Behavior analysis of RSK2 deficient mice: An animal model for the cognitive impairment in the Coffin-Lowry syndrome.

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Abstract **In human, mutations in the RSK2 gene leads either to the Coffin-Lowry syndrome, i.e., mental retardation associated with characteristic physical features, or to a form of X-linked non-specific mental retardation (XLMR). RSK2 is one of the p90 ribosomal S6 kinases (RSK) which are serine-threonine mitogen-activated protein kinases acting in the Ras/MAPK signal transduction pathway. To understand how the lack of RSK2 protein could be involved in cognitive functions, we generated a targeted null mutation in the murine homologous gene. The mutant mice are viable, fertile; 5 to 10 % smaller than wild type littermates but with no obvious physical abnormalities.**

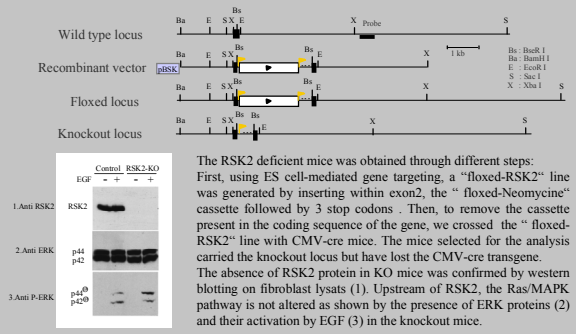
RSK2 deficient mice have normal activity and motor coordination as measured in activityscope cages and rotarod. Mutants showed altered behavior in emotional and exploratory tests such as dark-light box and emergence test.

In this study we focused on the memory abilities in different tasks. Analysis of spatial long-term memory (reference and spaced versions of the water maze) revealed a delay in acquisition due to thigmotaxic strategy. Therefore, mutant mice showed behavioral impairment that influenced their ability to perform adequately reference spatial tasks.

Working and episodic-like memories, assessed by 8-arm radial maze and a matching-to-place (DMP) version of the water maze, were impaired during acquisition, suggesting a short-term memory deficit.

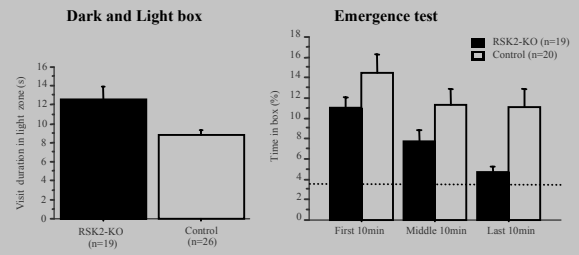
These results suggest a role of the RSK2 protein in cognitive functions in mice that resemble some of the deficits observed in humans. Thus, RSK2 deficient mice appear to be a promising model to analyze the causes of the mental retardation component of human syndromes.

1. Generation of RSK2 deficient mice



The RSK2 deficient mice was obtained through different steps:
First, using ES cell-mediated gene targeting, a "floxed-RSK2" line was generated by inserting within exon2, the " floxed-Neomycine" cassette followed by 3 stop codons . Then, to remove the cassette present in the coding sequence of the gene, we crossed the " floxed-RSK2" line with CMV-cre mice. The mice selected for the analysis carried the knockout locus but have lost the CMV-cre transgene.
The absence of RSK2 protein in KO mice was confirmed by western blotting on fibroblast lysats (1). Upstream of RSK2, the Ras/MAPK pathway is not altered as shown by the presence of ERK proteins (2) and their activation by EGF (3) in the knockout mice.

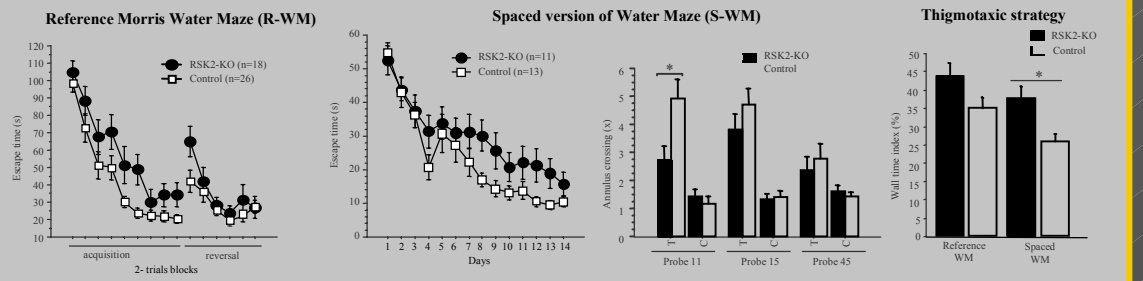
2. Emotion and exploratory behaviors



Altered behavior in emotional and exploratory tests

KO mice showed atypical behaviors:
In the dark/light box, KO mice were more often in the illuminated compartment than control animals ($F(1,43)=7.942$, $p=0.0073$).
In the emergence task, KO mice spent less time in the box (familiar box) ($F(1,38)=12.178$, $p=0.0012$).

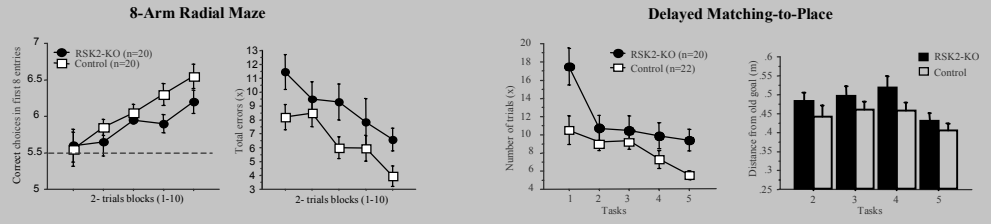
3. Spatial long-term memory



Delay in aquisition due to thigmotaxic strategy

- Escape latencies in R-WM reveal a significant difference only in aquisition phase (aquisition: $F(1,42)=6.123$, $p=0.0175$; reversal:$F(1,42)=1.958$, $p=0.1691$)
- In S-WM mutant mice showed a significant difference in escape latencies ($F(1,22)=5.212$, $p=0.0325$) confirmed by a deficit in probe 11 occuring in aquisition phase ($F(1,22)=6.509$, $p=0.0182$) whereas no significant differences appear in the probes trials occurring at the end of the acquisition (probe 15) and after a delay (probe 45).
- Performance of KO mice in R-WM and S-WM during aquisition is mainly determined by thigmotaxis (wall hugging) (R-WM: $F(1,42)=3.153$, $p=0.083$; S-WM: $F(1,22)=12.225$, $p=0.002$)

4. Working and episodic-like memories



Impairment in Short-term memory

- In 8-arm radial maze, correct choices in the first 8 entries reveal a significant difference between genotype ($F(1,38)=4.398$, $p=0.0427$), and also for the total number of errors ($F(1,38)=9.457$, $p=0.0039$). The decrease in the number of errors from beginning to the end of the test indicates that KO mice were able to assess the procedure, but only marginally significantly (KO: $F(19,4)=2.122$, $p=0.0862$). Their number of correct choices was barely above chance level (5.5).
- In delayed matching-to-place version of the water maze, both genotypes were able to acquire the new position of the platform in each task but KO mice were less efficient since they needed more trials per task ($F(1,39)=10.076$, $p=0.0029$) and they swam at a larger distance to the previous goal in the first trial of each new task ($F(1,39)=5.377$, $p=0.0257$)

Discussion

- RSK2 deficient mice exhibit atypical behavior, shown in emotional and exploratory tests, which could be also the cause of the delay observed in acquisition of water maze tasks. Nevertheless the spatial long-term memory is not altered as shown by their capacity to learn the position of the hidden platform.
- Working and episodic-like memories involve acquisition of memory across short time intervals. Therefore the deficit observed in KO mice is probably due to a defect in short-term memory.
- An impairment in short-term memory was also described in Coffin-Lowry patients. Our model did not show most of the anatomical features of the human patients but the cognitive impairment appears to be at least partially correlated with the mental retardation of patients.

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