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PHYSIOLOGY

The Ability to Solve Elementary Logic Tasks in Mice with the Knockout of Sodium–Calcium Exchanger Gene 2 (*NCX2*)[1]

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Abstract—Mice with a knockout of the sodium–calcium exchanger 2 (*NCX2*) gene were statistically significantly more successful than wild-type controls in the solution of two cognitive tasks, the test for the capacity to extrapolate the direction of the stimulus movement and the "puzzle-box" test for the capacity to find a hidden route to safe environment, which were based on food and aversive motivations, respectively. In both tests, the success of task solution was based on the animal's ability to use the object's "permanence" rule (according to J. Piaget). The data confirm that the knockout of this gene, which is accompanied by modulation of the temporal pattern of calcium membrane flux, also induces changes in mouse CNS plasticity.

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Gene knockouts (KOs) that affect brain physiology usually have deleterious effects on brain functions. Therefore, the cases when a brain function is enhanced as the result of gene knockout arouse vivid interest. Sodium–calcium exchangers (NCXs) are a group of evolutionary conservative proteins which control the intracellular calcium concentration [6]. Mice knocked out for one of these genes, *NCX2,* which is actively expressed in brain tissue, were more successful in spatial and passive avoidance learning [6].

The paper presents data on the ability of male mice with an *NCX2* KO (aged four to six months) to solve two elementary logical tasks in comparison to control mice with the wild-type genotype (WT). A successful solution of these tasks is based on the animal's ability to use the rules (empirical laws) that connect objects and events of the external world [2].

The mouse ability to extrapolate the direction of food movement when it disappears from the animal's view was analyzed, as well as the ability to find the underpass into a safe box compartment when this route was hidden [2, 3]. The animal's reaction to new food in a new environment was also tested in these mice (the hyponeophagia test). The extrapolation (Ex) test (as described in our previous communication [2]) was performed in a special box in which a hungry and thirsty mouse could receive reinforcement (milk) via a small opening from the small cup located behind this opening. After the animal began to drink milk from this cup, the cup started to move gradually to the right or to the left, and the animal could follow the cup movement over a distance of 1.5–2 cm; then, the mouse could not see the cup any more. Now the milk would be available only if the animal moved to the side opening, which was at the side of the box towards which the cup moved (a correct solution, while the approach to the opposite side opening was an incorrect solution). The test was presented either 6 times (during one experimental day), or 18 times (three experimental days). The rates of success of the Ex task solution for all animals (KO, $n = 48$; WT, $n = 53$) is presented in the table. The proportion of correct Ex task solutions for KO mice was significantly above the 50% random level of solutions, which was true both for the first trial and for the total scores of one to six trials. In WT mice, the proportion of correct Ex task solutions was not significantly different from the random level.

As at the moment of the first Ex task the animals had no individual experience of such solutions, the non-random performance by the group of KO mice meant that most of these animals are able to "under-

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The proportion of correct extrapolation task solutions in male mice of two groups: KO for *NCX2* and WT

Genotype, number of animals	Percentage of correct solutions upon the first task presentation	Significance of the difference from the 50% random level*	Percentage of correct solutions upon the first to sixth task presentations	Significance of the difference from the 50% random level
NCX2 KO, 48	70	$p < 0.05$	57	$p < 0.01$
WT, 53	58.7	$p > 0.05$	63.6	$p < 0.001$

* Calculated as the difference from the 50% random level using Fisher's φ method.

stand" the logical structure of the test: the food could be reached if the mouse moved to the side opening to which the cup moved. In the control group, the performance did not differ from the random level. Estimation of the performance during three experimental days (18 task presentations) also revealed the "superiority" of KO mice. The data on one of the experimental series are presented in Fig. 1. It shows that, starting from the fourth presentation, the scores for KO mice were higher than those for WT mice and, in almost all cases, significantly above the random level ($p < 0.001$, exact Fisher test). In the course of successive Ex tasks, the memory of previous events is formed; i.e., the experience of solutions accumulates. This "experience" includes at least two components. The first one is the instrumental learning of the approach to a side opening (to any one of them, because the 50% level of correct choices provides the food reinforcement in about one half of cases). Figure 2 shows that the time to approach the food cup (TAF) decreases gradually in mice of both groups. During the 18th task presentation, the TAF is significantly shorter than that during the first presentation ($p < 0.001$, Wilcoxon–Mann–Whitney test). Mice with KO approached the side opening in most cases more quickly than WT mice. The second component of experience accumulated is of "cognitive" nature. It is the gradual acquisition of the "rule" that food could be found at the side of the box to which it moved recently. This component is based on "understanding" this rule and could be revealed as gradual learning (KO mice acquired this rule more successfully).

The puzzle-box test [2] was performed in a plastic box divided into two compartments. The mouse was placed into the larger, brightly lit part, which the animal tended to escape. The smaller dark part of the box was connected with the brightly lit one by an "underpass," an opening in the wall deepened into the floor. The test was performed during two days (eight task presentations). The underpass was either free (the first and second trials), or hidden with wood shavings or with a light plastic plug in the most difficult trials of the test (which the mouse could remove by either shifting it aside or taking it away with its teeth) [2], or the wood shavings covered all the lower part of the wall with the underpass. The success of the test solution was estimated by the time required to enter the safe part of the box. This time did not differ in two groups of mice, although the proportion of the animals that solved the most difficult "plug" trials of this test within the 4-min time limit was significantly higher in the KO group (Fig. 3). This is also an indication of higher cognitive abilities of these mice.

Two behavioral characteristics were observed in KO mice that could be interpreted as deterioration of species-specific behavior. The first one was an almost

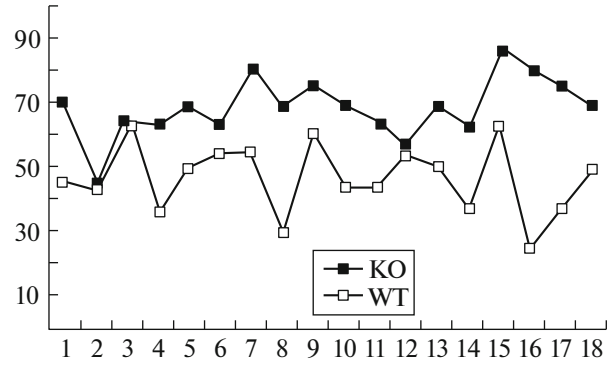

Fig. 1. The extrapolation test success in mice of two groups during 18 presentations. Ordinate, the proportion of correct solutions (%); $n = 16$ (KO) and $n = 13$ (WT).

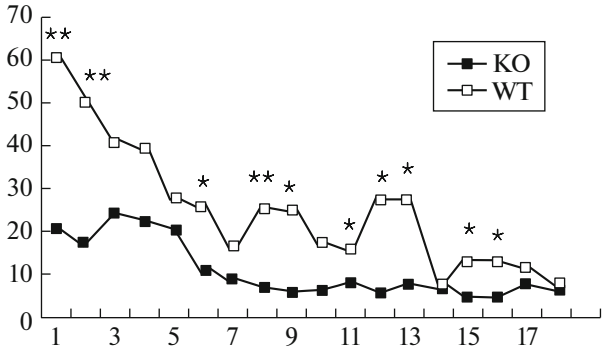

Fig. 2. The mean latencies of extrapolation task solution in the course of 18 presentations (irrespective to correctness of the solution); $n = 16$ (KO) and $n = 13$ (WT). Ordinate, time, s; abscissa, task presentations. Significant differences from the respective scores for KO and WT mice, respectively: * $p < 0.05$, ** $p < 0.01$.

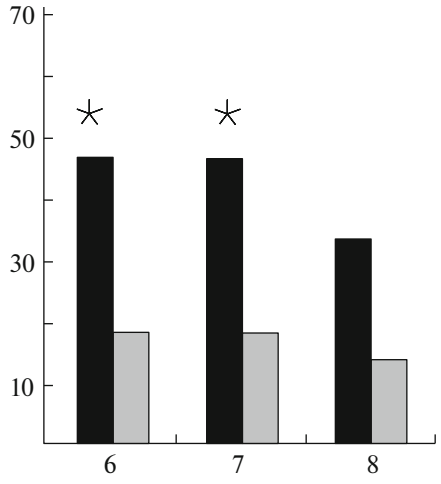

Fig. 3. The solution of the "puzzle box" test by KO ($n = 22$) and WT ($n = 22$) mice (when the underpass was blocked by the plug, which should be either shifted or removed by teeth). Ordinate, the proportions (%) of mice (here and in Fig. 4: black bars, KO mice; gray bars, WT mice) that were able to solve the most complicated "plug" trials of the puzzle-box test (trials 6 and 7) and trial 8, when the underpass was masked by wood shavings covering the length of the lower part of the box wall. * $p < 0.01$.

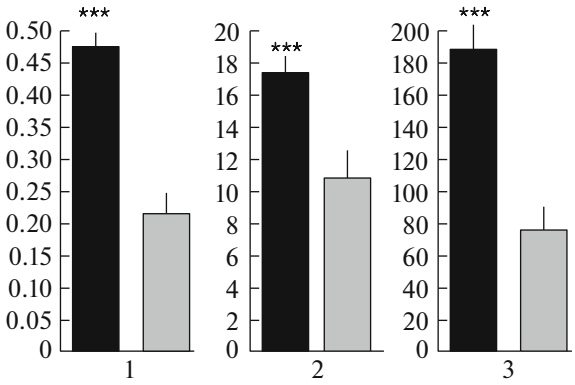

Fig. 4. Hyponeophagia test performance by *NCX2* KO ($n = 10$) and WT ($n = 13$) mice. Bar labels are the same as in Fig. 3. Abscissa: *1,* food eaten (g); *2,* number of approaches to food; *3,* time occupied by eating (s). *** $p < 0.001$.

total absence of rearing postures (while they were observed in WT mice). The second one was the absence of digging paw movements, which mice usually perform in order to remove the wood shavings from the underpass in the attempt to enter the dark compartment of the puzzle-box, and which WT mice displayed. These peculiarities could be the influence of this KO on the neurological substrate of these innate reactions.

The hyponeophagia test was used in order to evaluate the reaction to novelty in these two groups of mice, in which the reaction of the hungry mouse to new food (cheese) in a novel environment was evaluated. The experiments were performed on a circular plate (diameter, 40 cm), surrounded with an opaque wall (35 cm high) [1]. One-way ANOVA demonstrated a significant influence of the "genotype" factor with interstrain differences in the mass of food consumed ($F(1, 21) = 4.5370$, $p = 0.045158$), the number of food approaches ($F(1, 21) = 9.2682$, $p = 0.00616$), and the time occupied by eating during the 10-min test ($F(1, 21) = 22.9469$, $p = 0.000098$). All these variables were higher in KO mice, which means that WT mice feared the novelty more than KO mice did (Fig. 4). WT mice were found to be aggressive towards cage mates when they were returned to the home cage after the experiment, they also made systematic attempts to bite the experimenters' hand when being manipulated, which could be interpreted as a fear aggression sign. There are data that the intensity of reaction to novelty (which was higher in KO mice) is influenced not only by the level of anxiety [10, 11], but also by "cognitive" variables [4, 5, 8, 9].

Thus, testing the behavior of *NCX2* KO mice in comparison to WT controls demonstrated that (1) mice with KO showed significantly higher cognitive abilities than WT mice (as revealed in two independent tests); (2) the anxiety level was higher in WT mice than in KO ones; (3) practically no species-specific movements (fixed action patterns according to ethological classification) of exploratory (rearing postures) or comfort (digging) behavior were found in KO mice, which was rather unexpected. Earlier, the slower "clearance" of elevated Ca^{2+} concentrations in hippocampal neurons after the depolarization was reported for *NCX2* KO mice [6]. The decrease in the frequency thresholds of LTP and LTD in the hippocampal CA1 area, which promoted the LTP, was also described.

Thus, the functional exclusion of *NCX2* changed the dynamics of calcium homeostasis, which was accompanied by an enhanced hippocampal-dependent learning and memory processes [6]. These data [6], as well as our data, evidence that the *NCX2* gene encodes the protein influencing the brain plasticity, and its knockout changes the behavior. It should be noted that the knockout of the *NCKX*2 gene (potassium-dependent sodium–calcium exchanger) exerts the opposite influence on behavior, inducing, distinct decrease in learning capacity and a decrease in hippocampal LTP [7].

NCX2 KO mice exhibited a significantly higher success in solving both cognitive tests analyzed, the test for extrapolation ability and the puzzle-box test, than WT mice, although in the former one the animals were food-motivated, while the latter one was based on aversive motivation. It is postulated that in both cases the success of solution was based the animals' ability to "understand" the object permanence rule (according to Piaget's definition) [12], which means

that the object that is not perceived any more still exists, and its search is possible. In the Ex test, it is the food that moves away from the view of a hungry mouse, while in the puzzle-box this is the existence of the underpass, although it is obstructed. In both tests, the animal meets this situation for the first time in its life, although many mice were able to solve these tasks and they were more numerous among mice with KO in comparison with WT animals.

These data demonstrate a high degree of complexity in cognitive processes and reveal the important role of membrane processes and of the fine control of the calcium flux function.

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