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Changes in Cognitive Abilities of Laboratory Mice as a Result of Artificial Selection

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Abstract. Two selection experiments with mice are in progress in the laboratory: one for large and small relative brain weights (LB and SB lines) and another for success in cognitive task solutions. Data are presented on the cognitive abilities of these animals during the solution of two tasks which are based on food (extrapolation of the direction of food stimulus movement as the food disappears from view) and aversive motivations (avoidance of the brightly lit part of a box or puzzle-box), respectively. LB mice scored higher in comparison to SB mice in both tasks, while mice selected for the high scores of the extrapolation test were more successful in the puzzle-box solution only.

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Introduction

Two selection experiments with mice are in progress at our laboratory: (1) the selection for high and low relative brain weight (LB and SB lines) (Perepelkina, Golibrodo, Lilp, & Poletaeva, 2013), and (2) selection for the ability to solve an extrapolation task (extrapolation of the food stimulus movement) (Perepelkina, Golibrodo, Lilp, & Poletaeva, 2015) — line EX and control population CoEX.

Brain weight is traditionally used as an index of brain development, revealing not only interspecies but intraspecies variability. Relative brain weight scores are regarded as the index of brain complexity along the evolutionary scale. This could mean that the search for brain-behavior causal relationships, namely brain size and the size of definite brain structures, is a promising method for the investigation of animals' elementary cognitive abilities and for analysis of the biological prerequisites of human cognitive capacities (Poletaeva & Zorina, 2014).

Cognitive processes in animals have been investigated intensely, although many aspects of their neurogenetics are not clear. It is possible that the investigation of behavior in mice selected for high scores on an elementary logic task solution (namely, an extrapolation task) could reveal new information along this line of research. In our work, "cognitive abilities" implies the capacity of an animal to understand the most simple empirical laws which connect objects and events in the external world (Krushinsky, 1990). L.V. Krushinsky, who first coined the term "animal elementary reasoning", introduced several respective tests into laboratory practice. The ability of an animal to extrapolate the direction of movement of a food stimulus, which subsequently disappeared from the animal's view, was among them and was the most simple test. In this task, an animal has to grasp (i.e., to apprehend the law of object permanence and the law of movement) that an object which has started to move, continues its movement even though it is not seen any more.

A much earlier attempt to select rats for their ability to solve extrapolation tasks was made by Krushinsky and his team (1975), wherein they found a drastic increase in animals' anxiety and fear in the experimental box. After only three generations of selection, it became obvious that the selected animals were so fearful that it was not possible to perform the tests. Keeping in mind this experience, the present mouse selection experiment for high extrapolation scores was performed using the concomitant selection against fear reactions during the extrapolation test presentation.

In this paper, data are presented for the ability of mice to solve two cognitive tasks. The first was an extrapolation test in which hungry and thirsty mice face a small cup of milk which shifts to the left or to the right as soon as the mouse starts to drink, and then disappears from view. The second task (named by the authors the "puzzle-box"; Ben Abdallah et al., 2011) implies the ability of an animal to find the escape route to the darker part of a box, despite this underpass being masked in various ways. The choice of this test for the present investigation was determined by the fact that previous learning (memorizing the position of the underpass) could not be of use if the animal does not see the underpass anymore (as it is hidden) and thus the solution requires the necessity of an animal to understand the "object permanence" rule. Thus, the puzzle-box test is partly related to the extrapolation test by the basic logic of the task. The animals used in the experiments described below belong to two pairs of lines, as mentioned above: LB and SB pairs, and EX-CoEX pairs.

Material and Method

Selection for Large and Small Relative Brain Weight (LB and SB Lines)

Three experiments in which mice were selected for this trait were done in the laboratory during recent decades. Two of these selections started using a heterogeneous population: hybrids of A/HeSto, BALB/cJLacSto, CBA/CaLacSto, C3H/HeSto, C57BL/6JSto, and CC57BR/MvRa inbred strains, while the third experiment started from a F2 hybrid LB × SB population. Half of the mice from a given litter were sacrificed at the age of 60–70 days, and the values of their brain and body weights were used to check the deviation of given litter scores from the regression curve. If these values fell above (for LB) or below (for SB) the confidence interval of the regression curve for the line, the other half of those litter members were bred to generate a subsequent generation. After the F23 of the third experiment, the selection *per se* was stopped and mice of both lines (LB and SB) were bred randomly inside each line (Perepelkina et al., 2013). The brain-body weight scores for this experiment are presented in Table 1. In all three selections, the significant changes in relative brain weight occurred at F3 – F4, with the difference being about 15 percent.

Selection of Mice for Extrapolation Ability (EX and CoEX lines)

Mice of line EX were bred from the F2 – F4 hybrid population between the LB and SB lines. The control population (CoEX) was maintained by random breeding. Two criteria for selection were used: (1) the correct extrapolation task solution at the first task presentation and correct solutions (five or six out of six presentations); the latter criterion was chosen since not many animals demonstrated these scores, while four correct choices was the value of the mean population score; and (2) the animals were rejected from selection if they demonstrated anxiety behavior during task performance. The signs of mouse anxiety were: display of the chaotic run in the experimental box and "refusals" to solve the task due to apparent fear of the test environment. Animals were not taken for breeding if they approached the food but were not eager to search for the food bait after it disappeared from view. This means that the most fearful and anxious animals were excluded from breeding.

Extrapolation Test

Mice were deprived of food and water for 18 hours and placed in a special box, in which they began to drink milk from the central opening (see Figure 1a). After a few seconds, the experimenter shifted the milk cup to the left or to the right and put it close to one of the side openings from which it was possible to drink. The correct task solution was an approach to the side opening where the milk was. The control milk cup was displaced in the opposite direction, invisible to the animal, which served to "balance" the milk olfaction cues. The test was given six times (empirically established optimal number, as at the 6th presentation the mouse satisfaction still does not influence task performance). The direction of cup movement alternated in a quasi-random manner. Results were estimated by the percentage of correct solutions by each animal group during the first task presentation and as the mean percentage of trails 1 through 6. The Fisher test for alternative proportions (φ-method) was used to estimate the statistical significance of line score differences from the 50% chance level of performance (see also Poletaeva & Zorina, 2014).

The data for extrapolation ability tests are presented for all animals of a given generation.

Puzzle-box Test

This test is based on an animal's motivation to escape from a brightly lit area of the box into a dark compartment via a small underpass (1.5 cm deep, 4.5 cm wide) which was placed in the partition between dark and lit areas at the floor level (see Figure 1b). The test contained eight stages during two days. Four stages took place on the first day. In stages 1 and 2, the underpass was free (unobstructed); in stages 3 and 4, the underpass was covered by wood shavings at the floor level. The next four stages were held on the second day: stage 5 repeated the events of stages 3 and 4, while during stages 6 and 7 the underpass was covered by a light plug (made from plastic and carton), which could be removed by the animal using its paws and teeth. In stage 8, the whole floor of the box wall which contained underpass was covered by wood shavings to a level of 4–5 cm.

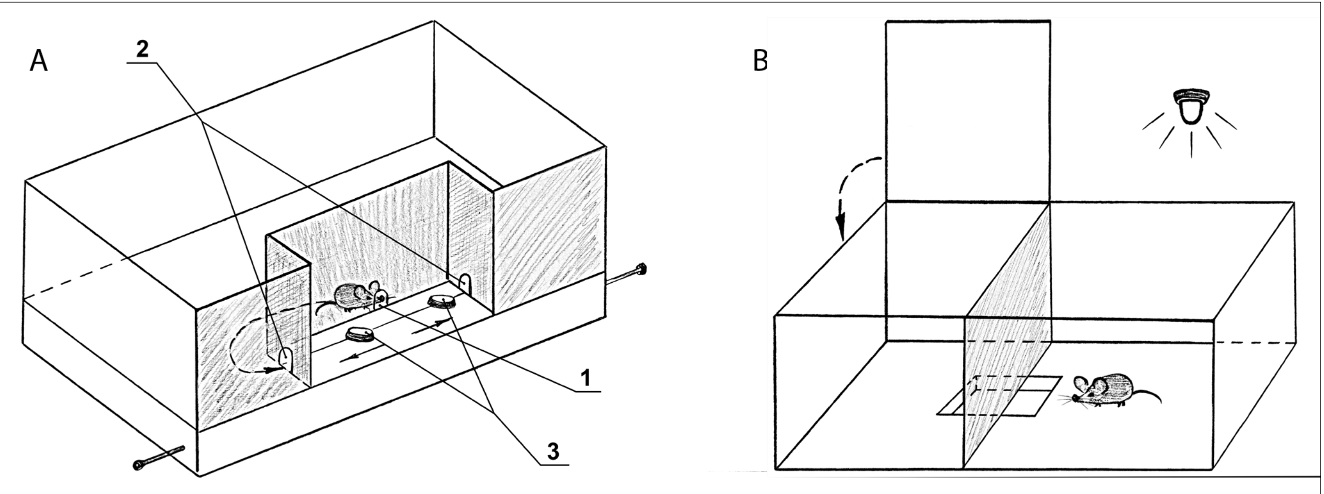

Figure 1. Schematic drawing of experimental devices. **A**: An extrapolation box (50×23×18 cm) contained central (1) and side (2) openings (10×12 and 10×10 mm), and drinking cups (3) were driven manually using special rods (not shown). Adapted from Poletaeva and Zorina (2014), Figure 4. **B**: The "puzzle box" device, consisting of two compartments, one brightly lit (right, 30×28×27.5 cm) and one dark (left, 14×28×27.5 cm), which were interconnected by an "underpass" (4.5×1.5×11.5 cm). Both devices were made of opaque plastic.

Table 1. Brain and body weight values for large brain (LB) and small brain (SB) mice in several selection generations and after the discontinuation of selection

Generation	Line	*n*	Body weight, g	*p*≤	Brain weight, mg	*p*≤
F5	LB	54	22.06±0.26	0.99	438±3.66	0.042
	SB	30	22.03±0.34		426.43±4.75	
	SB	65	23.96±0.42		433.9±3.03	
F10	LB	36	24.68±0.66	0.052	472.94±4.23	0.000
	SB	25	25.41±0.82		424.66±5.09	
F14	LB	51	29.32±0.56	0.04	486.92±5.05	0.0000
	SB	38	26.23±0.71		425.13±3.68	
F17	LB	37	28.03±0.71	0.0051	489.86±5.97	0.00000
	SB	27	23.48±0.73		406.51±3.93	
F19	LB	44	26.3±0.5	0.0003	504.1±5.3	10^{-7}
	SB	36	23.2±0.6		409.3±3.6	
F22	LB	23	21.1±0.5	0.00002	481.9±5.8	10^{-6}
	SB	22	17.3±0.4		400.5±3.5	
F25*	LB	28	28.4±1.1	0.2	490.2±5.9	10^{-7}
	SB	34	28.3±0.8		402.1±3.9	
F26–27*	LB	12	30.0±0.7	0.09	465.2±6.4	10^{-5}
	SB	16	28.5±0.5		414.4±4.5	
F28*	LB	11	29.7±1.2	0.6	485.8±11.6	10^{-6}
	SB	17	29.9±0.7		391.7±3.2	
F31*	LB	27	29.96±0.50	0.4	473.7±5.5	10^{-6}
	SB	21	30.6±0.6		420.3±6.2	

*** brain and body weight scores in generations when selection protocol was not used**

Table 2. The proportions of EX and CoEX mice which solved the puzzle-box test at the stages of "plug" in at least one of two "plug" presentations; *n* — number of animals

Generation	EX		CoEX	
	n	proportion in %	*n*	proportion in %
F9	42	76.2 ***	39	28.2
F10	19	100 ***	15	46.7
F11	43	68.2 *	29	41.4
F12	36	91.7 ***	34	26.4

*, *** significantly different from CoEX score; $p < .05$ and $p < .001$, respectively (Fisher ϕ-test)

The success of task solutions was estimated by the time (latency) required for an animal to enter the dark compartment. In case an animal failed to solve the task, the respective time scores were ascribed as 180 seconds for stages 1 through 5 and 8, and 240 seconds for stages 6 and 7 (the stages with the "plug"). The proportion of animals which were able to solve the most difficult test stages ("plug" stages) was also evaluated. Stages 3 through 8 were those which had the cognitive component, as the animal should be able to understand the object permanence rule (Zucca, Milos, & Vallortigara, 2007). The statistical significance of latency differences was tested using ANOVA with LSD Fisher post hoc analysis, while for differences in respective proportions of individuals which solved the "plug" stages, the Fisher φ-method was used.

Results

Extrapolation Task Solution in LB and SB Mice

The proportion of correct test solutions for the first selection and for presentations 1 through 6 in the LB line was significantly above the 50% chance level in several selection generations (F8, F9, F12 and F19 of the second selection; F11, F14 and F19 of the third selection). At the same time, the prevalence of correct task solutions in the SB line was found in F9 and F12 of the second selection only.

After the selection process was stopped, the prevalence of correct solutions in LB mice was preserved and these mice solved the test at a significantly non-random level in F27 and F31. Thus, in spite of a generally low level of extrapolation ability in laboratory mice (Poletaeva & Zorina, 2014), our data demonstrated that mice of the LB line are superior in this respect over the SB line.

Extrapolation Task Solution in EX and CoEX Mice

In F3 through F12 (with the exception of F8), the EX mice extrapolation performance in the first task presentation was above the chance level. However, in CoEX F4 through F6, F9, and F11 through F13, this score was also above the chance level. In F10 only the proportion of correct task solutions in EX mice was significantly different from that of CoEX mice (64% and 54%, respectively; $p < .001$). It is worth mentioning that in the course of selection, sex differences in task success were revealed. Thus the selection for such a complex trait as the ability to solve the cognitive task did not increase the capacity to solve it, while the demonstrated sex differences could also have an impact on this phenomenon. Several non-mutually exclusive explanations could be suggested (see Discussion below).

Puzzle-box Test

LB and SB lines of mice were tested in the puzzle box in F31 (the 8th generation after the selection protocol stopped). LB mice latencies were significantly shorter during stages 3 through 5 and 8, compared to SB mice, meaning that LB mice solved the task more successfully than SB mice. The times for solutions to stages 6 and 7 (when there was a "plug" in the underpass) were not significantly shorter in LB than in SB mice. LB mice latencies were long enough, but the proportion of mice which "mastered" this stage was much higher in the LB line: not a single SB mouse solved the "plug" stages, while one half of LB mice did so (although with longer latencies).

All generations of mice of the EX line (F9–F12) were more successful in puzzle-box solutions. Specifically, the latency values in the "cognitive" stages of this test were significantly lower than those of the CoEX controls. The proportions of mice which were able to solve the "plug" stages of this test were also higher in the EX line in comparison to the CoEX line (see Table 2).

Discussion

Differences in cognitive task solutions were demonstrated in mice during two independent selection experiments in which the traits for selection belonged to discrete domains: brain morphology (LB and SB lines) and complicated behavioral traits (EX and CoEX). The cognitive tests used in our experiments — the extrapolation test and the puzzle-box test — both imply the use of elementary logic operations. The puzzle-box test suggests that an animal is able to use the object permanence rule (Zucca et al., 2007). Both the LB and SB lines demonstrated a concordance of differences in the capacity to solve these two tests. LB mice were superior in both tasks.

There was another pattern of differences between the EX-CoEX pair of lines. The prevalence of EX mice was obvious only for puzzle box scores. The extrapolation test is more complicated in its structure than the puzzle box test, as it requires: (1) the formation of effective short-term memory traces (remembering the direction of food movement), (2) the ability to understand that the food stimulus which started the movement continues this movement on an invisible trajectory, (3) an understanding that the object which disappeared from view still exists (object permanence rule), and (4) performance of the instrumental skill: to move to the respective side opening in order to obtain the food. Thus, we suggest that the extrapolation task requirements are more difficult and may be (to a certain extent) contradictory for laboratory mice, and that this could be the reason why there was no direct response to the selection for high extrapolation scores. It could be that genetic elements which underlie this pattern of "requirements" cannot be selected for as the whole complicated trait.

At the same time, the response to selection was obvious in that the puzzle box performance in EX mice improved in comparison to controls. Notably, the relative brain weights in EX line mice measured in F9 and F11 were significantly higher than the values for CoEX mice (Perepelkina et al., 2015). This means that brain weight association with cognitive abilities is not a matter of simple correlation but reflects a causative relationship.

Another important issue in the analysis of mouse cognitive abilities is the problem of animal anxiety during the extrapolation test. A low anxiety level was one of two criteria for EX selection, and the anxiety indices of the elevated plus maze test (EPM) for EX mice decreased in initial selection generations. Later, the EX-CoEX difference in EPM scores was not as clear, as significant sex differences emerged (Perepelkina et al., 2015). The relevance of anxiety in laboratory tests is widely discussed as it is important for pharmacology (Enaceur, 2014).

We suggest that success in the extrapolation task, as a manifestation of the animal's cognition, is determined by multiple genetic factors with non-additive interactions. In any case, the existing data demonstrate an association between elevated relative brain weight and a capacity for elementary logic task solutions, as well as the existence of a genetic underpinning of elementary reasoning capacities.

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спецвыпуск

Изменение когнитивных способностей лабораторных мышей в результате искусственного отбора

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Аннотация. В лаборатории физиологии и генетики поведения (биологический ф-т МГУ) проводятся два селекционных эксперимента: искусственный отбор мышей на большой и малый относительный вес мозга и селекция мышей на способность к решению теста на экстраполяцию направления движения пищевого стимула. Анализ поведения животных селектированных линий в двух когнитивных тестах, основанных на пищевой (способность к экстраполяции направления движения пищевой приманки, исчезнувшей из поля зрения) и оборонительной (избегание ярко освещенной части камеры) мотивациях, показал превосходство мышей с большим весом мозга в решении обеих задач, а показатели мышей EX (отбор на высокую способность к экстраполяции) были выше только в тесте на поиск входа в укрытие.

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