Behavioural Brain Research 131 (2002) 1–7

BEHAVIOURAL BRAIN RESEARCH

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Research report

Early life environment modulates 'handedness' in rats

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Received 20 May 2001; received in revised form 27 June 2001; accepted 27 June 2001

Abstract

Right handedness is one of the most prominent markers of human functional brain asymmetry. Deviation from this norm appears to be associated with certain developmental disorders. While many studies have dealt with the genetic contribution to the determination of handedness, few have examined whether environmental factors that are subtler than forced hand switching can modulate the development of handedness. In this study, we exposed rats to a novel environment for 3 min daily during their first 3 weeks of life and found that their paw preferences during both infancy and adulthood showed a leftward shift compared with the controls. This result suggests that 'handedness' can be modified by rather subtle early environmental manipulation. Since exposure to a novel environment does not involve a direct asymmetric activation of the sensory–motor system underlying paw-use, mechanisms beyond this paw-specific system must exist to mediate the observed modulation of 'handedness'. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Handedness; Laterization; Novelty; Handling; Neonatal environment; Rats; Paw reaching; Asymmetry

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1. Introduction

Approximately 90% of the human population shows a right hand preference [13]. This preference is paralleled by asymmetric processing in the underlying motor system [7]. In rodents, a weaker population level right 'handedness' has been reported since the 1930s in studies of two different strains of rats and 29 strains of mice [14,31,34]. Unilateral lesions of the motor system underlying paw-use have been shown to impair reaching and grasping in the contralateral paw [36]. Genetic models of human 'handedness' [1,23], as well as genetic manipulation of rodent paw preference [6,2] have received considerable attention. In contrast, studies concerning environmental influences on handedness have been limited to the effects of forced selective paw use [15,36].

Environmental modulation of handedness can be of two types. First, by limiting the use of one hand or by selectively damaging the sensory motor system in one hemisphere, one can directly change the symmetry in the sensory motor system underlying hand use [36] (mechanism of direct asymmetric activation). Examples of direct asymmetric activation could also be found in studies of chicks in which asymmetric embryo posture lead to visual lateralization [26]. Alternatively, environmental influences could initiate a modification in symmetry through a general neuromodulatory system that is non-specific to the direct control of hand or paw use, but interacts with and modifies the symmetry of the hand-specific system (mechanism of asymmetric modulation).

To investigate whether early life environment can modulate 'handedness' through a mechanism non-specific to the sensory-motor system, we designed a neonatal novelty exposure procedure [30] based on the 'handling' method [20]. We exposed neonatal rats to a novel cage for 3 min daily during their first three weeks

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PII: S0166-4328(01)00330-8

of life. This novelty procedure has been shown to selectively enhance neural plasticity in the right hippocampus [40] and to produce a relative increase of right hippocampal volume [33]. Since this novelty manipulation does not involve a direct manipulation of paw use, nor does it involve any direct asymmetric stimulation of the sensory–motor system that controls paw use, any difference in paw preference between the novelty and control groups must be accounted for by the mechanism of direct asymmetric modulation.

2. Methods

2.1. Animals

Eight pregnant Long Evans hooded rats arrived in our laboratory 7–11 days prior to giving birth (Harlan Sprague–Dawley Company, Indianapolis, IN). A total of 52 male (46) and female (six) pups born of these dams (four to nine pups per litter) participated in this study. Pups were housed with the dams until weaning on postnatal day 21. The dams and post-weaning pups were housed separately in translucent plastic cages (51 × 25 × 22 cm) with a 07:00–19:00 h light–dark cycle. Animals were given water and food ad lib except for 4 days prior to and during the reaching test, during which access to food was restricted to maintain 90–95% of baseline body weights.

2.2. Materials

We designed a novel reaching apparatus for evaluating paw preference. Animals were individually tested in a metal housing cage with two small openings in the front separated by 1 cm (Fig. 1b). The FrootLoop rewards were placed outside of the cage, 1 cm in front of the two small openings. The testing cage was surrounded by a wall of cardboard to provide symmetric outer visual background. The symmetry of the reward presentation, i.e., the symmetry of visual and olfactory cues, were manipulated to allow assessment of their possible roles in paw preference (see below). A small opening in the wall of the cardboard, symmetric with respect to the two openings, was cut to allow video recording of the testing procedure. The openings in the testing cage were large enough to allow access to food rewards by the forepaws but small enough to prevent the animal from obtaining the reward using its snout.

3. Procedures

3.1. Novelty exposure

Using a split-litter design, we assigned the two halves of the pups within each litter to the experimental (E) and control (C) groups on postnatal day one (P1) (Fig. 1 a). We used a toe clipping procedure to mark the pups' group identity. All toe clippings were done on the hind-paws to avoid possible interference with the use of forepaws in the reaching task. The E and C pups were indicated by different digit combinations (e.g. E, left little finger and right thumb; C, right little finger and left thumb). The combinations were counterbalanced between the E and C groups. This procedure did not seem to produce any noticeable behavioral deficits.

Each day from P1 to P21, the dam was transferred to a separate cage placed next to the home cage. The E pups were then transferred to their individual new cages lined with fresh sawdust, which were also placed next to the home cage, and they remained there for 3 min. The C pups were left in the home cage. Each time an E pup was picked up by the experimenter, a C pup was also picked up and replaced to its original location. Except for the exposure to a novel environment, E and C rats received identical treatment. Fig. 1 a shows the sequence of pups' separation from the dam, exposure of the E rats to the new boxes and their return to the home cage, and the dam's return to the home cage.

3.2. Reach task

On each day, a total of eight successful trials was completed. A successful trial consisted of one or more attempted reaches followed by the retrieval of the reward with either paw. Since the target was presented laterally to the animals, this procedure was similar to the lateral paw reaching task [34] in contrast to the medial paw reaching task [6]. Stability over repeated measures is critical in establishing an animal model of 'handedness' and in evaluating the effect of the neonatal manipulation. Therefore, animals were tested first on 4 consecutive days at 6 weeks of age and again on a 5th day 6 months later. The 5 days of testing differed in developmental stage (infancy vs. adulthood), in the choice condition (free choice vs. forced alternation), and in the side where the first reward was placed (different initial conditions). These variations allowed us to evaluate the robustness of paw preference under different testing conditions and developmental stages.

On the first day of testing, food rewards were available at both openings (free choice). The same testing condition was repeated on the second day to allow the animal to reach a stable level of performance. From the second day on, all rats displayed stereotypical behavior in the reaching chamber and the total amount of time required to complete the eight trials also reached the same level (approximately 10 min) as in the following

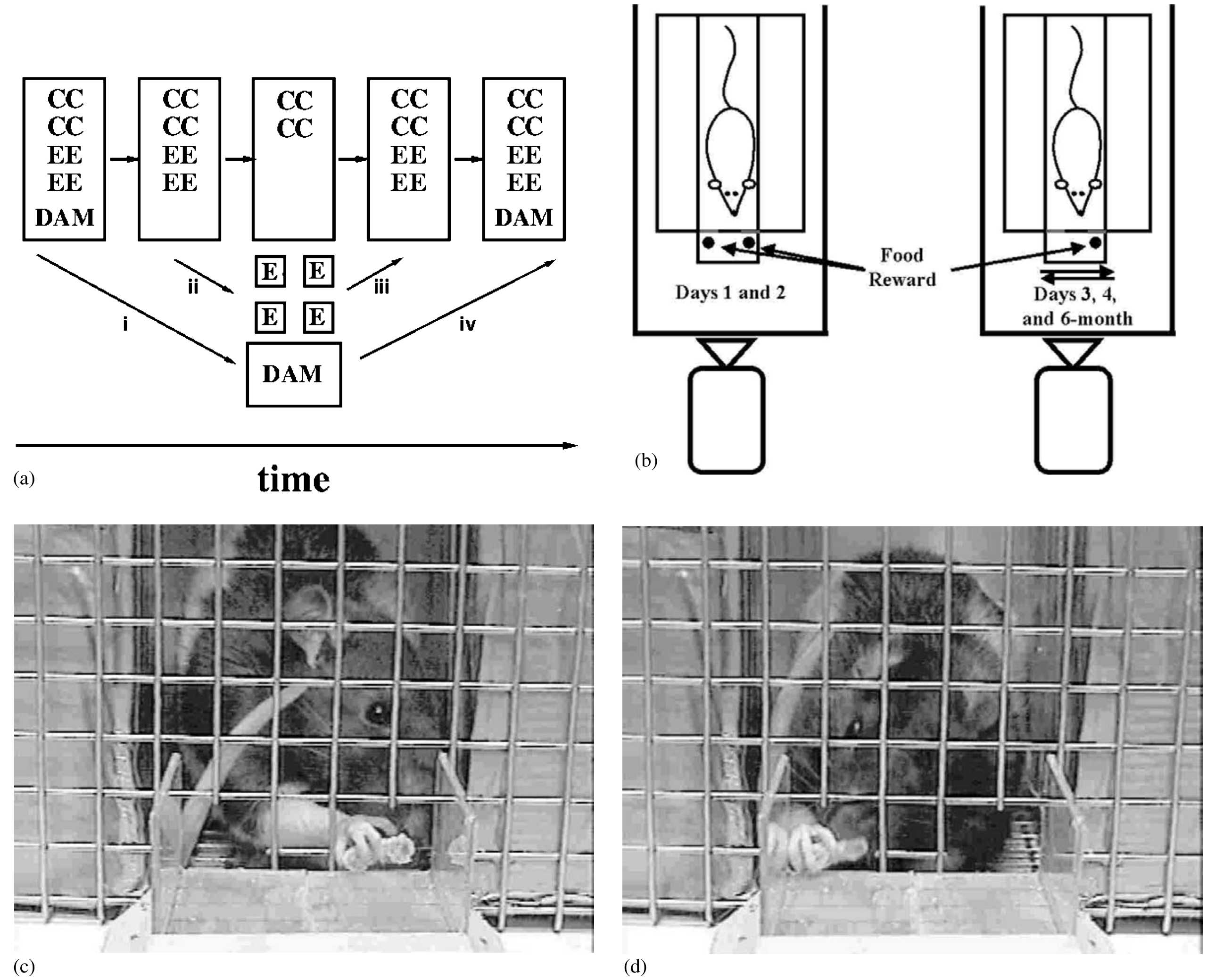

Fig. 1. a. The split-litter design: on postnatal day one, the two halves of each litter were randomly assigned to the experimental (E) and control (C) conditions. Steps of the daily neonatal novelty exposure, (i) separation of the dam from the litter, (ii) transfer of the experimental pups (E) to their novel individual cages where they remained for 3 min, (iii) return of the E pups to their home cage, (iv) return of the dam to her pups in the home cage. b. The paw reaching apparatus and the reaching task. On each of the 5 testing days, the rats were trained in eight trials to obtain one reward (a half of a FrootLoop) per trial. Day 1 and 2, free choice condition. The animal could retrieve from either the left or right opening. Day 3, 4, and retest 6 months later: forced alternation condition, in which reward was only available at one of the two openings. c–d. Examples of reaches made by a right-pawed rat through the left and right openings of the reaching apparatus.

testing days. On the third day, rewards were only presented at one of the two openings on any given trial and the locations were alternated between trials (forced alternation). If the paw preference was due to an asymmetry in the visual or olfactory cues, this forced alternation between the two openings should lead to a change in the number of left and right reaches from those observed under free choice condition on day 1 and 2. On the fourth day, the side of the first reward was altered from day 3 to test for any possible influence of the reward location on the first trial. On both day 3 and 4, half of the animals started with a left and the other half with a right reward. If the first trial paw choice is critical in determining the daily paw preference, then the paw preference should switch between day 3 and day 4. Six months later, long after the animals entered adulthood, the same testing condition as day 4 was used. This was done to evaluate long-term stability of paw preference.

4. Results

The direction of paw preference was measured by a daily directional lateralization score (directional L score) computed as $(L-R)/(R+L)$, where R and L are the number of reaches made by the right and left paws, respectively. A score of $+1$ and -1 corresponds to a perfect left and right-paw preference. A score of zero indicates ambidexterity (i.e. a lack of either left or

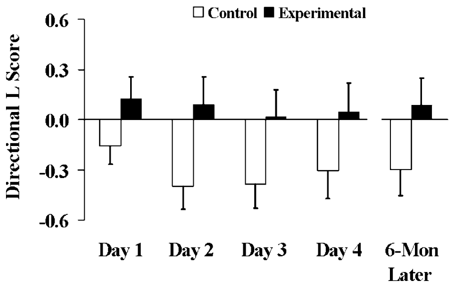

Fig. 2. Directional paw preference in experimental (E) and control (C) rats. C rats (grey bars) showed significant right-paw bias. Paw preference in E rats (black bars) differed significantly from that of C rats.

right preference). A change from a more negative to a more positive score indicates a right to left shift in paw preference and vice versa. As no significant sex difference was found (most likely due to the small number of female rats in the study), we combined the male and female data in the following analysis. One and two sample t-tests and Spearman's rank order correlations were performed on these directional lateralization scores. Significance levels were reported for one-tailed test because previous studies [8,10,33,40] predict that the novelty manipulation induces an increase in right hemisphere dominance and, therefore, a left-shift in paw preference.

4.1. Directional asymmetry

In the C rats, we found a right paw preference based on the average of the directional L scores for all 5 days of performance ($t = -2.275$, $P < 0.025$) (Fig. 2). Similar results were also found for all but the first day of testing across the varying testing conditions (day 2, free choice, $t = -2.896$, $P < 0.005$; day 3, forced alternation, $t = -2.653$, $P < 0.010$; day 4, forced alternation, $t = -1.826$, $P < 0.050$; day 5, adulthood, $t = -1.915$, $P < 0.050$)[2]. This consistent right paw bias, despite changing experimental conditions and developmental stages, suggests that the observed right-paw population preference is developmentally stable. In the E rats, the directional lateralization score was not significantly different from zero.

4.2. Stability of asymmetry

We further examined both short-term and long-term consistency in individual paw preference by computing the correlations among the lateralization scores of all days of testing (Table 1). The directional L scores across all 5 days were highly correlated with a Spearman's rank order correlation (R_s) ranged between 0.734 and 0.885 for any 2 days of comparison. Despite a 6 month delay between the last 2 days of testing, strongly left and right-pawed rats remained left and right-pawed and ambidextrous remained ambidextrous (Fig. 3). These results indicate that the paw-preference was also stable at the level of individuals across different testing conditions and different developmental stages.

4.3. Effect of novelty on asymmetry

We examined the effect of neonatal novelty exposure on paw preference. Directional L scores of the E rats were significantly greater than those of the C rats based on the average from all 5 days of testing (Fig. 2, $t = 1.917$, $P < 0.030$). Similar results were also found on individual days of testing (day 1, $t = 1.640$, $P < 0.054$; day 2, $t = 2.262$, $P < 0.0140$; day 3, $t = 1.859$, $P < 0.036$; day 4, $t = 1.492$, $P < 0.071$; 6 months later: $t = 1.771$, $P < 0.047$). These daily differences suggest that the effect of the novelty exposure on paw preference remained robust across testing conditions and developmental stages.

As there exist three subgroups of rats (left and right pawed and ambidextrous) (Fig. 3) and the control rats were right-paw biased, we computed a difference score for each litter: $N_{\mathrm{Right,C}} - N_{\mathrm{Right,E}}$. A Chi-square test showed that the number of right-pawed animals was significantly reduced in the E group ($P < 0.05$). The general trend is that E rats had a smaller proportion of right-pawed animals and greater proportion of left-pawed or ambidextrous animals than the C rats.

Table 1
Correlations of lateralization scores across 5 days of testing

	Day 1	Day 2	Day 3	Day 4	6 months later
Day 1	1.000	0.792	0.757	0.737	0.734
Day 2		1.000	0.824	0.823	0.788
Day 3			1.000	0.876	0.844
Day 4				1.000	0.844
6 months later					1.000

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[2] ANOVA revealed no litter effects. Therefore, litters were pooled in all analysis.

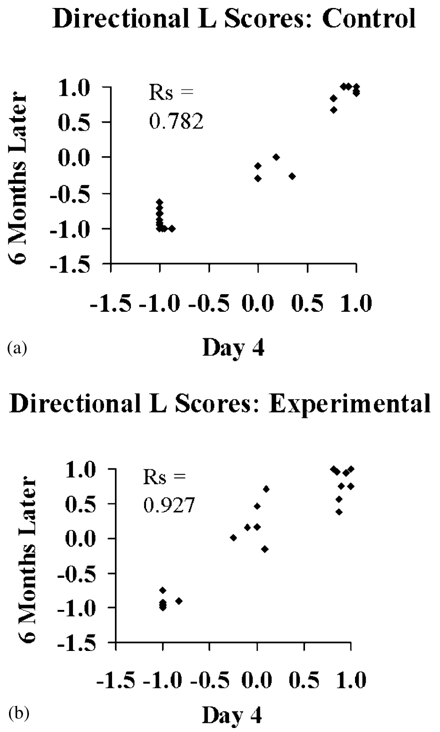

Fig. 3. Correlation of directional lateralization scores in the experimental (E) and control (C) groups between infancy (6 weeks old) and adulthood (7.5 months old). (a) E rats. (b) C rats.

5. Discussion

Using a novel reaching apparatus, we were able to demonstrate a long-lasting population level right paw preference in the rat. By modifying the classic neonatal 'handling' procedure [20], we were able to reduce the number of factors involved in the original design. This novelty procedure [30] allowed us to show that, in the absence of maternal separation, maternal stress, and experimenter handling per se as possible founding factors, neonatal novelty exposure lead to a modification of population asymmetry in paw preference. It is important to recognize that the role of neonatal novelty exposure should be considered as a triggering event that not only provides stimulation to the novelty exposed pups early in life, but also initiates a chain of events in the subsequent pup-to-pup and dam-to-pup interactions that can further influence the original treatment effects [9,21].

5.1. Right population level paw preference

Population level asymmetries in non-humans have been demonstrated among vertebrates (see reviews: [16,33]. In rodents, a population level asymmetry in 'handedness' has also been demonstrated [14,31,34]. Our finding of a right population level paw-preference in the control rats is in agreement with these findings, providing further support for the claim that humans are not unique in preferential hand use and that a homology exists between human and rat reaching movement [37]. However, negative findings on rodent population level paw preferences have been reported when a medial paw reaching test [6] was used or when the relative position of the reach target to the animal's body was not constrained [35]. We believe that these seemingly inconsistent results in the literature can be explained in terms of the differences among testing methods. It has been shown that the original Collin's test, a medial paw reaching test, appeared to be less sensitive than the lateral paw reaching test introduced by Waters and Denenberg [34]. The reaching apparatus used in Whishaw's study [35] allowed a great deal of variability in the angle of approaches, which could strongly influence the choice of paw use as was the case in studies of reaching in primates [19]. Among other possible causes, this variability may have prevented the detection of any population level paw preference. The minimized sources of variance afforded by our reaching apparatus may be the key to our improved ability in detecting a population asymmetry, even in a small number of reach trials.

5.2. Reduction in right-paw preference

The experimental rats differed significantly from the controls in that the experimental animals were less right-pawed. This result indicates that 'handedness' at adulthood can be modulated by subtle early life environmental manipulation, such as the 3 min daily novelty exposure. The observed short and long stability in paw preference also suggest that the effect of early environmental influence on 'handedness' is stable and long-lasting. The direction of modification is consistent with previous studies in which similar neonatal stimulation resulted in an increase in right-hemisphere dominance manifested in the control of open field activity [8,10], in the increase in neural plasticity and the influence of corticosterone on such neural plasticity [40], and in the increase in the relative hippocampal volume [33]. An increase in right-hemisphere dominance is consistent with a reduction in right paw preference.

5.3. Conclusions

Our observed difference in paw preference between the novelty exposed and the control animals suggests that a subtle symmetric manipulation very early in life can produce a shift in 'handedness' and this effect is stable, lasting well into adulthood. As the treatment of the E rats did not involve a lateralized stimulation of the sensory and motor systems directly underlying paw use, the observed change in paw preference could not be mediated solely by a direct alteration of asymmetry within the hand-specific system. Thus, our finding is consistent with the hypothesis that a general modulatory system, non-specific to the direct control of hand or paw use, must be involved in this observed environmental modulation of rodent 'handedness'. A number of lateralized neuromodulatory systems [3,4,11,18, 24,29,38] might be the candidates for the observed modulation of 'handedness' because of the roles that ACh, NE, and corticosterone play in an animal's response to novel [25,27] or stressful environment [22] and because of the involvement of DA in expectancy, prediction, and surprise [28]. Last but not least, deviations from normal brain lateralization, such as extreme handedness, have been associated with several forms of learning, affective, and developmental disorders [5,12,17,39]. Our results may suggest an animal model for investigating a possible role of environment in pathologies associated with abnormal brain asymmetry.

Acknowledgements

We thank Linda Alvarado, Leona Zamora, Tina Urbanki, and Jennifer Jones for assistance. We thank Ian Whishaw, Victor Denenberg, Robert Sutherland, and Dennis Feeney for discussions. We thank George Cowan for his generous gift.

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