Transgenic overexpression of MMP-9 in rats results learning and memory alterations

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Matrix metalloproteinases are a major group of enzymes regulating cell-matrix composition, which are essential for many biological phenomena. Matrix Metalloproteinase-9 (MMP-9) has recently emerged as an important molecule in control of extracellular proteolysis in synaptic plasticity. Using conventional transgenesis, we have created rats with overexpression of MMP-9 limited to neurons (MMP-9-gene is under control of synapsin-1 promoter).

The animals, along their wild-type siblings, were exposed to a variety of behavioral tasks. To analyze anxiety, we employed Elevated Plus Maze (EPM) test [1, with modifications] that showed 12-15-months old transgenic rats spending more time in the center zone, while the wild type rats stayed in the closed arms (none of the rats entered the open arms, see Figure 1A, B). Lack of entrance to the open arms was unexpected, and prompted us to repeat the test on 3-months old animals. The young rats were entering the open arms, and no significant difference between transgenic and control group was observed (see Figure 1C, D). Boguszewski and Zagrodzka [2] as well as Bessa et al. [3] reported similar age-related decline in entering the open arms. However, in their experiments the old animals still entered the open arms. This apparent discrepancy can be explained by differences in the experimental set-up employed. We used white curtains hanging around and near plus maze (rats oriented toward environment without extra-apparatus cues) and center light which might be a mild stressor for the animals. The other authors used dim red light or fluorescent lamps mounted above the maze so that all arms were equally illuminated, so their light conditions were quite different.

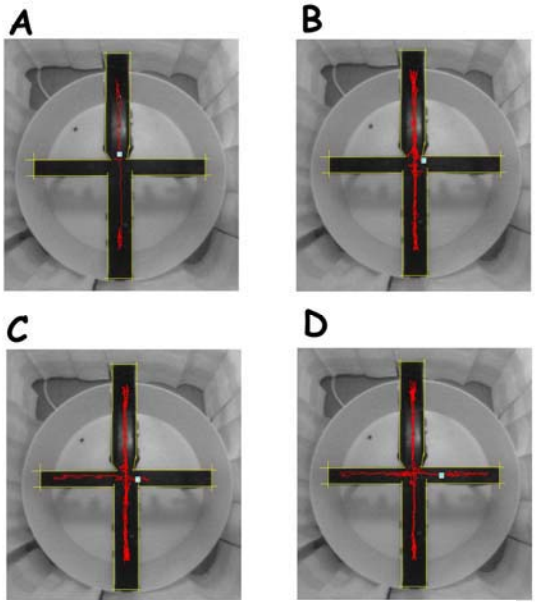

Figure 1*. Exemplary records of rat's footpath in Plus Maze recorded by Ethovision. A – old wild type rat, B – old transgenic rat, C – young wild type rat, D – young transgenic rat. In Plus Maze test old transgenic rats were more active than control rats and none of old rats entered to the open arms.*

In additional experiments, we noticed differences in general locomotor activity between old transgenic vs. wild type rats. In the Open Field (OF) analyzed by Ethovision, no significant differences between young transgenic and control rats were revealed. However, the old transgenic rats were more active in OF (see Figure 2A, B) and, in addition, performed better on a RotaRod than controls.

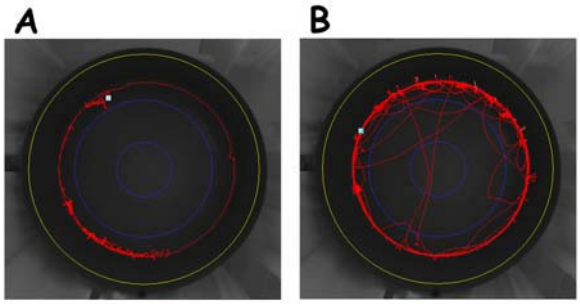

Figure 2. *Exemplary records of rat's footpath in Open Field recorded by Ethovision. A – old wild type rat, B – old transgenic rat. Transgenic rats were more active than control in Open Field test.*

We have also carried out a Flavor Preference (FP) test that is connected with appetitive learning involving flavor-reward associations [4, with modifications]. During the first 4 days of the experiment each rat was presented in the morning with water for 15 min, followed 5 hrs later by 1 hr water exposure. Then, the animals were divided into two groups. The first one was exposed to sweet orange and non-sweet apple juice on every second day in the morning and the second group to non-sweet orange and sweet apple juice under the same time regime. The juices were presented in dimmed bottles to avoid recognizing the color of the liquid. All the animals were exposed to water in the afternoon, as before. On the days 11-14, rats had a choice between non-sweet orange and non-sweet apple juice. The amount (ml) consumed of each solution was recorded daily and was used to measure % of consumed juice with positive flavor-association (that is memory connected with flavor). In this experiment we showed, that the old transgenic rats had a better taste preference than their wild type siblings. Rentiera et al. [5] showed that rats develop preference for the sweetened juice (drink more) and that the ability to form flavor-reward associations declines with age, resulting in impaired conditioned flavor preference, as we showed.

These results strongly suggest that MMP-9 may have an important role in control of the behavior of animals.

References

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