Brain Research Bulletin 87 (2012) 383–386

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Spontaneous alternation and spatial learning in *Dab1*^{scm} (*scrambler*) mutant mice

C. Jacquelin[a], C. Strazielle[a], R. Lalonde[b,*]

[a] *Université Henri Poincaré, Nancy I, Laboratoire de Nutrition Génétique et Exposition aux Risques Environnementaux, INSERM U954, Service de Microscopie Electronique, Faculté de Médecine, 54500 Vandoeuvre-les-Nancy, France*
[b] *Université de Rouen, Faculté des Sciences, Dépt. Psychologie, 76821 Mont-Saint-Aignan Cedex, France*

ARTICLE INFO

Article history:
Received 13 September 2011
Received in revised form
20 December 2011
Accepted 1 January 2012
Available online 8 January 2012

Keywords:
Reelin
Cerebellum
Hippocampus
Spontaneous alternation
Morris water maze
Spatial learning

ABSTRACT

Homozygous *Dab1*^{scm} mutants with cell ectopias in cerebellar cortex, hippocampus, and neocortex were compared with non-ataxic heterozygous and wild-type controls in spontaneous alternation and Morris water maze tests. Although there were no group differences in alternation rates, wild-type and heterozygote groups alternated above chance levels, whereas homozygous *Dab1*^{scm} mutants did not. In the Morris water maze, *Dab1*^{scm} mutants were impaired in both hidden and visible platform subtests. The deficits in spontaneous alternation and water maze measures reproduce the phenotype previously described in *Reln*$^{rl-Orl}$ mutants, attributed to disturbance of the same molecular pathway involving reelin.

Reelin is disrupted in the spontaneous autosomal recessive *reeler* mutation of the *Reln* gene, encoding an extracellular matrix protein involved in neural adhesion and migration [7,9,20], of which two alleles exist: Edinburg (*Reln*$^{rl-Ed}$) and Orleans (*Reln*$^{rl-Orl}$) [7]. In each case, *reeler* mutants display architectonic disorganization and cell ectopias in cerebellum, inferior olive, hippocampus, and neocortex, but with preserved anatomical connections between these regions and others [3,8,14,15,21,31,42,43]. In the cerebellum, granule cells are the most severely depleted, Purkinje cells to a lesser extent [21], along with the inferior olive [2,41]. Despite cell ectopias, the zonal pattern of climbing fiber projections to cerebellum is maintained [3], though with multiple as opposed to the normal monoinnervation of Purkinje cells [31,32].

The spontaneous autosomal recessive *scrambler* mutation of the *Dab1* gene situated on chromosome 4 causes a deficiency of the gene product, disabled-1, involved in reelin signaling [38,44]. As a result, homozygous *Dab1*^{scm} mutants possess a loss-of-function *reeler*-like phenotype with cell malpositioning in cerebellar cortex, hippocampus, and neocortex [18,38,40,44,46]. The same cell ectopias occur in the *Dab1* knockout [12,22]. Like *Reln*^{rl} [30], and despite normal *Reln*^{rl} mRNA levels [17], Purkinje and granule cell degeneration in *Dab1*^{scm} mutants results in ataxia and deficits in motor coordination [28], indicating that disabled-1 acts downstream of reelin. Despite Purkinje cell degeneration, the deep cerebellar nuclei appear intact [6]. The mutant Purkinje cells are not rescued by wild-type Purkinje cells in *Dab1*^{scm} chimeras [47].

In addition to ataxia and motor deficits, *Reln*^{rl} mutants were deficient in two spatial-related tasks: spontaneous alternation and the hidden platform version of the Morris water maze [4,30]. Low spontaneous alternation rates may be due to poor short-term spatial retention or to altered inhibitory responses, as the animal perseverates arm choices in contrast to the normal rodent response of exploring a novel maze arm [27]. Moreover, *Reln*$^{rl-Orl}$ mutants were impaired in the visible platform subtest of the Morris maze, often described as a visuomotor deficit, whereby the animal has a difficulty in coordinating whole-body movements towards a specific target. In the present study, we examined whether *Dab1*^{scm} mutants display the same phenotype as *Reln*^{rl} in these two tests. Since *Reln* and *Dab1* are involved in the same molecular pathway, we expected *Dab1*^{scm} mutants to be deficient relative to heterozygotes and wild-type controls in both.

1. Materials and methods

1.1. Mice

Dab1^{scm}/+ breeders on the A/A (agouti) genetic background were purchased from Jackson Laboratory (Bar Harbor, Maine, USA) and crossed to obtain ataxic *Dab1*^{scm}/*Dab1*^{scm} mutants (n = 9, 4 males, 5 females), non-ataxic *Dab1*^{scm}/+ heterozygotes (n = 6, 2 males, 4 females), and +/+ wild-type (n = 7, 3 males, 4 females), determined by genetic history. To facilitate survival of mutant homozygotes, food pellets were spread on the cage floor and the overhead bin. The mice were tested at

* Corresponding author. Tel.: +33 02 35 14 61 08; fax: +33 02 35 14 63 49.
E-mail address: robert.lalonde@univ-rouen.fr (R. Lalonde).

0361-9230/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2012.01.001

Table 1
Cumulative spontaneous alternation rate (%) and choice latencies (s) over 5 or 10 days in *Dab1*^{scm}, heterozygous, and wild-type mice.

Days and measures	Statistical parameters	Wild-type	Heterozygous	*Dab1*^{scm}
First 5 days				
Alternations (%)	Mean ± SEM	60 ± 8**	70 ± 9*	54 ± 8
	Median (range)	60 (20–80)	80 (40–100)	40 (20–80)
Latencies (s)	Mean ± SEM	35 ± 9	45 ± 17	48 ± 8
Full 10 days				
Alternations (%)	Mean ± SEM	63 ± 6	72 ± 6**	58 ± 6
	Median (range)	50 (50–90)	80 (50–90)	60 (30–80)
Latencies (s)	Mean ± SEM	66 ± 17	76 ± 19	101 ± 14

* $p = 0.05$ vs chance level, Mann–Whitney U test.
** $p < 0.05$ vs chance level, Mann–Whitney U test.

about 10 months of age (range: 8–12 months) in a protocol adhering to guidelines of the European Council Directive (86/609/EEC) and animal care regulations at the local university.

1.2. Methods and procedures

Spontaneous alternation was tested in a T-maze made of plywood, containing a central stem and 2 side-arms, 30 cm in length and 10 cm in width, surrounded by 20 cm-high walls. Naive mice with no previous experience in the maze but accustomed to handling were used. On the initial trial, the mice were placed in the stem with the right arm blocked by a plastic barrier (forced choice). After entering the left arm (4-paw criterion), the mice were kept inside for 1 min, retrieved, and placed back in the stem for a free-choice trial. On the following 9 days, the same procedure was repeated, except that the blocked arm on the initial trial was changed alternatively from right to left each day. The number of alternations and the latencies before responding were measured in 1-min trials. To obtain a response after the cut-off period had expired, the mice were briefly prodded from behind, usually not more than once and only when situated far from the choice-point.

The Morris water maze consisted of a basin (diameter: 86 cm, wall height: 30 cm) made of white plastic and filled with water (22 °C) at a height of 21 cm. Yellow plastic beads were evenly spread over the water surface to camouflage the escape platform (diameter: 8 cm) made of white plastic and covered with a yellow wiremesh grid to ensure a firm grip. The pool was contained in a room with several extramaze visual cues, such as light fixtures and laboratory instruments. The mice were placed next to and facing the wall successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NW quadrant. An experimenter followed their swimming trajectories on a videomonitor, on which the image of the pool was separated into 4 equally spaced quadrants. The quadrant entries (4-paw criterion) and escape latencies were measured in 4-trial sessions for 5 consecutive days with a 15-min intertrial interval. The mice remained on the escape platform for at least 5 s. Whenever the mice failed to reach the platform within the 1 min cut-off period, they were retrieved from the pool and placed on it for 5 s. After their swim, the mice were kept dry in a plastic holding cage filled with paper towels. The day after the acquisition phase, a probe trial was conducted by removing the platform and placing the mouse next to and facing the N side. The time spent in the previously correct quadrant was measured in a single 1-min trial. One hour later, the visible platform version was evaluated, with the escape platform lifted 1 cm above water level and shifted to the SE quadrant. A 13-cm high pole was inserted on top of the escape platform as a viewing aid. As with the place learning task, quadrant entries and escape latencies were measured for 4 trials, the animals stayed on the platform for 5 s, and a 1-min cut-off period was imposed with a 15-min intertrial interval, except that the test was conducted in a single session.

1.3. Statistical analyses

Intergroup differences were estimated by analyses of variance (ANOVAs) and Fisher's least significant difference test. For spontaneous alternation and probe tests, the groups were compared with the Kruskal–Wallis test and each group was compared by the Mann–Whitney U test to a theoretical group performing at chance (50% for alternations, 15/60 s for probe) with zero variance.

2. Results

Although there were no intergroup differences in spontaneous alternation rates [Krusal–Wallis, $p > 0.05$], the more sensitive method of comparing each group to chance reached significance. Indeed, during the first 5 days of spontaneous alternation (Table 1), wild-type [$U = 7$, $p < 0.05$] and heterozygote [$U = 5$, $p = 0.05$] mice alternated above chance, whereas *Dab1*^{scm} mutants did not [$U = 36$, $p > 0.05$]. This pattern held up for all 10 days of testing in heterozygotes [$U = 3$, $p < 0.05$] and *Dab1*^{scm} mutants [$U = 27$, $p > 0.05$], but wild-type mice wound up only at borderline significance [$U = 14$, $p = 0.06$]. In contrast, there was no intergroup difference in choice latencies at either the midway point or throughout the testing period [$p > 0.05$].

During acquisition of the hidden platform version of the Morris water maze (Fig. 1), there was a main group effect [quadrants: $F_{2,19} = 60.43$, $p < 0.001$; latencies: $F_{2,19} = 76.13$, $p < 0.001$], but no day effect [quadrants: $F_{4,76} = 0.43$, $p > 0.05$; latencies: $F_{4,76} = 0.29$, $p > 0.05$] or group × day interaction [quadrants: $F_{4,76} = 0.93$, $p > 0.05$; latencies: $F_{4,76} = 0.88$, $p > 0.05$]. Despite the relatively poor performance of both control groups as manifested by the lack of a day effect, *Dab1*^{scm} mutants had higher quadrant entries and escape

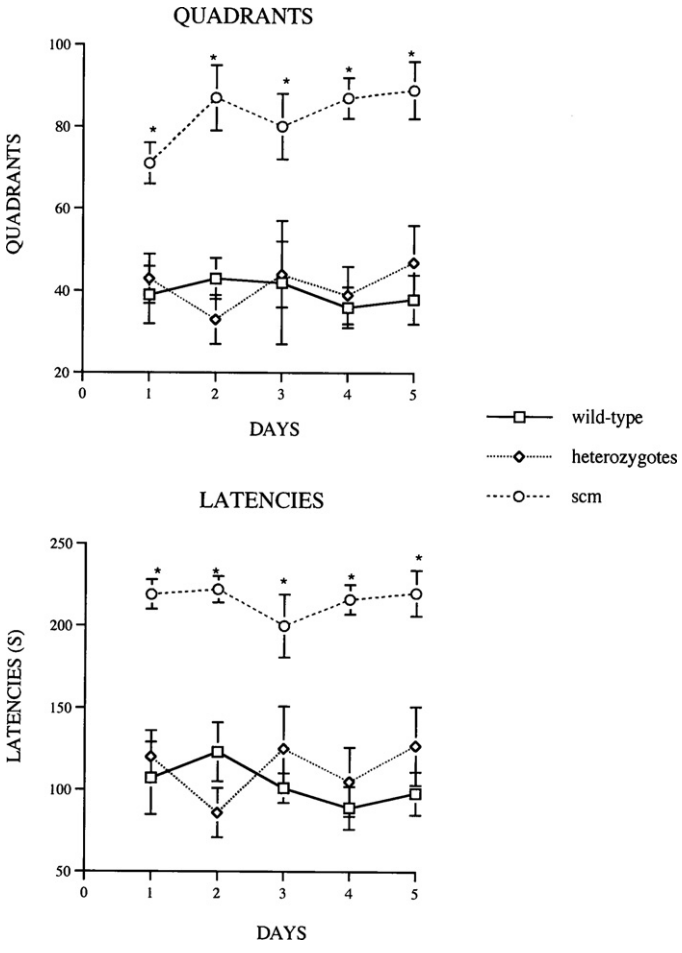

Fig. 1. Hidden platform acquisition in the Morris water maze as measured by quadrant entries and escape latencies (s); cumulative score over 4 trials during 5 days of training in *Dab1*^{scm}, heterozygous, and wild-type mice (means ± SEM). *$p < 0.01$, *Dab1*^{scm} mutants vs either heterozygotes or wild-type.