Behavioural Brain Research 293 (2015) 162–165

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Right-but not left-paw use in female rats provides advantage in forced swim tests

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HIGHLIGHTS

- Behavioral laterality including paw preference in rats reveals hemispheric asymmetry.
- Left-pawed female rats are more susceptible to induction of depression by means of forced swim tests than right-pawed rats.
- Paw preference in female rats does not affect spatial learning in the Morris water maze.
- Paw preference is stable and may provide a model of lateralization in depression.

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 6 July 2015
Accepted 6 July 2015
Available online 26 July 2015

Keywords:
Paw preference
Depression
Laterality
Forced swimming
Water maze

ABSTRACT

Left- and right-pawed adult female Wistar rats were subjected to forced swimming on two consecutive days. Compared to the right-pawed group, left- pawed rats displayed significantly increased immobility from the first to the second swim test and remained significantly more immobile in the second swim test. Both groups performed similarly in spatial learning in the Morris water maze suggesting that left-pawed rats are differentially and specifically susceptible to depressogenic treatment.

There is strong evidence that the two cerebral hemispheres are differentially involved in the etiology, symptomatology and possibly the therapy of depression [1–3]. Handedness in humans not only provides valuable insights into cerebral asymmetry and lateralized hemispheric functions but is also a potential indicator of differential susceptibility to mood disorders. In particular, left-handed people are more likely to suffer from depression or to score higher in measures of depression [4–6]. Moreover, intracranial stimulation in epileptic patients undergoing brain surgery indicated a strong lateralization in mood with right-sided stimulation inducing dysphoric mood more frequently than left-sided stimulation [7].

Because findings related to cerebral lateralization in mood disorders in humans are correlational in nature, research with animals may provide the basis for investigating the mechanisms related to cerebral lateralization and depression. A large number of animal studies on side biases such as turning, rotation or paw preference have uncovered underlying hemispheric laterality involving neuroanatomical [8,9], neurochemical [10–14] as well as neurophysiological differences [15] in the rodent brain paralleling findings in humans.

Since handedness in humans is indicative of hemispheric asymmetry and susceptibility to mood disorders, paw preference in rats may also be a behavioral marker for the asymmetric involvement of the two hemispheres in depression. While there is no clear population bias for paw preference in rats unlike the overwhelming right hand bias in humans, individual rats do show clear preference for paw usage. A survey of the literature indicates that there is no direct assessment of paw preference in an animal depression model. It is therefore important to investigate the relationship between paw

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http://dx.doi.org/10.1016/j.bbr.2015.07.027
0166-4328/

preference and response to a depressogenic manipulation. In the present study, rats displaying right or left paw preference were subjected to forced swim tests to assess the potential effect of paw preference on behavioral despair induced by two forced swim tests separated by 24 h. Increased immobility in the second swim test compared to the first is a sign of behavioral despair [16]. The present study therefore aimed to investigate the effect of forced swim tests on rats determined to have preference for the left or the right paw. Studies with rodents indicate a stronger activation of the hemisphere contralateral to the preferred side or paw [11,14,17]. Since left hemispheric activation in rats has been shown to be as effective an antidepressant treatment as bilateral stimulation [18], it was hypothesized that right-pawed rats will be less susceptible to the depressogenic effect of forced swim tests than left-pawed ones.

Comorbidity of depression and cognitive impairment is frequently observed in depressed patients and in animal models of depression [19,20]. The present study therefore also investigated the possible differential effect of paw preference in learning as assessed by means of a Morris Water Maze (MWM) task [21]. Because the MWM test involves a swim-related stress protocol [22], comparison of performances in the forced swim tests and the water maze task may indicate whether the potential differential behavioral manifestations of paw preference in the present study is a general phenomenon related to stressful situations or is specific to depressogenic manipulations.

Adult female Wistar rats weighing 188–227 g at the beginning of the experiment were housed in a temperature controlled environment (22 ± 2 °C) in cages of four with a 12 h L:12 h D lighting schedule (lights off at 7:00 PM). Food and water were freely available except during paw preference tests when rats were deprived of food for 48 h before the first paw preference session and for 24 h before the subsequent ones. The study was approved by and conducted in accordance with the regulations of the Bogazici University Ethics Committee on Animal Maintenance and Experimentation.

Paw preference was determined in a test where food-deprived rats could obtain food from a tube narrow enough to be obtained only by a single paw but equally accessible by either paw [23]. This procedure was administered 4 times over 8 days (50 trials/day) and animals were classified as left-or right-pawed if they used the left or the right paw, respectively, in at least 70% of the 100 trials in the last two days of testing. The day after the second swim test, rats were administered one more paw preference session in order to determine whether forced swimming stress altered paw pReferences

Four days after the last paw preference test session, eight left- and nine right-pawed rats were subjected to two consecutive forced swim tests separated by 24 h. Swim tests were performed in a Plexiglas cylinder with a height of 45 cm and 30 cm diameter filled with water (25 °C ± 0.1 °C) up to the height of 30 cm. Animals were allowed to swim for 15 min and 5 min in the first and second swim tests, respectively.

Seven weeks after the second forced swim test, animals were tested over five days (5 trials/day with an intertrial interval of 10 min) in spatial navigation test using a water maze (WM) tank with a hidden platform. The tank was a circular Plexiglas pool (120 cm diameter, 60 cm height) filled to a height of 40 cm with water at 24 °C ± 0.2 °C temperature and was surrounded by a large number of objects providing distinct cues. A transparent platform (10 cm × 10 cm) was fixed in position with the top 2.0 cm beneath the water surface. On each trial, the animal was randomly immersed into water from one of four designated starting points and was allowed to swim until it climbed onto the platform. The duration of this swimming interval was recorded as escape latency. Animals were allowed to stay on the platform for 10 s before being removed from the tank. When an animal failed to climb onto the platform within 60 s, it was gently guided by hand to the platform and allowed to stay there for 10 s. A two-minute probe trial was conducted on the sixth day of testing with the platform removed.

Preliminary analyses revealed that the data was normally distributed (Shapiro–Wilk tests, all $ps > 0.30$) and all variances were equal (Levene's tests, all $ps > 0.08$) except for one comparison ($p = 0.02$), which was analyzed via a *t*-test corrected for violation of homogeneity (see below). Two-way analyses of variance (ANOVA) with repeated measures were used to assess durations of immobility in the two forced swim tests (Paw Preference × Swim Tests) and spatial learning performance (latency to step on the platform) over the five days of water maze testing (Paw Preference × Days) followed by independent- and paired-samples *t*-tests as post hoc evaluation of significant differences of $p < 0.05$. An independent-samples *t*-test was used to evaluate the time spent in the quadrant where the platform was previously placed in the probe session of the water maze.

The percentages of food retrieval (mean ± sem) by the preferred paw for the left- and right-paw groups were, respectively, 85.5 ± 3.3 and 94.6 ± 2.1 before and 85.0 ± 5.1 and 91.8 ± 3.5 after the forced swimming tests. Paired-samples *t*-tests indicated no significant change between the paw preferences determined before and after swimming in either group (left-pawed: $t(7) = 1.02$, $p = 0.3389$; right-pawed: $t(8) = 0.10$, $p = 0.9199$). The left-pawed group had a significantly lower percentage of paw preference compared to the right-pawed group before ($t(15) = 2.42$, $p = 0.0287$) but not after the forced swim tests ($t(15) = 1.15$, $p = 0.2670$).

Fig. 1 shows the durations of immobility (mean ± SEM) in the first five minutes of the first swim test and during the second swim test. ANOVA indicated a significant effect of Swim Tests ($F(1,15) = 12.58$, $p = 0.0029$), no significant main effect of Paw Preference ($F(1,15) = 3.79$, $p = 0.0704$), but a significant Paw Preference × Swim Tests interaction ($F(1,15) = 28.40$, $p = 0.0001$). Post hoc paired-samples *t*-tests revealed a significant increase in the duration of immobility from the first to the second swim test for the left-pawed ($t(7) = 4.98$, $p = 0.0016$) but not the right-pawed group ($t(8) = 1.73$, $p = 0.1218$). Moreover, left-pawed rats displayed significantly longer duration of immobility in the second swim test ($t(15) = 3.19$, $p = 0.0100$, equal variances not assumed) but not the first ($t(15) = 0.10$, $p = 0.9224$) compared to the right-pawed ones.

Fig. 2 shows the mean latencies (mean ± SEM) to reach the platform in the five days of the water maze task and the time spent in the quadrant containing the platform in the probe test. ANOVA indicated a significant effect of Days of testing in the water maze ($F(4,60) = 41.69$, $p = 0.0001$), but no significant main effect for Paw Preference ($F(1,15) = 1.12$, $p = 0.3070$) nor a significant Paw Preference × Days interaction ($F(4,60) = 0.41$, $p = 0.8010$). An independent-samples *t*-test revealed no significant difference between the two paw groups in the probe test for the duration spent in the quadrant previously harboring the hidden platform in the training days ($t(15) = 0.93$, $p = 0.3651$).

The results indicated that left-pawed rats are more vulnerable to behavioral despair while the water maze tests showed no differential effect of paw preference in cognitive function in a spatial task.

The results point to a significant difference in the way left- and right-pawed rats respond to behavioral despair induction in the form of two forced swim tests. Specifically, left-pawed rats showed a significant increase in the duration of immobility in the second swim test compared to the right-pawed rats. The finding that left-pawed rats are more susceptible to behavioral despair induction is bolstered by the fact that the left- but not the right-pawed rats significantly increased their duration of immobility in the second swim test compared to the first, a well-established sign of behavioral despair. Thus, the present findings not only point to the vulnerability of left-pawed rats to depression inducing stress,