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Nogo-A downregulation impairs place avoidance in the Carousel maze but not spatial memory in the Morris water maze

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ABSTRACT

Nogo-A protein is an important inhibitor of axonal growth, which also regulates neuronal plasticity in the CNS. Mutations in the gene encoding Nogo-A or abnormalities in Nogo-A expression are linked to neuropsychiatric disorders such as schizophrenia. The present study assesses the impact of constitutively reduced expression of Nogo-A on place navigation in a novel transgenic rat model. Two spatial paradigms were used: (1) A battery of tests in the Carousel maze requiring continuous processing of spatial information with increasing demands for the segregation of reference frames and behavioral flexibility and (2) a delayed-matching-to-place version of the Morris water maze (MWM), which requires place navigation and is sensitive to deficits in one-trial-encoded place representation. The Carousel maze testing revealed a subtle but significant impairment in management of reference frames. Matching-to-place learning in the Morris water maze was unaffected, suggesting an intact representation of an unmarked goal. Our results show that Nogo-A deficiency leads to cognitive deficit in processing of the reference frames. Such a deficit may be the result of neuro-developmental alterations resulting from Nogo-A deficiency.

1. Introduction

In pathological conditions involving loss of neurons or axonal damage, the very restricted regenerative plasticity of the adult mammalian CNS severely hinders functional recovery. The search for growth-inhibiting factors has led to the discovery of several classes of such inhibitors, including Nogo proteins. The Nogo-A isoform is widely recognized as a major myelin-associated inhibitor of axon growth and regeneration (for recent reviews, see Akbik, Cafferty, & Strittmatter, 2012; Pernet & Schwab, 2012; Schwab, 2010).

Strong inhibition of axonal growth and regeneration can be alleviated by interfering with the Nogo-A signaling cascade, facilitating re-growth of damaged axons and/or compensatory plasticity in spared CNS structures. Very promising results have been reported after the application of Nogo-A specific antibodies, which led to remarkable functional recovery after spinal cord lesion (Liebscher et al., 2005; Schnell & Schwab, 1990) and stroke (Wiessner et al., 2003) in rodent models. These results have been already validated in primate models (Fouad, Klusman, & Schwab, 2004; Freund et al., 2009). Human anti-Nogo-A antibody (ATI-355; Novartis) is undergoing clinical testing in human patients with spinal cord injury (http://ClincalTrials.gov: NCT00406016) (Zörner & Schwab, 2010).

Beside oligodendrocyte expression, Nogo-A is also expressed by neurons (Huber, Weinmann, Brösamle, Oertle, & Schwab, 2002; Josephson, Widenfalk, Widmer, Olson, & Spenger, 2001; Wang et al., 2002), regulating cell migration, axon growth and guidance during development (Petrinovic et al., 2010; Schwab & Schnell, 1991; Wang, Chan, Taylor, & Chan, 2008). After birth, neuronal Nogo-A expression usually decreases, except for neurons retaining a high plasticity of their connections, including hippocampal neurons (Huber et al., 2002; Mingorance et al., 2004). Nogo-A in the hippocampus regulates synaptic plasticity (Delekate, Zagrebelsky, Kramer, Schwab, & Korte, 2011; Lee et al., 2008).

Several studies have implicated Nogo signaling pathways in human psychiatric disorders (Willi & Schwab, 2013). On the genomic level, Nogo-A or NgR chromosomal loci were linked to predispositions to the development of schizophrenia or bipolar disorder (Budel et al., 2008; Hsu et al., 2007; Jitoku et al., 2011; Novak,

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Kim, Seeman, & Tallerico, 2002; Sinibaldi et al., 2004; Tan, Chong, Wang, Chew-Ping Lim, & Teo, 2005; Voineskos, 2009). Behavioral alterations demonstrated in Nogo-A *knockout* mice were proposed as endophenotypes of schizophrenia-like behavior (Willi, Aloy, Yee, Feldon, & Schwab, 2009; Willi et al., 2010). Disruption of the Nogo-A regulatory function in synapse development and their plasticity might offer a link between schizophrenia-like phenotypes in Nogo-A *knockout* mice and human pathology.

The present study explores behavioral effects of reduced Nogo-A protein expression in a transgenic rat model. Rats have been shown to be generally superior to mice as *in vivo* models (Gill, Smith, Wissler, & Kunz, 1989). Furthermore, the *knockdown* model should be more comparable to human pathological conditions (Willi & Schwab, 2013) and does not exhibit compensatory overexpression of Nogo-B (Tews et al., 2013), reported in some KO mice studies (Simonen et al., 2003). The specific aims are to test the effect of Nogo-A deficiency on spatial avoidance in a battery of tasks in the Carousel maze with increasing demands for segregation of reference frames and flexibility, and one-trial learning in the delayed-matching-to-place version of the Morris water maze. We hypothesized that the Nogo-A knockdown resulting in possible neurodevelopmental alterations in the brain could impair spatial frame segregation in the Carousel Maze more severely than the general spatial navigation and memory in the Morris Water Maze or sensorimotor performance, as would be expected after manipulation affecting hippocampal function, which is generally very sensitive to experimental disruption.

2. Materials and methods

2.1. Generation of the transgenic model

Transgenic rats with down-regulated Nogo-A expression (designated SD-Tg(CAG-RNAi: Nogo-A,EGFP)L2ZI, short L2; standing for line 2) were generated in the Central Institute of Mental Health (CIMH, Mannheim, Germany), in cooperation with M. Schwab from the Swiss Federal Institute of Technology (ETH Zürich). The parental subjects were obtained from Charles River, Germany. The target gene expression was reduced using small interfering RNAs (siRNAs) targeting Nogo-A-specific exon 3 of *Rtn4*. Quantification of processed miRNA and analyses of the endogenous mRNA expression in transgenic and WT animals were determined by qPCR using RNA preparations of various brain regions. Protein concentrations of Nogo-A were measured qualitatively and quantitatively by Western blotting and immunohistochemistry employing epifluorescence and confocal microscopy.

The L2 transgenic line with Sprague Dawley (outbred) genetic background showed about a 50% reduction of Nogo-A protein expression in the CNS. The *knockdown* was more prominent in neurons as compared to oligodendrocytes, with neuronal levels reduced to 60% of WT in the hippocampus and to 30% of WT in cortex. Preliminary assessment revealed significant increase in long-term potentiation in both the hippocampus and motor cortex. The L2 rats exhibited prominent schizophrenia-like behavioral phenotypes such as perseveration, disrupted prepulse inhibition and strong withdrawal from social interactions. For detailed description of the model on molecular, neurophysiological and behavioral level, see Tews et al. (2013). We have independently checked that the expression of Nogo-A, at both mRNA and protein level, is decreased in the L2 animals used in this study, by means of rtPCR and Western blotting (Petrasek et al., in preparation).

2.2. Animals

Adult male rats (3–4 months old, weighing 450–550 g) either with Nogo-A *knockdown* (L2) or age-matched but unrelated wild-type Sprague Dawley controls, were used in this study. First group ($n = 20$) was used for the cognitive experiments, beam walking test was done with another group ($n = 18$).

The rats were housed in groups of two or three in standard transparent animal cages ($30 \times 40 \times 30$ cm), and maintained on a regular 12/12 light/dark cycle in an air-conditioned animal room with a stable temperature of 21 °C and humidity (40%). All experiments were performed during the light phase, between 9 a.m. and 4 p.m. The animals had access to food *ad libitum*, except during the Carousel maze training, when they were maintained on 85% of their normal weight by food restriction with daily weighing and monitoring. Water was always freely available. All animal experimentation complied with the Animal Protection Code of the Czech Republic and international guidelines including EU directives (86/609/EEC and 2010/63/EC). Experiments were approved by local Animal Care Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

2.3. Behavioral tasks

2.3.1. Carousel maze testing

The apparatus consisted of a smooth circular metallic arena (82 cm in diameter) surrounded by a 30-cm-high transparent Plexiglas wall, located in the middle of a room with abundance of visual cues. The animals had to avoid a directly imperceptible 60-degree to-be-avoided sector, with each entrance (error) punished by mild electric foot-shocks (Blahna, Svoboda, Telensky, & Klement, 2011; Prokopova et al., 2012; Wesierska, Dockery, & Fenton, 2005). The intensity of current was individually adjusted for each rat to provoke escape reaction, but not freezing, ranging between 0.4 and 0.7 mA (50 Hz). The shock lasted 0.5 s, and was repeated after 1.5 s if the animal did not leave the sector. The task demands could be modified by switching lights on and off, rotating the arena and covering the arena surface by shallow water, enabling manipulation with different sources of environmental information coming from the arena and the room, respectively. Beside avoidance, food-deprived animals (see Section 2.2.) were accommodated to explore the arena homogenously by dropping small food pellets (Nesquik, Nestlé, Czech Republic) on the arena floor at regular, 30-s intervals.

The battery of place avoidance variants was a set of behavioral paradigms performed in the Carousel maze, based on concepts from previous experiments (Abdel Baki, Kao, Kelemen, Fenton, & Bergold, 2009; Burghardt, Park, Hen, & Fenton, 2012; Wesierska et al., 2005). The battery aimed at testing continuous place avoidance efficiency under different conditions, probing various aspects of reference frame segregation (Table 1). The main concept was the dissociation of two spatial reference frames, i.e., arena and room frames. In subsequent stages of the experiment, information from either frame could be either present or hidden, or even made relevant or irrelevant for the task solution; the frames could be continuously dissociated by slow arena rotation.

Initially, four pretraining sessions were given to the rats (10 L2 and 10 wildtype), during which the animals were habituated to the arena and trained to collect Nesquik pellets dropped onto its floor. Five stages of avoidance training followed, each stage consisting of three daily 20-min sessions (separated by 24-h intervals). In the first stage, the rats were required to avoid a stable sector on the stable arena in light (Stage 1, (Room/Arena)+ avoidance). This task, which is essentially a passive place avoidance task (Cimadevilla, Kaminsky, Fenton, & Bures, 2000), has relatively low cognitive demands as both the arena and room frames are stable and yield congruent information (Cimadevilla et al., 2000; Wesierska et al., 2005). Subsequently, avoidance training on the rotating arena in darkness with a to-be-avoided sector defined with respect to the rotating arena surface (Stage 2, Arena + avoidance) tested egocen-

Table 1
Stages of the Carousel maze battery and their cognitive demands.

Stage	Foraging	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Condition		(Room/Arena)+	Arena+	Room+	(Room)+/(Arena)−	(Room)+/(Arena)−
Number of daily sessions	4	3	3	3	3	3
To-be-avoided sector	Absent	Present	Present	Present	Present	Present
Arena	Stable	Stable	Rotating	Rotating	Rotating	Rotating
Conflict of reference frames	Absent	Absent	Absent	Minimized	Present	Present
Arena-frame cues	Present	Present, relevant	Present, relevant	Hidden, irrelevant	Present, irrelevant	Present, irrelevant
Room-frame cues	Present	Present, relevant	Hidden, irrelevant	Present, relevant	Present, relevant	Present, relevant
Notes	No avoidance	Passive avoidance	Passive avoidance, darkness	Active avoidance, water	Active avoidance	Active avoidance, sector position changed

tric memory and passive place avoidance with only one reference frame available (i.e., arena frame). It was followed by testing of avoidance of a stable room-frame defined sector on the lighted rotating arena covered with 1-cm layer of water suppressing the intramaze cues (Stage 3, Room + avoidance), which tests active allocentric avoidance with minimized conflict between frames. Note that the arena frame cues were suppressed by shallow water; however, self-motion information still provided input about the arena frame (Stuchlik, Fenton, & Bures, 2001). This stage was followed by testing in similar settings except without water (rotating dry arena with stable to-be-avoided sector and light on), (Stage 4, (Room) + /(Arena)−). This condition requires selecting the room as the relevant coordinate frame and ignoring the arena frame information (which is present but misleading in this configuration). Finally, testing in the (Room) + /(Arena)− version with position of to-be-avoided sector reversed to opposite room side aimed at assessing both frame segregation and behavioral plasticity (Stage 5; Burghardt et al., 2012).

2.3.2. Water maze testing

The Morris water maze experiments were undertaken in a metallic circular tank (180 cm in diameter, 50 cm high) filled with water (20 °C). An escape platform 10 cm in diameter was placed in the pool, submerged 1.5 cm below the water surface. The Delayed-matching-to-place (DMP) task (O'Carroll et al., 2006; Steele & Morris, 1999) is a test of one-trial-acquired place representation, which permits study of spatial working memory and engram persistence after a single learning episode. The test was administered over daily sessions consisting of four swims (trials) to a hidden platform with variable delays (15 s, 20 and 120 min) between first and second trial (inter-trial interval, ITI). ITIs between trials 2 and 3 and between trials 3 and 4 were kept at 15 s only to maintain win-stay intra-session strategy. Platform position was changed daily in a pseudorandom order. Being essentially a one-trial learning test, the DMP presumes minimal interference between particular sessions (Steele & Morris, 1999); therefore, there were eight daily sessions with unique platform location. ITIs and release positions were pseudo-randomized by partial Latin square method. Testing in the DMP version of the MWM was done after the Carousel maze battery, on the same group of subjects.

2.3.3. Beam walking

The beam walking test is a commonly used test of motor coordination (Goldstein, 1993) and is critically dependent on intact function of the sensorimotor areas. The test used a 2-m-long wooden beam stretched between blinded end and a homecage. Animals initially explored the beam during habituation trials on the 5-cm-wide beam (four habituation trials, animal starting first from ¼, once from ½ and two times from the blinded end; i.e., whole beam had to be traversed in the last case) but soon learned to traverse the beam from the blinded end to the homecage. Two habituation trials (one run from the ½ of the length and one from the blinded end) were repeated on the 1.5 cm-wide beam (narrow beam). Footslips and falls were recorded manually during both habituation periods (wide and narrow beam). Test trials on the narrow beam were conducted immediately after the habituation, and consisted of four testing runs, in which animals were released from the blinded end; latency to reach the homecage, number of falls, and number of footslips were visually recorded by two experimenters, each viewing the animal from an opposite side. Beam walking test in this study was performed with different groups of rats (9 wildtype and 9 L2 rats) than the Carousel maze and DMP experiments. The animals were of the same age and origin.

2.4. Measured parameters and statistical design

2.4.1. Carousel maze

In Carousel maze stages, several measures of performance were assessed. **Total distance** measured overall path walked by a subject during a session and was measured as a sum of linear distances of points selected every 1 s (passive motion introduced by the rotation of the arena in some stages was subtracted by the tracking software). **Number of errors** measured the number of entrances into the to-be-avoided sector during a session. **Maximum time avoided** was defined as the maximal continuous duration without an entry into the sector. Finally, **latency to the first error** measured the time from the beginning of a session to the first entrance into the to-be-avoided sector.

Since distributions of total distance, number of errors and latency to first error were positively skewed, we used logarithmic transformation to reduce the skew. It was possible to avoid the sector during the whole session without an entrance; therefore, we added a constant 1 to number of errors before the transformation. Several data points were missing (about 1.8% of all) due to procedural and technical errors. In order to avoid difficulties caused by these missing data, we averaged data across three sessions for every stage of the Carousel maze. Before doing so, we standardized data for each measure and session to ensure comparability of performance between different sessions of a particular stage. Then, data for all days had the same mean and variance and it was possible to compute average of every measure for each subject and stage. Without the standardization an animal with a missing data point from the first session of the given stage, where performance is generally worse, would seem to have better performance than animals without a missing data point (similar reasoning can be applied for missing data points from the last session of the stage). We averaged only second and third days from each stage for latency to first error, since the animals could not know the position of the sector during the first session of a stage, which made the latency of the first error in the first session essentially random. Next, we standardized resultant measures again to ascertain comparability between stages. This allowed us to analyze differences between

groups across all stages as well as development of differences between groups during consecutive stages. As a result of the aforementioned data transformations, we obtained four measures of performance for five different stages for every subject. Henceforth, we use these transformed measures for statistical analysis. For simplicity, we report the original parameter names in both transformed and raw data. Both raw and transformed data are shown in the figures.

The mixed-design analysis of variance (ANOVA) was conducted for each measure. Group (L2 vs. wildtype) served as a between-subject factor and stage (five stages of Carousel maze ordered according to their succession) as a within-subject factor. The stages were ordered according to their cognitive demands, therefore, we used polynomial contrasts for the stage factor. Third and fourth order polynomials are not readily interpretable, therefore, we report only linear and quadratic contrasts. Since the mean for every stage was zero (in every measure) due to the standardizations, stage was included as a factor only to assess interaction of stage and group factors. Furthermore, for the same reason and because both groups contained the same number of animals, means for both groups had the same value and opposite sign. Therefore, only one of the means is always reported. While group effect can be interpreted as a general difference between groups across all stages, interactions between group and stage factors may suggest variation of group differences between stages.

Previous research found increased perseveration in Nogo-A knockdown rats. Perseveration could have occurred during Stage 5, when the position of the punished sector changed from the previous stage. Therefore, we attempted to test for perseverative behavior during Stage 5 by comparing the time spent in the former to-be-avoided sector (directly opposite to the new to-be-avoided sector) with time spent in "neutral" sectors, which had never been punished. This measure was computed in a similar way as the other measures (i.e. it was standardized for every day, averaged for each animal, and standardized again). Since this measure was computed only for one stage, we obtained one value for each animal. The resultant measure was compared between groups with a Student's *t*-test.

2.4.2. Morris water maze

Performance on the DMP task in the MWM was measured by escape latency and total distance to reach the platform. Both measures can be used for single trials (1–4) and for the calculation of savings between trials. Second trial performance and savings between first and second trial were used as an indicator of one-trial learning. The performance during the first two daily sessions was worse than during remaining days and therefore the data for the first two days (serving essentially for learning the rule) were not included in subsequent analysis. Rats showed good performance at trials 2–4 since the third daily session. Performance measures were averaged for every ITI for each subject. Measures for single trials were logarithmically transformed before averaging in order to reduce positive skew. The averaged measures were used for subsequent computations. Average speed of L2 and control group for the first trial differed; means (M) = 19.8 cm/s and 23.5 cm/s for L2 and control group respectively; $t(18) = 2.99$, $p = 0.008$, however, there was no difference in average speed for trials 2–4; $ts(18) < 0.79$, $ps > 0.49$. Due to the difference in average speed between groups for the first trial and generally small correlations between measures of performance for different trials in the same day, we used only the performance in second trial as an indicator of one-trial learning. Therefore, we conducted mixed-design ANOVA with average *log*-transformed total distance in the second trial as a dependent variable, group (L2 vs. control) as a between-subject factor and delay as a within-subject factor. We used polynomial contrasts for the delay factor.

Apart from analyzing performance, we assessed the proportion of time spent in the 45-deg sector containing the platform during previous day. This measure was used as a measure of perseveration and was computed only for the first trial and averaged across all ITIs. The groups were compared with a Student's *t*-test.

2.4.3. Beam walking

Since latencies to reach the homecage for four trials were positively skewed, we used a logarithmic transformation. We then standardized the transformed measures for each trial and computed mean for every animal across the four trials. We thus obtained one measure assessing latency to reach the homecage for each rat. We standardized the resultant measure for the ease of interpretation. The difference in latency to reach the home-cage for the two groups was compared by a Student's *t*-test. To compare the number of slips and number of falls between the two groups, we summed all slips and falls respectively for each animal across trials. If an animal fell, the number of slips in a given trial was computed as a maximum number of slips obtained by the worst animal (3) plus one, which added to four. The resultant measures were compared with a Mann–Whitney *U*-test.

3. Results

3.1. Carousel maze

Visual inspection of the animals showed no signs of excessive discomfort during the Carousel maze testing. During the habituation phase (d1–d4; see Table 1) rats quickly adopted foraging for Nesquik pellets and after the introduction of the to-be-avoided sector they generally responded to electric foot-shocks with rapid escape reactions. During testing in passive variants of the Carousel maze (the to-be-avoided sector stable relative to the arena floor), the rats performed generally well. However, when a shallow layer of water was added to the arena in Stage 3, some animals from both groups were agitated and exhibited higher locomotion and decreased avoidance. During progressive training in the active versions of Carousel maze, they decreased the number of errors.

Results for total distance (Fig. 1a) showed that L2 rats walked slightly more ($M = 0.26$) than control rats, however, the difference was not significant, $F(1,18) = 2.00$, $p = 0.17$, $\eta^2 = 0.10$. The interactions of stage contrasts and group factor were not significant, $Fs(1,18) < 1.42$, $ps > 0.25$. Results for maximum time avoided (Fig. 1c) showed a significant effect of group, $M_{L2} = -0.29$, $F(1,18) = 5.43$, $p = 0.03$, $\eta^2 = 0.23$. This result reveals general impairment of ability of shock avoidance in L2 rats. While a graphics review indicated a linear increase in difference between groups toward the more demanding stages with L2 rats performing worse during late stages but not during early stages, the interaction of group and linear contrast for stage was not significant $F(1,18) = 1.54$, $p = 0.23$, partial $\eta^2 = 0.08$. Neither was the interaction of group and quadratic contrast for stage, $F(1,18) < 1$. Therefore, statistical analysis did not show differential impairment across stages, even though it was indicated by pattern of group means. Maximum time avoided and number of errors (Fig. 1b) were highly correlated (median correlation for the same stage was −0.86) and therefore it was not surprising that the results of the ANOVA for number of errors were similar. Effect of group on number of errors was marginally significant, $M_{L2} = 0.25$, $F(1,18) = 3.81$, $p = 0.07$, $\eta^2 = 0.17$. Graphics review again suggested a linearly increasing trend for the differences. Again the interaction of group and linear contrast for stage was not significant, $F(1,18) = 2.06$, $p = 0.17$, partial $\eta^2 = 0.10$. The interaction of group and quadratic contrast for stage was also not significant, $F(1,18) < 1$. Finally, an ANOVA for latency to first error (Fig. 1d)

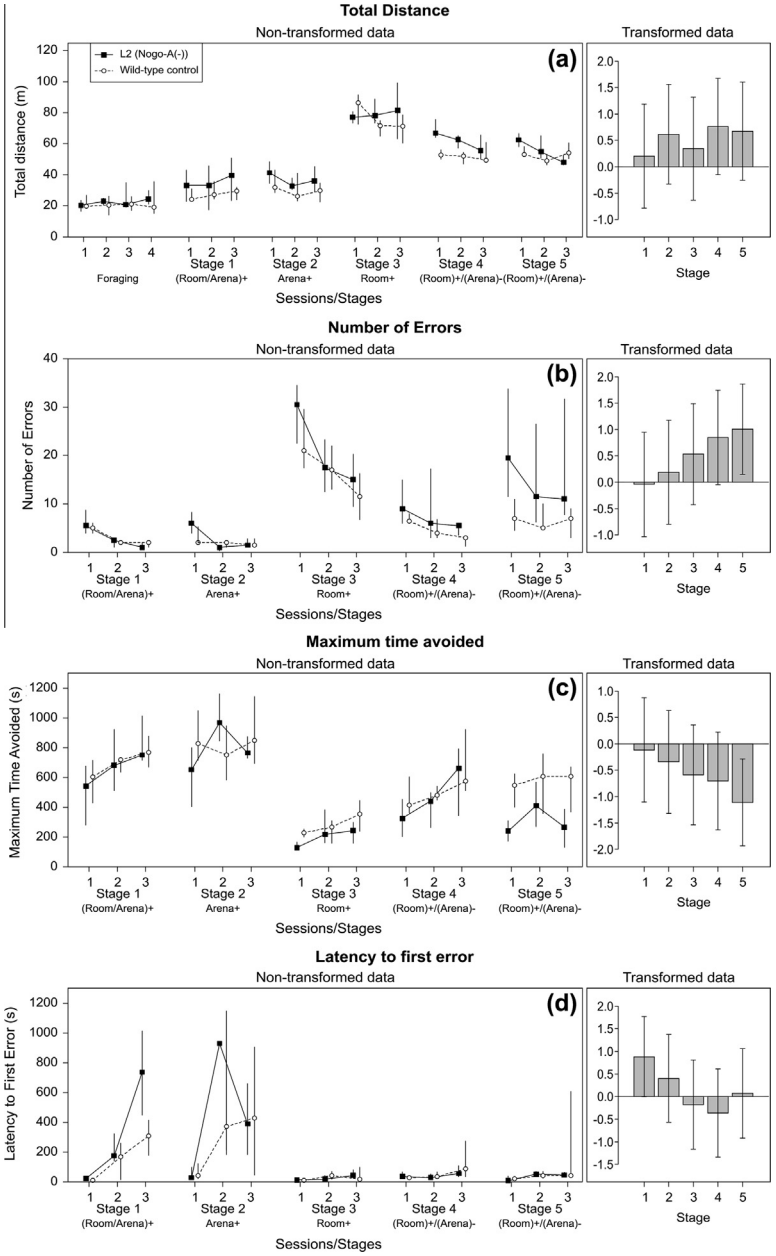

Fig. 1. Carousel maze results. Panels on the left represent daily median values and ends of lines represent first and third quartiles of raw non-transformed data. Note that the quartile values depicted are computed from single sessions. Panels on the right show results for subsequent stages of the task (each stage included three sessions). The plot shows differences in group means ($M_{L2} - M_{Wildtypes}$) and 95% confidence intervals for the differences, all for transformed parameters. Confidence intervals are not adjusted to show between-stage difference in differences of means, therefore, statistical significance only for a difference between groups in a single stage can be properly inferred from confidence intervals (confidence interval, that does not include zero, is equivalent to significant *t*-test for differences between group means for the given stage with $\alpha = 0.05$). Note that ends of ordinate differ between panels (a–d). Total $n = 20$ (10 L2 and 10 control animals). (a) Total distance walked. In Stages 3–5, locomotion increased because of the need to actively avoid the to-be-avoided sector. During Stage 3, the presence of water probably disturbed the animals, leading to higher locomotion. Note the mildly increased locomotor activity apparent in the L2 group during most sessions. (b) Number of errors (entrances into the sector). In stages 1 and 2 (passive place avoidance), the number of errors was rather low. In Stage 3, when active avoidance became necessary, the number of errors sharply increased. Stage 4 introduced the conflict of reference frames, but the sector position remained the same as learned during the Stage 3, thus no increase in number of errors is seen. In Stage 5, the sector position was changed, resulting in a deterioration of performance. L2 animals made marginally significantly more errors ($p = 0.07$) and there was a trend for the aggravation of this deficit towards the last, and presumably most difficult, stages. The trend was, however, not significant, $p = 0.17$. (c) Maximum time avoided. The results generally mirror the Number of Errors, with a drop in performance in Stage 3. In stages 3–5, an immobile rat would enter the to-be-avoided sectors every 60 s, but animals in both groups quickly learned to achieve much longer periods of avoidance. In Stage 3, arena-frame cues (scent marks, feces) were suppressed by shallow water, but a conflict between the relevant room-frame cues and idiothetic information was already present. Removal of water from the arena in Stage 4 made more irrelevant arena-frame cues accessible, which did not notably affect the avoidance of the previously-learned sector position, and the animals continued to improve. In Stage 5, when the sector position was changed, the performance again dropped noticeably. L2 animals avoided for significantly shorter periods of time than the controls ($p = 0.03$), and pattern of group means suggested aggravation of this deficit during the last, and presumably most difficult, stages. The trend was, however, not significant, $p = 0.23$. (d) Latency to first error. In Stages 1 and 2 (passive place avoidance), the latency to first error was high in both groups. In stages 3–5, latency to first error was generally close to the random level of 60 s. Some animals managed to attain non-random performance, which was more common in the controls (notice the lines representing third quartile by the last days of Stage 4 and Stage 5). Results did not reveal general difference between groups.

did not show any difference between groups, $F(1,18) < 1$. However, there was an indication of interaction between group and stage factors, $F(1,18) = 2.63$, $p = 0.12$, partial $\eta^2 = 0.13$ for linear contrast and $F(1,18) = 3.51$, $p = 0.08$, partial $\eta^2 = 0.16$ for quadratic contrast. While L2 rats exhibited higher latency to the first error than control rats in the first two stages of the Carousel maze battery, $M_{L2} = 0.32$, they showed slightly lower latency to the first error in the next two stages, $M_{L2} = -0.14$.

While L_2 rats spent slightly less time in the previously punished sector relative to controls in Stage 5, the difference was not significant, $t(18) = 1.15$, $p = 0.27$, $r = -0.26$, $M_{L2} = -0.25$, which suggests that the groups do not differ in their tendency to perseverate. Perseverative behavior occurred in some individuals only and was not the typical behavioral strategy in either group. No other difference or even a trend was apparent in the data.

To summarize the results, L2 rats performed generally worse than control rats as was shown by differences in maximum time avoided and number of errors. Development of group means in consecutive stages of the Carousel maze suggested worse performance of L2 rats in late stages than in early stages when taken in comparison to control rats. While this pattern was seen in all three parameters measuring performance, it was not statistically significant in any case.

3.2. DMP test in the MWM

Results from the DMP showed a significant linear contrast for delay between first and second trials, with higher delays resulting in greater total distance, $F(1,18) = 7.06$, $p = 0.016$, partial $\eta^2 = 0.28$. This indicates that the rats performed worse when they had to recall the location of the platform after longer delays, as could be expected. No other effect (group, interaction of delay and group, quadratic trend for delay) was significant, all $Fs(1,18) < 1.2$, $ps > 0.29$. A mixed-design ANOVA using *log*-transformed escape latency in the second trial as a dependent variable yielded virtually the same results and therefore is not reported. Additionally, there was no between-group difference in performance during the last two trials. Results of the performed analysis might not show group differences, even if one-trial learning differs between groups, when one group is worse in the first trial and both groups have similar results in the second trial. However, there was no indication for the difference in performance between groups during the first trial. Furthermore, analyses for savings also did not show any significant difference (Fig. 2).

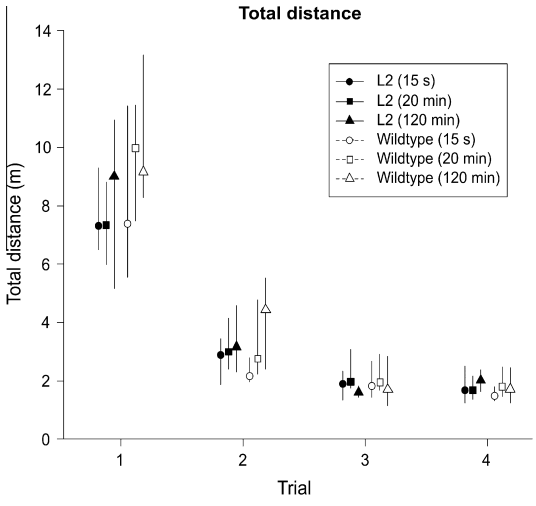

Fig. 2. Water maze results. The graph shows total distance to reach a hidden platform in the delayed-matching-to-place task. Points represent median values and ends of lines represent first and third quartiles of the raw data. All values were computed from performance from 3rd to 8th day and averaged for each animal across a given delay and trial. The performance of L2 and control animals is comparable in all parameters recorded.

There was no sign of difference in proportion of time spent in the sector containing the platform during previous day between the L2 (M = 0.123) and control ($M = 0.134$) group, $t(18) = 0.55$, $p = 0.59$, $r = -0.13$, which shows that the groups did not differ in terms of perseveration.

3.3. Beam walking

The results did not suggest any difference between the L2 ($M = 0.20$) and control ($M = -0.20$) group, $t(16) = 0.83$, $p = 0.42$. Additionally, the groups did not differ in the number of slips (Mann–Whitney $U = 38.5$, $n_{L2} = n_{control} = 9$, $p = 0.89$) or in the number of falls (Mann–Whitney $U = 31.0$, $n_{L2} = n_{control} = 9$, $p = 0.45$).

4. Discussion

Our findings show that the reduced Nogo-A expression indeed affects behavior and cognitive abilities. In the Carousel maze, the L2 rats exhibited consistently and significantly impaired performance, as measured by the number of errors and maximum time avoided. Visual inspection of data for the maximum time avoided, the number of errors and the latency to the first error suggests that the impairment of L2 rats was more accentuated in the later stages, demanding segregation of spatial frames (as is apparent from Fig. 1b, c and d). However, this notion should be taken with caution since the effect did not achieve the traditional level of significance. The latency to the first error did not turn out to be a very reliable measure of cognitive performance. Animals often entered the sector accidentally very early during the session, even in cases when they apparently knew its position well, introducing additional noise into the data. During the first two stages ((Room/Arena)+ and Arena+), when the to-be-avoided sector was stable with respect to the arena floor, the Nogo-A *knockdown* animals tended to exhibit longer latencies to the first error, although the total number of errors in this group was similar to controls or even higher. This may suggest initial attempts to avoid punishment by immobility in L2 rats. During the later three stages, when active avoidance was required, L2 rats showed comparable or even shorter latencies to the first error relative to controls. We must note that for stages 3–5, the median value of latency to the first error generally did not exceed 60 s (one rotation period of the arena).

The animals acquired the procedural aspects of the task and were highly motivated. The eyesight of both groups was good enough, as proven by efficient performance in the MWM, and motor coordination was at a level sufficient to manage beam-walking performance, suggesting that possible procedural influences could be excluded. However, results from Stage 3, when the arena floor was covered with water, should be interpreted with caution, as the animals were disturbed by the presence of water, and even their perception of electric punishment might have been altered. Total distance traveled in the Carousel maze was slightly but consistently increased in the L2 group (Fig. 1a), but the difference was not significant. Total distance in the Carousel maze is probably related mainly to the spatial strategy used by the animals and the stressful nature of the task, although spontaneous hyperlocomotion has been observed in Nogo-A deficient mice by Willi et al. (2009).

Maintaining continuous active avoidance on a rotating arena is a demanding task (Wesierska et al., 2005), requiring not only having intact memory, but also paying sustained attention to distant, room-frame cues and separating them from the irrelevant,

arena-frame cues. To solve this problem, the animals must encode and use two distinct, mutually conflicting representations. This ability has been described as cognitive coordination or cognitive control (Kubik & Fenton, 2005; Lee et al., 2012; Wesierska et al., 2005). Even during the Stage 3, when most of the arena frame cues were hidden, idiothetic input (i.e., path integration) remained in conflict with the distant room frame cues. In Stage 4, even more profound conflict between reference frames was introduced (distal cues vs. path integration + local scent cues). The room-frame defined sector remained in the same position as during the previous stage, enabling the animals to use the previously learned avoidance response. In Stage 5, the sector location was changed, while other conditions remained the same. This shift markedly disrupted performance in both groups, but the L2 group was affected to larger extent, suggesting a deficit in cognitive flexibility. We attempted to test for perseverative behavior during Stage 5 of the Carousel Maze training. The results do not suggest perseveration as the prevailing behavioral mode in either group, and the difference between L2 and control animals was not significant. Therefore, we must assume that the deficit in L2 animals comprised primarily of an inability to deal with the newly defined sector, rather than persisting avoidance of the old and no longer reinforced one.

In the DMP test in the MWM, rats from both L2 and wildtype groups were able to locate the learned hidden platform position and performed well, showing their capability to use distant landmarks for representation of the hidden goal position. This suggests that the ability of both groups to acquire a one-trial place representation was comparable, as well as memory persistence, even after the longest delay tested (120 min). Similarly as in Stage 5 of the Carousel maze, we did not observe any sign of perseveration in L2 rats in the DMP task.

The beam walking test suggested that the Nogo-A deficiency did not adversely affect locomotor coordination, which is in accordance with the literature describing Nogo *knockout* mice. No effect of Nogo-A absence on performance in the rotarod test was found by Kim, Li, GrandPre, Qiu, and Strittmatter (2003) and Willi et al. (2009) reported even improved motor coordination in Nogo-A *knockout* mice.

We interpret our results as a mild cognitive deficit in L2 rats compared to wildtypes in continuous spatial avoidance in the Carousel maze battery. The observed pattern suggests that it included, but was not limited to, impaired cognitive coordination and flexibility, while spatial navigation and memory (assessed by the MWM) was spared. Impaired cognitive coordination is characteristic for schizophrenic patients (Phillips & Silverstein, 2003) and has been reported in animal models of the disease (Lee et al., 2012). Cognitive coordination assessed by Carousel maze tasks depends strongly on an intact hippocampus (Wesierska et al., 2005) and is more sensitive to hippocampal lesions than the MWM (Kubik & Fenton, 2005). Inflexibility and impairments in reversal learning are also typical for schizophrenia (Lee et al., 2012) and hippocampal lesion models (Kimble & Kimble, 1965). We propose that at least some effects observed in the Carousel maze in the L2 rats might be attributed to dysfunction of the hippocampus, a structure characterized by prominent expression of the Nogo-A, and may belong to a wider complex of schizophrenia-like endophenotypes reported in Nogo-A deficient rodents by Tews et al. (2013), Willi et al. (2010).

On the cellular level, the decrease in Nogo-A expression was demonstrated (1) to facilitate hippocampal and neocortical long-term potentiation (LTP) (Delekate et al., 2011), the neurophysiological substrate of memory trace formation including spatial learning (Pastalkova et al., 2006; Serrano et al., 2008), and (2) to modulate other forms of synaptic plasticity within the hippocampus (Zagrebelsky, Schweigreiter, Bandtlow, Schwab, & Korte, 2010). Complementing this result on the behavioral level, enhanced Nogo signaling (by means of increased NgR1 expression) was found to impair lasting long-term memory (after retention times of 30 and 60 days, but not one day or less) in both passive avoidance and the MWM (Karlen et al., 2009). Correlation between cognitive decline and increase in Nogo-A expression in the hippocampus was found in aged rats by Vanguilder et al. (2011). One would, thus, expect the L2 rats to exhibit facilitated, rather than impaired, learning and memory, but the opposite is true. It is conceivable that the normal level of Nogo-A dependent signaling in the hippocampus is fine-tuned to provide optimal levels of synaptic plasticity, and an artificial increase, as well as decrease, may lead to compromised hippocampal function.

In the literature, studies of the consequences of altered Nogo-A signaling on cognitive functions are rather sparse. Willi et al. (2010) demonstrated that Nogo-A *knockout* mice exhibited perseveration behavior during reversal learning in a water T-maze, while acquisition was not affected, suggesting specific impairment of behavioral flexibility. This parallels the findings of Tews et al. (2013). A previous study by Willi et al. (2009) reported intact learning in the reference-memory version of the MWM, even after reversal (changed platform position). Out results revealed perseveration neither in Carousel maze, nor in DMP test in MWM. This might suggest that the manifestation of the cognitive flexibility impairment is dependent either on the animal model or the behavioral paradigm used or both factors. A working memory deficit, typical for schizophrenia, was described previously in mice with Nogo receptor deletion in a delayed alternation task (Budel et al., 2008). Interestingly, the L2 rats have demonstrated a deficit in short-term object recognition and object relocation memory, tested in spontaneous object-exploration paradigms (Tews et al., 2013). On the other hand, our results obtained using the DMP test do not show any impairment in spatial working memory. It must be emphasized that the MWM testing in general involves high levels of motivation (Morris, 1981). Therefore, the working memory capabilities in this model may depend upon experiment configuration and level of motivation.

To sum up, Nogo-A *knockdown* results in a selective cognitive impairment, which is not apparent in the DMP version of the Water Maze, focused on precise place representation and spatial working memory, but expresses itself in the Carousel maze battery, where on-line management of spatial reference frames is required. This supports the hypothesis linking Nogo-A-dependent signalization disruption with neuropsychiatric symptoms and cognitive disorders.

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