

The Stability and Intertest Consonance of Lateral Postural-Motor Biases in Rats: Results and Implications

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Each of six different tests of lateral postural-motor asymmetries was repeatedly administered to 126 rats. Directional reliability was found for rotatory swimming, open-field exploration, and stepping down from a beam. Neonatal posture, turn in an unbaited T maze, and orientation to tail pinch proved not to be reliable across days. The behavioral asymmetries in the open-field and step-down tests were directionally consonant with each other, but neither was related to the asymmetry exhibited in rotatory swimming, implying the existence of at least two independent asymmetrical neural substrates underlying the behaviors. Neither sample-wide directional biases nor major sex differences in bias were found. The sexes were, however, differentially influenced in direction on some tests by the number of males in their natal litters, implying a role for intrauterine exposure to androgens in predisposing rats toward some left- or right-biased behaviors.

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In humans, such traits as handedness and eyedness are enduring characteristics of individuals, and it is precisely their stability that accounts for their central place in the development of theories about hemispheric differences in man. Similar stability of left-right behaviors in the rat would likewise carry implications for attempts to understand cerebral asymmetries in that species. In a recent review, Robinson, Becker, Camp, and Mansour (1985) identified seven different tests of left-right behavioral bias in rats, and Kolb and Whishaw (1985) have suggested

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several others; most of the tests involve observation of body turning in varying circumstances. The behavior elicited by each test can be viewed as an index of the laterality of the animal; however, the legitimacy of a test in providing such an index depends upon the directional stability of the behavior across test administrations.

The issue of directional reliability has already been addressed for some indices. Peterson's (1934) data indicate substantial stability across days in rats' paw choice in both a food-retrieval task ($N = 60$) and a latch-box task ($N = 7$), but much less stability for the side chosen in escaping from a water tank ($N = 6$). Glick, Jerussi, and Zimmerberg (1977) found complete test-retest directional consonance of *d*-amphetamine-induced turning in a spherical "rotometer" in 15 of 15 rats. Considerable test-retest stability of the direction of turn in an electrified T maze—over trials within a day and for intertest intervals of up to a month—has been reported by Zimmerberg, Glick, and Jerussi (1974) ($N = 24$ rats showing preferences on their initial test); by Camp, Robinson, and Becker (1984) ($N = 78$); and by Castellano, Diaz-Palarea, Rodriguez, and Barroso (1987) ($N = 70$). The direction in which the neonate's tail was deviated was found by Ross, Glick, and Meibach (1981) to be consistent in single observations made on 2 successive days during the first 3 days of life in 11 of 15 rat pups. Myslobodsky and Braun (1980) report that, for three indices (head deviation when pressure was applied to the tail, body curvature in orienting to the pinching produced by a clip placed on the tail, and general directional bias in walking on a long narrow platform), but not for a fourth (the rat's body curl when suspended by the tail), the preferred direction was the same before and after injection of amphetamine in 18 or more of 20 animals; however, less test-retest reliability resulted when additional subjects under different conditions were observed (Myslobodsky & Braun, 1981). Kolb and Whishaw (1985) report that normal rats ($N = 15$) showed little or no day-to-day consistency in the direction of five behaviors: curl assumed by the body when the animal was suspended by its tail; turn during righting when dropped from an upside-down position; turn when placed with the forepaws overhanging the edge of a table; turn in leaving a cul-de-sac; and first turn when placed on the floor of an animal-colony room. Finally, Axelrod and Noonan (1989) reported that the laterality of postures assumed by rats when resting in their home cages did not show any reliability across observations.

It is clear that despite considerable interest and effort, several problems remain in this field. First, some studies are based on behavioral indices whose legitimacy with respect to reliability has yet to be unequivocally established. Second, the wide variety of testing procedures employed makes it difficult to compare and integrate findings from different laboratories. Third, those tests which appear to produce reliable direction-

ality have disadvantages: the determination of pawedness is quite time consuming and difficult to carry out; the electrified T maze requires delivery of painful stimulation and is confounded by the fact that shock offset is likely to reinforce the animal's initial turn(s), thereafter altering the probabilities of left and right turning; and the rotational bias revealed under the influence of amphetamine depends on the dose and timing of drug administration. By assessing the reliability of several easily administered tests in a large sample of untreated subjects ($N = 126$), the work presented here is intended to address these problems by providing reliable alternatives which can serve as a basis for future procedural standardization of tests of lateral bias in rats.

For those behaviors which do show directional stability, it is important to ask whether they are directionally consonant with each other. In humans, directions of handedness, footedness, and earedness are correlated (e.g., Coren & Porac, 1978; Noonan & Axelrod, 1981), presumably because all three reflect a single neocortical hemispheric-dominance relationship. Correlations in rats among left-right biases in different tests would likewise imply common neural underpinnings of those biases. Relevant work has been reported by Ross et al. (1981), who found neonatal tail position to be predictive of the direction of rotation induced by injection of amphetamine at age 85 days in 28 of 33 rats. The direction of amphetamine-induced rotation was also found by Glick and Jerussi (1974) to be consonant in 9 of 10 animals with the preference for the left or right lever in bar pressing for water reinforcement and by Zimmerberg et al. (1974) ($N = 18$) to be highly correlated with side preference in the electrified T maze.

Glick and Shapiro (1985) review evidence that such lateral behavioral biases are underlain by asymmetries in nigrostriatal dopaminergic activity and raise the possibility that the dopamine asymmetry relates to spatial behavior in general. However, Camp et al. (1984) found no consistent relationship between dopamine concentrations in the contralateral and ipsilateral striatum ($N \leq 31$) or nucleus accumbens ($N \leq 26$) and the direction of four postural-motor asymmetries (amphetamine-induced rotation, electrified-T-maze turning, orienting to clip-induced tail pinch, side preference in leaving a corner of an open-field apparatus); and none of those behavioral asymmetries predicted directional bias in any of the others ($N = 78$). Similarly, Peterson (1934) found that paw choice in reaching for food was predictive neither of paw used on a latch-box nor of side chosen in water-tank escape ($N = 7$). Further, Myslobodsky and Braun (1980, 1981) found that none of their four postural-motor indices reliably predicted preferred direction in a rotometer ($N = 20$). Such failures to find intertest consonance for side preference, although of course only when each test's legitimacy has been established by demonstration of its reliability, imply the existence of more than one separate

underlying neural substrate (cf. Glick & Shapiro, 1985; Robinson et al., 1985).

By assessing the degree of intertest directional consonance in the present project, we were able to further address this question of whether different left-right behavioral biases in rats reflect common neural underpinnings. The behaviors examined were neonatal posture, open-field exploration, turn in an unbaited T maze, direction taken in stepping down off a beam, side of turn when orienting toward tail pinch, and direction of rotation while swimming.

GENERAL PROCEDURES

Subjects

Twelve male-female pairs of black-hooded Long-Evans rats were mated, and the 24 parents and 102 of their offspring served as subjects. The cages of the female rats were examined for pups daily, and all pups in a litter were considered to have been born on the day ("Day 1") during which the first pup in the litter was seen. In the morning of Day 2, the pups in each litter were sexed according to their anogenital distance, and the pattern of pigmentation on the skin of the dorsum of each pup was sketched to permit subsequent identification of individuals within each litter. A total of 118 pups was born. In 16 cases, sex and pigment pattern combined were judged insufficiently distinct to confidently distinguish two pups within a litter; in these cases, one of the pair was randomly eliminated from the study. On Days 45-48, the pigmentation pattern of the hair of each pup in each litter was successfully matched with a Day 2 drawing of the skin-pigment pattern of a like-sexed pup in its litter. Each pup was thereafter housed separately and tested by observers blind to relatedness and to the neonatal-posture findings.

Sequence of Testing

The animals were maintained on ad lib food and water, under a 12:12 light:dark cycle, and all observations were made during the light phase. The rats received the tests in the sequence specified in Table 1.

Counterbalanced Variables

To guard against the possibility that asymmetries in handling or in the testing environments might influence the left-right behavior of the subjects, several potentially contaminating procedural variables were deliberately varied and distributed across days. On successive days, we alternated the hand used to grasp the rats for placement into a test apparatus (hand of day); thus, all rat handlers were "left handed" on one day, "right handed" on the next day, and so on. Furthermore, except in the neonatal-posture test, the orientation of each testing apparatus to be described below was counterbalanced across days with respect to

TABLE 1
Sequence of Testing

Test	No. test days	Age range (days) when tests began			
		Mothers	Fathers	Offspring	
				Cohort 1	Cohort 2
Neonatal posture	4	—	—	2-5	2-5
Open field→T maze →step down	5	139-175	100-147	63-68	78-82
Tail pinch→ rotatory swimming	5	146-182	107-154	70-75	85-89

asymmetries of the testing room (e.g., windows, doors). The direction from which the animal was placed into the apparatus and the position from which the behavior was observed were likewise counterbalanced across days. In addition, the order in which the rats were tested was counterbalanced across days, as were observers across rats.

Statistics

The degree of correspondence between two sets of scores was assessed on the total sample by correlation coefficients: Pearson r when neither measure was dichotomous; point-biserial r when one of the two was dichotomous; and ϕ when both were dichotomous. For tests of directional consonance, the probability values presented are derived from one-tailed tests, the prediction having been of a positive relationship. Where both variables were dichotomous, the significance of ϕ was derived from the corresponding $2 \times 2 \chi^2$, uncorrected for continuity (Fienberg, 1977). For logistical reasons, it occasionally happened that a test administration for one or another rat did not yield usable results; therefore, there are slight variations in the number of rats represented in the results presented.

METHODS AND RESULTS

Neonatal Posture (NP)

Method. On each of 4 consecutive days, beginning with Day 2 (24-48 h after birth), the pup was picked up by the skin on the nape of the neck, using the thumb and forefinger of the hand of the day (HD), and placed ventral side down with the head away from the observer in the center of a 36×36 -cm test compartment. Immediately following release of the infant, its initial tail position (NPITP) was recorded as (1) deviated fully left (the tail resting in a position more than 90° left of the vertebral axis, i.e., with the tail close to or touching the left side of the body), (2) deviated slightly left (less than 90° to the left), (3) straight, (4) slightly

right, or (5) fully right. Then, in a procedure modeled on that of Ross et al. (1981), the pup was held straight on the floor of the compartment for 5 s. The thumb and forefinger of the HD held the tip of the tail, while the non-HD reached around to grasp the head with its thumb and forefinger; thus both forefingers were on one side of the pup and both thumbs on the other. The pup was then released and the first deviation made by the tail (NPT), the head (NPH), and the body (NPB) were recorded on a similar five-position scale.

Results. On all four measures (NPITP, NPT, NPH, and NPB), "straight" was usually the modal, although rarely the majority, response. This contrasts with the reports of both Ross et al. (1981) and Denenberg, Rosen, Hofmann, Gall, Stockler, and Yutzey (1982), who found straight responses to be absent or rare. The proportion of pups showing lateralized (i.e., not straight) tail postures did not vary significantly over days (.70, .76, .70, and .68 for Days 2 through 5, respectively), a finding at variance with the assertion of Ross et al. (1981) that tail asymmetry diminishes and is no longer apparent by Day 4.

When deviating from straight, pups tended to make organized flexions, with the head and tail deviated to the same side, as was revealed by the substantial consonance among the directional biases of the three body parts observed following the 5-s forced-straight positioning; all 12 correlation coefficients (three body-part pairings \times 4 days) were positive and significant, $p < .001$. The body curling on the first day of testing (Day 2) was less coherent than on subsequent days: the correlation coefficients among parts on Day 2 ranged from .34 to .43, whereas on Days 3 to 5 they ranged between .52 and .83.

No significant sample-wide biases were found (mean across days: NPITP = 2.96, NPT = 2.97, NPH = 3.03, NPB = 3.07), nor did males and females show any consistent pattern of difference. With particular reference to tail position (NPT), the absence of sample-wide bias, and of a sex-by-direction interaction, is at variance with the reports both of Denenberg et al. (1982)—who found significantly higher proportions of leftward than of rightward tail deviations in large samples of day-old pups of both sexes, the bias being significantly larger in females—and of Ross et al. (1981)—who found, in pups observed once during the first 3 postnatal days, significantly more right-biased than left-biased tail positions among their females, and nonsignificantly more left-biased than right-biased positions among their males.

The differences in sample-wide bias may be attributable to differences in the strain of rat tested (Denenberg et al., 1982); but because the pup-handling procedure can significantly influence the results and may well vary from laboratory to laboratory, we are inclined to reserve judgment until different strains are tested under identical conditions. In our study, although every effort was made when positioning the pups to align their

vertebral axes straight regardless of the hand used, the hand-of-day variable proved to affect the pups' behavior. There was a significant HD effect on NPT for Day 2 (the first testing day), although not for the mean across days: when the forefinger and thumb of the left hand were used to straighten the pup's tail before release, 65% of the lateralized pups assumed leftward tail positions, whereas when the Day 2 HD was right, 77% of those lateralized assumed rightward positions ($p < .01$). There was also a significant effect of the variation in the disposition of the hands across days for NPH and NPB, reflecting a tendency in the pups to move their heads and bodies toward the thumb of the hand holding the head. These findings have important implications. Denenberg et al. (1982), who found an overall leftward tail bias in Day 1 pups, would appear to have exhaustively addressed the question of population-wide bias in rats by testing 2223 animals. However, they mention no controls for asymmetries in handling. If this source of asymmetrical influence was not controlled, conclusions about population-wide bias in neonatal posture are unwarranted.

Within-day directional consonance might have been expected between NPITP and NPT, two observations of tail deviation made only seconds apart. Yet, the within-day correlations between them achieved statistical significance only on Days 3 and 5, and never exceeded .36.

Furthermore, there was no evidence at all of reliability when each day's results were correlated with those of the next day, nor was there any consistent pattern of correlations when each day's results were compared with the mean for the other days (Table 2). Consonance across days did not emerge even when analyses were confined only to those pups that had scores of 1 or 5, i.e., pups showing full deviations, nor did it when the data were analyzed for each sex separately.

Open Field (OF)

Method. One 3-min open-field observation was made on each of 5 successive days. The floor of the compartment was 80 cm square, ruled into an 8 × 8 grid of 64 10-cm squares; the walls were 30 cm high. With the HD grasping the tail, the observer placed the animal into one of the corners, grasped the rat's trunk with the (gloved) non-HD, aligned the rat diagonally so that it was facing the center of the compartment, and immediately released it. The direction of the animal's initial turn (OFIT) from the diagonal position and the wall (OFIW) along which it first exited from the corner (here defined as the immediate 20-cm-square area) were recorded. Thereafter, throughout the 3-min period, the direction of every entry made into any of the 64 squares was recorded, an entry being defined by the tip of the animal's nose crossing a ruled line into a square.

OFIT and OFIW were coded as 1 for left and 5 for right. In addition,

TABLE 2
Percentages of Concordance and Correlations: Each Day vs Mean on Other Days

Test/measure	Comparison				
	First—others	Second—others	Third—others	Fourth—others	Fifth—others
Neonatal posture					
Initial tail position					
Tail					
Head					
Body					
Open field					
Initial turn					
Initial wall					
Wall hugging					
T maze					
Head					
Forefeet					
Back feet					
Step down ^a					
Head					
Forefeet					
Back feet					
Tail pinch					
Flexion					
Rotatory swimming					
Circling					

Note. Concordances are given in the form (percentage lateralized on both scores and concordant)/(percentage lateralized on both scores but discordant). Correlation coefficients are point-biserial *r* or Pearson *r*, as appropriate; see text.
^a For step down, the values are derived from the means of the two test administrations on each day.
 * *p* < .05, one tail. ** *p* < .01, one tail. *** *p* < .001, one tail.

of the entries into the 28 squares immediately adjacent to the walls (always the majority of entries), a "wall-hugging" ratio (OFRATIO) was computed of entries made with the right side adjacent to the wall divided by the total, $R/(L + R)$.

Results. There were no significant sample-wide directional tendencies (means: OFIT = 3.04, OFIW = 3.01, OFRATIO = 0.48), findings which are compatible with other observations of open-field behavior (Camp et al., 1984; Sherman, Garbanati, Rosen, Hofmann, Yutzey, & Denenberg, 1983; Sherman, Garbanati, Rosen, Yutzey, & Denenberg, 1980). With respect to sex, both the Sherman and the Camp groups found that female rats which were not handled during infancy, when released from the confines of a temporary barrier, tended significantly to first explore along the left wall, handled females having a nonsignificant trend in the same direction. But for males, only rats which had had daily 3-min-long handling experiences on Days 1–21 of age showed a significant leftward bias; nonhandled males showed nonsignificant trends rightward. In our rats, which had received only the 4 days of neonatal handling constituted by the NP tests, when released by the gloved hand, females turned more often to the left (mean OFIT = 2.73) and males more often to the right (3.30), $p = .009$; thus, these results most closely correspond with those found by the other researchers in their nonhandled conditions. The sex difference was similar for OFIW, but failed to reach statistical significance. The two sexes had virtually identical and unbiased scores on OFRATIO.

There was directional consonance among the three measures within testing days. Of the 15 correlation coefficients (three measure pairs \times 5 days), 12 were significant, and, as with the neonatal-posture test, there was a slight tendency for consonance to increase over days, the correlations ranging on the first day between .11 (OFIT vs OFRATIO) and .29 (OFIT vs OFIW), and on the fifth day between .36 (OFIT vs OFRATIO) and .39 (OFIT vs OFIW).

All three measures (OFIT, OFIW, and OFRATIO) proved to possess a degree of reliability across days. When the scores on each measure on each of the first 4 days were correlated with those from the next day, all 15 coefficients were positive, and all but 2 were significant, and when each day's score was correlated with the mean score for the remaining days, all 15 coefficients were statistically significant (Table 2). The most reliable of the three measures was OFRATIO, and the least was OFIW. There was evidence that the rats "settled in" to an increasingly consistent pattern over days, the correlation coefficients being somewhat smaller for the first than for subsequent days.

There were no systematic effects on open-field measures of variations in HD, starting corner, observer, or location of observer.

Initial Turn in Unbaited T Maze (TM)

Method. The direction in which the animal turned at the choice point of an enclosed unbaited T maze was observed for one trial per day over 5 days. The width of the starting box, runway, and arms was 15 cm; wooden walls were 18 cm high, and metal mesh constituted the floor and ceiling; the runway was 65 cm long, and the cross-piece of the T was 167 cm long. After its release, the animal was left free to move at its own pace throughout the maze, and when it reached the T junction, the side(s) into which it first moved its head (TMH), forefeet (TMFF), and back feet (TMBF) was recorded; direction was coded as 1 for left and 5 for right.

Results. The sample-wide means over the 5 days were TMH = 3.07, TMFF = 3.23, TMBF = 3.14; this rightward bias was significant only for TMFF ($p < .01$). No sex differences in bias were found.

It is not surprising that there was substantial directional consonance among the three measures (TMH, TMFF, and TMBF) within testing days. All 15 ϕ coefficients (three measure pairs \times 5 days) were significant ($p < .001$), ranging between .34 and .90. There was no suggestion of any change over days in intermeasure consonance.

No consistent evidence emerged for directional reliability when each day's scores were correlated with those of the next day. Likewise, when each day's scores were compared to the mean of the other days' (Table 2), only the TMH directions were significantly consonant and only on the third, fourth, and fifth testing days; no such pattern was obtained for the forefeet or back feet. No sex differences were found for any of these cross-day assessments of consonance. No systematic influences were found on any TM measure of variation in HD, handler/observer, or orientation of the maze within the room.

Step Down (SD)

Method. The direction taken in stepping down from a narrow platform was observed for 5 consecutive days in a procedure adapted from that of Giehrl and Distel (1980). The apparatus consisted of a horizontal beam 9 cm wide, 7.5 cm high, and 53 cm long, centered in the open-field compartment previously described, with one end of the beam abutting a wall of the compartment. A 36-cm-long slide, set at 37° to the horizontal, was positioned at the opposite wall with its bottom edge resting on the beam. The slide was 22 cm wide at its upper end and tapered to 9 cm as it joined the beam. The slide had high walls, and both slide and beam were painted with high-gloss enamel. With the HD grasping the tail, the observer placed the rat, facing downward, at the top of the slide, and released it, allowing it to slide on its feet down to the beam. The rat then typically walked some distance along the beam and within a few seconds stepped down onto the floor of the compartment. This slide

procedure was employed because, in pilot attempts, we had found that a rat placed directly onto the beam invariably began to step down before its tail was released; the slide to some degree distanced the lateral choice of the animal from a possible influence of handling and served to "funnel" the animal into an approximately symmetrical head-on arrival onto the beam.

We recorded the side on which the rat first extended its head over the edge of the beam (SDH), first placed its forefeet on the floor of the compartment (SDF), and first placed its back feet on the compartment floor (SDBF). For all three measures, left and right were coded as 1 and 5, respectively. Two trials per day were given, about 30 min apart.

Results. Giehl and Distel (1980) found that hamsters showed a sample-wide bias toward stepping down rightward off the centerboard of a visual-cliff apparatus. By contrast, our rats had a slight overall tendency to step down leftward (mean values across all 10 test administrations: SDH = 2.88, SDF = 2.84, SDBF = 2.85). This tendency reached statistical significance, however, only for SDF ($p < .05$, two-tailed). Males and females did not differ significantly in average bias.

Again unsurprisingly, there was considerable concordance among the three measures (body parts) within trials: all 30 ϕ coefficients (three measure pairs \times two trials \times 5 days) were statistically significant, $p < .001$. On this test, the cross-measure associations did not increase across days.

The 15 ϕ coefficients (three measures \times 5 days) relating responses made on the two trials on each day ranged only between .02 and .26, and only 6 were significant. Yet, despite this low degree of directional correspondence between successive trials on the same day, the correlations between the mean of the two trials on any day and the two-trial mean on the next day, and between the mean on any day and the mean of all other days (Table 2), revealed a modest degree of reliability for this test. For both sets of comparisons, 13 of the 15 coefficients were significant. As with the open-field test, the rats settled in across days, in that the laterality scores obtained on the first day were less strongly correlated with the mean of the remaining days than were those obtained on the second through fifth days.

Male and female rats did not differ systematically in reliability; and hand-of-day, orientation of the apparatus within the room, observer, and location of observer did not exert any systematic influence on the direction taken.

Tail Pinch (TP)

Method. On each of 5 successive days, the direction in which the animal turned to orient to a clip fixed to its tail was observed, using a procedure modeled after those of Myslobodsky and Braun (1980) and

Camp et al. (1984). The tests took place in the open-field compartment, the floor of which was now covered with wood-chip bedding material. The animal was placed in the center of the compartment, and, after a 60-s acclimation period had elapsed, a spring-loaded clip with padded jaws was placed approximately midway between the base of the tail and its tip; the clip weighed 4.1 g, and its spring was adjusted so that it held firmly to the tail but did not evoke vocalization. For the next 3 min, the time during which the animal turned and touched the clip with its nose, mouth or forepaws was accumulated separately for turns to its left and to its right. From these times, a directional flexion ratio of $R/(L + R)$ was computed (TPRATIO).

Results. Sample wide, the times spent deviated left and right were almost identical, the mean TPRATIO over the 5 days being 0.496, and no sex differences were found.

The total time ($L + R$) oriented toward the tail irrespective of direction, presumably reflecting the rat's general sensitivity to having its tail pinched, proved to be reliable over days, being significantly correlated on any day with each of the other days (range of $r = .23$ to $.48$). However, the tail-pinch test showed no reliability for *direction* (TPRATIO) as indexed either by day-with-next-day correlations or by day-with-mean-other-days correlations (Table 2), either sample wide or within either sex, even when analyses were confined to those rats which on any day had TPRATIO scores more than one standard deviation either leftward (<0.19) or rightward (>0.81) from 0.5. HD, observer, and location of observer were not found to have any influence.

Rotatory Swimming (RS)

Method. On each of 5 successive days, in a test based on that described by Collins (1985), the animal was placed into a 55-cm-high plastic bucket 34 cm in diameter, filled with room-temperature water (25°C) to a depth of 33 cm. In this situation, rats spend most of their time swimming along the bucket wall in an apparent attempt to escape. The number of times during a 2-min period that the animal's nose (more accurately, the anterior edge of the animal's midsagittal plane) crossed a vertical line inscribed on the bucket wall while moving either leftward (counterclockwise swimming) or rightward (clockwise swimming) was recorded, and these values were converted into a circling ratio, $R/(L + R)$ (RSRATIO).

Results. There was no sample-wide bias in direction (mean over the 5 days = 0.508), nor were any sex differences found.

Table 2 reveals performance on this test to have been quite stable across days. Reliability coefficients were never lower than .27 and reached as high as .77. A similar pattern emerged for day-with-next-day correlations (range .25-.75). As with the open-field and step-down tests, the cross-day correlations were lower for the first day than those for

subsequent days. Male and female rats did not differ systematically in reliability, and HD, orientation of the apparatus, observer, and location of observer did not have any influence on the behavior.

GENERAL FINDINGS

Intertest Correlations

The pattern of intercorrelations among our three reliable tests (open field, step down, rotatory swimming) revealed neither overall directional consonance nor the opposite, a complete absence of intertest consonance. The means across days from the open-field test were directionally consonant with those from the step-down test: all nine Pearson r 's relating the three OF measures (OFIT, OFIW, and OFRATIO) with the three SD measures (SDH, SDFF, and SDBF) were positive, six significantly so. The strongest correlations occurred between the mean initial turn taken in the open field (OFIT) and the mean turn made in the step-down test (all three SD measures) (.30-.35, p 's < .001). On the other hand, the mean across days of RSRATIO (indexing directional bias in the swimming test, our most reliable measure) did not correlate significantly with the means across days of any of the OF or SD measures; indeed, the correlations were uniformly near zero.

Direction-Independent Strength of Bias

Collins (1977, 1985) has stressed the usefulness of considering the strength of lateral bias as distinguished from its direction. To examine the dimension of strength, we computed the absolute value of the deviation of scores from the no-bias value (3.0 or 0.5, as appropriate). This transformation was performed for each day on NPITP, NPT, NPH, NPB, OFRATIO, TPRATIO, and RSRATIO (the nondichotomous measures) and for the mean across days for all measures. We performed analyses on the strength values analogous to those performed on the directional scores, examining (a) sex differences, (b) day-to-day reliability for the nondichotomous variables, and (c) intertest associations. The findings can be summarized as follows:

(a) No significant sex differences in strength were found for any measure on any test.

(b) The day-to-day Pearson r reliability coefficients of the strength values were positive and significant for both OFRATIO and RSRATIO, although somewhat smaller than those of the untransformed directional scores. The sexes did not differ systematically in day-to-day reliability of strength.

(c) The strengths of the means across days for the neonatal-posture test (NPT, NPH, and NPB) conducted during infancy were *negatively*

correlated with the strengths of the means across days of four tests conducted during adulthood, namely, TM, SD, TP, and RS; however, we note that NP itself did not show evidence of reliability across days for strength, nor, it will be recalled, for direction.

Frequency Distributions

If for a given behavior most animals are either strongly right biased or strongly left biased, rather than ambilateral or unbiased, then the curve relating the number of animals to behavioral scores will be U shaped. Collins (1968, 1985) found such a U-shaped frequency distribution for pawedness in mice; and the well-documented J-shaped distribution for human handedness (e.g., Leiber & Axelrod, 1981) is the variation of the U that results when there is a pronounced population bias. By contrast, in the present study the frequency distributions for the means across days for all measures from all tests, and for OFRATIO, TPRATIO, and RSRATIO on each of the 5 days separately, were unimodal and symmetrical (approximately bell shaped). In other words, although animals tended to be reliably left or right biased on some tests, the biases were characteristically only slight, relative to the possible range of responses. Caution would therefore seem appropriate in drawing analogies between lateral biases in the postural-motor behaviors studied here and forelimb preference in man or mouse.

Effects of Male Littermates

Ross et al. (1981) reported a significant negative correlation in 19 litters between the number of males in a litter and the percentage of female pups which adopted rightward neonatal tail positions, a finding not replicated by Denenberg et al. (1982). In the present study there was likewise no relationship between number of male littermates and litter means for either of our measures of tail position (NPITP and NPT) on any day or for the mean across days. We did, however, find significant differential effects on the two sexes of the number of male littermates on neonatal head and body positions. Additionally, several relevant findings emerged from our results for some of the other tests when the average mean across days was computed separately for each sex within each litter, and those values were correlated with the number of male littermates (see Table 3). The correlations were negative for females and positive for males, with the difference between them being significant, on measures derived from the open-field (OFIW and OFRATIO) and step-down (SDFP and SDBF) tests; that is, on these measures, increasing numbers of male littermates were associated with increasing leftness in females and increasing rightness in males. By contrast, for the rotatory-swimming test (and for NPH and NPB), the differential effects on the sexes were again significant, but reversed in sign: increasing exposure to male lit-

TABLE 3
Correlations between Mean Directional Scores and Number of Male Littermates, by Measure and Sex

Test/measure		Sex	<i>r</i>	Significance of sex difference ^a
Neonatal posture				
Initial tail position	NPITP	F	.44	
		M	-.26	ns
Tail	NPT	F	.36	
		M	-.25	ns
Head	NPH	F	.17	
		M	-.71**	.05
Body	NPB	F	.31	
		M	-.76**	.01
Open field				
Initial turn	OFIT	F	-.53	
		M	.10	ns
Initial wall	OFIW	F	-.28	
		M	.61*	.05
Wall hugging	OFRATIO	F	-.58*	
		M	.56	.01
T maze				
Head	TMH	F	.22	
		M	-.40	ns
Forefeet	TMFF	F	.51	
		M	-.26	ns
Back feet	TMBF	F	.53	
		M	.04	ns
Step down				
Head	SDH	F	-.34	
		M	-.06	ns
Forefeet	SDFF	F	-.75**	
		M	.39	.01
Back feet	SDBF	F	-.73**	
		M	.25	.05
Tail pinch				
Flexion	TPRATIO	F	.27	
		M	.24	ns
Rotatory swimming				
Circling	SBRATIO	F	.14	
		M	-.79**	.01

^a This column presents the significance level (two tails) of the critical ratio testing the difference between the *r* for the females and the *r* for the males.

* *p* < .05, two tails.

** *p* < .01, two tails.

termates was associated with increasing rightness in females and increased leftness in males.

DISCUSSION

Our data bear on two sets of issues, methodological and biobehavioral.

Methodological

Reliability. Current investigations of lateral behavioral asymmetries in rats have relied on numerous and diverse tests; the results obtained here indicate that not all such tests are of equal value. A test which does not possess reliability across administrations at best presents information only about the momentary state of the animal, whereas a reliable test provides information about an enduring characteristic. We did not find reliability for two tests (neonatal posture and tail pinch) upon which a number of previous investigations have depended. It clearly behooves future investigators to ascertain the degree to which their tests reflect stable directional dispositions.

Of the tests examined here, the rotatory-swimming test proved to be the most reliable; less reliability was found for the open-field test, and still less for the step-down test. The other three tests (neonatal posture, turn in unbaited T maze, and tail pinch) were unreliable. It is ironic that Collins' rotatory-swimming test, the best of those we examined, has rarely been used by workers in the field. We can highly recommend this test; it is inexpensive, easy to administer, and brief, and, as long as the water and ambient air are kept warm, it appears not to be unduly stressful to the subject.

Increasing consistency over time. There was a general pattern for the rats' behavior to settle in over time, this showing itself in two ways. First, on many tests there was a tendency toward more coherence among the component measures of a test on later test days than on earlier ones (neonatal posture and open field). Second, the reliability across days of many measures tended to increase from the earlier to the later days (Table 2). The latter phenomenon in particular has implications for studies in which the behavioral results form the basis of subsequent subject selection; the categorization of animals as left or right biased on the basis of scores derived from a single testing session is likely to be less valid as an index of laterality than categorization based on the combined scores over several sessions. Collins has observed a similar settling in for mice tested in his rotatory-swimming test and has adopted the practice of administering five daily testing sessions but using only the data from the last three (personal communication). A similar practice seems advisable when indexing directional tendencies in rats.

Control of potential procedural contaminants. It will be recalled that we distributed and assessed the effects of variation of such potentially

biasing factors as the orientation of the test apparatus in the testing environment and the hand used in grasping the animal (HD). As has been shown in other contexts (Noonan & Axelrod, 1981), even subtle exogenous variables can influence lateralized behavior, and indeed, in the present study, significant effects on neonatal posture of left–right variation in experimenter hand use were found. Because lack of control of such variables can call into question the conclusions drawn from research on lateral asymmetries, we recommend that such control be routinely included in study protocols.

Wall hugging in the open field. A rat in an open field characteristically spends most of its time walking (apparently exploring) very close to the walls, the laterality of which was quantified as OFRATIO. It was not apparent to us at the outset whether to predict consonance or dissonance between OFRATIO and the turning tendency reflected in OFIT. An animal hugging the right wall, for example—unless, as rarely occurs, it makes a U turn—consistently makes only left turns every time it reaches a corner, and thus would appear to be a characteristic left turner. Alternatively, it may be viewed as being prone to go rightward but being prevented from doing so by the wall, as if the rat is searching the wall for an opportunity to go beyond it. The positive correlation found between OFIT and OFRATIO favors the latter interpretation, which is further buoyed by the finding of consistent positive correlations between the turning scores on the step-down test and all of the open-field measures, including OFRATIO. In other words, in rats, open-field wall hugging is associated with tendencies to turn in the direction of the wall which is hugged, despite the fact that the net effect of such persistent wall hugging is repeated 90° turns in the opposite direction.

Biobehavioral

Sample-wide bias? One of the fundamental themes is human neural organization is the population-wide asymmetrical pattern of left–right differences, and much recent effort has focused on a search for sample-wide neural and/or behavioral asymmetries in rats (e.g., Denenberg, Garbanati, Sherman, Yutzey, & Kaplan, 1978; Glick & Ross, 1981). The results of the present work suggest that rats do not possess a population-wide general postural–motor bias: of the 15 measures assessed by the six behavioral tests administered, sample-wide means differed significantly from chance expectations for only two, one leftward (SDFF) and one rightward (TMFF), the remaining measures trending nonsignificantly leftward as often as rightward.

Sex differences. Several reports have suggested that the lateral neural organization of female and male rats may differ (see Diamond, 1985; Robinson et al., 1985). Nevertheless, the present project provides little evidence of sex differences in behavioral bias. The differences between

the sexes were slight on all 15 measures and reached statistical significance on only one (OFIT).

However, our finding of differential directional influences on the sexes of the number of male littermates, although not directly corresponding to that found by Ross et al. (1981), suggests, as does theirs and the experiment findings of Rosen, Berrebi, Yutzey, and Denenberg (1983), a role for prenatal exposure to androgens in the ontogeny of postural-motor biases in rats.

Intertest consonance. Our failure to find general directional correspondence across tests is reminiscent of similar absences reported by Camp et al. (1984) and Myslobodsky and Braun (1980, 1981). We caution, however, that noncorrespondence is all one can expect between tasks which do not themselves assess stable lateral biases as reflected by reliability across days. Nevertheless, our failure to find directional correspondence between our two most reliable tests (open field and rotatory swimming) suggests that more than one independent asymmetrical neural mechanism operates in the brains of rats. We join Camp et al. (1984), Myslobodsky and Braun (1980), and Robinson et al. (1985) in cautioning against an assumption that any single asymmetry in behavior reflects left or right brainedness in general.

We did find a modest degree of directional correspondence between the open-field and step-down tests, which suggests that the biases they reveal are at least partially underlain by a common asymmetrical neural mechanism—one evidently independent of a second mechanism underlying directional bias in rotatory swimming. Fitting in nicely with this interpretation is the fact that the direction of the difference between the sexes in the association between the number of male littermates and the behavioral bias is the same for OF and SD, but opposite for RS.

In both the open-field and step-down tests, the rats' behavior seemed to us primarily exploratory in nature, whereas in the rotatory-swimming test the rat seemed clearly to be attempting to escape from an aversive situation. We wonder to what degree commonality in motivational state relates to directional consonance. We would consider it important in the future to ask if behaviors shown on other reliable tests show directional consonance with OF and SD when similarly exploratory, or with RS when the behavior is similarly escape driven. Such findings would have clear implications for the search for the neural substrates underlying these behavioral biases.

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