



Impaired behavioral control and altered processing of spatial information in mice deficient for the X-chromosomal mental retardation gene *Arhgef6*

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Σ Summary

The *ARHGEF6* gene (also known as alphaPIX or Cool-2), encodes a protein with homology to guanine nucleotide exchange factors (GEF) for Rho GTPases. Mutations in this gene have been recently identified in patients with X-linked mental retardation (Kutsche et al. Nat Genet 26:247-250, 2000). We report the cognitive and behavioral characterization of male mice carrying a targeted deletion of *Arhgef6*. These mice are healthy and have normal appearance. They were evaluated in a water-maze place navigation task, in a spatial working memory procedure on the 8-arm radial maze, as well as in a set of tests assessing locomotor activity, anxiety, exploratory behavior and their reaction to novel stimuli.

In the water maze (1), *Arhgef6* deficient mice produced navigation errors during acquisition and showed spatial perseverance when required to adapt to a new goal position. Spatial reference memory as such was normal (2). Spatial working memory as well as day to day habituation to a novel arena were intact (not shown) and we found no indication of altered basal activity, anxiety, or abnormal adaptation to stressful situations. Detailed analysis of locomotion and swim patterns did not reveal neurological deficits. However, the reaction of *Arhgef6* deficient mice to a novel stimulus within in a familiar environment was clearly disinhibited (3).

Conclusions

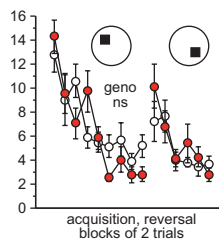
Arhgef6 null mice displayed specific behavioral alterations suggesting deficient behavioral control and altered processing of spatial information. The behavioral profile of *Arhgef6* null mice is reminiscent of phenotypic changes observed in other mouse models with impaired function of the hippocampus and connected cortical regions.

Methods

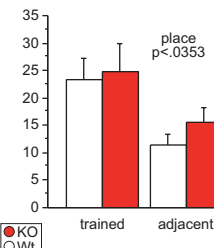
R1 ES cell line, 6 generations backcrossing to B6 followed by matings to produce littermate KO and Wt for behavioral testing. 12/12h inverted light cycle, single housing during testing period. Testing during dark phase, 12 lux in test room. 12 KO + 9 Wt (males, 5.5 months). Test sequence: place navigation in the WM (3 days x 6 trials acquisition, 2 days x 6 trials reversal, max 120 s, ITI 30-60 min, pool diameter 1.5 m, probe trial = first 30 s of first reversal trial), 8-arm radial maze (10 days x 1 trial, arm length 38 cm), open field (1.5 m diameter, 2x10 min), O-maze (10 min, diameter 46 cm), emergence test (30 min, arena 50x50 cm, home box 12x8x4 cm), object exploration (30 min habituation, 30 min object = Falcon tube, arena 50x50 cm). Additional 13 KO + 14 Wt (9.1 months) tested in radial maze, emergence test, object exploration. Videotracking by Noldus Ethovision 3.0, analysis by Wintrack 2.4.

1 Navigation errors and perseverance

a Water-maze, training swim path length (m)

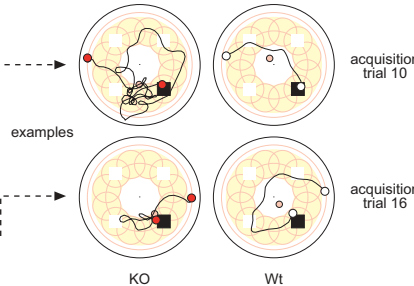
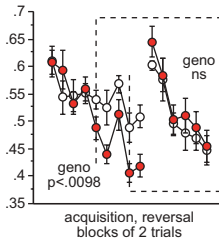


b Water-maze, probe time in zone (%)

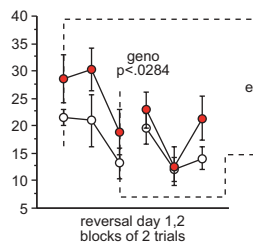


Performance of KO in the place navigation task was normal according to standard measures of path length during training (a) and zone time during the probe trial (b). But during late acquisition, KO missed the goal often and spent more time than controls searching in its vicinity (c). During reversal training, KO had trouble switching to the new goal and focused their searching perseveratively on the old goal area (d).

c Water-maze, training distance to goal (m)



d Water-maze, reversal time in old goal zone (%)



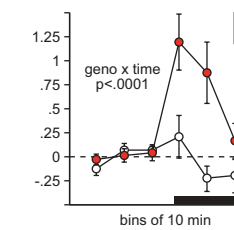
2 Normal spatial reference memory

	Factor 1 "navigation errors"	Factor 2 "memory"	Factor 3 "perseverance"
<i>Acquisition training</i>			
time in current goal quadrant	0.930	0.075	0.136
average distance to goal	-0.924	-0.116	-0.144
Wishaw's error	0.803	0.080	-0.172
<i>Reversal training</i>			
time in old goal quadrant	0.325	-0.050	0.909
time in old goal zone	0.276	-0.043	0.926
crossing index old goal	-0.032	-0.157	0.883
<i>Probe trial</i>			
time in trained quadrant	0.011	0.981	0.003
time in trained zone	0.085	0.950	-0.219
genotype effect	-0.191	ns	0.495
variance explained	44% ↑	28%	17% ↑

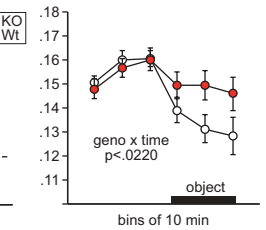
Factor analysis with varimax rotation extracted 3 factors. ANOVA on individual factor scores shows significant genotype effects on the "navigation errors" and "perseverance" factors but not on the "memory" factor, indicating that spatial memory per se is intact in KO mice.

3 Disinhibited object exploration

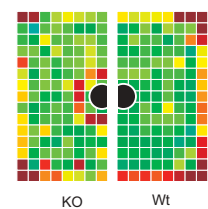
a Object exploration episodes (x/min)



b Object exploration distance to corner (m)



c Occupancy change



occupancy change after object introduction:



KO approached the novel object more often than Wt in which avoidance and approach response were balanced (a) and they showed less preference for the arena corners (b). KO and Wt were indistinguishable in absence of the object. After introduction of the object, occupancy in its vicinity increased strongly in KO but not in Wt (c).