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Recovery of a Motor Habit after Lesioning of the Caudate Nucleus in Rats with Different Forelimb Preferences

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One manifestation of asymmetry between the hemispheres of the brain is interhemisphere asymmetry in compensatory capacities. This question has received almost no study, with the exception of a number of reports on the motor areas of the cortex [1, 4]. We elected to investigate asymmetry in the recovery of a motor habit after lesioning of the head of the caudate nucleus, a structure known to be associated with performance of this habit [5].

Studies were performed on male Wistar rats weighing 250–300 g. The motor habit (MH) consisted of a reaching response, which has been used for studies of motor preference in rats. Initially, three sunflower seeds were placed on the floor of the experimental chamber, the animals being able to reach these with no hindrance. Rats then had to extract seeds from the opening of a tube 13 mm in diameter and located outside the chamber at a height of 5 cm. The first seed was placed at the edge of the tube and the rat could reach it with its tongue. The next piece of food was pushed in or held on a needle such that the rat could reach it only with its paws. At the final stage, sees were positioned in the tube to a depth of 17 mm. Performance of the response was developed over five steps [1, 2]: the first was from placing the rat in the chamber to collection of the first seed; the second was from eating the three seeds from the floor to obtaining the seed at the end of the tube with the tongue; the third was from taking the seed with the tongue to the first collection with the paw; the fourth was the first collection by scraping to the first performance of collecting food from a depth of 17 mm into the tube; the fifth consisted of 10 collections from 17 mm. According to the hypothesis presented in [1], the first and second stages characterize the emotional state of the animal and are conveniently termed the "emotional" stages; the second to fifth stages relate to the formation of the MH and are termed the "motor" stages." The times taken to complete all stages were measured during testing. Paw preference was identified at the fifth stage, i.e., ten collections from a depth of 17 mm. This was expressed in terms of the coefficient of asymmetry (K_{as}), where $K_{as} = (R - L)/(R + L)$, where R is the number of collections with the right paw and L is the number with the left paw. K_{as} was used to divide the rats into those preferring the right or left paws (termed "left-handed" and "right-handed"); $0.4 < K_{as} \leq 1$ identified right-handed rats and $-0.4 < K_{as} \leq -1$ identified right-handed rats; those with $-0.4 \leq K_{as} \leq 0.4$ were ambidextrous animals, which used both paws [2]. The criterion ±0.4 corresponded to seven collections of 10, and this discriminator has often been used in the literature [1, 2, 4]. The set of rats studied here consisted of 27 left-handed rats and 22 right-handed rats. Some of the rats were sacrificed during the study, such that 20 and 13 survived to the end of the study. After testing, animals were anesthetized with Kalipsol and Rometar (1.5 ml/kg of each) and electrodes were inserted into two points (L 2.5 and 3.0 mm from the bregma; A 1.5 mm from the bregma; H 5.5 mm) stereotaxically and anode currents (4 mA, 40 sec) were applied to produce electrolytic lesions to the head of the caudate nucleus (CN) contralateral to the preferred paw. The same procedure was performed in a control group (six rats), and electrodes were also inserted into the CN, though no current was passed.

Studies of the recovery of the habit of the "sick" paw were started seven days later. Walking, grooming, and collection of food from the floor showed no external changes, though testing for the habit acquired in the experiment showed that all rats had substituted the preferred paw for the "healthy" paw. Use of the "healthy" paw was prevented by

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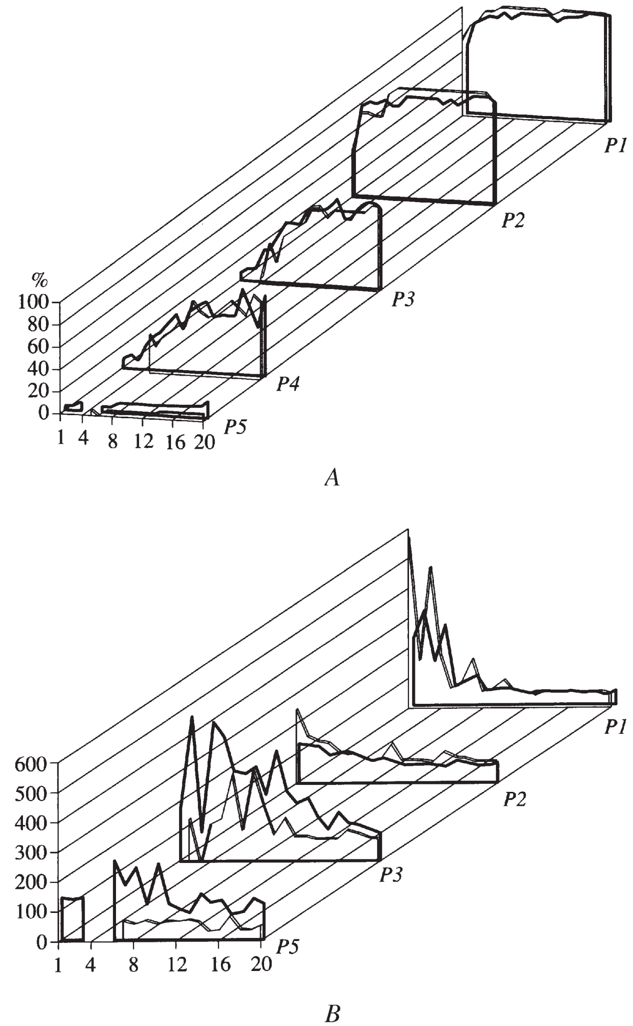

Fig. 1. Dynamics of the recovery of different stages of the habit in right-handed and left-handed rats during post-operative testing. *A*) Proportions of animals performing the habit; *B*) relative durations of different stages of habit performance, %. *P1–P5*) Stages of recovery of the habit. The abscissa shows weeks of post-operative testing; the ordinate shows the relative duration of the stage, %. Thick lines show left-handed rats and thick lines show right-handed rats.

attaching a bracelet to the wrist [4]. Re-training was avoided by testing once weekly over five months.

Animals were kept in the experimental chamber for 10–20 min during post-operative testing, depending on the extent of recovery of the habit. If the animal had not collected the seed from the edge of the tube with the paw by 10 min, the experiment was stopped. If collection with the paw did occur, the experiment was continued for a further 10 min from this time point. The relative duration (RDu) of the above stages was measured (ratio of durations at each stage to the corresponding measure before surgery, which was taken as 100%, was determined for each animal independently, along with the proportion of animals performing the given stage of MH. On insertion of the paw into the tube at the fourth stage, the relative depth (RDe) was also measured, as the ratio of the depth achieved to the maximum possible (17 mm).

Results were analyzed using a PC-486 DX using the Mann–Whitney, Fisher, χ^2, and Student's tests. After experiments, animals were anesthetized and brains were fixed with 10% formalin. Lesion volumes were measured on series of frontal sections of thickness 20 μm, stained using the Nissl method; each fifth section was examined. Further processing was performed by computer using the IP3DemoPlus program.

First and Second Stages. Recovery of the emotional stages (Fig. 1, *A*) occurred in the first weeks of the study. The proportion of "functioning" right-handed rats was slightly greater than that of left-handed rats. However, left-handed rats generally performed these stages more quickly than right-handed rats (Fig. 1, *B*), and this difference was significant for the first stage at 19 weeks and for the second at 4, 11, 13, and 14 weeks (Mann–Whitney test, $p < 0.05$).

Third Stage. Recovery of the operant limb movement was much slower. Unlike the situation with the first and second stages, the proportions of left-handed rats performing the movement was slightly greater than the proportion of right-handed rats (Fig. 1, *A*). The RDu for the third stage decreased from week to week, though less than for the first and second stages. The mean RDu for performing the movement at the third stage, unlike that for the emotional stages, was slightly lower in right-handed rats (Fig. 1, *B*).

Fourth Stage. Performance of this seed-procuring movement suffered the most. As only an insignificant number of rats could collect the seed from the maximum depth (17 mm), measurements for the fourth stage were made of its duration and the maximum depth of insertion of the paw into the tube over a fixed period of time (10 min from the end of the third stage). As shown in Fig. 1, *A* and Fig. 2, *A*, right-handed rats started to perform (partial) collections of food only from five weeks of post-operative testing, while some left-handed rats performed partial collections from the first week. These differences were significant at four weeks of post-operative testing ($p < 0.05$; Fisher's test). In general, there were no significant differences between the two groups of rats in terms of the depth of paw insertion. However, there were significant differences in the time taken to achieve the maximum depth of paw insertion. Figure 2, *B* shows that a greater proportion of right-handed rats achieved the maximum depth of paw insertion at 1–5 weeks of post-operative testing ($p < 0.05$), while this occurred in left-handed rats at 11–15 weeks ($p < 0.05$; χ^2 test). Thus, left-handed rats started to recover the fourth stage earlier than right-handed rats, but achieved the maximum depth at later stages of testing.

Fifth Stage. This stage was characterized by animals which had made complete recoveries, i.e., those animals which could use the "sick" paw to collect ten seeds from a

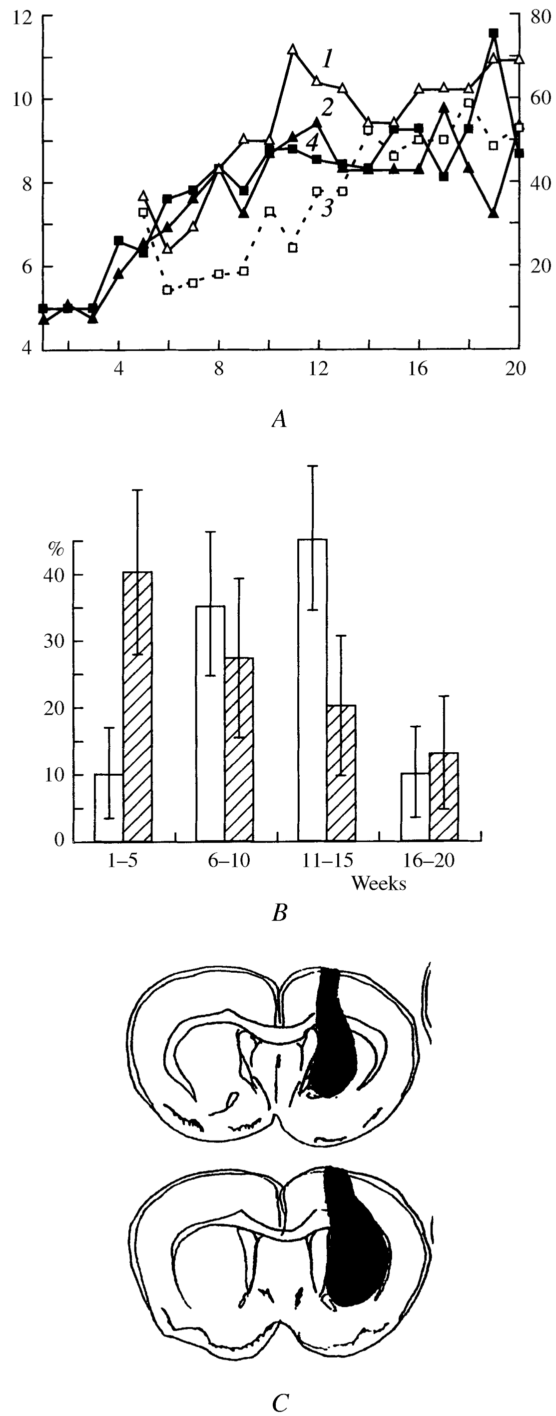

Fig. 2. Dynamics of performance of the fourth stage of the habit in right-handed and left-handed rats. *A*) Mean relative depth of paw insertion into the tube (left vertical axis, cm; curve *3* shows right-handed rats and curve *4* shows left-handed rats) and percentage of animals (right vertical axis; curve *1* shows right-handed rats and curve *2* shows left-handed rats) performing (partially) the habit at stage 4. The horizontal axis shows weeks of post-operative testing. *B*) Time taken to achieve the maximum depth of paw insertion in right-handed (shaded columns) and left-handed (white columns) rats. The horizontal axis shows weeks of post-operative testing (mean values are shown for blocks of 5 weeks); the vertical axis shows the percentage of animals achieving the maximum depth of paw insertion into the tube for that animal. *C*) Frontal sections of the brain of an experimental rat showing an example lesion.

depth of 17 mm. Few rats reached this level of recovery: three left-handed rats and two right-handed rats, such that the proportion of left-handed rats completely performing the MH was greater than that proportion of right-handed rats (Fig. 1, *A*). The mean RDu for performance of the fifth stage was greater in left-handed than in right-handed rats, though the difference was insignificant in terms of the Mann–Whitney test (Fig. 1, *B*).

Thus, complete recovery of the habit occurred in five animals and partial recovery occurred in 28 animals; five animals showed no recovery of the MH (three right-handed and two left-handed).

At the end of the study, all animals were tested for limb preference during free behavior, without the bracelet. Completely recovered animals continued to use the initially preferred, "sick" limb, i.e., it was clear that adequate compensation had been achieved and the rats experienced no discomfort in performing the MH. The other rats used the previously non-preferred, "healthy" limb in the no-bracelet test.

In the control group, in which electrodes were inserted into the CN without passage of current, the habit was completely recovered by seven days after surgery.

The results obtained here show that at the "emotional" stages, the proportion of functioning animals was generally somewhat larger among right-handed rats, i.e., animals with left hemisphere motor dominance, while there were no significant differences in this measure between groups at the "motor" stages. The mean RDu of performing the emotional stages was lower in left-handed animals, while the mean RDu of the motor stages (stages 3 and 5) was lower in right-handed rats. As regards the fourth stage, there were no differences in the depth of paw insertion between the groups of rats, though left-handed animals started to recover the fourth stage significantly earlier than right-handed rats, even though left-handed animals achieved the maximum insertion depth later.

Morphological monitoring showed that the CN was lesioned in all animals. The motor cortex and corpus callosum were also affected to some extent. The mean volumes of lesions in the head of the CN in right- and left-handed animals were not significantly different, and were 12.5 ± 5.3 and 14.1 ± 6.3 mm^3 respectively. Lesions to the head of the CN in control animals, due to electrode insertion, were significantly smaller than in experimental animals (significant difference, $p < 0.05$). Among the experimental animals, a critical level of lesion volume was reached, as supported by the absence of any correlation between the extent of recovery and the volume of the lesion. Figure 2, *C* shows an example of a lesion.

Thus, these experiments demonstrated asymmetry in the compensation for disruption of a lateralized motor habit following lesioning of the head of the caudate nucleus. The authors of [5] observed severe disruption to reaching reactions after lesioning the head of the CN, though they did not report any differences in recovery related to motor preference. It is interesting to compare our data with those on the recovery of this reaction after lesioning of the motor cortex contralateral to the preferred paw, as reported by Miklyaeva et al. [4] and Vasil'eva [1]. Lesioning of the motor cortex (MC) was followed by much faster recovery: complete recovery of all stages of the habit was seen by six weeks in 80% of animals, though it might be expected that damage to the MC, as compared with damage to underlying structures, would lead to longer-lasting motor disturbance. It may be that the CN is one of the structures involved in the recovery of the habit after damage to the MC, and that the more severe disruption of the habit is associated with combined lesioning of the MC and CN, as shown by the morphological monitoring in the present experiments. It is also important to note the fact that in rats, the CN receives inputs from almost the entire cortex; unlike the situation in, for example, carnivores, not all cortical efferents pass through the internal capsule but go directly through the striatum [3]. The question of the reasons for the more severe disturbances after lesioning of the CN as compared with the MC is of great interest and clearly requires its own experimental study.

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