nature neuroscience

Spatial navigation impairment in mice lacking cerebellar LTD: a motor adaptation deficit?

Eric Burguière[1], Angelo Arleo[1,4], Mohammad reza Hojjati[2,3], Ype Elgersma[2], Chris I De Zeeuw[2], Alain Berthoz[1] & Laure Rondi-Reig[1]

L7-PKCI transgenic mice, which lack parallel fiber–Purkinje cell long-term depression (LTD), were tested with two different mazes to dissociate the relative importance of declarative and procedural components of spatial navigation. We show that L7-PKCI mice are deficient in acquisition of an adapted goal-oriented behavior, part of the procedural component of the task. This supports the hypothesis that cerebellar LTD may subserve a general sensorimotor adaptation process shared by motor and spatial learning functions.

Spatial navigation offers a suitable framework to study the ability of animals to adapt their behavior to a specific context, defined here as the combination of the multimodal information sensed by the animal and its internal state at a specific time. Spatial navigation requires at least two complementary processes: (i) the development of a spatial representation of the environment (declarative component), enabling the animal to encode the spatiotemporal relationships among environmental cues or events, and (ii) the acquisition of a motor behavior adapted to the context in which navigation takes place (procedural component), permitting the execution of optimal (direct) trajectories toward rewarding locations[1]. Testing of several cerebellar animal models in spatial navigation tasks[2] (**Supplementary Note**) has suggested that the cerebellum has a role in mediating the procedural component of spatial navigation. In this study, we focused on the cellular mechanisms subserving the contribution of the cerebellum in spatial learning. Our working hypothesis is that cerebellar LTD that occurs at the parallel fiber–Purkinje cell synapses and is required for the acquisition of classical conditioning tasks[3] may also be necessary for the acquisition of efficient trajectories toward a spatial goal through a basic and common process of sensorimotor adaptation.

We used the L7-PKCI transgenic mice model[4], which allows specific inactivation of parallel fiber–Purkinje cell LTD, to investigate the potential role of this cellular mechanism during spatial navigation (**Supplementary Methods**; all experiments were performed in compliance with European Union Council animal ethics guidelines). L7-PKCI mutants are known to have intact motor capabilities and normal electrophysiological properties of Purkinje cells[5,6] (**Supplementary Note**). Likewise, we did not observe any abnormalities in the sensorimotor reflexes, physical characteristics or general behavior of L7-PKCI mice (**Supplementary Table 1**). Hippocampal functions (synaptic transmission and plasticity), known to be essential for navigation tasks, were normal in L7-PKCI mice (**Supplementary Fig. 1**).

In order to dissociate the relative importance of the declarative and procedural components of navigation, we used two different behavioral tasks: the Morris water maze and a new task called the 'starmaze' (**Fig. 1**). In both cases, the animal has to find a fixed hidden platform from random departure locations, which requires the declarative capability to learn a spatial representation of the environment. Yet, in contrast to the Morris water maze task, the starmaze allows the animal to swim only within alleys guiding its movement. This helps the animal to execute goal-directed trajectories effectively, and reduces the procedural demand of the task.

To compare the navigation performances of L7-PKCI mice ($n = 14$) and their control littermates ($n = 15$) when solving the hidden-platform version of the Morris water maze, we used three standard parameters: (i) the mean escape latency, which measures the time the animal takes to reach the target and estimates its ability to learn the navigation task; (ii) the search score, which describes the goal-oriented trajectory quantitatively[7], and (iii) circling behavior, defined as the time spent in a 10-cm annulus near the wall of the pool, already interpreted as a deficit in the procedural component of the spatial task[8].

Both the mean escape latency and the search score of L7-PKCI mice were significantly higher than those in wild-type mice (ANOVA, $F_{1,27} = 14.2$, $P < 0.001$; and ANOVA, $F_{1,27} = 19.3$, $P < 0.001$; **Fig. 2a**). The

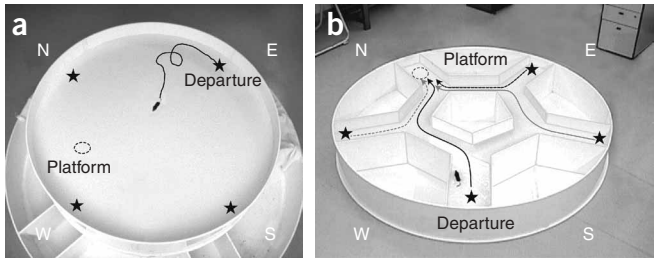

Figure 1 Comparison of two spatial navigation tasks. Both the Morris water maze (**a**) and the allocentric version of the starmaze task (**b**) require mice to find an escape platform (dashed circle) submerged under opaque water. To locate the platform efficiently, animals have to use the configuration of cues located outside the apparatus. In both tasks, animals are trained to reach the platform from four randomly selected departure points (black stars). Similar to the Morris water maze, solving the starmaze task implies spatial learning capabilities. In contrast, in the starmaze, animals are constrained to swim within alleys that guide their movements, which reduces the possible deviations from an ideal trajectory.

[1]Laboratoire de Physiologie de la Perception et de l'Action, UMR CNRS 7152, 11 place Marcelin Berthelot, Collège de France, 75005 Paris, France. [2]Department of Neuroscience, Erasmus MC, 3000 DR, Rotterdam, The Netherlands. [3]Department of Physiology, Shahrekord University of Medical Sciences, Iran. [4]Present address: Neuroscience Group, Sony Computer Science Laboratory, 6 rue Amyot, 75005 Paris, France. Correspondence should be addressed to L.R.-R. (laure.rondi-reig@college-de-france.fr).

Received 27 June; accepted 3 August; published online 28 August 2005; doi:10.1038/nn1532

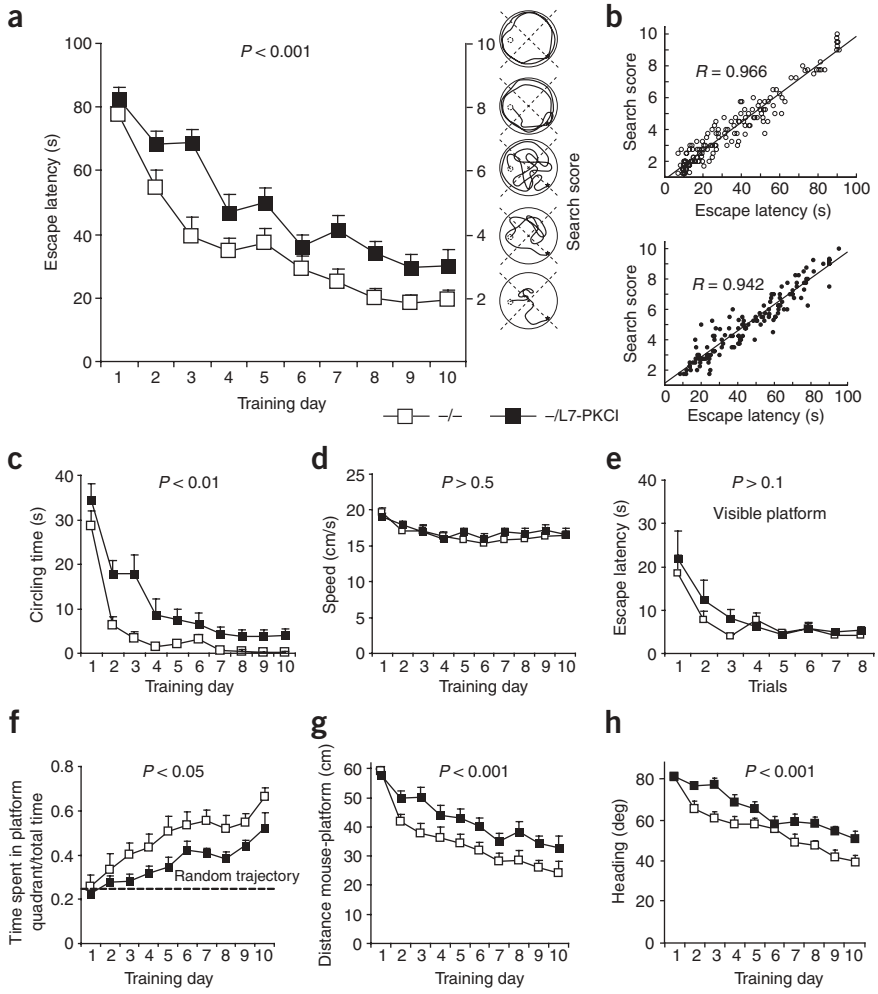

Figure 2 Inactivation of LTD in L7-PKCI mice affected their performance in the Morris water maze task. (**a**) The mean escape latencies of controls and mutants (open and filled squares, respectively) were similar at the beginning of training (day 1). However, controls improved their performance significantly better than mutants over time. The behavioral patterns corresponding to the search scores are illustrated by the cartoon trajectories on the right side: the lower the score, the better the searching behavior. The search scores of L7-PKCI mice were significantly higher than those of control animals. (**b**) The search scores and the escape latencies were highly correlated for both control (top) and mutant (bottom) mice, suggesting that the longer time-to-goal needed by L7-PKCI mice was due to non-optimal searching trajectories. (**c**) L7-PKCI mutants showed a significantly larger amount of circling behavior over training, which has been interpreted as a deficit in the procedural component of navigation[7]. (**d**) The mean swimming speeds of controls and mutants remained comparable over the entire training period. (**e**) Mutants are not impaired when solving the visible platform version of the Morris water maze (that is, the visual guidance navigation task). (**f**) The mean ratio of the time spent within the target quadrant to the total duration of the trial of both controls and mutants increased significantly above the random trajectory level during training. However, controls improved their ratio significantly better than L7-PKCI mice. (**g**) The mouse-platform distance parameter demonstrated that mutants followed significantly longer trajectories than controls. (**h**) The mean angular deviation between ideal and actual trajectory showed that mutants had a deficit in maintaining their body locomotion oriented toward the platform during navigation.

escape latency measure and the search score method were highly correlated for both groups of animals ($R = 0.966$, $P < 0.001$, and $R = 0.942$, $P < 0.001$, for wild-type mice and mutants, respectively; **Fig. 2b**). The circling times of the LTD-deficient mutants were also significantly longer than those of wild types (ANOVA, $F_{1,27} = 11.6$, $P < 0.01$; **Fig. 2c**). These results showed that L7-PKCI mice were impaired in solving the Morris water maze task. This spatial navigation impairment was due neither to a deficit in swimming speed (ANOVA, $F_{1,27} = 0.46$, $P > 0.5$; **Fig. 2d**) nor to a deficit in visual guidance abilities (ANOVA, $F_{1,27} = 1.2$, $P > 0.1$; **Fig. 2e**).

The ratio between the time spent in the target quadrant and the duration of the trial provides an estimate of the ability of an animal to locate the platform. Both groups of mice spent significantly more time in the target quadrant than in any other quadrant (ANOVA, $F_{1,9} = 15$, $P < 0.0001$), which suggested that both control and L7-PKCI mice were able to acquire a memory of the localization of the platform. However, an inter-group comparison of this ratio as a function of learning showed that L7-PKCI mice spent significantly less time than controls within the target quadrant (ANOVA, $F_{1,27} = 5.1$, $P < 0.05$; **Fig. 2f**). To investigate this difference and to assess the accuracy of the mice's goal-oriented behavior during learning, we calculated the average mouse-to-platform distance during a trial for both control and L7-PKCI mice (**Fig. 2g**). L7-PKCI mice showed a longer mean distance relative to the platform than control animals over the entire training period (ANOVA, $F_{1,27} = 16.6$, $P < 0.001$). This result indicated that the trajectories of L7-PKCI mice toward the platform were less direct than those used by control mice. This issue was further investigated by computing the ongoing egocentric angle ($\phi(t) \in [0°, 180°]$) between the optimal direction towards the target and the actual motion direction of the animal. The larger the angle ϕ, the bigger the

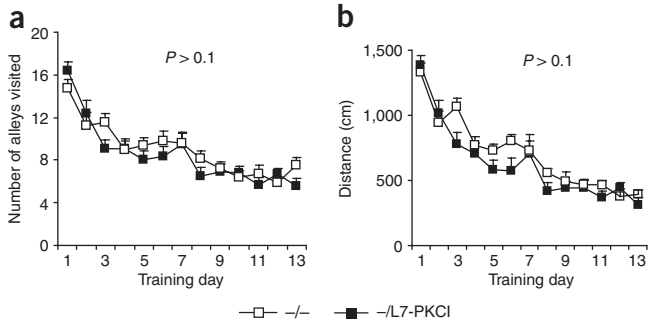

Figure 3 L7-PKCI mutants were not impaired in solving the allocentric version of the starmaze task. (**a**) The mean number of alleys visited during a trial was not significantly different between control and mutant mice. (**b**) The distance swum to reach the target was not significantly different between the two groups.

deviation between the ideal goal-directed trajectory and the actual trajectory. Both groups significantly decreased their mean angular deviation from the ideal trajectory over training (ANOVA, $F_{1,9} = 29.4$, $P < 0.0001$; **Fig. 2h**). However, L7-PKCI mice showed significantly higher deviations than control mice (ANOVA, $F_{1,27} = 14.9$, $P < 0.001$). The statistical correlation between the mean angular deviation measure and the search score parameter was significant for both wild-type mice and mutants ($R = 0.871$, $P < 0.001$, and $R = 0.839$, $P < 0.001$, respectively).

The results obtained with the Morris water maze may suggest that L7-PKCI mice could learn to locate the platform (declarative component) but that they executed non-optimal goal-directed trajectories (procedural component; see **Supplementary Fig. 2** for a qualitative comparison between the searching behavior of controls and mutants over training). To test this hypothesis, we used a task requiring declarative capabilities but with a lower procedural demand than the Morris water maze: the allocentric starmaze task (**Supplementary Methods**). This task, similar to the Morris water maze, has been shown to depend on intact hippocampal function (L. Rondi-Reig *et al.*, *Soc. Neurosci. Abstr.*, 329.2, 2004). To assess the ability of mice to learn the starmaze task, we observed the number of visited alleys and the mean distance swum before finding the platform. Over time, both L7-PKCI mice ($n = 15$) and control mice ($n = 11$) significantly improved their ability to reach the platform quickly (ANOVA, $F_{1,24} = 7.45$, $P < 0.0001$). No statistical difference was observed between the two groups in the number of visited alleys (ANOVA, $F_{1,24} = 0.64$, $P > 0.1$, **Fig. 3a**) or the mean distance swum to reach the platform (ANOVA, $F_{1,24} = 2.56$, $P > 0.1$, **Fig. 3b**; see **Supplementary Fig. 3** for a qualitative representation of the similar behavior of controls and mutants).

The results obtained with the starmaze strengthened our hypothesis that the declarative component was not affected in L7-PKCI mice. The absence of a deficit when the trajectory was guided corroborated the results obtained with the Morris water maze, which suggested that L7-PKCI mice were unable to adapt their goal-oriented behavior effectively.

The parallel fiber–Purkinje cell LTD mechanism is likely to constitute a core process underlying cerebellar learning, and it has been proposed to contribute to both motor and cognitive learning[9]. It has been demonstrated that L7-PKCI mutants have response deficits in both adaptation of the vestibulo-ocular reflex[4] and eyelid conditioning tasks[10]. In addition, cerebellar LTD seems most prominently involved in rapid learning of well-timed movements with specific amplitudes. Our results corroborate previous spatial navigation studies with other cerebellar models[8,11] and suggest that parallel fiber–Purkinje cell LTD participates in the procedural component of navigation (**Supplementary Note**).

How could the same cellular mechanism (that is, parallel fiber–Purkinje cell LTD) be involved in motor learning as well as more cognitive processes such as spatial learning? Several cognitive processes can be considered on the basis of the same sensorimotor coupling scheme observed in classical motor learning[12]. Spatial navigation requires a linkage between the spatial context (including sensory inputs and internal state information) and the explorative response (motor output) characterized by the animal's trajectory. Although the spatial context may be conveyed by the mossy fiber–granule cell–parallel fiber pathway relaying information from the pontine nuclei[13], errors in the explorative response may be mediated by the climbing fiber signals that originate from the olivary subnuclei that are innervated by descending projections from the mesodiencephalic junction and cerebral cortex[14]. As induction of parallel fiber–Purkinje cell LTD requires conjunctive activation of the parallel fiber and climbing fiber pathways, this form of synaptic plasticity could be responsible for the establishment of this linkage[12]. Thus, the cerebellum may mediate a general learning function to create a context-response linkage adapted to the task[15]. During spatial learning, the subject could establish an appropriate context-response coupling resulting in effective motor behavior (for example, in the execution of optimal trajectories to the target). At the cerebellar level, procedural learning may result from a classical control learning scheme in which the feedback loop allows the system to converge towards an adapted context-motor linkage (**Supplementary Fig. 4**). The absence of parallel fiber–Purkinje cell LTD in L7-PKCI mice could result in an accumulation of errors over time (that is, during the execution of a goal-directed trajectory) due to the absence of continuous context-dependent corrections of the motor signals. From a behavioral point of view, the accumulation of these errors would lead to a drift during unconstrained (not guided) navigation as in the Morris water maze task, but it would be irrelevant in the starmaze task owing to the reduced number of possible goal-directed trajectories.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

The authors thank M. Rutteman, V. Gautheron and F. Maloumian for technical support. L.R.-R. is supported by the Action Concertée Initiative 'Neurosciences intégratives et computationnelles' (NIC 0083), the Institut Fédératif de Recherche 52 'Transduction du signal: molécules à action centrale et périphérique', and the 'Groupement d'Intérêt Scientifique Longévité' (grant L0201). A.B. is supported by the Laboratoire Européen des Neurosciences de l'Action (LENA). C.I.D.Z. is supported by Nederlandse Organisatie voor Wetenschappelijk Onderzoek, the European Economic Community and Neuro-Bsik.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureneuroscience/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Schenk, F. & Morris, R.G. *Exp. Brain Res.* **58**, 11–28 (1985).
2. Rondi-Reig, L. & Burguiere, E. *Prog. Brain Res.* **148**, 199–212 (2005).
3. Thompson, R.F. *et al. Int. Rev. Neurobiol.* **41**, 151–189 (1997).
4. De Zeeuw, C.I. *et al. Neuron* **20**, 495–508 (1998).
5. Goossens, H.H. *et al. Eur. J. Neurosci.* **19**, 687–697 (2004).
6. Goossens, J. *et al. J. Neurosci.* **21**, 5813–5823 (2001).
7. Petrosini, L., Molinari, M. & Dell'Anna, M.E. *Eur. J. Neurosci.* **8**, 1882–1896 (1996).
8. Leggio, M.G. *et al. Exp. Brain Res.* **127**, 1–11 (1999).
9. Ito, M. *Physiol. Rev.* **81**, 1143–1195 (2001).
10. Koekkoek, S.K. *et al. Science* **301**, 1736–1739 (2003).
11. Martin, L.A., Goldowitz, D. & Mittleman, G. *Eur. J. Neurosci.* **18**, 2002–2010 (2003).
12. Ito, M. *Rev. Neurol. (Paris)* **149**, 596–599 (1993).
13. Thach, W.T., Goodkin, H.P. & Keating, J.G. *Annu. Rev. Neurosci.* **15**, 403–442 (1992).
14. De Zeeuw, C.I. *et al. Trends Neurosci.* **21**, 391–400 (1998).
15. Thach, W.T. *Int. Rev. Neurobiol.* **41**, 599–611 (1997).