

# Different Spatial Learning Performance of 5-Htt Knockout Mice on Land or Water

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## Introduction

The serotonin transporter (5-HTT) as a key regulator of central serotonergic activity has been linked to neuropsychiatric disorders like anxiety and depression. The evidence of a connection between such mood disorders and genetic variations of the Serotonin transporter led to the generation of 5-HTT knockout mice with a targeted inactivation of the 5-HTT function. These mice, contingent upon their genotypes, differentially express functional 5-HTT. Homozygous knockouts completely lack the 5-HTT, heterozygous mice show a reduced 5-HTT density of about 50%, while wild-type controls express normal levels of 5-HTT. Behavioral phenotyping revealed that 5-HTT knockout mice display a number of phenotypic changes especially regarding anxiety related behavior. On the one hand it is known that 5-HTT as well as 5-HT receptor subtypes are abundant in the cortical areas involved in cognitive functions, implying also an important role in learning and memory. On the other hand, cognitive processes are well known to be strongly influenced by emotionality, possibly modulated by stress-related adrenal steroid hormones. In the present study we evaluated whether spatial memory is affected by the genotype per se or if there is an interference of differences in the aversiveness of the testing conditions.

We therefore conducted different learning and memory tasks in order to measure how the performance is affected by the 5-HTT genotype and differences in the aversiveness of testing conditions.

## Methods

Male 5-HTT knockout mice (n=28), heterozygous 5-HTT mice (n=28), and wild-type controls (n=28) have been subjected to a 5 day series of repeated trials in either a water maze or a Barnes maze. An additional group of male mice of all three genotypes (KO n=32, HET n=36, WT n=30) were used to measure how the different testing procedures are related to plasma corticosterone concentrations, the main stress hormone of the murine hypothalamic-pituitary-adreno-cortical axis. These mice were sacrificed 15 minutes after a single session of the respective tests or, as a control, without any test experience. The water maze apparatus consisted of a circular pool (1 m in diameter) made of blue plastic. The pool was filled with water (21 °C) to a height of 33 cm. An escape platform made of transparent plastic was submerged 0.5 cm under the water surface. Three-dimensional visual cues were placed around the water maze. We measure the time until the mice found the submerged platform by means of an automated tracking system (<http://www.phenotyping.de/digital.html>). The test was conducted on four consecutive days with three trials per day using the same platform position. On the fifth day a different position had to be found. The Barnes maze apparatus consisted of a round white platform (1 m in diameter) that was elevated 127 cm above the floor. The platform was surrounded by three-dimensional visual cues. From the platform the mice could escape to their home cage (placed directly beneath the table, not visible for the mice) via one of 12 holes (3 cm diameter) at the border of the platform. The escape hole was connected to the home cage by a wire-mesh tunnel. The other holes led to little blind-ending mesh tunnels. We measured the time to find the correct hole that leads back to the home cage of the mouse by means of an automated tracking system (see above). The test was conducted on four consecutive days with two trials per day using the same escape hole. On the fifth day a different escape hole had to be found.

## Results and Discussion

An ANOVA calculated for the areas under the learning curves as well as a non-linear mixed effect model did not reveal any significant difference between the genotypes in the Barnes maze ( $p > 0.1$ ). A search strategy analysis revealed also no differences regarding the strategy used to solve the maze between the genotypes. However, the same statistical analyses performed for the water maze identified significant differences between the genotypes ( $p < 0.001$ ) with regard to learning this test. Post hoc analysis revealed significant differences between 5-HTT knockout mice and both other genotypes (both:  $p < 0.0001$ ). Moreover, knockout mice significantly differed from both other genotypes regarding the strategy used to solve the water maze by relying on random search strategies rather than on direct navigation. Both learning tests led to significantly increased plasma corticosterone concentrations compared with basal values in all three genotypes. Corticosterone concentrations measured after a single trial in the water maze did not differ from concentrations measured after a single trial in the Barnes maze in heterozygotes and wild-types but corticosterone titers of 5-HTT knockout mice were noticeably higher in the water maze. We suggest that this exaggerated stress reaction contribute to the performance differences between the genotypes that were found in water maze learning.

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