8 between 8 a.m. and 12:30 p.m. After separation dam and pups were reunited in their home cage and brought back into the *vivarium.*

Fig. 17 Maternal separation. *Pubs are placed in single compartments (covering sheet removed)*

Behavioral tests

Between PD 21 and PD 23 the pups were weaned and separated from their mother. Bodyweight was taken; a 1-2 mm long tip of the tail was cut to collect a biopsy for genotyping and one ear was punched to distinguish single mice. On PD 30 mice were housed in groups of 2 to 4 siblings in Type II long cages (15 x 20 x 30 cm). For the behavioral tests maximal 2 KO or WT per sex and litter were taken to avoid a litter effect. Every tenth day bodyweight was measured and the cages were changed with clean ones. Starting at the age of 9–12 weeks, WT and KO mice underwent the behavioral paradigms (Table 6).

Table 6

Day	Age	Experiment
1	9 - 12 w	Open field
3 - 4	9 - 12 w	Elevated Plus Maze
9 - 17	10 - 13 w	Spontaneous alternation 1
15 - 25	12 - 15 w	Social preference
19 - 26	12 - 15 w	Isolation + Novel Object
25 - 27	13 - 16 w	Urine Marking (males only)
28 - 30	13 - 16 w	Resident /Intruder (males only)
32 - 37	14 - 17 w	Step through passive avoidance
37 - 43	14 - 17 w	Pole test
39 - 45	15 - 18 w	Rotarod
72 - 95	21 - 25 w	Spontaneous alternation 2
106 - 127	25 - 28 w	Tail suspension + blood sampling

Open field

The open field test was performed as described in part A with an illumination of 100 Lux.

Elevated Plus Maze

The elevated Plus maze (EPM) was performed as described in part A with the only difference that the test was done in darkness and video-recorded by an infrared video-camera. In addition to the parameters analyzed in part A, the behavior of head dipping was subdivided in protected (from the center or closed arms) and unprotected (from the open arms) head dipping.

Spontaneous Alternation

T-maze. The maze consisted of 3 transparent Plexiglas arms of the same size (34 × 5 cm and 30 cm high): 2 opposing and 1 central arm connected to build a T. Two sliding doors controlled the access to the opposing arms (Fig. 18A). Mice were tested over 2 days with 1 session per day until they performed 14 trials or 20 min had elapsed. The session started by placing the mouse at the dead end of the central arm allowing the mouse to freely enter one of the two opposing arms. Once the mouse entered one arm, access to the opposing arm was occluded by the sliding door. When the mouse returned to the central arm the next trial was started by opening the door. The set-up was illuminated with dim white light (5 Lux). Data were analyzed as percentage of alternations over trials. Moreover, to assess possible

differences in exploratory behavior, average time required to conclude one trial (duration per trial) was evaluated. The results from the first and second day were combined.

Y-Maze. The maze consisted of 3 symmetrical arranged gangways of 30 x 7 cm connected to build a Y, walled by non transparent Plexiglas (30 cm high) (Fig. 18B). Mice were tested over 2 days with 1 session per day and maximum 20 trials or 15 min per session. The session started by placing the mouse in the center of the gangways junction. The mouse was allowed to move freely within the 3 gangways. An entry was noted when the mouse stepped with 4 paws more than 5 cm into one gangway. The set-up was illuminated by 2-3 Lux. As for the T-maze spontaneous alternation test, percentage of alternations over trials and duration per trial were analyzed. The results from the first and second day were combined.

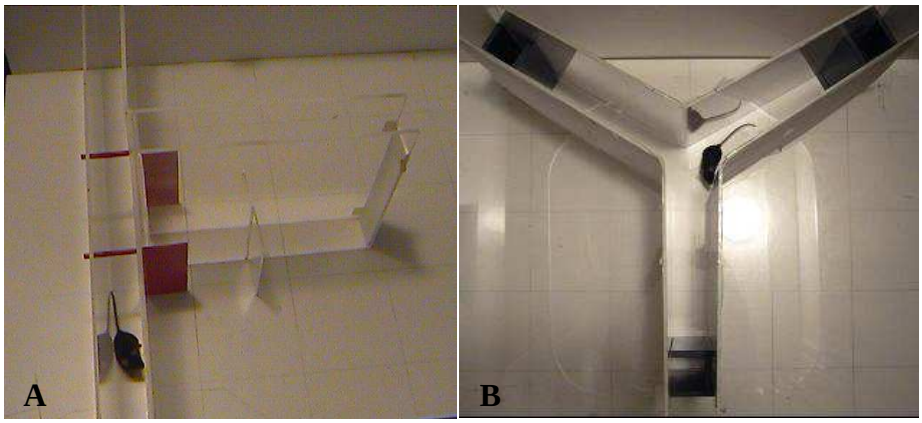

Fig. 18 Spontaneous alternation. *(A) T-form. (B) Y-form.*

Social preference

The arena used for the open field test (50 x 50 cm) was divided into two identical compartments by a 40-cm-high wall with a circular starting box (diameter of 15 cm, 30 cm high) in the middle. The starting box had two openings to allow access to the two compartments. A transparent plastic cup (diameter of 9 cm, 11 cm high) was located in one corner of each compartment containing either one familiar or unfamiliar mouse. Familiar mice were recruited from heterozygous siblings living in the same cage. In few cases WT or KO siblings had to be taken, this was counterbalanced between genotypes. Unknown C57Bl/6J mice were taken as unfamiliar mice.

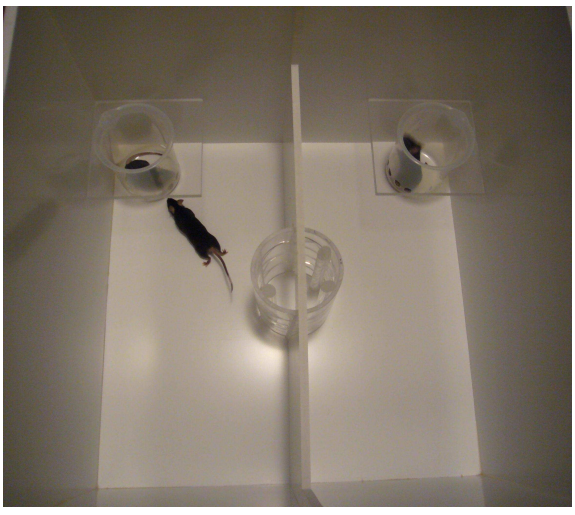

Fig. 19 Social preference box.

The cups had holes (diameter of 0.8 cm) at their bottom allowing olfactory exploration between mice (Fig. 19). Placement of familiar and unfamiliar mice was counterbalanced between the two compartments to avoid any bias due to the location. The experimental mouse was placed into the starting box and allowed to move freely between compartments for 15 min. The set-up was illuminated with 5 Lux. Distance moved, mean velocity, time spent in each compartment, and time spent in proximity of the two cups were analyzed with the software EthoVision.

Novel Object

Mice were placed into a cage (38 × 22 × 15 cm) with fresh bedding and food and water *ad libitum*. After 24 hrs a novel object (a 7 × 7 cm and 10 cm high plastic water bottle for rodents with the bottom cut off and with an entrance of 3 × 4 cm on one side) was introduced into the cage. The entrance was facing the center of the cage (Fig. 20). Behavior was video-recorded for 5 min after introducing the novel object. Following parameters were analyzed using The Observer: self-grooming, immobility, rearing at the cage, rearing at the object, digging, climbing at the top grid of the cage, stretch attend posture (SAP) towards the object, staying at the wall opposite to the object (within 20 cm at the opposite wall), and time spent approaching the object (forward motion towards the object).

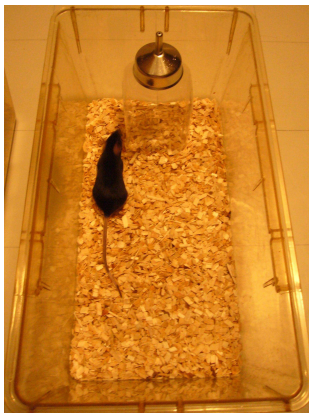

Fig. 20 Novel object

Urine marking test

The floor of the open field box (see above) was covered with Whatman filter paper # 4. Three C57BL/6J female mice were placed in one corner of the box surrounded by Plexiglas walls (18 × 18 cm and 30 cm high) with holes (diameter of 0.8 cm) allowing visual and olfactory perception but limited body contact between females and the experimental male mouse (Fig. 21). Male mice could freely move for 30 min. Urine spots were counted under UV light. Distance moved, mean velocity and time spent in the quadrant containing the female mice were analyzed with EthoVision.

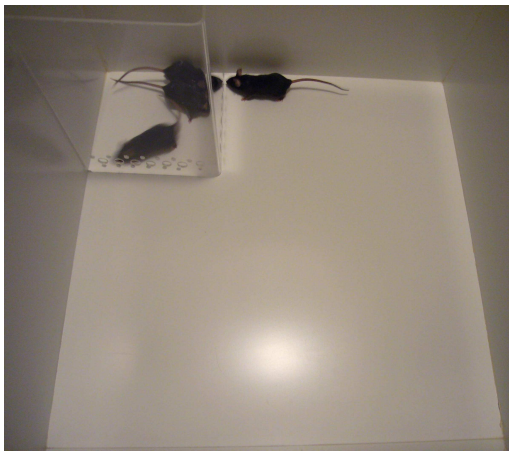

Fig. 21 Urine marking. *Male approaching females box.*

Resident/Intruder test

The test was performed as described in part A.

Step through passive avoidance test

The test was performed as described in part A, except that the familiarization protocol was left out and the illumination of the lit compartment was 100 Lux.

Pole test

The test was performed as described in part A with the difference that the maximum duration per trial was 120 s.

Rotarod

The test was performed as described in part A, except that the maximum duration of the accelerating trials was 5 min.

Tail suspension

The mouse was suspended with the tip of its tail from a shelf. The tail was fixed with detachable tape. Behavior of the mouse was recorded for 6 min. In case the mouse was able to climb its own tail and reach the shelf it was returned to the hanging position. In the rare events a mouse was able to free itself and fell down (the floor was covered with foam to avoid injuries) it was suspended again. The set-up was illuminated with red light and following parameters were analyzed with The Observer: moving, immobile, climbing the own tail, falling.

Analysis of behavioral parameters

Analysis was performed as described in part A. With exception of the Rotarod and T-maze spontaneous alternation tests, all tests were video-recorded. The arena of the social preference test was subdivided into 4 equally sized quadrants (each 25 x 25 cm) to obtain information about time spent in the different quadrants. Furthermore a 15 x 15 cm field around the two cups was designed to calculate latency to enter, number of entries and time spent at the cups. The arena of the urine marking test was virtually subdivided into a 30 x 30 cm field around the females' chamber and a 20 x 20 cm field in the corner opposite to the females to obtain information about the percentage of time spent at the females' side *versus* the side opposite to the females.

Blood sampling and ELISA

30 min after start of the tail suspension mice were sacrificed and trunk blood was collected in heparinized collection tubes (Sarstedt, Nürnbrecht Germany), and centrifuged for 20 minutes at 4°C at 1700 **g**. Plasma was collected and stored at –20°C. Plasma corticosterone levels were measured using a commercially available corticosterone ELISA kit (IBL, Hamburg Germany).

Statistics

Data relative to male and female mice were analyzed separately. For each gender the 2-way ANOVA with Genotype and Treatment as between factors was performed, followed by a Newmann-Keuls post-hoc analysis when appropriate. Taking into account that samples were not always normally distributed, the Mann-Whitney test as a non-parametric test was applied to compare the CON *versus* the MS group, thereby putting an emphasis on the impact of the treatment. To judge whether the mice alternated in the spontaneous alternation tests group values were compared to the chance level of 50% by using the non-parametric Wilcoxon signed-rank test. Comparison of the proportion of mice showing a particular performance was tested with Fisher's exact probability test. All tests were performed two-tailed and level of significance was set at $p < 0.05$.

Results

Breeding of mice and maternal separation

Thirty-four females in 18 breeding cages produced 22 litters composed of 83 males and 80 female pups. Eleven litters were assigned to the CON group and 11 litters underwent maternal separation (MS). All pups survived the MS procedure without any apparent detraction as judged from the general appearance and agility. Table 7 shows the composition of groups arising after genotyping. Heterozygous animals were not included in the behavioral tests.

Table 7 Experimental groups

	males		females	
	WT	CHL1 KO	WT	CHL1 KO
CON	10	6	8	10
MS	9	7	12	9

Bodyweight

There was no effect of MS on the body weight of males or females of neither genotype as measured regularly from weaning till PD 90 and before isolation and 2 days after the Rotarod. KO male and female mice were slightly but consistently lighter than their WT littermates (3-way ANOVA for repeated measures, effect of Genotype: $F_{1,24} = 8.21$; $P < 0.01$; $F_{1,22} = 6.54$; $P < 0.05$ for males and females respectively, Fig. 22).

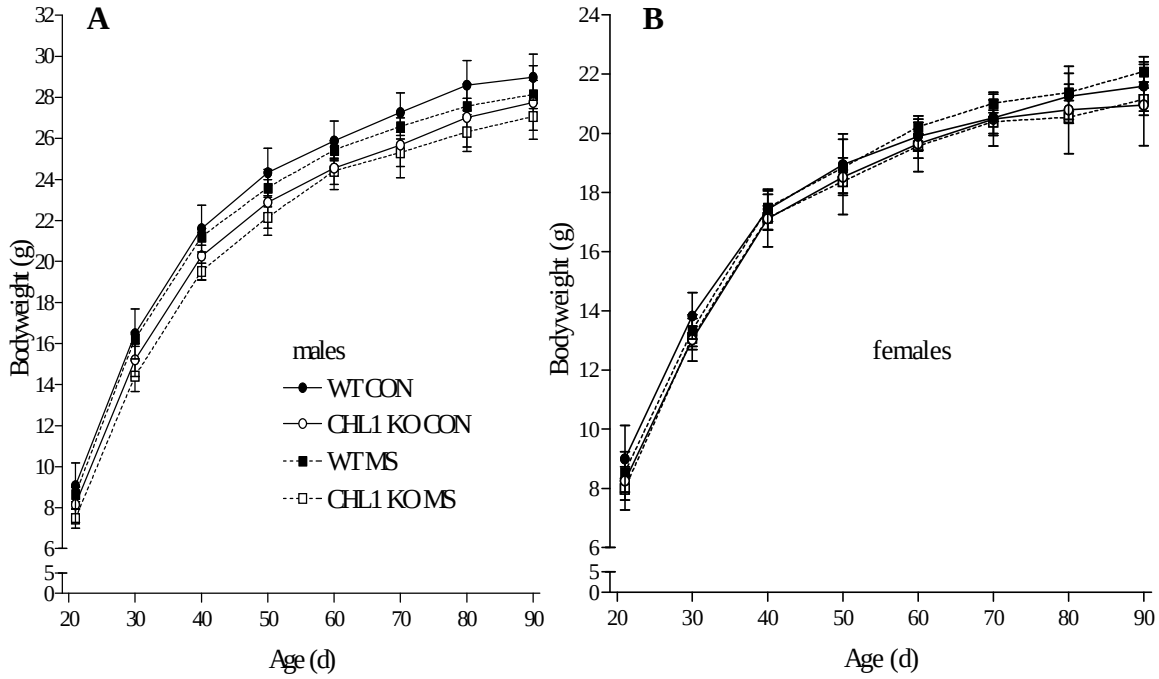

Fig. 22 Maternal separation has no influence on the bodyweight of WT and KO mice. *Data are expressed as mean ± SEM (A) Bodyweight of WT and CHL1 KO males under control situation (CON) and after maternal separation (MS). (B) Bodyweight of WT and CHL1 KO females.*

Open field

WT and KO male mice increased their locomotion activity in the open field after MS. In female mice of both genotypes MS led to a later start of rearing.

Open field males

Two-way ANOVA having Genotype and Treatment as between factors showed an effect of Treatment on the percentage of time spent moving ($F_{1,28} = 5.43$; $P < 0.05$) and on total distance moved ($F_{1,28} = 5.25$; $P < 0.05$). Mice in the MS group moved more and covered a longer distance, revealing an increase in motor activity in the OF compared to the CON group (Fig. 23A,B). When distance moved was analyzed for 3 discrete 5 min intervals all mice showed a similar pattern of habituation to the OF (3-way ANOVA for repeated measures, effect of Time: $F_{2,56} = 18.41$; $P < 0.001$). Activity was high in the first 5 minutes and declined till the end of the test. 2-way ANOVA revealed that WT males displayed a significantly higher mean velocity compared to KO males ($F_{1,28} = 6.67$; $P < 0.05$, effect of Genotype) (Fig. 23C). MS had no effect on the pattern of exploration as accessed by parameters like percentage of time in the center and mean distance to the wall (Fig. 23D). 2-way ANOVA revealed a decreased thigmotaxis in KO mice compared to WT mice as indicated by higher distance to the wall ($F_{1,28} = 4.58$; $P < 0.05$; effect of Genotype; Fig. 23D). When behavioral analysis of the first five min was performed, no differences concerning rearing or grooming behavior between groups were detected.

Open field females

Two-way ANOVA having Genotype and Treatment as between factors revealed no effect of MS on locomotion- and thigmotaxis-related parameters (Fig. 23E,F). 2-way ANOVA revealed a significant effect of Treatment on the latency to start rearing ($F_{1,35} = 5.59$; $P < 0.05$). Mice in the MS group performed the first rearing later than CON mice (Fig. 23G). Accordingly, a strong tendency ($F_{1,35} = 3.85$; $P = 0.06$, effect of Treatment) to decrease the amount of rearing events (on and off wall) was found in the MS compared to CON group (Fig. 23H).

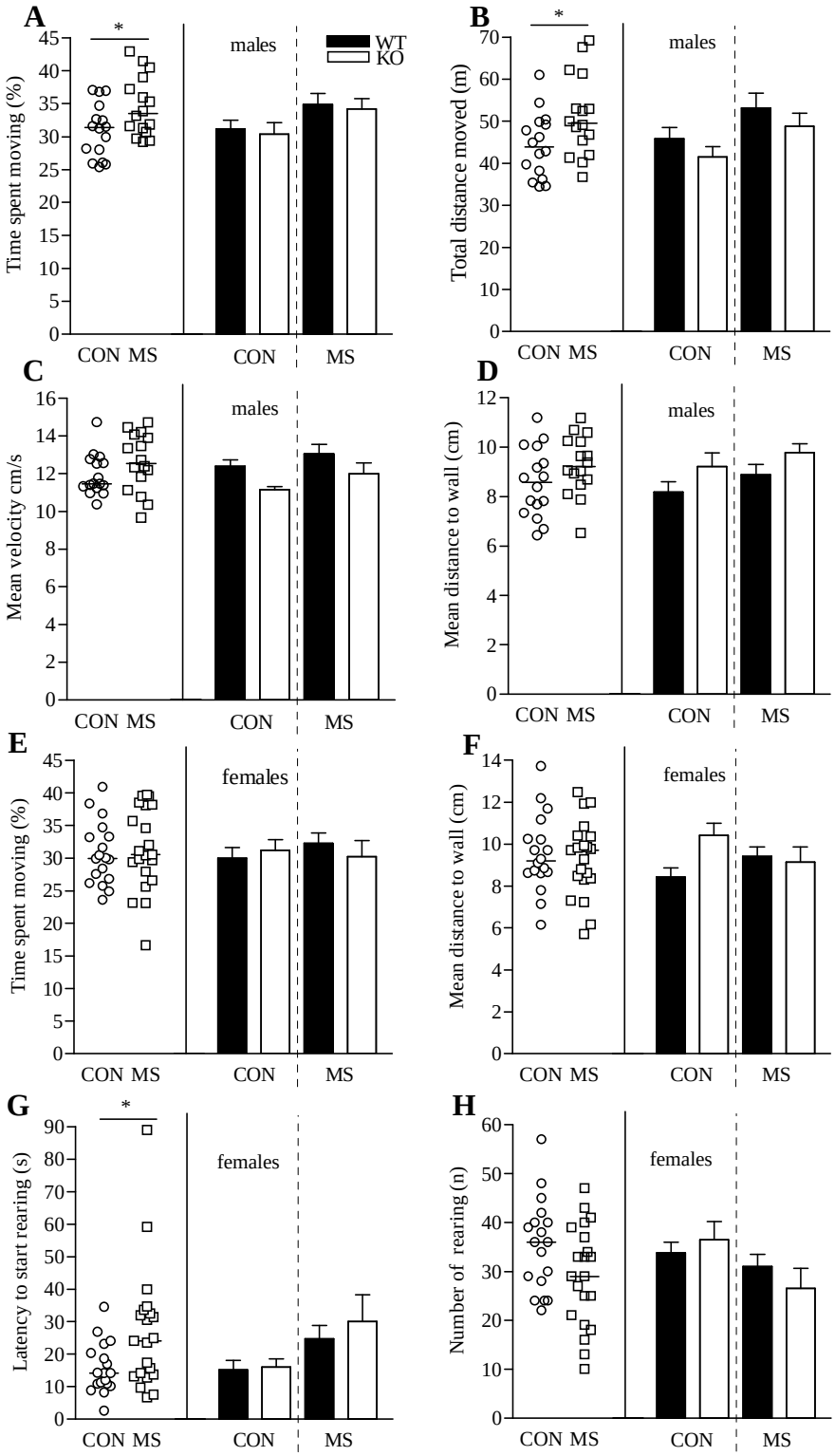

Fig. 23 Performance of WT and KO mice in the open field. *The scatter plots represent single values of WT and KO combined and the median. The columns display means ± SEM.(A) – (D) male mice, (E)-(H) female mice. (A) Time spent moving. (B) Total distance moved. (C) Mean velocity. (D) Mean distance to the wall. (E) Time spent moving. (F) Mean distance to wall. (G) Latency to start rearing. (H) Number of rearings (on and off wall).CON: Control group; MS: Maternal separation group; * : P < 0,05 as analyzed with the Mann-Whitney test.*

Elevated Plus Maze

MS increased the locomotion activity in males, particularly in KO mice compared to CON KO mice and led to subtle changes in the exploration of female mice compared to the CON group.

EPM males

Two-way ANOVA having Genotype and Treatment as between factors revealed that MS increased the number of closed arm entries in WT and KO males ($F_{1,28} = 8.34$; $P < 0.01$; effect of Treatment). Post-hoc analysis showed a significant increase in the number of closed arm entries in the KO-MS group compared to KO-CON group (Fig. 24A). Accordingly, 2-way ANOVA showed a strong tendency towards increased total transitions after MS ($F_{1,28} = 3.66$; $P = 0.07$; effect of Treatment), accompanied by a tendency towards decreased % of open arm entries ($F_{1,28} = 3.08$; $P = 0.09$; effect of Treatment; Fig. 24B,C). No effect of Treatment was detected for time spent on open *versus* closed arms and time spent in the center. An effect of Genotype revealed that KO mice had lower latencies to enter the open arm edges ($F_{1,28} = 6.13$; $P < 0.05$) compared to WT mice, and this differences between genotypes appeared to be more pronounced in the MS group compared to the CON group (Fig. 24D). Mice in the MS group performed more rearing bouts than mice in the CON group, as indicated by a significant effect of Treatment ($F_{1,28} = 12.03$; $P < 0.01$). This effect persisted, when frequency of rearing was normalized by the time in closed arms ($F_{1,28} = 4.64$; $P < 0.05$; Fig. 24E). No effect was detectable for the other parameters analyzed.

EPM females

Two-way ANOVA having Genotype and Treatment as between factors showed a tendency for increased total transitions in the MS group compared to CON group ($F_{1,35} = 3.23$; $P = 0.08$, effect of Treatment; Fig. 24F). Furthermore an effect of Treatment on the time spent in the center was detected ($F_{1,35} = 4.53$; $P < 0.05$), indicating reduced time spent in the center in the MS group compared to CON mice (Fig. 24G). There was a tendency towards lower latencies to enter the open arms in MS group compared with CON group ($F_{1,35} = 2.91$; $P = 0.1$; effect of Treatment; Fig. 24H). Moreover, a higher proportion of mice in the MS group entered the open arm within the first 5 seconds of the test (CON: 2/18; MS: 9/21: $P < 0.05$ Fischer's exact probability test). The percentage of open *versus* closed arm entries or the time spent on the different arms was not affected by Treatment. Females in the MS group did less protected head dipping than CON females ($F_{1,35} = 6.19$; $P < 0.05$, effect of Treatment). The amount of head dipping as expressed per minute in center did not differ between groups.

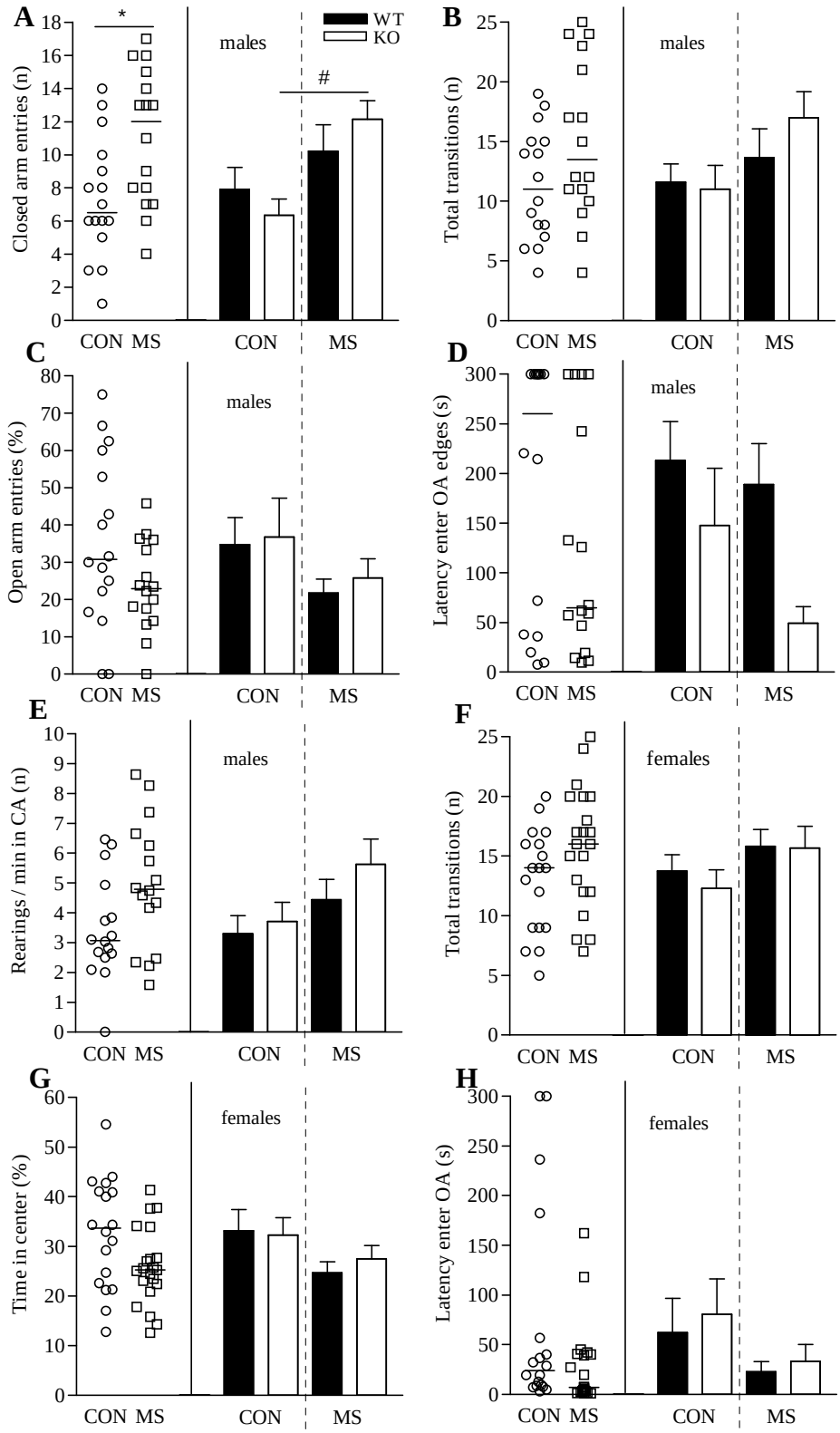

Fig. 24 Performance of WT and KO mice in the elevated plus maze*. The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A)-(E) male mice; (F)-(H) female mice. (A) Number of CA entries. (B) Number of total transitions. (C) Percentage of OA entries. (D) Latency to enter an OA edge (E) Number of rearings per minute in CA.(F) Number of total transitions. (G) Percentage of time spent in the center. (H) Latency to enter an OA. CA: closed arm; CON: Control; MS: Maternal separation; OA: open arm. * : P < 0 .05 as analyzed with the Mann-Whitney test; # : P < 0.05 as analyzed by post-hoc analysis (Newman-Keuls test) after 2-way ANOVA that showed an effect of Treatment.*

Spontaneous alternation

Male KO mice of the MS group showed reduced alternation rate compared to male KO mice of the CON group. There was a slight tendency towards higher locomotion activity in males of both genotypes after MS compared to the CON males. No major impact of MS on the alternation rate of WT and KO female mice was found. In order to eliminate a possible influence of the sliding door on the alternating behavior of the mice, a second spontaneous alternation test with a Y-Form was performed.

Spontaneous alternation 1 T-Form males

Males in the CON and MS group displayed alternating behavior when values were compared to the chance level of 50% (Wilcoxon signed rank test). When values were assessed separately for WT and KO, only the KO in the MS group failed to reach significant values (P = 0.23; Fig. 25A). Two-way ANOVA having Genotype and Treatment as between factors revealed a slight tendency towards faster transitions after MS in both genotypes. ($F_{1,28}$ = 2.91; P = 0.1; effect of Treatment, Fig. 25C).

Spontaneous alternation 2 Y-Form males

Male mice in the CON and MS group displayed alternating behavior when values were compared to the chance level of 50% (Wilcoxon signed rank test). When values were calculated separately for WT and KO, only the KO in the MS group failed to reach significant alternation rate, but revealed a tendency (P = 0.07; Fig. 25B). There where no differences between groups concerning the duration of transitions.

Spontaneous alternation 1 T-Form females

Three females (1 WT-CON, 1 KO-CON and 1 WT-MS mouse) did less than 8 transitions and thus were excluded from the calculation of the alternation-rate. Females in the CON and MS group displayed alternation as compared to the chance level of 50% (Wilcoxon signed rank test). By investigating KO and WT separately it was found that WT in the CON group did not show alternation (P = 0.16) and KO after MS showed only a tendency towards alternation (P = 0.07; Fig. 25D). There was no influence of maternal separation on the duration per trial as evaluated with 2-way ANOVA.

Spontaneous alternation 2 Y-Form females

Females in the CON and MS group showed alternation compared to chance levels of 50% (Wilcoxon signed rank test). When values were assessed separately for WT and KO all groups displayed significant alternation. However, the alternation rate was significantly lower

in the MS group compared to the CON group (P < 0.01; Mann-Whitney-U test; Fig. 25E). No differences concerning the duration of transitions were found.

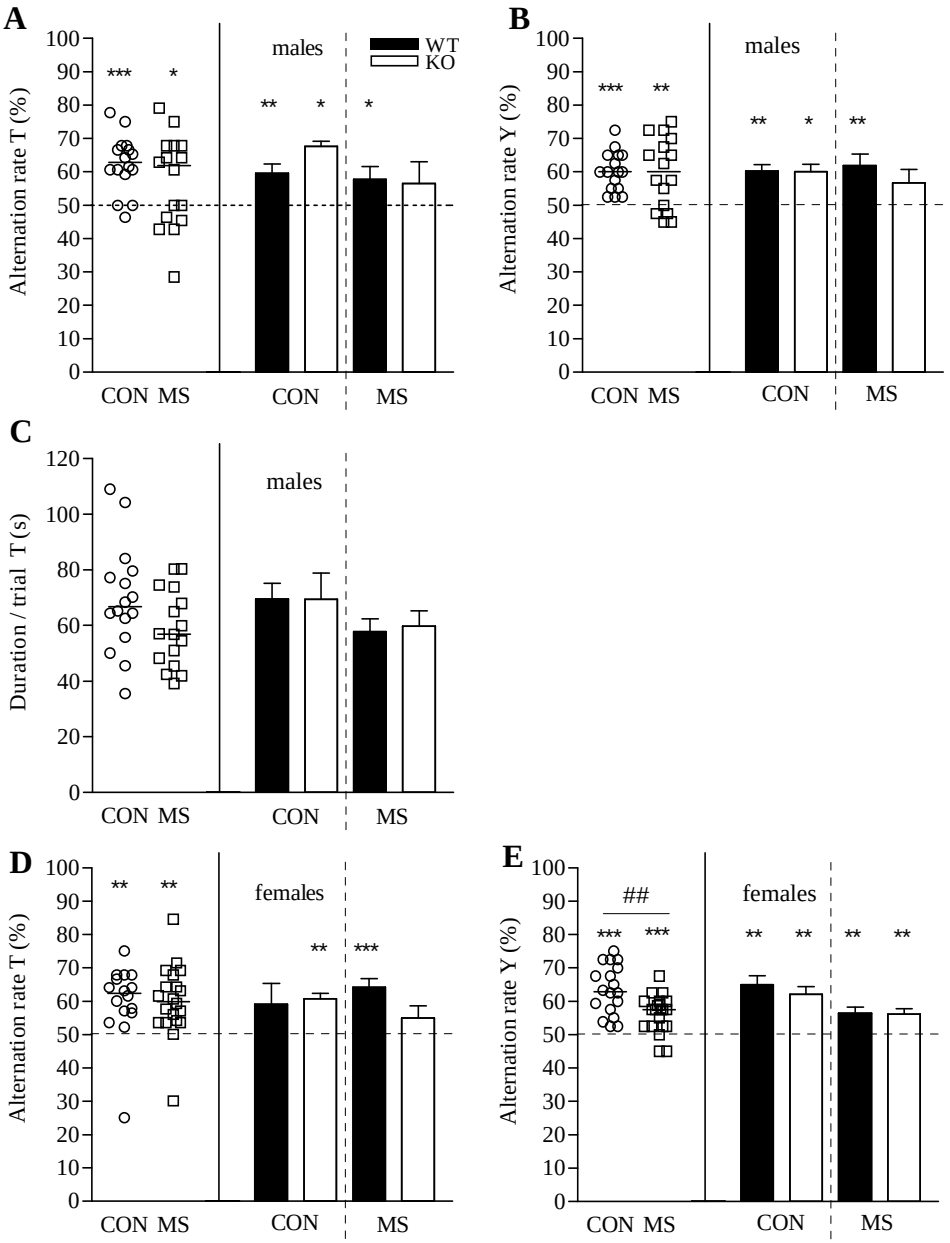

Fig. 25 Performance of WT and KO mice in the Spontaneous alternation tests (SA). *The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A)-(C) male mice; (D)+(E) female mice. (A) Percentage of alternation in SA 1 T-form. (B) Percentage of alternation in SA 2 Y-form. (C) Duration per trial in SA 1 T-form. (D) Percentage of alternation in SA 1 T-form. (E) Percentage of alternation in SA 2 Y-form. CON: Control group; MS: Maternal separation group. ##: P < 0.01, as analyzed with the Mann-Whitney U-test. . *, **, *** P < 0.05, 0.01, 0.001, respectively, as comparing values with the chance-level of 50% with the Wilcoxon signed-rank test.*

Social preference

MS resulted in an increase of locomotor activity and a decrease of social investigation in male mice. MS had no major influence on the behavior of female mice of both genotypes in the social preference test.

Social preference males

Two-way ANOVA having Genotype and Treatment as between factors indicated that mice in the MS group had higher mean velocities than mice in the CON group ($F_{1,28} = 9.43$; $P < 0.01$; effect of Treatment). As indicated by an effect of Genotype KO males displayed a lower mean velocity than WT ($F_{1,28} = 17.64$; $P < 0.001$), but both genotypes increased their velocity likewise after MS ($F_{1,28} = 0.03$; $P = 0.87$; no interaction of Genotype X Treatment and revealed by post-hoc analysis Fig. 26A). MS did not have an effect on total distance moved. Regarding the parameters related to the investigation of the familiar (F) and unfamiliar (UF) conspecific mouse, 2 way-ANOVA revealed a tendency towards less time spent investigating the cups after MS ($F_{1,28} = 3.05$; $P = 0.091$; effect of Treatment Fig. 26B). There was no effect of MS on the number of visits to the cups (Fig. 26C), but a decreased time spent at the cup per visit in the MS group ($F_{1,28} = 5.31$; $P < 0.05$; effect of Treatment Fig. 26D). Comparison of the time mice spent at the UF versus the F cup to the chance level of 50% revealed that only males from the MS group spent significantly more time at the UF versus F conspecific (Wilcoxon signed-rank test; Fig. 26E). Furthermore, in the CON group a higher proportion of mice visited the F cup first and in the MS group a higher proportion of mice visited the UF cup first ($P < 0.05$; Fisher's exact probability test). This change was particularly pronounced in the KO groups (Fig. 26F). There was no effect of MS on the latency to contact the cups (UF or F). Two-way ANOVA revealed a tendency towards fewer visits at the cups (UF and F) in KO versus WT males (effect of Genotype: $F_{1,28} = 3.86$; $P = 0.059$; Fig. 26C).

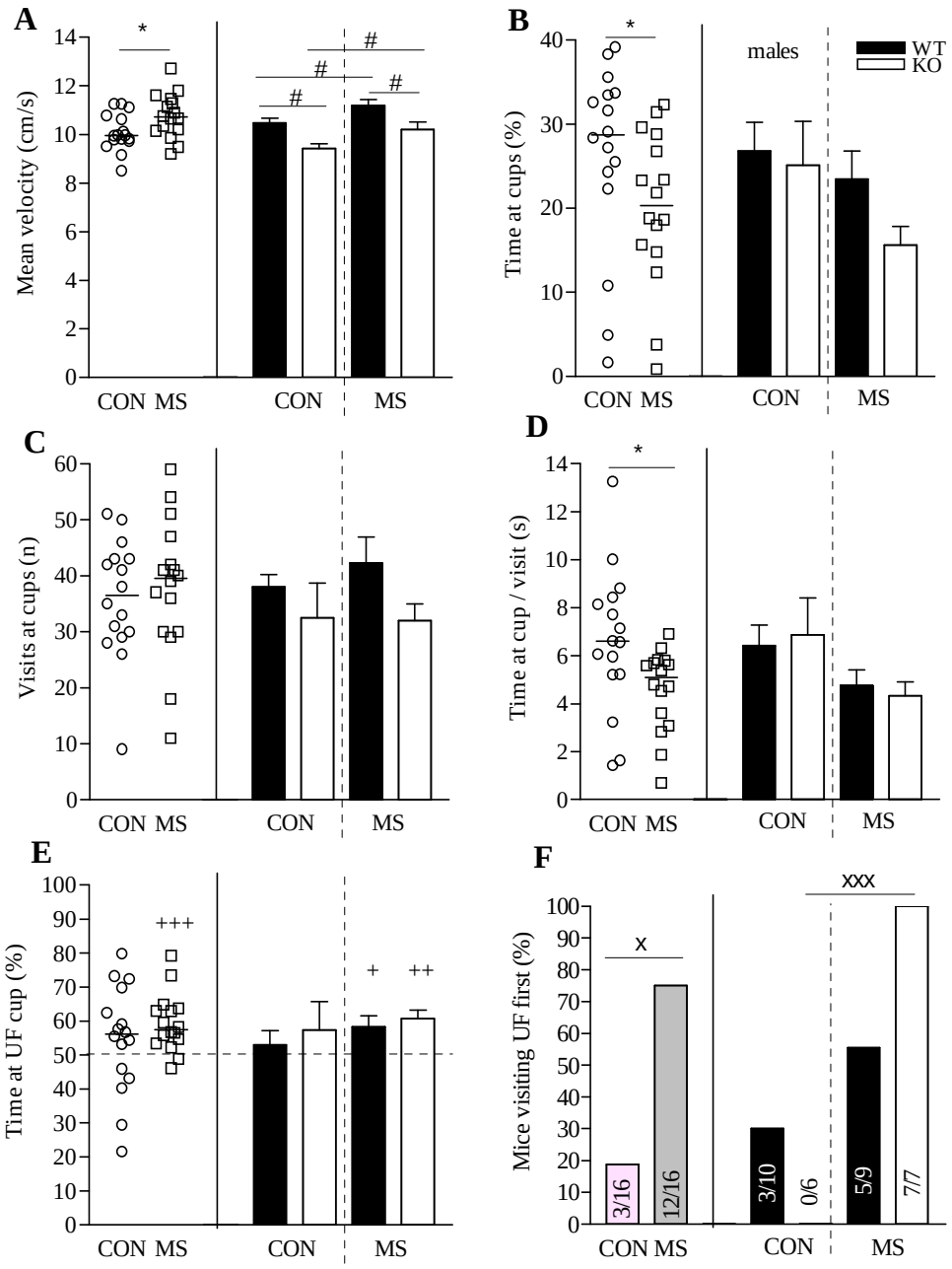

Fig. 26 Performance of WT and KO male mice in the Social preference test*. The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM and percentages for (F). (A) Mean velocity. (B) Percentage of time spent at the cups. (C) Number of visits at the cups. (D) Time spent at a cup per visit. (E) Percentage of time spent at the UF cup (calculated from total time at the cups). (F) Percentage of mice visiting the UF cup first. CON: Control group; MS: Maternal separation group. *, P < 0.05, as analysed with the Mann-Whitney U-test.; # : P < 0.05 as analysed by post-hoc analysis (Newman-Keuls test) after 2-way ANOVA that showed an effect of Treatment. +, ++, +++: P < 0.05, 0.01, 0.001, respectively, comparing values with the chance-level of 50% with the Wilcoxon signed-rank test. x, xxx, P < 0.05, 0.001, respectively, as analyzed with Fisher´s exact.*

Social preference females

Two-way ANOVA having Genotype and Treatment as between factors revealed no effect of MS on the mean velocity or total distance moved. KO females had significantly lower mean velocities compared to WT females ($F_{1,35}$ = 4.49; P < 0.05, effect of Genotype; Fig. 27A). There was no effect of MS on the percentage of time spent at the cups or number of visits to the cups. KO females spent less time at the cups and tended to perform fewer visits compared to WT females ($F_{1,35}$ = 4.70; P < 0.05; $F_{1,35}$ = 3.6; P = 0.07, effect of Genotype Fig. 27B,C). Only the KO CON females spend significantly more time at the UF cup versus F cup (P < 0.01 compared to chance level with Wilcoxon signed–rank test). The other groups showed a nearly balanced allocation (Fig. 27D). There was also no preference for visiting the UF or F cup first in any of the groups. Furthermore, there was no effect of MS on the latencies to contact the cups (F or UF).

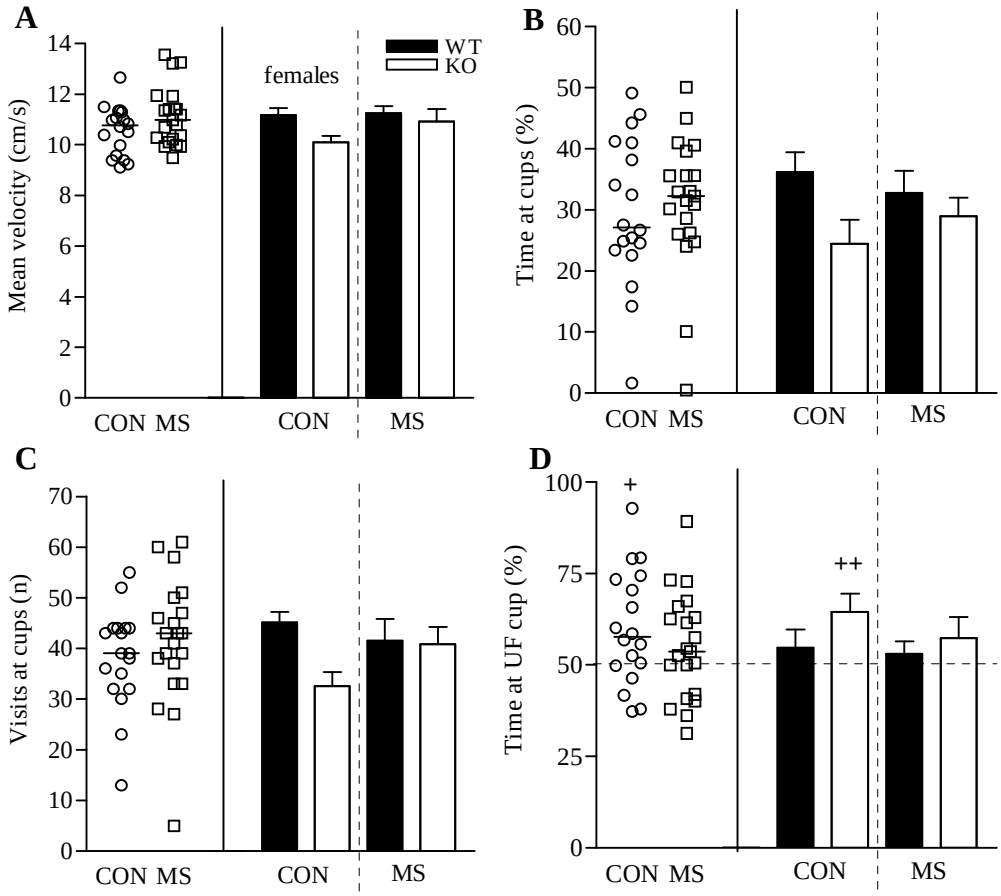

Fig. 27 Performance of WT and KO female mice in the social preference test*. The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A) Mean velocity. (B) Percentage of time spent at the cups. (C) Number of visits at the cups. (D) Percentage of time spent at the UF cup (calculated from total time at the cups). CON: Control group; MS: Maternal separation group. +, ++: P < 0.05, 0.01, respectively, comparing values with the chance-level of 50% with the Wilcoxon signed-rank test.*

Novel Object

MS did not change the behavior of male mice in the novel object test and had only a minor effect on the females. In general all mice did not show relevant amounts of interaction with the new object.

Novel object males

Two-way ANOVA having Genotype and Treatment as between factors revealed no differences between groups concerning latency to perceive the object, % of time spent approaching the object, % of time being opposite the object, latency contacting the object, time spent contacting the object, number of rearing events, number of stretch attend posture (SAP), % of time spent self grooming and % of time spent climbing. In general, all mice showed only very little interaction with the new object. Only 12 out of 32 male mice contacted the new object and none of the mice entered the new object, 2 important parameters that have been observed regularly in former novel object tests.

Novel object females

Two-way ANOVA having Genotype and Treatment as between factors showed that WT females from the MS-group decreased the time spent opposite the object, while KO females showed similar values in both groups (effect of the interaction of Genotype x Treatment: $F_{1,35} = 5.14$; $P < 0.05$ and post-hoc analysis, Fig 28). WT females performed more climbing at the cage in comparison to KO females regardless of the treatment ($F_{1,35} = 5.77$; $P < 0.05$; effect of Genotype). No other investigated parameter uncovered significant differences. As observed for the males, also the females of all groups showed extremely few investigation of the new object, thus only 3 out of 39 females were directly contacting the object.

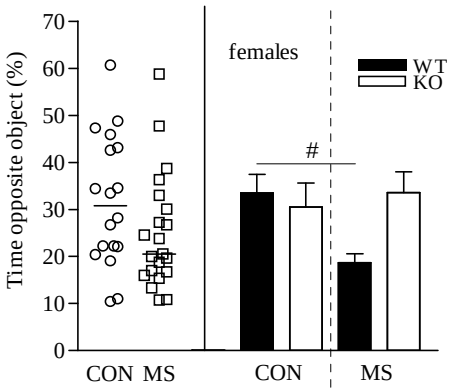

Fig 28. Time spent opposite the object in the novel object test. *The scatter plot represents single values of WT and KO females combined and the median. The columns display mean ± SEM. Percentage of time spent within a 20 cm area opposite the new object. CON: Control; MS: Maternal separation. #: P < 0.05 as analysed by post-hoc analysis (Newman-Keuls test) after 2-way ANOVA that showed an effect of the interaction Genotype x Treatment.*

Urine marking

Maternal separation had no major effect on the behavior of males of both genotypes in the urine marking test.

In 3 cases the females boxes came loose and the females were freely moving in the open field box. Therefore the papers with the urine-marks from these males (all 3 KO from the MS group) had to be excluded from the counting. Analysis of other parameters was performed up to the time the females got free. Counting of urine marks showed that, despite one WT from the MS-group which produced 340 spots, males from all 4 groups performed extremely poor in this test, producing only 20 spots as overall average and 2-way ANOVA revealed no differences between groups. Analysis of the moving pattern revealed that KO males displayed a lower mean velocity and moved lower distances compared to WT males ($F_{1,25} = 7.99$; $P < 0.01$ and $F_{1,25} = 4.7$; $P < 0.05$, respectively, effect of Genotype Fig. 29A). Furthermore KO males spent less time around the females' box compared to WT males ($F_{1,25} = 5.1$; $P < 0.05$, effect of Genotype Fig. 29B).

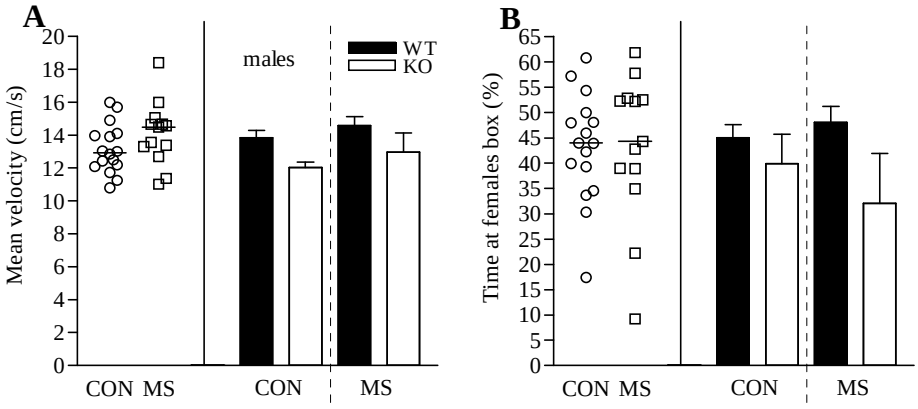

Fig. 29 Performance of WT and KO males in the urine marking test. *The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A) Mean velocity. (B) Percentage of time spent at the females box. CON: Control group; MS: Maternal separation group.*

Resident intruder

Maternal separation had no major effect on the behavior of males of both genotypes in the resident intruder test.

When analyzed for their behavior in the resident intruder test, WT and KO in both groups spent similar amount of time investigating the intruder and showed similar patterns of habituation. The intruders did also not show different investigation of any of the groups. There were also no differences detected concerning the amount of rearing, time spent self-grooming or allogrooming, and time spent digging in the cage. Remarkably none of the residents attacked the intruder, pointing towards very low aggressiveness in this mouse line.

Step through passive avoidance test

MS had no effect on the performance of male mice in the step through paradigm. Females of both genotypes in the MS group displayed higher latencies to step through on day 1.

Step through passive avoidance test males

Two-way ANOVA having Genotype and Treatment as between factors revealed that KO mice had higher latencies to step through the door than WT mice on the first day of acquisition ($F_{1,28}$ = 5.5; P < 0.05; effect of Genotype Fig. 30A). One male in the WT-MS group did not step through (latency 120 s) and was not retested the next day. On the second day, when retention was tested, WT males from the CON group provided the highest amount of mice stepping through (WT-CON: 7/10; KO-CON: 0/6; WT-MS 2/8; KO-MS: 2/7) and a significant difference between WT and KO of the CON group was detected (P < 0.05, Fisher's exact probability test).

Step through passive avoidance test females

Two-way ANOVA having Genotype and Treatment as between factors showed that females in the MS group had higher latencies to step through the door than females in the CON group on the first day of acquisition ($F_{1,35}$ = 4.86; P < 0.05; effect of Treatment Fig. 30B). Two KO females, one from the CON and one from the MS group did not step through and in one case (1 female from the KO CON group) the shock did not work correctly, thus these mice were excluded on day 2. On the second day of retention all 4 groups provided similar fractions of mice stepping through (WT-CON: 3/8; KO-CON: 4/8; WT-MS 5/12; KO-MS: 4/8) with similar latencies.

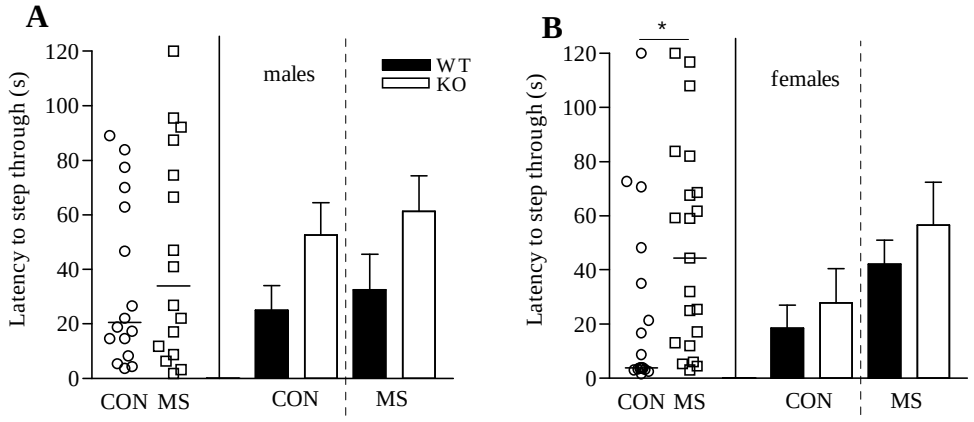

Fig. 30 Performance of WT and KO mice in the step through*. The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A) Latency to step through on day 1 males. (B) Latency to step through on day 1 females. CON: Control group; MS: Maternal separation group. *: P < 0.05, as analyzed with the Mann-Whitney U-test.*

Pole test

KO mice of the MS group tended to have slight, albeit not significant, difficulties to turn at the vertical pole.

Pole test males

In trial 1 males from the MS group displayed the lowest number of mice turning themselves 180° and climb down the rod head foremost (Fig. 31A), although the Fisher's exact probability test did not reveal significant differences between groups (P = 0.1 and P = 0.3 comparing KO-MS with KO-CON and WT-MS respectively). Two-way ANOVA revealed that KO mice started their first attempt to turn later and hence had higher latencies to climb down compared to WT mice ($F_{1,28}$ = 13.79; P < 0.01, $F_{1,28}$ = 14.37; P < 0.001 effect of Genotype for first attempt and latency respectively).

Pole test females

No differences in % of mice turning at the rod between the 4 different groups at any trial were found. Interestingly, when the level of turning was taken into consideration, it was detected that in the first trial a lower proportion of KO females from the MS group were turning on the upper levels 1 or 2 and instead turned at level 3, the one next to the ground (Fig. 31B), thus exhibiting a similar pattern as observed for the males (P = 0.15 and P = 0.12 comparing KO-MS with KO-CON and WT-MS respectively; Fisher's exact probability test). There were no differences detected concerning the duration to climb down or the latency till the first turning attempt was made.

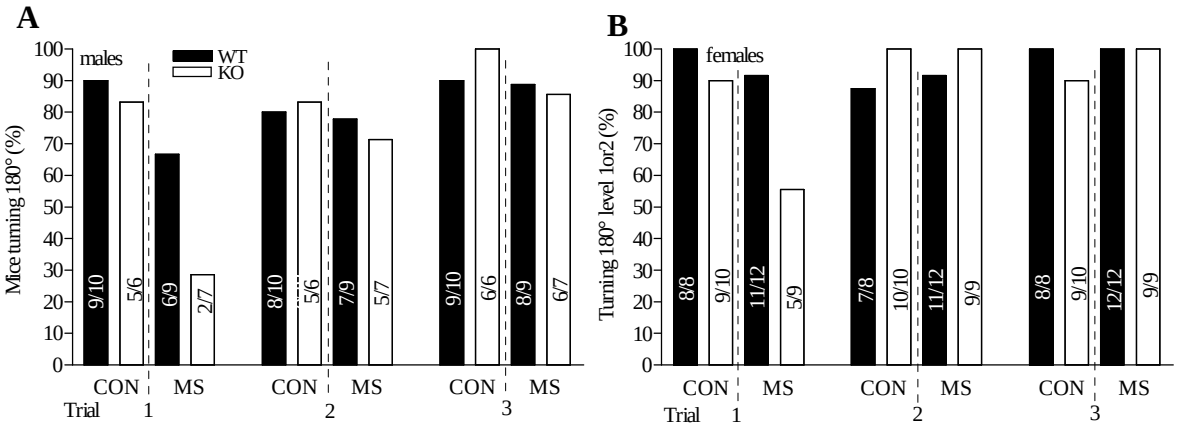

Fig. 31 Performance of WT and KO male and female mice in the pole test. *(A) Percentage of male mice turning 180°. (B) Percentage of female mice turning 180° on level 1 or 2.*

Rotarod

Both genotypes of both genders and in both treatment groups performed likewise in the Rotarod.

Tail suspension

In both genders KO mice spent more time immobile under CON situation, whereas no difference between genotypes was observed in the MS group.

Tail suspension males

Two-way ANOVA having Genotype and Treatment as between factors revealed no effect of MS on time spent immobile. However, an effect of Genotype showed that KO males spent more time immobile than WT males ($F_{1,28} = 6.26$; $P < 0.05$). Post-hoc analysis revealed a significant difference between KO and WT in the CON group ($P < 0.05$), but not in the MS group ($P = 0.57$). Indicating that after MS both genotypes spent a similar amount of time immobile ($F_{1,28} = 2.83$; $P = 0.1$ no effect of the interaction between Genotype x Treatment (Fig. 32A). 1 WT from the CON group managed to climb its own tail.

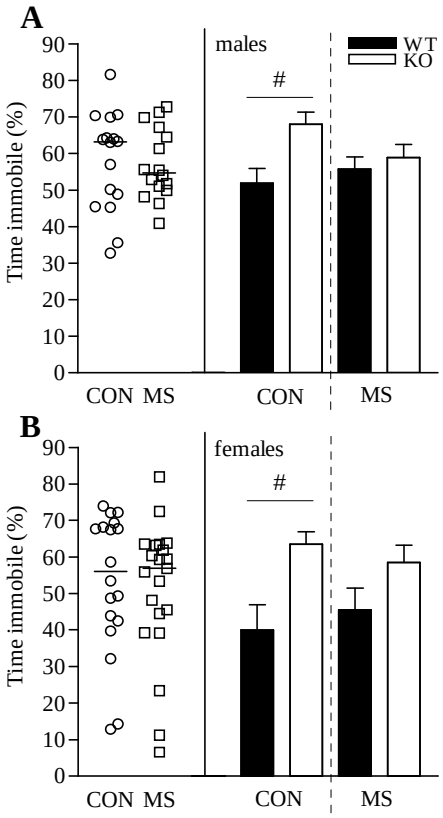

Fig. 32 Time spent immobile during tail suspension. *The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A) Percentage of time spent immobile males. (B) Percentage of time spent immobile females CON: Control; MS: Maternal separation. #: P < 0.05 as analyzed by post-hoc analysis (Newman-Keuls test) after 2-way ANOVA that showed an effect of Genotype.*

Tail suspension females

Two-way ANOVA having Genotype and Treatment as between factors revealed that KO females spent more time immobile than WT females ($F_{1,35} = 10.94$; $P < 0.01$, effect of Genotype). Post-hoc analysis showed a significant difference between KO and WT in the CON group ($P < 0.05$), but not between KO and WT in the MS group (Fig. 32B). Twelve females climbed the own tail during tail suspension, some individuals even up to 20 times during the 6 min. Interestingly 75 % of the WT mice in the CON group and 50 % of the WT mice in the MS group performed climbing, but not a single KO mouse did, resulting in a significant difference between WT-CON and KO-CON ($P < 0.01$) and between WT-MS and KO-MS ($P < 0.05$) as analyzed with Fisher's exact probability test.

Corticosterone

MS had no influence on the plasma corticosterone level of male and female mice of both genotypes.

Corticosterone males

Two-way ANOVA having Genotype and Treatment as between factors revealed no effect of MS on the plasma corticosterone level of KO and WT male mice measured 30 min after the beginning of the tail suspension test. All four groups displayed similar concentrations of plasma corticosterone (Fig. 33A).

Corticosterone females

Two-way ANOVA revealed no effect of MS on the plasma corticosterone level of KO and WT female mice measured 30 minutes after the beginning of the tail suspension test. All four groups displayed similar concentrations of plasma corticosterone (Fig. 33B).

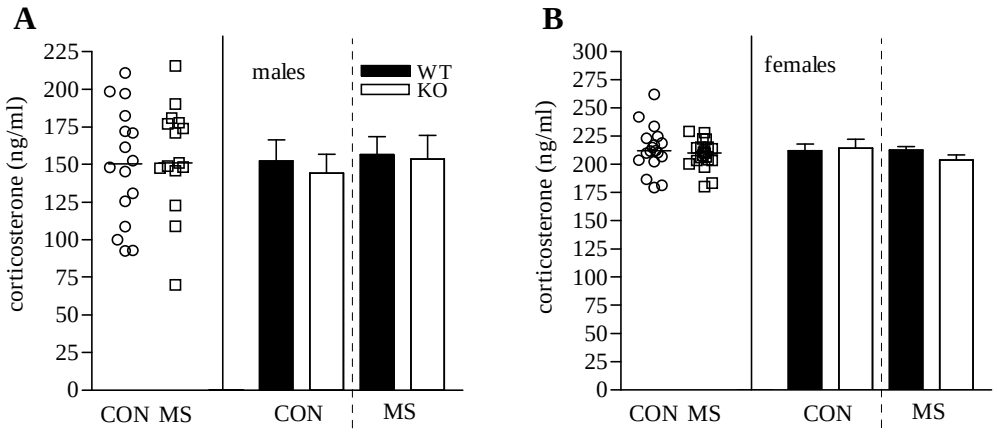

Fig. 33 Plasma corticosterone concentration of male and female mice 30 min after onset of tail suspension. *The scatter plots represent single values of WT and KO combined and the median. The columns display mean ± SEM. (A) Plasma corticosterone concentration (ng/ml) males. (B) Plasma corticosterone concentration (ng/ml) females.*

Summary of the results

Table 8 Effect of MS

exploration + anxiety	males	females
OF time moving (%)	↑	↔
OF total distance moved	↑	↔
OF latency to start rearing	↔	↑
OF rearing (n)	↔	(↓)
EPM closed arm entries (n)	↑	↔
EPM total transitions (n)	(↑)	(↑)
EPM open arm entries (%)	(↓)	↔
EPM rearing (n)	↑	↔
EPM time in center	↔	↓
EPM latency to enter open arm	↔	(↓)
NO time opposite object	↔	↓WT
cognition		
SAT alternation rate	↓KO	↑WT;↓KO
SAY alternation rate	(↓KO)	↓
STPAT latency step day 1	↔	↑
social behavior		
SP mean velocity	↑	↔
SP time at cups	(↓)	↔
SP time at cup per visit	↓	↔
SP preference for UF	↑	↔
motor function		
Pole turning trial 1	((↓KO))	↔
Pole turning 1or 2 trial 1	((↓KO))	((↓KO))

Table 8 Summary of the effects of Maternal separation (MS).
Only parameters where there was an effect in either gender are mentioned. ↔: no alteration; ↑, ↓: significant alteration (P < 0.05); (↑), (↓): tendency of alteration (P < 0.1); ((↑)), ((↓)): tendency of alteration (P < 0.2). WT behind the arrows indicates that the alteration was only observed in wildtyp mice; KO behind the arrow indicates that the alteration was only observed in CHL1 deficient mice. EPM: elevated plus maze; NO: novel object; OF: open field; SAT: spontaneous alternation T form; SAY: spontaneous alternation Y form; SP: social preference; STPAT: step through passive avoidance test; UM: urine marking; UF unfamiliar.

Discussion

This study investigated the influence of early maternal separation (MS) on different aspects of the behavior of WT and CHL1 deficient mice. The MS paradigm was chosen in order to apply a stressor at very early stages of postnatal development. The mouse as an altricial mammal depends crucially on the maternal care; therefore it seems reasonable that a regular disruption of the maternal care may have an influence on the expression of behavior. However, as revealed in the literature, behavioral and endocrinological alterations induced by MS in mice are to some extend inconsistent (Millstein and Holmes 2007). Based on the hypothesis that a synergic interaction between genetic predisposition and environmental insult contribute to the development of schizophrenia in humans (Tandon et al., 2008) we wondered whether deficiency in CHL1 may render the CNS more vulnerable to early adverse stimuli so that, as a consequence, MS may elicit behavioral alterations in CHL1 deficient mice that are not, or only to a minor extent, observed in WT mice.

In this present study a 3 hrs daily MS paradigm from PD 2 to PD 8 was performed. All mice survived the MS without apparent influences on general health or appearance. However MS did influence some aspects of the behavior of male and female mice of both genotypes, as discussed in the following.

Exploration

In both WT and KO mice, MS enhances locomotion in male mice and impulsivity in female mice.

Rodents have an innate curiosity to explore and investigate novel environments and objects. However, confrontation with a new situation can also elicit anxiety-like behavior, which is, to a certain extend, of adaptive value. Displaying behavior that can be interpreted as being cautious or anxious in an unknown situation or environment may protect from possible danger or harm. Thus behavior of mice in the open field, elevated plus maze and novel object depends crucially on the interplay between the drive to explore and the level of anxiety. The present study investigated whether the experience of early maternal separation has an impact on the exploration and anxiety displayed by CHL1 deficient and WT mice in the above mentioned tests.

Increased thigmotaxis and the avoidance of the central region as the presumably most anxiogenic area can be indicative of increased state anxiety. MS had no influence on the pattern of exploration of male mice in the open field, for the amount of thigmotaxis and visits

to the center were not altered. Thus the results propose that MS did not alter the level of anxiety under these specific aspects. Independent of the treatment, it was found that CHL1 deficient males display less thigmotaxis than WT mice, which reproduces the findings reported by Morellini et al. (2007). In the elevated plus maze as a classical test for anxiety behavior in rodents (Belzung and Griebel 2001) the choice between the open and closed arms, emphasizes the ambivalence of exploring a novel unprotected terrain versus being protected within an enclosed location. Indeed, open arm entries and time on the open arm are accepted measures for the anxiety level of rodents (Rodgers and Johnson; 1995). MS had no influence on the time male mice of both genotypes spent on the open arms, in the center, and on the latency to enter the open arms. Thus, the results again lead to the interpretation that MS did not influence the anxiety level of male mice of neither genotype under these specific conditions. On the other hand, an effect of MS in the open field was found regarding the locomotion activity, namely MS male mice increased the time spent moving and accordingly the distance moved in the arena. There where no differences between CHL1 deficient and WT mice detected, indicating that MS affected KO and WT likewise in this parameter. Increased motion in the arena could point to increased exploration, but the amount of rearing, an ethological marker for novelty induced exploratory behavior (Crusio, 2001; Lever et al., 2006), was not affected by the treatment. This raises the question, whether the increase in locomotion was linked to real exploration in terms of gaining information of the new environment, or whether it represents a general non context specific hyperactivity.

As in the open field test, also in the EPM male mice showed increased locomotion as indicated by a tendency towards more total transitions and a significant increase in closed arm entries after MS. The number of total transitions is commonly used to determine the general activity, though the number of closed arm entries offers a more accurate measure, since it is not biased by a decreased number of open arm entries due to an altered anxiety level (Rodgers and Johnson; 1995). The increased closed arm entries performed by males of both genotypes after MS are indicative of increased locomotion. Interestingly, also the number of rearings where elevated after MS, even when the number was corrected by the time spent in the closed arms (rearing is commonly performed only when the mouse is in the closed arms). This could refer to an increase in exploration after MS, unlike in the open field, where the number of rearing events was not altered. As observed also for locomotion in the open field test, MS separation equally enhanced exploratory behavior of both genotypes in the elevated plus maze test. The increase in locomotion after MS was furthermore affirmed in the social preference test, where males of both genotypes increased the mean velocity after MS.

The novel object uses the extensive drive of rodents to explore an unknown object that is inserted into a familiar environment, i.e. the home cage. Unfortunately, on the contrary to what usually observed in WT mice, all male mice showed extremely low investigation and interaction with the new object. In fact, the behavior directed towards the object was reduced to an extend not allowing a reasonable comparison between groups. As it will be discussed in detail in the paragraph about WT mice (see below), it seems that specific behavioral responses usually observed in the mouse (as the investigation of a new object in the home cage) are not expressed in the cohort of mice investigated in this study, possibly as a consequence of epigenetic alterations occurred in the original C57BL/6 mouse line on which the CHL1 had been backcrossed.

In female mice of both genotypes the MS treatment had no effect on the locomotor activity or on the thigmotactic pattern of exploration in the open field, as none of the activity related parameters like time spent moving and velocity, or thigmotactic measures where altered after MS. Solely the rearing behavior was affected such, that after MS the first rearing was performed later, thus after a longer phase of habituation. This is probably indicative of a delayed response to a novel environment. Moreover a tendency to decrease the total amount of rearing was detected. This changes where observed likewise in females of both genotypes and point to an altered exploration after MS. Contrary to what observed in the open field test, in the elevated plus maze the rearing behavior of females was not affected by the MS, whereas the time spent in the center was reduced in comparison to the control group. The center of the EPM is the important area of decision making, where risk assessment is performed usually towards the more anxiogenic unprotected open arms. Mice can distinguish between the open and closed arms and consequently decide to stay protected or to enter an open arm. The reduced time in the center after MS could thus point towards reduced exploration and reduced risk assessment. This would also explain the tendency towards faster entries on the open arms after MS. In this case the faster open arm entries would not be a sign of decreased anxiety, but rather result from an increased impulsivity. Indeed, a high proportion of females in the MS group entered the open arms within the first 5 seconds of the test, which does not leave much time for thorough investigation of the open arms and proper assessment of potential risk. Analogue to the males, the females showed nearly no interaction with the new object, in the NO test, thereby making it impossible to evaluate possible differences (see paragraph about WT mice below).

Summing up the results obtained from the three tests of exploration it can be stated that MS had no influence on the anxiety level of male and female mice of neither genotype under

the tested conditions, but lead to an increased locomotion in males and increased impulsivity in females.

It has been reported that variations in maternal care influence the development of behavioral and endocrine responses to stress in the adult offspring (Levine et al., 1957; Francis and Meaney 1999; Francis et al., 1999). As a consequence, several studies showed that disruption of maternal care via separation can increase the anxiety level of rats (McIntosh et al., 1999; Wigger and Neumann., 1999; Huot et al., 2001; Champagne and Meaney; 2001; Holmes et al., 2005), although negative results have also been reported (Lehmann and Feldon 2000; Pryce et al., 2001). Concordant with the findings that MS has an impact on the anxiety behavior, theories about the underlying mechanisms have been postulated, which can be summarized as follows: MS at early postnatal stages deprives the pups of the otherwise regularly administered maternal care and thereby induces stress at stages when the hypothalamus - pituitary gland - adrenal (HPA) axis, the main system processing stress responses, is thought to be hypoactive. In the absence of the mother the glucocorticoid feedback inhibition of HPA activity of the pups is reduced, leading to a greater HPA response, which, as a consequence, enhances the responsiveness of the HPA axis when confronted to stressors in adulthood. This greater responsiveness of the HPA axis can in turn result in increased anxiety-related behavior (van Oers et al., 1998; Francis and Meaney 1999). An influence of MS on the HPA axis has also been described for mice (Schmidt et al., 2004). And also an increase in anxiety related behaviors of male, but not female mice after MS in the open field and EPM have been reported (Romeo et al., 2003). However, an elaborate study performed by Millstein and Holmes (2007), where the impact of MS on the anxiety-like behavior of five different mouse strains was investigated, led the authors to the conclusion that MS cannot be used as a robust model for increasing anxiety in mice because it produced no clear alterations in the anxiety-related parameters of any of the strains tested. This outcome is in accordance with the results obtained in this present study and can be extended to the CHL1 deficient mice. Interestingly, Millstein and Holmes (2007) also observed an increase in closed arm entries in the EPM after MS in the C57BL/6J strain. The mice used in this present study also have a C57BL/6J background and, as reported by Millstein and Holmes (2007), increased the entries into the closed arm of the EPM when subjected to MS. In the present study, the MS-induced enhanced locomotion in the EPM was confirmed by enhanced locomotion in the open field and social preference tests, strongly suggesting that MS robustly induces hyperactivity in male mice of the C57BL/6J strain. Whereas, in female mice the main effect of MS seems to be a form of more impulsive or disinhibited behavior when confronted

with a new, challenging situation. This is revealed by the reduced time in the center of the EPM and the faster entries to the open arms. Regarding the question weather the CHL1 mice are more vulnerable to the disturbance of MS it can be stated that in the open field, elevated plus maze and novel object test the CHL1 deficient mice displayed the same alterations to a similar extend as observed in WT mice, thus rejecting the hypothesis that the lack of CHL1 predisposes to the effects of MS.

Memory

MS separation decreased working memory performances of male KO mice whereas one-trial learning and long-term memory were unaffected

Exposure to early stressful adverse life events may increase the vulnerability towards the development of neuropsychological disorders. It is known that mental illnesses also influence cognitive abilities (Lewis and Liebermann, 2000). Especially deficits in working memory have been described as one of the core symptoms of schizophrenia (Forbes et al., 2008). In this present study was tested whether maternal separation has an influence on cognitive abilities of WT and CHL1 deficient mice, namely working memory in the spontaneous alternation test and one-trial learning and long term memory in the step-through passive avoidance test.

Rodents tend to visit places they have not explored before, therefore it is to be expected, that mice alternately visit the arms of the T- and Y-mazes in the spontaneous alternation test. In order to alternate, the mouse should remember in which arm it has just been. Therefore, this test is meant to address working memory abilities (Gerlai, 2001). During performance of the T-form of the test it occurred that some mice where distracted by opening and closing of the doors, thus the behavior could have been biased by this variable, a point also discussed by Gerlai (2001). We, thus, decided to perform also a spontaneous alternation test with a Y-maze, similar to the T-maze but with no doors. Analyzing the performance of male mice it was found that only CHL1 deficient males from the MS group failed to reach the criteria for significant alternation, and instead performed an enhanced number of re-entries into the just visited arm in the T- and Y-mazes, although it has to be underlined, that in the Y-maze CHL1 deficient males showed a tendency for alternation. The performance of the females in the T maze is somehow puzzling. Although alike the males, the CHL1 deficient females from the MS group failed to reach significant alternation, also the WT females from the CON group did not alternate. Possibly, female mice were disturbed to a greater extend by the sliding doors than males. Thus the performance of the females was supposedly strongly biased and

does not allow for a conclusion about working memory abilities. Congruent with this idea, female mice alternated in the Y maze. Here females from all four groups displayed alternation, although the MS reduced the alternation rate significantly in females of both genotypes. This suggests that MS had a slightly decreasing effect on the performance of females, suggesting a slight reduction in working memory due to MS.

The step through passive avoidance task is a one-trail learning task that has been suggested to require hippocampus function and that can be applied to test long-term (e.g., 24 hours) memory consolidation and retrieval. In this test, a mouse should associate an unconditioned stimulus (one foot shock) with the act of stepping from a dark starting box into a brightly lit adjacent compartment. When placed into the dark compartment 24 hours after conditioning, a mouse that avoids stepping into the lit compartment is supposed to have learnt the association and to consolidate and recall this information after a long-term interval (McGaugh, 1966). There was no effect of MS on the performance of male mice in the step through passive avoidance test, although under control situation a higher proportion of WT males entered the dark compartment during the recall trial than KO males. Also the memory performance of the females was not influenced by the MS as indicated by similar results for all groups. However it is interesting that during the conditioning trial on day one WT and KO females of the MS group displayed significant higher latencies to step into the dark compartment than control WT and KO females. This holds true for both genotypes. Colorado et al., (2006) used the time spent in the light, versus dark compartment as a measure for impulsivity after MS in rats. Since bright light is supposed to be an anxiogenic stimulus for nocturnal animals, more time in the light compartment was interpreted as an indicator of increased impulsivity. Therefore the longer time in the bright chamber displayed by female mice after MS may add on the finding of increased impulsivity as already concluded from the results of the tests of exploration (see above). Taken together the results suggest that after MS CHL1 deficient male mice showed impairment in the spontaneous alternation paradigm. For female mice a minor reduction of working memory in both genotypes was observed after MS. Long term memory, as evaluated with the passive avoidance test, seems to be unaffected by the MS.

It is known that stress can have an impact on cognitive abilities (Arnsten, 1998). Combining this with the proposed hyper activation of the HPA-axis due to MS as described by Francis and Meaney (1999) (see above), leads to the assumption that MS can also have an influence on the cognitive abilities and hence on memory functions of the separated pups. Still there are only very few studies that focus on the impact of MS on cognitive abilities of rodents. It has been described that early MS in rats leads to cognitive impairments in the

Morris water maze and novel object recognition tests (Aisa et al., 2007). Fabricius et al., (2008) investigated the impact of a 24 hour-long maternal separation at PD 9 on the performance of mice in the Barnes maze and found normal learning during acquisition, but increased perseverance in the reversal phase.

Our data suggest a decline in working memory due to MS specifically in male CHL1 deficient mice, what would be first evidence that under this specific postnatal condition the lack of CHL1 renders male mice more vulnerable towards the negative effects of MS on working memory functions. However, this impairment was only striking in the T maze, since in the Y maze CHL1 deficient males displayed a tendency for alternation after MS. Two hypotheses can be proposed to explain the fact that the performance of CHL1 deficient mice was impaired in the T-maze and almost normal in the Y-maze. The Y-maze was performed, with the intent to reduced possible anxiogenic stimuli intrinsic in the T-maze, namely the constant intervention of the experimenter while moving the sliding doors. Therefore the reduced performance of CHL1 deficient males in the T-maze may indeed be related to altered exploratory behavior and not to impaired working memory of CHL1 deficient mice. Alternatively, it is possible, that the Y-maze is easier to accomplish for mice, since the intervals between single entries are much shorter than for the T-maze, making the test less demanding in terms of memory performance. Moreover, a possible habit of mice to constantly turn to one side (e.g., always turning left) would lead to high frequency of alternations without requiring that mice remember which arm they had just visited, thus, mice could alternate in the Y-maze also with an impaired working memory. Since working memory impairments are typically observed in schizophrenic patients (Lewis and Liebermann, 2000; Manoach, 2003, Forbes et al., 2008), further detailed analysis of the extent and quality of the possible working memory deficits of maternally separated CHL1 deficient mice by means of different tests and paradigms will be of paramount importance.

Social behavior

MS decreased the motivation to investigate social stimuli in both WT and KO mice.

In the wildlife, depending on population density, mice live in social groups of several male and female individuals that share a common territory (Bronson, 1979). Successful group organization and defense of the habitat depends crucially on proper interaction with other conspecifics and on the expression of territorial behaviors (Bronson, 1979). It was investigated whether the experience of early maternal separation has an influence on social interactions of male and female mice in the social preference test. Furthermore a possible

influence of MS on the expression of typical territorial behaviors of male mice was investigated by performing the urine marking and resident intruder tests.

After MS male mice tended to spend less time investigating the cups containing a familiar or unfamiliar conspecific than males from the control group. Moreover a significant decrease in time spent at the cup per visit revealed that after MS males went as often to the cups, but discontinued the investigations faster than males from the control group. This holds true for males of both genotypes, although the effect seems to be more pronounced in the CHL1 deficient compared to WT mice. The decreased time spent at the cups per visit could reflect less interest in the conspecific mice or, congruent with results obtained by other tests, result from increased impulsivity and hyperactivity induced by MS. MS tended to increase the preference for the unfamiliar mouse. This was reflected not only by the prolonged time spent at the unfamiliar versus the familiar cup in the MS group, but also by the fact that MS mice first approached the unfamiliar mice while CON mice first approached the familiar mice as expected for untreated wild type mice (Morellini et al., 2007). This effect of MS was particular evident in CHL1 deficient mice since all KO mice of the CON group first approached the familiar mice, whereas all KO mice of the MS group first approached the unfamiliar mice. Usually, mice tend to first approach familiar individuals and then direct their attention to the unfamiliar subjects and finally show more interest (i.e., enhanced investigation) towards unfamiliar versus familiar mice (Morellini et al., 2007). The increased preference for and the first visit of the unfamiliar after MS could again hint towards decreased inhibition and increased impulsivity. In this context, visiting the unfamiliar mice before the familiar ones could have the same underlying causes as the fast entries on the open arms in the EPM (see above), namely enhanced impulsivity and decreased risk assessment.

No effects of MS were detected in the urine marking and resident intruder tests, suggesting that MS had no influences on the expression of territorial behavior of male mice. However it is important to mention, that males in all four groups (including WT-CON mice) showed very little territorial behavior: None of the males attacked the intruder and amount of urine marking was extremely low, much lower than what expected from male mice of a similar genetic background (Morellini et al., 2007). Thus, a conclusive statement on the impact of MS on this behavior is not possible. This problem, together with the possibly already increased basal anxiety level of the mice is discussed in the paragraph about the behavior of WT mice. For the females no influence of MS on the behavior in the social preference test could be detected for either genotype.

Taken together, male mice of both genotypes showed a slight decrease of social investigation and a slightly changed preference for the unfamiliar versus the familiar conspecific after MS in the social preference test. No influence of MS on the expression of territorial behaviors of males or on the social investigations of females could be detected.

The majority of studies using MS as an early adverse treatment focus on anxiety and depression like behavior and there is paucity of investigations about the influence on social behaviors. Nonetheless, this is an important issue since early life stress is supposed to be a risk factor for altered adult emotionality including impaired social behavior, enhanced aggression and violence (Dodge et al., 1990). Furthermore HPA-axis abnormalities that may result from early life stress have been associated with changes in male aggression and reduced social interactions in rats (Haller et al., 2004). Accordingly it has been reported that MS leads to increased offensive play-fighting and consequently to increased adult male aggression in rats (Veenema et al., 2006; Veenema and Neumann, 2008). Contradictory to the rat Veneema et al., (2007)) did not observe increased aggressiveness after MS in the mouse. Venerosi et al., (2003) observed an enhanced aggressive profile of male mice after MS. Unfortunately, the lack of aggressive interactions displayed by the control WT mice in this present study does not allow the detection of a reduced expression of territorial behavior induced by CHL1 ablation and or MS. Only the results obtained from the social preference point towards a possible decrease in social interactions after MS in male mice of both genotypes, although the behavior of shorter time at the cups per visit could also be a consequence of hyperactivity and enhanced impulsivity as already observed in the tests of exploration.

Motor function

MS impairs coordination in KO mice.

The pole test and Rotarod were performed in order to check for motor function and motor abilities of the mice. There was no effect of MS on the performance of male and female mice of both genotypes in the Rotarod. All mice performed equally well and moreover showed the same improvement over trials. Thus motor function and motor learning does not seem to be influenced by MS. Also analysis of the results from the pole test did not reveal any significant differences between groups. However, although the performance of all mice on trials 2 and 3 was similar, it is striking that after MS only very few CHL1 deficient males where able to turn 180° in trial 1, in contrast to CHL1 deficient males in the CON group or to WT males. Moreover although nearly all females in all four groups managed to turn 180° on the first trial, a smaller proportion of CHL1 deficient females after MS managed to turn on the upper

levels of the rod, but slide down sideways until they managed to turn. This observation suggests that MS causes a slight impairment in fine coordination specifically in CHL1 deficient mice. Apparently there are no reports about the influence of MS on the motor behavior of mice or rats up to date and the results from the present study also suggest that there is no influence of the treatment on the motor function as tested in the Rotarod test. On the other had, it should be underlined that the pole test challenges the coordination skills of mice since they are asked to performed a three-dimensional rotation of the body using fine coordinated movements of the four limbs to which mice are not familiar in their standard housing conditions. Especially the first trial requires a quick perception and analysis of the situation, a high motivation to climb down, a low level of anxiety and fine motor skill (Freitag et al., 2003). Thus, it is not surprising that a motor impairment is detected in the pole but not Rotarod test which, contrary to the pole test, requires that mice use motor skills similar to those used during ambulation. Regarding the impairment of CHL1 deficient mice of the MS group, one may speculate that after MS CHL1 deficient mice are delayed in reacting appropriate to the novel situation, leading to a poorer performance in the first trial of the pole test.

Tail suspension and corticosterone

MS did not affect learnt-helplessness in the tail suspension test, though it tended to decrease the genotype-dependent differences observed in control mice.

Stressful events at early stages are thought to contribute to the development of anxiety disorders and depression (de Wilde et al., 1992; Heim et al., 2008). In the present study it was tested whether MS has an influence on depression like behavior of CHL1 deficient and WT mice in the tail suspension test. Since antidepressants reduce the time mice spend immobile while being suspended at the tail, this parameter is used to assess depressive like behavior in rodents (Cryan et al., 2005).

No effect of MS on the time spent immobile could be detected in male mice, although in the control group CHL1 deficient mice spent significant more time immobile in comparison to WT mice and this difference between CHL1 deficient and WT male mice tended to be attenuated in the MS group. Interestingly, similar results are obtained for the females. In the control group CHL1 deficient female mice spent more time immobile than WT females, whereas this difference tended to be diminished in the MS group. Taken together there was no clear effect of MS on the behavior of mice in the tail suspension test, although the diminished

differences between CHL1 deficient and WT mice after MS point towards a possible effect of MS on the two genotypes in this paradigm.

Anxiety and depression like behavior belong to the most extensive studied effects of MS in rats and mice. This arises from the observed influence of adverse early experiences on the development on anxiety and mood disorders in humans (Heim et al., 2004) and the aim to model this situation in rodents. Although the main tenor seems to be increased depression like behavior after MS, the results are not consistent. Most studies have conducted the forced swim test (FST) where, analogue to the tail suspension, the time spent immobile is interpreted as a sign of despair and hence depressions like behavior. Wigger and Neumann (1999) report no changes in the time rats spent immobile in the FST after MS, whereas other studies report increased immobility after MS (Aisa et al., 2007; Lee at al., 2007). In another study increased immobility was found only after additional chronic stress (Marais et al., 2008). In mice, MacQueen et al., (2003) found increased immobility after MS on a second trial in the FST and Macri and Laviola (2004) found increased immobility after a single 24 hrs separation paradigm. In the comparative study from Millstein and Holmes (2007), none of 5 different mouse strains displayed alterations in the FST after MS. Also this present study did not reveal a clear effect of MS on the behavior in the tail suspension test. Only the convergence of behavior between CHL1 deficient and WT male and female mice after MS may point towards a slight effect that may be diverse in the two genotypes.

Stress-induced plasma corticosterone was not affected by MS.

Activation of the HPA axis ultimately results in secretion of plasma corticosterone, the main glucocorticoid of rodents. Corticosterone acts on metabolism, the cardiovascular system and the nervous system and thereby mediates the physiological responses to acute stress (for review see Lightman, 2008). Since MS has been reported to affect the activity of the HPA axis, we tested whether MS has an influence on the plasma corticosterone concentration 30 minutes after exposure to a stressor, namely the tail suspension test. After the onset of acoustic stress C57BL/6J mice show an increase of plasma corticosterone after 15 minutes, an elevated level after 30 minutes and a return to baseline after 60 minutes (Parfitt et al., 2004). Thus, 30 minutes was considered as a significant time to test for a possibly altered corticosterone secretion due to MS.

CHL1 deficient and WT males in both treatment groups revealed similar levels of corticosterone, thus MS had no effect on the plasma corticosterone level of male mice as measured 30 minutes after onset of the acute stressor (i.e., tail suspension). Also for female mice no differences in plasma corticosterone were detected. Females displayed higher

corticosterone concentrations than males, which is in concordance with the literature (Wigger and Neumann 1999; Desbonnet et al., 2008).

Measurement of corticosterone after MS is frequently accomplished, but the results are inconsistent and sometimes contradictory, which can in part be attributed to differences in methodology (reviewed by Lehmann and Feldon 2000). In rats, higher levels of corticosterone after acute stress in maternal separated pups versus handled or control treated rats have been found (Meaney et al., 1994; Liu et al., 1997; Francis et al., 2002). The same has been reported for mice by Parfitt et al. (2004): handling pups for 15 minutes daily resulted in a blunted corticosterone response to stress, whereas MS mice showed elevated corticosterone response compared to non handled mice. In a sequel study the same authors stated that the effects on corticosterone secretion are not as robust as initially thought since MS during the last 3 hrs of the light phase resulted in blunted stress induced corticosterone concentration (Parfitt et al., 2007). Taken together, effects of maternal separation on corticosterone level seem to be diverse and strongly influenced by methodological differences like frequency, duration and time of separation, type of stress experienced prior to measurement, age and gender of the animals and on the point of time the blood samples are taken. In this present study MS did not have an impact on the plasma corticosterone concentration. Of course a possible influence may have been missed due to the wrong choice of the time interval after the stressor. Thus, it cannot be excluded that MS leads to a prolonged HPA activation, which would lead to the same corticosterone secretion at the peak of activation, but a slower decrease that would lead to higher corticosterone concentrations in MS animals, e.g., 60 min after the stressor. For instance, Parfitt et al., (2004) found significant elevated corticosterone concentration only 60 minutes after the onset of acoustic stress in maternal separated versus control mice. It is also possible, that mice displayed an increased basal corticosterone level that has been masked by the extreme activation of corticosterone due to the stress of tail suspension.

Remarks on the behavior of WT mice

A problem occurring in this study was the altered behavior displayed by WT mice of the control group in several tests when compared to other cohorts of wild type animals previously tested in our laboratory (Freitag et al., 2003; Brandewiede et al., 2005; Morellini and Schachner, 2006; Morellini et al., 2007). For instance in the novel object test all mice showed nearly no interaction with the novel object that was inserted into the home cage. Furthermore in the urine marking test males performed extremely little urine marks in response to the females and moreover did not exhibit any aggressive interaction with a male intruder. These

behavioral responses strongly deviate from the behavior usually observed in laboratory animals of the genetic background used in this study, which is commonly a mix between the 129 and C57BL/6 strains, with a marked predominance of the C57BL/6 genetic background (Freitag et al., 2003; Morellini and Schachner, 2006; Morellini et al., 2007). Indeed, it is common practice to backcross genetic engineered mutant mice with a defined inbred mouse strain in order to gain a uniform genetic background except for the gene of interest. Mice used in this study had six backcrosses to C57Bl/6J mice that where derived from a breeding facility at the UKE. Interestingly the behavior of adult C57BL/6J mice from this animal facility has changed for several parameters over the last years when compared to mice of the same strain and origin tested in the years 2000 to 2002. Changes in behavior started to appear in 2005-2006, one year after this strain has been started to be housed in individually ventilated cages (IVC), whereas mice had been previously housed in normal open cages. Behavioral changes observed include: reduced distance moved and rearing in the open field, reduced time spent on open arms and number of total transitions in the elevated plus maze and enhanced emergence latency in the free choice open field. In addition, the expression of two typical territorial behaviors, aggressive behavior in the resident intruder and urine marking almost disappeared in this strain. Moreover, male mice show low levels of interest and even avoidance towards unfamiliar female mice when tested in the Reeperbahn test (Fabio Morellini, personal communication). It is possible that the IVC racks which prevent air exchange between different cages and quickly remove outgoing air also deprive the mice of olfactory and external acoustic stimuli and thereby reduce their behavioral repertoire. It has been shown that rat mothers deprived of the pups' odor perform less licking of the pups, a maternal behavior that seems to be essential for normal development (Moore and Power 1992; Lenz and Sengelaub 2006). Moreover, female pups that received reduced maternal care will themselves display reduced maternal behavior to their pups (Francis et al., 1999; Champagne and Meaney, 2001). Therefore the deprivation of odor could be an epigenetic disturbance that is transmissible from one generation to the next and consequently may have had also an effect on the behavior of the pups investigated in this study. This could even mean that the pups used in this study have been exposed to reduced maternal care already under standard control conditions when compared to the "normal" amount of maternal care expressed by C67BL/6 mice housed in normal cages. The fact that commonly observed adaptive behavioral responses disappeared in the strain of reference C57BL/6 is of great impact since it may have masked possible effects of the MS as well as possible differences between WT and CHL1 deficient mice. If the mice already display basically a high anxiety

level and reduced social behavior as a trait a further reduction may have been impossible. This was for instance the case of territorial behavior which had been described to be reduced in CHL1 KO mice compared to WT littermates in a cohort from 2005 with 3 backcrosses into C57BL/6 (Morellini et al., 2007): the difference was then reduced but still significant in a study performed in 2006, due to a decreased expression of territorial behavior by WT mice (Fang Kuang, personal communication). Finally, territorial behavior almost completely disappeared in male WT mice in the present study which was performed on the next generation compared to the study in 2006, so that no difference could be detected between WT and KO mice of the control group.

Remarks on the statistics

In this study a total of 71 mice were tested in several behavioral paradigms. Regarding the expenditure of time for the different tests and the tight time schedule, handling of a much larger amount of mice by one experimenter within one project would be getting difficult. However, since mice were assigned to 8 different groups according to their genotype, treatment and gender, resulting sample sizes are only between 6 and 12 mice per group. Sample sizes below 10 are suboptimal for appropriate statistical analysis of behavior though, since statistical tests are less powerful and minor changes, although present, will not be detected. In this present study several examples can be found where the behavior of the distinct groups consistently differed in terms of mean group values but only tendency (P values between 0.05 and 0.1) for a significant effect could be detected. For instance the selective impairment of CHL1 deficient mice after MS in the pole test becomes only significant if males and females are pooled together, thus if sample size is increased, whereas only a tendency can be detected when males and females are analyzed separately. However general grouping of the genders cannot be used to increase the N, since in many parameters behavior of males and females differed. Consequently also observed tendencies should be taken into consideration, when looking for possible influences of MS in this study, but to prove differences, replications of the experiment with an increased sample size will indeed be absolutely necessary.

Concluding remarks

This study was based on the hypothesis that CHL1 deficient mice may be more vulnerable towards postnatal environmental perturbations compared to WT mice. The hypothesis was based on the fact that CHL1 has been related to schizophrenia in humans (Sakurai et al., 2002; Chen et al., 2005) and CHL1 deficient mice showed some behavioral alterations reminiscent

of schizophrenic symptoms (Braff et al., 2001; Irintchev et al., 2004). Since schizophrenia is supposed to result from the interaction between genetic predisposition and environmental insult during perinatal development (Tandon et al., 2008), we tested the effect of maternal separation on CHL1 deficient mice and their WT littermates.

The main effect of maternal separation was a stable induction of hyperactivity in male mice and a more impulsive or disinhibited behavior in female mice of both genotypes. Anxiety like behavior and depression like behavior of WT and CHL1 deficient mice remained largely unaffected by the treatment. However, the possible decline in working memory performance of male CHL1 deficient mice in the spontaneous alternation test is interesting but remains to be validated and further investigated. This result could provide first preliminary evidence that indeed CHL1 deficient mice may be affected to a greater extend by adverse environmental factors and as a consequence, react with alterations that can also be observed in schizophrenic patients.

In the context of the effects of maternal separation on the development of mouse behavior in general, we conclude that early daily maternal separation of 3 hours is not sufficient to induce severe, pathological behavioral alterations in WT and also not in CHL1 deficient mice. Mice appear to be able to compensate to a great extend for the adverse influences of maternal separation, thus a more severe interference or application of additional stress seems to be necessary to elicit greater disturbances. Nevertheless maternal separation stably induced hyperactivity in male mice and increased impulsive behavior in female mice. In the wildlife, these features could turn out to be maladaptive, since they constrain the animal from proper investigation of the environment and may lead to a disadvantageous exposure to possible danger.

General Discussion

In this study two approaches are presented to investigate the influence of a certain molecule on the behavior of mice. In the first part behavioral phenotyping of a tenascin-R deficient mouse has been performed. The outcome indicates that ablation of tenascin-R influences exploratory behavior, anxiety, and motor performance of mice. In the second part the effect of maternal separation on CHL1 deficient mice and their wildtype littermates was investigated. The results show an influence of the early manipulation on the behavior of adult mice of both genotypes.

There is ample evidence that environmental factors have profound effect on the development of behavior. For example intrauterine position, nutrition in utero, stress during pregnancy, maternal attention, endocrine factors, social status, housing conditions, isolation, training history and local environmental effects have been shown to influence the outcome of behavior (McIlwain et al., 2001; Meaney, 2001; Bohannon, 2002; Ryan and Vandenbergh, 2002; Wahlsten et al., 2003; Lathe, 2004). Also in the two present studies environmental factors played a crucial role for the expression of behavior. Thus it was shown that the anxiety level of tenascin-R deficient mice was more strongly influenced by environmental factors as compared to wild-type littermates. This indicates that the lack of tenascin-R does not only have a proximate effect on the behavior of mice. Moreover it seems that the lack of tenascin-R renders mice to be more influenceable by the environment. In the second study was demonstrated that environment, namely during the early postnatal period, influences the behavioral responses of WT and CHL1 deficient mice in adulthood.

The mouse is a highly adaptive animal displaying a great plasticity in the expression of behavior that is not only determined by genetics, but also shaped by environmental factors. This may also be the reason for the great success of the species mice that is found all around the world and in a great diversity of habitats as pointed out by Silver (1995). Finally although the diverse influences of environment complicate behavioral analysis, it also holds the positive feature that the outcome of behavior is not solely dependent on the genetics and that behavioral maladaptions can eventually be influenced positively by environmental alterations.

References

Aisa B, Tordera R, Lasheras B, Del Río J, Ramírez MJ (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology. Apr;32(3):256-66. Epub 2007 Feb 20.

Albelda SM, Buck CA (1990). Integrins and other cell adhesion molecules. FASEB J. Aug;4(11):2868-80.

Angeloni D, Lindor NM, Pack S, Latif F, Wei MH, Lerman MI (1999). CALL gene is haploinsufficient in a 3p- syndrome patient. Am J Med Genet. Oct 29;86(5):482-5.

Apostolova I, Irintchev A, Schachner M (2006). Tenascin-R restricts posttraumatic remodeling of motoneuron innervation and functional recovery after spinal cord injury in adult mice. J Neurosci. Jul 26;26(30):7849-59.

Arnsten AF (1998). The biology of being frazzled. Science. Jun 12;280(5370):1711-2.

Aspberg A, Miura R, Bourdoulous S, Shimonaka M, Heinegârd D, Schachner M, Ruoslahti E, Yamaguchi Y (1997). The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. Proc Natl Acad Sci U S A. Sep 16;94(19):10116-21.

Bardo MT, Donohew RL, Harrington NG (1996). Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res; 77(1-2):23-43.

Bartolomucci A, Palanza P, Gaspani L, Limiroli E, Panerai AE, Ceresini G, Poli MD, Parmigiani S (2001). Social status in mice: behavioral, endocrine and immune changes are context dependent. Physiol Behav; 73(3):401-410.

Bartsch U, Pesheva P, Raff M, Schachner M (1993). Expression of janusin (J1-160/180) in the retina and optic nerve of the developing and adult mouse. Glia. Sep;9(1):57-69.

Becker CG, Becker T, Meyer RL, Schachner M (1999). Tenascin-R inhibits the growth of optic fibers in vitro but is rapidly eliminated during nerve regeneration in the salamander Pleurodeles waltl. J Neurosci. Jan 15;19(2):813-27.

Becker CG, Schweitzer J, Feldner J, Becker T, Schachner M (2003). Tenascin-R as a repellent guidance molecule for developing optic axons in zebrafish. J Neurosci. Jul 16;23(15):6232-7.

Becker CG, Schweitzer J, Feldner J, Schachner M, Becker T (2004). Tenascin-R as a repellent guidance molecule for newly growing and regenerating optic axons in adult zebrafish. Mol Cell Neurosci. Jul;26(3):376-89.

Becker T, Anliker B, Becker CG, Taylor J, Schachner M, Meyer RL, Bartsch U (2000). Tenascin-R inhibits regrowth of optic fibers in vitro and persists in the optic nerve of mice after injury. Glia. Feb 15;29(4):330-46.

Belzung C, Griebel G (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav Brain Res; 125(1-2):141-149.

Bohannon J (2002). Animal models. Can a mouse be standardized? Science. Dec 20;298(5602):2320-1.

Braff DL, Geyer MA, Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl). Jul;156(2-3):234-58.

Brandewiede J, Schachner M, Morellini F (2005). Ethological analysis of the senescence-accelerated P/8 mouse.Behav Brain Res. Mar 7;158(1):109-21.

Brandon EP, Logue SF, Adams MR, Qi M, Sullivan SP, Matsumoto AM, Dorsa DM, Wehner JM, McKnight GS, Idzerda RL (1998). Defective motor behavior and neural gene expression in RIIbeta-protein kinase A mutant mice. J Neurosci; 18(10):3639-3649.

Brenneke F, Bukalo O, Dityatev A, Lie AA (2004). Mice deficient for the extracellular matrix glycoprotein tenascin-r show physiological and structural hallmarks of increased hippocampal excitability, but no increased susceptibility to seizures in the pilocarpine model of epilepsy. Neuroscience.;124(4):841-55.

Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL (1993). Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. J Cell Biol. Jul;122(1):265-78.

Bronson FH (1979). The reproductive ecology of the house mouse. Q Rev Biol. Sep;54(3):265-99.

Brückner G, Brauer K, Härtig W, Wolff JR, Rickmann MJ, Derouiche A, Delpech B, Girard N, Oertel WH, Reichenbach A (1993). Perineuronal nets provide a polyanionic, glia-associated form of microenvironment around certain neurons in many parts of the rat brain. Glia. Jul;8(3):183-200.

Brückner G, Grosche J, Schmidt S, Hartig W, Margolis RU, Delpech B, Seidenbecher CI, Czaniera R, Schachner M (2000). Postnatal development of perineuronal nets in wild-type mice and in a mutant deficient in tenascin-R. J Comp Neurol; 428(4):616-629.

Buhusi M, Midkiff BR, Gates AM, Richter M, Schachner M, Maness PF (2003). Close homolog of L1 is an enhancer of integrin-mediated cell migration. J Biol Chem. Jul 4;278(27):25024-31.

Bukalo O, Schachner M, Dityatev A (2001). Modification of extracellular matrix by enzymatic removal of chondroitin sulfate and by lack of tenascin-R differentially affects several forms of synaptic plasticity in the hippocampus. Neuroscience; 104(2):359-369.

Bukalo O, Schachner M, Dityatev A (2007). Hippocampal metaplasticity induced by deficiency in the extracellular matrix glycoprotein tenascin-R. J Neurosci. May 30;27(22):6019-28.

Burns T, Patrick D (2007). Social functioning as an outcome measure in schizophrenia studies. Acta Psychiatr Scand. Dec;116(6):403-18. Epub 2007 Oct 17.

Cameron NM, Champagne FA, Parent C, Fish EW, Ozaki-Kuroda K, Meaney MJ (2005). The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. Neurosci Biobehav Rev.;29(4-5):843-65.

Capecchi MR (2005). Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. Nat Rev Genet. Jun;6(6):507-12.

Celio MR and Chiquet-Ehrismann R (1993). 'Perineuronal nets' around cortical interneurons expressing parvalbumin are rich in tenascin. Neurosci Lett. Nov 12;162(1-2):137-40.

Celio MR and Rathjen FG (1993). Restrictin occurs in 'perineuronal nets' of the adult brain. *Soc. Neurosci. Abstr.* 19, p. 689.

Celio MR, Blümcke I (1994). Perineuronal nets--a specialized form of extracellular matrix in the adult nervous system. Brain Res Brain Res Rev. Jan;19(1):128-45.

Champagne F, Meaney MJ (2001). Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. Prog Brain Res.;133:287-302.

Chen J, Wu J, Apostolova I, Skup M, Irintchev A, Kügler S, Schachner M (2007). Adeno-associated virus-mediated L1 expression promotes functional recovery after spinal cord injury. Brain. Apr;130(Pt 4):954-69.

Chen S, Mantei N, Dong L, Schachner M (1999). Prevention of neuronal cell death by neural adhesion molecules L1 and CHL1. J Neurobiol. Feb 15;38(3):428-39.

Chen QY, Chen Q, Feng GY, Lindpaintner K, Chen Y, Sun X, Chen Z, Gao Z, Tang J, He L (2005). Case-control association study of the close homologue of L1 (CHL1) gene and schizophrenia in the Chinese population. Schizophr Res. Mar 1;73(2-3):269-74.

Chiquet M, Fambrough DM (1984). Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. J Cell Biol. Jun;98(6):1937-46.

Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T (1986). Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell. Oct 10;47(1):131-9.

Colorado RA, Shumake J, Conejo NM, Gonzalez-Pardo H, Gonzalez-Lima F (2006). Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats.Behav Processes. Jan 10;71(1):51-8.

Crabbe JC, Wahlsten D, Dudek BC (1999). Genetics of mouse behavior: interactions with laboratory environment. Science. Jun 4;284(5420):1670-2.

Crews D (2008). Epigenetics and its implications for behavioral neuroendocrinology. Front Neuroendocrinol. Jun;29(3):344-57.

Crossin KL, Krushel LA (2000). Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily. Dev Dyn. Jun;218(2):260-79.

Crusio WE (2001). Genetic dissection of mouse exploratory behaviour. Behav Brain Res; 125(1-2):127-132.

Cryan JF, Mombereau C, Vassout A (2005). The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev.;29(4-5):571-625.

de Wilde EJ, Kienhorst IC, Diekstra RF, Wolters WH (1992). The relationship between adolescent suicidal behavior and life events in childhood and adolescence. Am J Psychiatry. Jan;149(1):45-51.

Demyanenko GP, Schachner M, Anton E, Schmid R, Feng G, Sanes J, Maness PF (2004). Close homolog of L1 modulates area-specific neuronal positioning and dendrite orientation in the cerebral cortex. Neuron. Oct 28;44(3):423-37.

Desbonnet L, Garrett L, Daly E, McDermott KW, Dinan TG (2008). Sexually dimorphic effects of maternal separation stress on corticotrophin-releasing factor and vasopressin systems in the adult rat brain. Int J Dev Neurosci. May-Jun;26(3-4):259-68.

Dityatev A, Schachner M (2003). Extracellular matrix molecules and synaptic plasticity. Nat Rev Neurosci. Jun;4(6):456-68.

Dodge KA, Bates JE, Pettit GS (1990). Mechanisms in the cycle of violence. Science. Dec 21;250(4988):1678-83.

Edelman GM (1987). CAMs and Igs: cell adhesion and the evolutionary origins of immunity. Immunol Rev. Dec;100:11-45.

Edelman GM, Crossin KL (1991). Cell adhesion molecules: implications for a molecular histology. Annu Rev Biochem.;60:155-90.

Erickson HP (1993). Tenascin-C, tenascin-R and tenascin-X: a family of talented proteins in search of functions. Curr Opin Cell Biol; 5(5):869-876.

Erickson HP (1994). Evolution of the tenascin family--implications for function of the C- terminal fibrinogen-like domain. Perspect Dev Neurobiol; 2(1):9-19.

Fabricius K, Wörtwein G, Pakkenberg B (2008). The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. Brain Struct Funct. Feb;212(5):403-16.

Forbes NF, Carrick LA, McIntosh AM, Lawrie SM (2008). Working memory in schizophrenia: a meta-analysis. Psychol Med. Oct 23:1-17.

ffrench-Constant C, Miller RH, Kruse J, Schachner M, Raff MC (1986). Molecular specialization of astrocyte processes at nodes of Ranvier in rat optic nerve. J Cell Biol; 102(3):844-852.

Francis DD, Meaney MJ (1999). Maternal care and the development of stress responses. Curr Opin Neurobiol. Feb;9(1):128-34.

Francis D, Diorio J, Liu D, Meaney MJ (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science. Nov 5;286(5442):1155-8.

Francis DD, Diorio J, Plotsky PM, Meaney MJ (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci. Sep 15;22(18):7840-3.

Francis DD, Szegda K, Campbell G, Martin WD, Insel TR (2003). Epigenetic sources of behavioral differences in mice. Nat Neurosci. May;6(5):445-6.

Freitag S, Schachner M, Morellini F (2003). Behavioral alterations in mice deficient for the extracellular matrix glycoprotein tenascin-R. Behav Brain Res. 2003 Oct 17;145(1-2):189-207.

Frints SG, Marynen P, Hartmann D, Fryns JP, Steyaert J, Schachner M, Rolf B, Craessaerts K, Snellinx A, Hollanders K, D'Hooge R, De Deyn PP, Froyen G (2003). CALL interrupted in a patient with non-specific mental retardation: gene dosage-dependent alteration of murine brain development and behavior. Hum Mol Genet. Jul 1;12(13):1463-74.

Fuss B, Wintergerst ES, Bartsch U, Schachner M (1993). Molecular characterization and in situ mRNA localization of the neural recognition molecule J1-160/180: a modular structure similar to tenascin. J Cell Biol. 1993 Mar;120(5):1237-49.

Gerlai R (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci; 19(5):177-181.

Gerlai R (2001). Behavioral tests of hippocampal function: simple paradigms complex problems. Behav Brain Res. Nov 1;125(1-2):269-77.

Golgi C (1893). Intorno all`origine del quarto nervo cerebrale e una questione isto-fisiologica che a questo argomento si collega. Rendiconti della Reale accademica dei Lincei (21 maggio), (2): 443-450.

Grant EC, Mackintosh JH (1963). A comparison of the social postures of some common laboratory rodents. Behavior; 21:246-257.

Griebel G, Belzung C, Misslin R, Vogel E (1993). The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs. Behav Pharmacol; 4(6):637-644.

Grumet M, Hoffman S, Crossin KL, Edelman GM (1985). Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. Proc Natl Acad Sci U S A.Dec;82(23):8075-9.

Grumet M, Mauro V, Burgoon MP, Edelman GM, Cunningham BA (1991). Structure of a new nervous system glycoprotein, Nr-CAM, and its relationship to subgroups of neural cell adhesion moleculesJ Cell Biol. Jun;113(6):1399-412.

Guntinas-Lichius O, Angelov DN, Morellini F, Lenzen M, Skouras E, Schachner M, Irintchev A (2005). Opposite impacts of tenascin-C and tenascin-R deficiency in mice on the functional outcome of facial nerve repair. Eur J Neurosci. Nov;22(9):2171-9.

Gurevicius K, Gureviciene I, Valjakka A, Schachner M, Tanila H (2004). Enhanced cortical and hippocampal neuronal excitability in mice deficient in the extracellular matrix glycoprotein tenascin-R. Mol Cell Neurosci. Mar;25(3):515-23.

Hagihara K, Miura R, Kosaki R, Berglund E, Ranscht B, Yamaguchi Y (1999). Immunohistochemical evidence for the brevican-tenascin-R interaction: colocalization in perineuronal nets suggests a physiological role for the interaction in the adult rat brain. J Comp Neurol; 410(2):256-264.

Haller J, Halász J, Mikics E, Kruk MR (2004). Chronic glucocorticoid deficiency-induced abnormal aggression, autonomic hypoarousal, and social deficit in rats. J Neuroendocrinol. Jun;16(6):550-7.

Haunsø A, Ibrahim M, Bartsch U, Letiembre M, Celio MR, Menoud P (2000). Morphology of perineuronal nets in tenascin-R and parvalbumin single and double knockout mice. Brain Res. May 2;864(1):142-5.

Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. Psychoneuroendocrinology. Jul;33(6):693-710.

Hillenbrand R, Molthagen M, Montag D, Schachner M (1999). The close homologue of the neural adhesion molecule L1 (CHL1): patterns of expression and promotion of neurite outgrowth by heterophilic interactions. Eur J Neurosci. Mar;11(3):813-26.

Hofer MA (1975). Studies on how early maternal separation produces behavioral change in young rats. Psychosom Med.. May-Jun;37(3):245-64.

Holm J, Hillenbrand R, Steuber V, Bartsch U, Moos M, Lübbert H, Montag D, Schachner M (1996). Structural features of a close homologue of L1 (CHL1) in the mouse: a new member of the L1 family of neural recognition molecules. Eur J Neurosci. Aug;8(8):1613-29.

Holmes A (2001). Targeted gene mutation approaches to the study of anxiety-like behavior in mice. Neurosci Biobehav Rev; 25(3):261-273.

Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C (2005). Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. Neurosci Biobehav Rev.;29(8):1335-46.

Hortsch M (1996). The L1 family of neural cell adhesion molecules: old proteins performing new tricks. Neuron. Oct;17(4):587-93.

Hortsch M (2000). Structural and functional evolution of the L1 family: are four adhesion molecules better than one? Mol Cell Neurosci. Jan;15(1):1-10.

Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl). Dec;158(4):366-73.

Irintchev A, Koch M, Needham LK, Maness P, Schachner M (2004). Impairment of sensorimotor gating in mice deficient in the cell adhesion molecule L1 or its close homologue, CHL1. Brain Res. Dec 10;1029(1):131-4.

Kruse J, Keilhauer G, Faissner A, Timpl R, Schachner M (1985). The J1 glycoprotein--a novel nervous system cell adhesion molecule of the L2/HNK-1 family. Nature. Jul 11-17;316(6024):146-8.

Lang DM, Monzon-Mayor M, Del Mar Romero-Aleman M, Yanes C, Santos E, Pesheva P (2008). Tenascin- R and axon growth-promoting molecules are up-regulated in the regenerating visual pathway of the lizard (Gallotia galloti). Dev Neurobiol.Jun;68(7):899-916.

Lathe R (2004). The individuality of mice. Genes Brain Behav. Dec;3(6):317-27.

Laviola G, Hannan AJ, Macrì S, Solinas M, Jaber M (2008). Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis.Aug;31(2):159-68.

Lee JH, Kim HJ, Kim JG, Ryu V, Kim BT, Kang DW, Jahng JW (2007). Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation. Neurosci Res. May;58(1):32-9.

Lee S van der, Boot LM (1955). Spontaneous pseudopregnancy in mice. Acta Physiol Pharmacol Neerl; 4:442-444.

Lee S van der, Boot LM (1956). Spontaneous pseudopregnancy in mice II. Acta Physiol Pharmacol Neerl; 5:213-215.

Lehmann J, Feldon J (2000). Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev Neurosci.;11(4):383-408.

Lenz KM, Sengelaub DR (2006). Maternal licking influences dendritic development of motoneurons in a sexually dimorphic neuromuscular system. Brain Res. May 30;1092(1):87-99.

Lever C, Burton S, O'Keefe J (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. Rev Neurosci. ;17(1-2):111-33.

Levine S, Alpert M, Lewis GW (1957). Infantile experience and the maturation of the pituitary adrenal axis. Science. Dec 27;126(3287):1347.

Lewis DA, Lieberman JA (2000). Catching up on schizophrenia: natural history and neurobiology. Neuron 28, 325-334.

Lightman SL (2008). The neuroendocrinology of stress: a never ending story. J Neuroendocrinol. Jun;20(6):880-884.

Lister RG (1990). Ethologically-based animal models of anxiety disorders. Pharmacol Ther; 46(3):321-340.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science. Sep 12;277(5332):1659-62.

Loers G, Schachner M (2007). Recognition molecules and neural repair. J Neurochem. May;101(4):865-82.

Lüthi A, Mohajeri H, Schachner M, Laurent JP (1996). Reduction of hippocampal long-term potentiation in transgenic mice ectopically expressing the neural cell adhesion molecule L1 in astrocytes. J Neurosci Res. Oct 1;46(1):1-6.

Macrì S, Laviola G (2004). Single episode of maternal deprivation and adult depressive profile in mice: interaction with cannabinoid exposure during adolescence. Behav Brain Res. Sep 23;154(1):231-8.

MacQueen GM, Ramakrishnan K, Ratnasingan R, Chen B, Young LT (2003). Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation. Int J Neuropsychopharmacol. Dec;6(4):391-6.

Marais L, van Rensburg SJ, van Zyl JM, Stein DJ, Daniels WM (2008). Maternal separation of rat pups increases the risk of developing depressive-like behavior after subsequent chronic stress by altering corticosterone and neurotrophin levels in the hippocampus. Neurosci Res. May;61(1):106-12.

McGaugh JL (1966). Time-dependent processes in memory storage. Science. Sep 16;153(742):1351-8.

McIlwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R (2001). The use of behavioral test batteries: effects of training history. Physiol Behav. Aug;73(5):705-17.

McIntosh J, Anisman H, Merali Z (1999). Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. Brain Res Dev Brain Res. Mar 12;113(1-2):97-106.

Maness PF, Schachner M (2007). Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nat Neurosci. Jan;10(1):19-26.

Manoach DS (2003). Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr Res.Apr 1;60(2-3):285-98.

Meaney MJ; Tannenbaum B, Francis D, Bhatnagar S, Shanks N, Viau V, O´Donnell D, PLotsky PM (1994). Early environmental programming hypothalamic-pituitary-adrenal responses to stress. Seminars in The Neurosciences, Vol 6, pp 247-259.

Meaney MJ (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci. 2001;24:1161-92.

Metz GA, Schwab ME, Welzl H (2001). The effects of acute and chronic stress on motor and sensory performance in male Lewis rats. Physiol Behav; 72(1-2):29-35.

Milev P, Chiba A, Häring M, Rauvala H, Schachner M, Ranscht B, Margolis RK, Margolis RU (1998). High affinity binding and overlapping localization of neurocan and phosphacan/protein-tyrosine phosphatase-zeta/beta with tenascin-R, amphoterin, and the heparin-binding growth-associated molecule. J Biol Chem. Mar 20;273(12):6998-7005.

Millstein RA, Ralph RJ, Yang RJ, Holmes A (2006). Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. Genes Brain Behav. Jun;5(4):346-54.

Millstein RA, Holmes A (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neurosci Biobehav Rev.;31(1):3-17.

Mittal VA, Ellman LM, Cannon TD (2008). Gene-environment interaction and covariation in schizophrenia: the role of obstetric complications. Schizophr Bull. Nov;34(6):1083-94.

Moffett MC, Vicentic A, Kozel M, Plotsky P, Francis DD, Kuhar MJ (2007). Maternal separation alters drug intake patterns in adulthood in rats. Biochem Pharmacol. Feb 1;73(3):321-30.

Montag-Sallaz M, Schachner M, Montag D (2002). Misguided axonal projections, neural cell adhesion molecule 180 mRNA upregulation, and altered behavior in mice deficient for the close homolog of L1. Mol Cell Biol. Nov;22(22):7967-81.

Moore CL, Power KL (1992). Variation in maternal care and individual differences in play, exploration, and grooming of juvenile Norway rat offspring. Dev Psychobiol. Apr;25(3):165-82.

Morellini F, Schachner M (2006). Enhanced novelty-induced activity, reduced anxiety, delayed resynchronization to daylight reversal and weaker muscle strength in tenascin-C-deficient mice. Eur J Neurosci. Mar;23(5):1255-68.

Morellini F, Lepsveridze E, Kähler B, Dityatev A, Schachner M (2007). Reduced reactivity to novelty, impaired social behavior, and enhanced basal synaptic excitatory activity in perforant path projections to the dentate gyrus in young adult mice deficient in the neural cell adhesion molecule CHL1. Mol Cell Neurosci. Feb;34(2):121-36.

Morgan C, Fisher H (2007). Environment and schizophrenia: environmental factors in schizophrenia: childhood trauma--a critical review. Schizophr Bull. Jan;33(1):3-10.

Morganti MC, Taylor J, Pesheva P, Schachner M (1990). Oligodendrocyte-derived J1-160/180 extracellular matrix glycoproteins are adhesive or repulsive depending on the partner cell type and time of interaction. Exp Neurol. Jul;109(1):98-110.

Nikonenko A, Schmidt S, Skibo G, Bruckner G, Schachner M (2003). Tenascin-R-deficient mice show structural alterations of symmetric perisomatic synapses in the CA1 region of the hippocampus. J Comp Neurol; 456(4):338-349.

Nishimune H, Bernreuther C, Carroll P, Chen S, Schachner M, Henderson CE (2005). Neural adhesion molecules L1 and CHL1 are survival factors for motoneurons. J Neurosci Res. Jun 1;80(5):593-9.

Nörenberg U, Wille H, Wolff JM, Frank R, Rathjen FG (1992). The chicken neural extracellular matrix molecule restrictin: similarity with EGF-, fibronectin type III-, and fibrinogen-like motifs. Neuron. May;8(5):849-63.

Nörenberg U, Hubert M, Brümmendorf T, Tárnok A, Rathjen FG (1995). Characterization of functional domains of the tenascin-R (restrictin) polypeptide: cell attachment site, binding with F11, and enhancement of F11-mediated neurite outgrowth by tenascin-R. J Cell Biol. Jul;130(2):473-84.

Nuechterlein KH, Pashler HE, Subotnik KL (2006). Translating basic attentional paradigms to schizophrenia research: reconsidering the nature of the deficits. Dev Psychopathol. Summer;18(3):831-51.

Parfitt DB, Levin JK, Saltstein KP, Klayman AS, Greer LM, Helmreich DL (2004). Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. Brain Res. Jul 30;1016(1):111-8.

Parfitt DB, Walton JR, Corriveau EA, Helmreich DL (2007). Early life stress effects on adult stress-induced corticosterone secretion and anxiety-like behavior in the C57BL/6 mouse are not as robust as initially thought. Horm Behav. Nov;52(4):417-26.

Pesheva P, Spiess E, Schachner M (1989). J1-160 and J1-180 are oligodendrocyte-secreted nonpermissive substrates for cell adhesion. J Cell Biol.Oct;109(4 Pt 1):1765-78.

Pesheva P, Gennarini G, Goridis C, Schachner M (1993). The F3/11 cell adhesion molecule mediates the repulsion of neurons by the extracellular matrix glycoprotein J1-160/180. Neuron. Jan;10(1):69-82.

Pesheva P, Gloor S, Schachner M, Probstmeier R (1997). Tenascin-R is an intrinsic autocrine factor for oligodendrocyte differentiation and promotes cell adhesion by a sulfatide-mediated mechanism. J Neurosci. Jun 15;17(12):4642-51.

Pesheva P, Probstmeier R, Lang DM, McBride R, Hsu NJ, Gennarini G, Spiess E, Peshev Z (2006). Early coevolution of adhesive but not antiadhesive tenascin-R ligand-receptor pairs in vertebrates: a phylogenetic study. Mol Cell Neurosci. Aug;32(4):366-86.

Pratte M, Rougon G, Schachner M, Jamon M (2003). Mice deficient for the close homologue of the neural adhesion cell L1 (CHL1) display alterations in emotional reactivity and motor coordination. Behav Brain Res. Dec 17;147(1-2):31-9.

Pryce CR, Bettschen D, Bahr NI, Feldon J (2001). Comparison of the effects of infant handling, isolation, and nonhandling on acoustic startle, prepulse inhibition, locomotion, and HPA activity in the adult rat. Behav Neurosci. Feb;115(1):71-83.

Probstmeier R, Stichel CC, Müller HW, Asou H, Pesheva P (2000). Chondroitin sulfates expressed on oligodendrocyte-derived tenascin-R are involved in neural cell recognition. Functional implications during CNS development and regeneration. J Neurosci Res. Apr 1;60(1):21-36.

Probstmeier R, Nellen J, Gloor S, Wernig A, Pesheva P (2001). Tenascin-R is expressed by Schwann cells in the peripheral nervous system. J Neurosci Res; 64(1):70-78.

Rampon C, Tsien JZ (2000). Genetic analysis of learning behavior-induced structural plasticity. Hippocampus.;10(5):605-9.

Rathjen FG, Schachner M (1984). Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. EMBO J. Jan;3(1):1-10.

Rathjen FG, Wolff JM, Chiquet-Ehrismann R (1991). Restrictin: a chick neural extracellular matrix protein involved in cell attachment co-purifies with the cell recognition molecule F11. Development. Sep;113(1):151-64.

Rodgers RJ, Johnson NJ (1995). Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 1; 52(2):297-303.

Rodgers RJ, Dalvi A (1997). Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev; 21(6):801-810.

Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG (2003). Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. Horm Behav. May;43(5):561-7.

Rutishauser U (1993). Adhesion molecules of the nervous system. Curr Opin Neurobiol. Oct;3(5):709-15.

Ryan BC, Vandenbergh JG (2002). Intrauterine position effects. Neurosci Biobehav Rev. Oct;26(6):665-78.

Saghatelyan AK, Gorissen S, Albert M, Hertlein B, Schachner M, Dityatev A (2000). The extracellular matrix molecule tenascin-R and its HNK-1 carbohydrate modulate perisomatic inhibition and long-term potentiation in the CA1 region of the hippocampus. Eur J Neurosci; 12(9):3331-3342.

Saghatelyan AK, Dityatev A, Schmidt S, Schuster T, Bartsch U, Schachner M (2001). Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R. Mol Cell Neurosci; 17(1):226-240.

Saghatelyan A, de Chevigny A, Schachner M, Lledo PM (2004). Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain. Nat Neurosci. Apr;7(4):347-56. Epub 2004 Mar 14.

Sakurai K, Migita O, Toru M, Arinami T (2002). An association between a missense polymorphism in the close homologue of L1 (CHL1, CALL) gene and schizophrenia. Mol Psychiatry;7(4):412-5.

Salzer JL, Colman DR (1998). Mechanisms of cell adhesion in the nervous system: role of the immunoglobulin gene superfamily. Dev Neurosci.;11(6):377-90.

Schachner M (1997).Neural recognition molecules and synaptic plasticity. Curr Opin Cell Biol. Oct;9(5):627-34.

Schmidt M, Enthoven L, van Woezik JH, Levine S, de Kloet ER, Oitzl MS (2004). The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. J Neuroendocrinol. Jan;16(1):52-7.

Schuster T, Krug M, Stalder M, Hackel N, Gerardy-Schahn R, Schachner M (2001). Immunoelectron microscopic localization of the neural recognition molecules L1, NCAM, and its isoform NCAM180, the NCAM-associated polysialic acid, beta1 integrin and the extracellular matrix molecule tenascin-R in synapses of the adult rat hippocampus. J Neurobiol. Nov 5;49(2):142-58.

Shapiro L, Colman DR (1998). Structural biology of cadherins in the nervous system. Curr Opin Neurobiol. Oct;8(5):593-9.

Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992). Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science. 1992 Jul 10;257(5067):206-11.

Silver LM (1995). Mouse Genetic, Concepts and Applications. Oxford University Press, Oxford.

Srinivasan J, Schachner M, Catterall WA (1998). Interaction of voltage-gated sodium channels with the extracellular matrix molecules tenascin-C and tenascin-R. Proc Natl Acad Sci U S A; 95(26):15753-15757.

Syková E, Vorísek I, Mazel T, Antonova T, Schachner M (2005). Reduced extracellular space in the brain of tenascin-R- and HNK-1-sulphotransferase deficient mice. Eur J Neurosci. Oct;22(8):1873-80.

Tacke R, Moos M, Teplow DB, Früh K, Scherer H, Bach A, Schachner M (1987). Identification of cDNA clones of the mouse neural cell adhesion molecule L1. Neurosci Lett. Nov 10;82(1):89-94.

Tandon R, Keshavan MS, Nasrallah HA (2008). Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr Res.Jul;102(1-3):1-18.

Tarantino LM, Gould TJ, Druhan JP, Bucan M (2000). Behavior and mutagenesis screens: the importance of baseline analysis of inbred strains. Mamm Genome; 11(7):555-564.

Taylor J, Pesheva P, Schachner M (1993). Influence of janusin and tenascin on growth cone behavior in vitro. J Neurosci Res. Jul 1;35(4):347-62.

Terranova ML, Laviola G, de Acetis L, Alleva E (1998). A description of the ontogeny of mouse agonistic behavior. J Comp Psychol; 112(1):3-12.

Tucker RP, Drabikowski K, Hess JF, Ferralli J, Chiquet-Ehrismann R, Adams JC (2006). Phylogenetic analysis of the tenascin gene family: evidence of origin early in the chordate lineage. BMC Evol Biol. Aug 7;6:60.

van Oers HJ, de Kloet ER, Li C, Levine S (1998). The ontogeny of glucocorticoid negative feedback: influence of maternal deprivation. Endocrinology. Jun;139(6):2838-46.

Veenema AH, Blume A, Niederle D, Buwalda B, Neumann ID (2006). Effects of early life stress on adult male aggression and hypothalamic vasopressin and serotonin. Eur J Neurosci. Sep;24(6):1711-20.

Veenema AH, Bredewold R, Neumann ID (2007). Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. Psychoneuroendocrinology. Jun;32(5):437-50.

Veenema AH, Neumann ID (2008). Maternal separation enhances offensive play-fighting, basal corticosterone and hypothalamic vasopressin mRNA expression in juvenile male rats. Psychoneuroendocrinology. Dec 2.

Venero C, Tilling T, Hermans-Borgmeyer I, Herrero AI, Schachner M, Sandi C (2004). Water maze learning and forebrain mRNA expression of the neural cell adhesion molecule L1. J Neurosci Res. Jan 15;75(2):172-81.

Venerosi A, Cirulli F, Capone F, Alleva E (2003). Prolonged perinatal AZT administration and early maternal separation: effects on social and emotional behaviour of periadolescent mice. Pharmacol Biochem Behav. Feb;74(3):671-81.

Volkmer H, Hassel B, Wolff JM, Frank R, Rathjen FG (1992).Structure of the axonal surface recognition molecule neurofascin and its relationship to a neural subgroup of the immunoglobulin superfamily.J Cell Biol. Jul;118(1):149-61.

Wahlsten D, Metten P, Phillips TJ, Boehm SL 2nd, Burkhart-Kasch S, Dorow J, Doerksen S, Downing C, Fogarty J, Rodd-Henricks K, Hen R, McKinnon CS, Merrill CM, Nolte C, Schalomon M, Schlumbohm JP, Sibert JR, Wenger CD, Dudek BC, Crabbe JC (2003). Different data from different labs: lessons from studies of gene-environment interaction. J Neurobiol. Jan;54(1):283-311.

Weber P, Montag D, Schachner M, Bernhardt RR (1998). Zebrafish tenascin-W, a new member of the tenascin family. J Neurobiol.Apr;35(1):1- 16.

Weber P, Bartsch U, Rasband MN, Czaniera R, Lang Y, Bluethmann H, Margolis RU, Levinson SR, Shrager P, Montag D, Schachner M (1999). Mice deficient for tenascin-R display alterations of the extracellular matrix and decreased axonal conduction velocities in the CNS. J Neurosci 1999; 19(11):4245-4262.

Whitten WK (1959). Occurrence of anoestrus in mice caged in groups. J Endocrin 18:102-107.

Wigger A, Neumann ID (1999). Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. Physiol Behav. Apr;66(2):293-302.

Wintergerst ES, Fuss B, Bartsch U (1993). Localization of janusin mRNA in the central nervous system of the developing and adult mouse. Eur J Neurosci. Apr 1;5(4):299-310.

Wintergerst ES, Vogt Weisenhorn DM, Rathjen FG, Riederer BM, Lambert S, Celio MR (1996).Temporal and spatial appearance of the membrane cytoskeleton and perineuronal nets in the rat neocortex. Neurosci Lett. May 17;209(3):173-6.

Wintergerst ES, Bartsch U, Batini C, Schachner M (1997). Changes in the expression of the extracellular matrix molecules tenascin-C and tenascin-R after 3-acetylpyridine-induced lesion of the olivocerebellar system of the adult rat. Eur J Neurosci. Mar;9(3):424-34.

Xiao ZC, Taylor J, Montag D, Rougon G, Schachner M (1996). Distinct effects of recombinant tenascin-R domains in neuronal cell functions and identification of the domain interacting with the neuronal recognition molecule F3/11. Eur J Neurosci. Apr;8(4):766-82.

Xiao ZC, Bartsch U, Margolis RK, Rougon G, Montag D, Schachner M (1997). Isolation of a tenascin-R binding protein from mouse brain membranes. A phosphacan-related chondroitin sulfate proteoglycan. J Biol Chem; 272(51):32092-32101.

Xiao ZC, Ragsdale DS, Malhotra JD, Mattei LN, Braun PE, Schachner M, Isom LL (1999). Tenascin-R is a functional modulator of sodium channel beta subunits. J Biol Chem; 274(37):26511-26517.

Yang H, Xiao ZC, Becker B, Hillenbrand R, Rougon G, Schachner M (1999). Role for myelin-associated glycoprotein as a functional tenascin-R receptor. J Neurosci Res. Mar 15;55(6):687-701.

Zacharias U, Rauch U (2006). Competition and cooperation between tenascin-R, lecticans and contactin 1 regulate neurite growth and morphology. J Cell Sci. Aug 15;119(Pt 16):3456-66.

Zamze S, Harvey DJ, Pesheva P, Mattu TS, Schachner M, Dwek RA, Wing DR (1999). Glycosylation of a CNS-specific extracellular matrix glycoprotein, tenascin-R, is dominated by O-linked sialylated glycans and "brain-type" neutral N-glycans. Glycobiology. Aug;9(8):823-31.

Abbreviations

CA	closed arm
CHL1	close homologue of L1
CNS	central nervous system
CON	control group
d	days
DM	distance moved
ECM	extracellular matrix
ELISA	enzyme linked immunosorbent assay
EPM	elevated plus maze
F	familiar
FCOF	free choice open field
FST	forced swim test
HET	heterozygous
HPA	hypothalamic-pituitary-adrenal
ITI	inter-trial interval
IV	interval
kD	kilo Dalton
KO	knockout
LTD	long-term depression
LTP	long-term potentiation
m	months
MDW	mean distance to wall
MS	maternal separation
OA	open arm
OF	open field
PD	postnatal day
PN	perineuronal net
PNS	peripheral nervous system
rpm	rounds per minute
SAP	stretch attend posture
SEM	standard error of the mean
TDM	total distance moved
TN	tenascin
UF	unfamiliar
w	weeks
WT	wildtype

Danksagung

Ich danke Frau Prof. Dr. Melitta Schachner für die Überlassung des Themas und die Möglichkeit die Arbeit am Institut für Biosynthese neuraler Strukturen unter hervorragenden Bedingungen durchführen zu können. Zusätzlich möchte ich mich dafür bedanken, daß Frau Prof. Dr. Schachner mir ganz selbstverständlich die Gelegenheit gegeben hat die Arbeit nach meiner Elternzeit halbtags weiterzuführen.

Mein ganz besonderer Dank gilt Dr. Fabio Morellini. Durch seine Fähigkeit Wissen mit Freude zu vermitteln und durch Geduld und Aufmunterung zur rechten Zeit, war die Betreuung für mich in jeder Hinsicht vortrefflich. Nicht zuletzt konnte ich von dem italienischen Optimismus im hohen Norden nicht nur etwas für das Laborleben lernen.

Mein herzlichster Dank gilt Herrn Prof. Dr. Hans-Joachim Bischof für seine Bereitschaft die externe Betreuung und Begutachtung dieser Arbeit zu übernehmen. Ich habe das als großes Entgegenkommen empfunden. Besonders gefreut habe ich mich über die unkomplizierte Wiederaufnahme nach vier Jahren Pause.

Meinen Kollegen “from the old days” und meinen “neuen” Kollegen danke ich für die freundschaftliche Atmosphäre und die perfekte Zusammenarbeit.
Aus vielen Kollegen sind Freunde geworden. Danke Anja, Annette, Astrid, Bettina, Fabio, Fang, Gila, Ia, Jörg, Jörn, Laetitia, Marius, Meike, Melanie, Mira, Silke und den vielen anderen Laborgefährten.

Ich danke Ali Derin für die Tierpflege und Achim Dahlmann für die Genotypisierung.

Großer Dank gebührt natürlich auch meiner Familie und meinen Freunden.
Meinen Eltern danke ich für ihre unermüdliche Unterstützung und ihr Vertrauen. Ohne die häufige und spontane Kinderbetreuung durch meine Mutter bei Windpocken und sonstigen Unpäßlichkeiten wäre mir die Fertigstellung der Arbeit nicht möglich gewesen.
Meinen Kindern Linda und Justus danke ich, daß sie bereitwillig jeden Tag in ihre Igel- und Pingufantengruppe gegangen sind und mir somit die Arbeit in meiner “Mausgruppe” ermöglicht haben.

Lars, Danke für Deine liebevolle Unterstützung und Zuversicht!

Curriculum vitae

Sandra Freitag (geb. Schmidt)
Geboren am 09.09.1972 in Bielefeld
Verheiratet, 2 Kinder

Bildungsweg:

1982 – 1992	Helmholtzgymnasium Bielefeld, Abschluss Abitur
1992 – 1997	Biologiestudium an der Universität Bielefeld
1997 – 1998	Diplomarbeit am Institut für Biosynthese neuraler Strukturen bei Frau Prof. Dr. Melitta Schachner, Zentrum für molekulare Neurobiologie Hamburg des Universitätskrankenhauses Eppendorf
1998 – 2002	Beginn der Promotion am oben genanntem Institut bei Frau Prof. Dr. Melitta Schachner
2003 – 2007	Elternzeit
2007 – 2009	Fortführung der Promotion

Publikationen:

Brückner G, Grosche J, Schmidt S, Härtig W, Margolis RU, Delpech B, Seidenbecher CI, Czaniera R, Schachner M (2000). Postnatal development of perineuronal nets in wild-type mice and in a mutant deficient in tenascin-R. J Comp Neurol. 2000 Dec 25;428(4):616-29.

Saghatelyan AK, Dityatev A, Schmidt S, Schuster T, Bartsch U, Schachner M (2001). Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R Mol Cell Neurosci. 2001 Jan;17(1):226-40.

Brückner G, Grosche J, Hartlage-Rübsamen M, Schmidt S, Schachner M (2003). Region and lamina-specific distribution of extracellular matrix proteoglycans, hyaluronan and tenascin-R in the mouse hippocampal formation. J Chem Neuroanat. Aug;26(1):37-50.

Nikonenko A, Schmidt S, Skibo G, Brückner G, Schachner M (2003). Tenascin-R-deficient mice show structural alterations of symmetric perisomatic synapses in the CA1 region of the hippocampus. J Comp Neurol. Feb 17;456(4):338-49.

Freitag S, Schachner M, Morellini F (2003). Behavioral alterations in mice deficient for the extracellular matrix glycoprotein tenascin-R. Behav Brain Res. 2003 Oct 17;145(1-2):189-207.

Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Ich habe keine anderen als die angegebenen Hilfsmittel und Quellen benutzt und habe die entnommenen Stellen als solche kenntlich gemacht.

Diese Arbeit ist zuvor keiner Prüfungsbehörde, weder in dieser noch in abgewandelter Form, zum Erwerb des Doktorgrades vorgelegt worden. Auch mit keiner anderen Arbeit habe ich mich zuvor um den Erwerb des Doktorgrades bemüht.

Hamburg, März 2009