ELSEVIER

Behavioural Brain Research 93 (1998) 51–62

BEHAVIOURAL BRAIN RESEARCH

Research report

Arousal mediates relations among medial paw preference, lateral paw preference, and spatial preference in the mouse

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Received 14 April 1997; received in revised form 2 September 1997; accepted 2 September 1997

Abstract

Rodents exhibit two well-documented behavioural lateralities: spatial preference and paw preference. Waters and Denenberg [36] have identified two seemingly independent factors of paw preference: medial and lateral paw preference. In the present work, the relations among spatial preference (SP), medial paw preference (MPP), and lateral paw preference (LPP) during states of high and low arousal were examined. These preferences were measured in terms of direction, which describes the side of the preference regardless of strength, and degree, which describes the strength of the preference regardless of direction. A strong positive correlation between LPP and SP was found during periods of high, but not low, arousal. A negative correlation between the degree components of LPP and MPP was found during the low, but not high, arousal periods. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Laterality; Paw preference; Mice; Arousal; Dopamine; Spatial preference; Handedness

1. Introduction

Rodents display two well-documented behavioural lateralities. Firstly, Collins [13] has shown that mice reliably exhibit a paw preference when reaching into a chest-high tube for food. This preference remains consistent across different types of reaching tasks [15]. It may be separated into two components: a *degree* component, which measures the strength of the preference regardless of the side; and a *direction* component indicating the side of the preference (left or right) regardless of its strength. Collins [15] was unsuccessful in his attempts to selectively breed mice for direction of paw preference, but was successful in developing two lines, the Collins HI and LO lines, that were distinguished by differences in the degree to which they used either the right or the left paw. Subsequent work, however, by Biddle and colleagues [4,5], and Waters and Denenberg [36] (see also [7]), who have revealed strain differences in the direction of paw usage, has cast doubt on Collins' conclusion that direction of preference is not heritable. In addition, as has already been pointed out [36], preferred direction of paw preference in mice appears to be related to heritable differences in a sensory system: the whisker-to-barrel pathway [2].

Paw preference can be altered by localized lesions to the sensorimotor cortex [33,37], the nigrostriatal dopaminergic system, and the caudate putamen [37]. Training on a paw preference task in young, post-weaning rats has been shown to increase cortical thickness in the sensorimotor cortex [17], and to increase dendritic branching in the motor cortex [26]. Training has also been shown to permanently change paw preference [32]. Paw preference has been linked variously to monoamine asymmetries in the amygdala and septum

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PII S0166-4328(97)00141-1

[34], the mesolimbic system (especially the nucleus accumbens septi) [8], and the bulbo-spinal region [3].

The second type of behavioural laterality observed in rodents is a spatial preference. This preference seems to be driven by a nigrostriatal dopamine asymmetry, and manifests itself in a variety of lateralized behaviours. Jerussi and Glick [27] have shown that rats injected with *d*-amphetamine, a drug that increases dopaminergic activity, turn circles in a consistent direction. Many rats spontaneously rotate in a like manner during the night, when the dopaminergic system is naturally most active [22]. Histological studies have shown that rats rotate in the direction contralateral to the hemisphere with the most dopamine, whereas those rats not exhibiting a consistent directional preference have no discernible difference in dopamine levels between hemispheres [24]. Other manifestations of the spatial preference include a reliable preference for choosing the branch of an electrified T-maze contralateral to the hemisphere with the most dopamine [1,40]; a preference for moving in a given direction in an open-field test [16]; and a preference for choosing the contralateral lever in a two-lever operant task [25]. All of these behaviours involve a preference for moving toward or manipulating objects in one side of space, relative to the animal, instead of the other.

This story is not completely clear-cut, however, as some researchers have failed to find some of the relations described above. For example, Camp, Robinson and Becker [9] measured performance on four tests of behavioural lateralization, including T-maze side preference, amphetamine-induced rotation, an open-field test, and tail-pinch induced postural asymmetries. They found no consistent relation between these behavioural measures and dopamine asymmetries in either the nucleus accumbens septi or the striatum. Furthermore, none of the behavioural measures correlated in direction of preference. However, all behavioural measures in this study were taken during the dark portion of a diurnal cycle, when dopaminergic activity is highest. Fluctuation in overall level of dopaminergic activity has been shown to alter behavioural side preferences; for instance, Castellano et al. [12] have shown that apomorphine, a DA-receptor agonist, reduces the degree of laterality displayed in several tests of side preference. Other researchers have also failed to find relations between various different measures of behavioural and postural side preference [12,28,29], though these studies generally examined either direction of preference only, or measures that combined degree and direction of preference; they did not examine correlations among measures of degree of preference only. Noonan and Axelrod [31] examined test-retest reliability in six postural-motor bias measurements in rats, and found that only three (rotational swimming, open-field circling, and beam step down) were reliable over time.

Although the two behavioural lateralities described above—paw preference and spatial preference—are widely documented, the relation between them is not well understood. A literature review yielded few papers directly comparing these behavioural measures; those that did found no relation between paw preference and spatial preference [30,37]. Peripheral evidence, however, suggests that these might be related in degree. Collins [15], for example, found that rotatory swimming preference correlated in degree, but not direction, with paw preference. Nielsen et al. [30] have examined the relation between rotational preference and paw preference in lines of mice bred to exhibit either strong (HI) or weak (LO) degrees of paw preference. They found no relation between degree of paw preference and rotational preference within a line, indicating that paw- and side-preference phenotypes are independent. However, between lines, LO mice exhibited a significantly lower degree of rotational preference as well as a lower degree of paw preference, suggesting that there may be a common genetic mechanism underlying these biases. Both lateralities have been linked to asymmetries in dopamine metabolism [8,9,12,30,34,37,38]. Spatial preference, however, seems to be most strongly linked to dopamine utilization in the nigro-striatal system, whereas paw preference does not appear to be related to striatal dopamine imbalances per se [3,8]. The first purpose of the present study was to examine the relation between spatial and paw preferences.

Waters and Denenberg [36] have demonstrated a second type of paw preference, independent of Collins' paw preference, and manifesting itself during lateral reaches for food, but not during medial reaches. This discovery arose from an effort to develop a less labour-intensive method for measuring paw preference [35]. The Waters method employs an apparatus that allows a mouse access to food from two hoppers. Food from the first hopper is most easily obtained by the left paw, whereas food from the second hopper is most easily obtained by the right paw. After allowing a mouse access to both hoppers for a period of time, a researcher may compare the amount of food consumed from each hopper. A measure derived from the difference in the amount of food eaten from each hopper describes paw preference in degree and direction: mice who have eaten more from the left or right hopper correspondingly prefer the left or right paw; and the greater the difference between hoppers, the stronger the preference.

Waters and Denenberg [35] have shown that their measure is reliable over time, as are its degree and direction components. Collins' measure and its degree and direction components are also reliable over time [13], though the degree score tends to increase significantly with subsequent measurements [14]. The two measures correlate only slightly, however; Spearman

rank-order correlations reported in [35] and [36] were $r = 0.127$ and $r = 0.186$, respectively. While this relation was significant with a sample size of 623 in [36], it shows that only 3% of the variation is shared between the two measures. From this evidence, Waters and Denenberg [36] suggested that their procedure measures a lateralized behaviour functionally independent of Collins' paw preference. We shall refer to this preference as 'lateral paw preference' (LPP), and to the behaviour measured by Collins' procedure as 'medial paw preference' (MPP). According to Waters and Denenberg, LPP is exhibited when a mouse reaches away from the midline, whereas MPP is exhibited when a mouse reaches toward the midline [35]. This model posits different factors of handedness similar to those found in humans [21]. However, Collins [15] reports that MPP does transfer across other types of reaching tasks. Is LPP functionally independent of MPP? The second purpose of the current study was to address this question.

A third goal of the study comes from the finding that water-deprived rats, when given the choice of two bars to press for water, prefer the bar contralateral to the hemisphere with more dopamine [39]. In other words, the spatial preference (hereafter SP) manifests itself in a preference to manipulate, or to move toward, or to notice objects in the preferred side of space. In the context of the Waters and Denenberg task, this finding suggests that mice faced with two holes from which food is available will choose to reach through the hole in the preferred side of space. The Waters apparatus makes it difficult to reach for food from a given hopper with the opposite paw. But if paw preference drives a mouse to prefer the hole most easily accessible to the preferred paw, while simultaneously spatial preference drives it to favour the hole on the preferred side of space, it is unclear which drive will dominate. Thus, Waters and Denenberg's LPP may be a confounded measure of MPP and SP; that is, there may be no need to differentiate between medial and lateral paw preference. The fact that MPP accounts for a trivial amount of variation in LPP, and that LPP is reliable over time (presumably measuring some stable phenomenon), suggests that LPP may be driven primarily by SP. If so, Waters and Denenberg's results may be explained without introducing different factors of handedness. The third and final purpose of this study is to test the hypothesis that LPP is related to SP.

2. Materials and methods

To reach the three ends described above, three measures were taken. Collins' procedure was performed first, to assess MPP. Next, a free-choice water T-maze test was administered to assess SP. Several researchers have shown that side preference in an electrified free-choice T-maze is highly reliable, and is related to dopamine asymmetries [11,18,39]. Finally, the Waters and Denenberg procedure was carried out as a measure of LPP.

2.1. Subjects

Forty-nine (25 female and 24 male) pigmented BALB/c mice were tested in the three procedures. These mice were housed at the University of Waterloo Psychology Department animal laboratories, and have been inbred for more than twenty generations. They are a congenic strain, designated C.B6$-{}^{+c/+c}$, descended from BALB/cWah2 [6]. Except as indicated, they had access to laboratory chow and water ad libidum, and were maintained on a 12-h light/dark schedule.

Of the total sample of 49 mice, two (both female) never reached for food in the MPP test. Three mice (two male and one female) did not eat from the hoppers in the LPP task. These mice were discarded from the analysis. Thus, 44 mice, half male and half female, were available for analysis on all three measures.

2.2. MPP

2.2.1. Apparatus

The apparatus was constructed to Collins' [13] specifications. It includes four chambers, each with a transparent plexiglass front, and opaque plexiglass sides and back. In the centre of the transparent wall of each chamber and 6 cm from the bottom, a plexiglass tube is set. The tubes are 7 mm in internal diameter and 2.5 cm long. The entire apparatus sits on a wire mesh platform through which droppings may fall. The top of the unit is covered with a transparent piece of plexiglass to prevent escape, with a gap left for air circulation.

2.2.2. Procedure

Mice were food-deprived for 18–24 h before performing the MPP test. Four mice were placed in the apparatus in a single session, one in each chamber. These could be observed by the researcher through the transparent front of the apparatus. Bits of sweetened rolled oats (Maypo, Quaker Maple Instant Oatmeal) were placed in the horizontal tubes, and were pushed up to the inside edge of the tube where the mice would notice them. Once a mouse had begun to eat the Maypo, the food was kept at a point in the tube where it could not be reached by the tongue. Most mice quickly learned to reach into the tube with a paw to obtain food. In order to be counted, a reach had to go far enough into the tube to pass the outside of the plexiglass wall, approximately 3 mm.

Four mice were left in the apparatus until 50 reaches had been observed from each. Other research has re-

vealed a very high correlation between measures obtained using this protocol, and measures obtained from observing the first 50 reaches [7]. Thus, the researcher recorded reaches as they were observed, with no concern for recording the first 50 reaches. Each reach was coded as either 'left' or 'right.' The few (4) mice that did not complete 50 reaches after 1 h and 15 min were replaced in their cages, and were tested again after a week to recuperate from food deprivation. Only two mice failed after the second attempt, and were discarded from the analysis as noted above.

The MPP score is the number of right paw reaches out of 50. This score is equivalent to Collins' RPE score, but as noted we designate it MPP here to distinguish it from lateral paw preference. Thus, an MPP score of 25 represents an ambidextrous mouse, whereas a score of 50 represents a perfectly right-pawed mouse, etc. From this score, degree and direction component scores were also calculated. The degree component (MPP-deg) score is the absolute value of the MPP minus 25:

$$MPP\text{-}deg = |MPP - 25|$$

Thus, a score of 0 MPP-deg describes an ambidextrous mouse, and a score of 25 describes a strongly handed mouse. The direction component (MPP-dir) is a dichotomous variable on which mice scoring an MPP of 25 or less were coded 'left-pawed', and those scoring greater than 25 were coded 'right-pawed'.

MPP, MPP-deg, and MPP-dir scores were also calculated separately for the first 25 reaches and the last 25 reaches, in order to obtain a test-retest reliability measure for each.

2.3. Water T-maze test

2.3.1. Apparatus

A water T-maze was used to measure spatial preference. The maze had a stem of 22.5 cm, and arms of 16 cm. Channels were 7.5 cm wide and 22.5 cm deep. The maze was composed of stainless steel and was not painted. The water was not clouded. Escape platforms were placed in both arms of the T, about 2 mm above the surface of the water. These platforms were visible from the decision-point. The water was maintained at 23°C, and at a depth of 14 cm.

2.3.2. Procedure

This procedure was adapted from Zimmerberg et al. [40]. Each mouse was placed at the base of the T, and allowed to swim until it found an escape platform. It was then placed in a cage lined with paper towel and heated from below by a heating pad. The mouse was allowed 30 s of rest in the heated cage before being placed back in the T-maze for the next trial. During this rest period, a second mouse was placed in the T-maze. Thus, two mice were run consecutively, alternating rest and swimming stages. After five trials, both mice were allowed a 90-s rest period in the heated cage, and then were replaced in their home cages while two more mice were run. Four pairs of mice were tested in a given session, so that after the fourth pair had finished, the first pair was given a second set of five trials. This provided approximately 20 min of rest between each block of five trials. Each pair performed four blocks of five trials, yielding 20 trials per mouse.

Trials were recorded as either 'left' or 'right,' depending on the direction the mouse turned at the decision point. If a mouse turned 270° to its left, ending up in the right-hand branch, the response was coded as 'left.' In almost every trial, however, the mouse ended up in the branch of the maze corresponding to the direction in which it had turned.

SP was calculated for each mouse, consisting of the number of times out of 20 it turned toward the right. Thus, a score of 10 indicates a mouse with no spatial preference, whereas a score of 0 indicates perfect preference for the left side of space. This score was also reduced to its degree (SP-deg) and direction (SP-dir) components, as in the MPP test, described above. In addition, SP, SP-deg, and SP-dir scores were calculated separately for the first and last 10 trials, to measure test-retest reliability.

2.4. LPP

2.4.1. Apparatus

The apparatus was built according to the specifications in Waters and Denenberg [35]. Two hoppers are housed in the main casing, and each is accessed through one of two holes in the casing. The outside edge of each hole is flush with the wall next to it, making it difficult for a mouse to reach in with the contralateral paw. Thus, food from the left-hand hopper is available most easily to the left paw, and food from the right-hand hopper most easily to the right paw. Although it is possible for a mouse to reach into a hopper with the contralateral paw, Waters and Denenberg [35] report that none of the mice in their sample was observed to do so. No data were collected in the present study to determine whether mice were obtaining food by any method other than using the appropriate paw, though as in [35], no mouse was observed to do so. Hoppers were loaded with Maypo for testing. For the last nine mice, Quaker Maple and Brown Sugar oatmeal was used.

2.4.2. Procedure

Three apparatus were built, and each was kept in a separate cage. During testing, mice were allowed access to regular food and water as well as to the sweetened oats. Before beginning a trial, the apparatus was

cleaned, and the hoppers were filled and weighed on a balance scale accurate to 0.1 g. One mouse was then placed in each cage with a Waters apparatus. After 12 h, the apparatus was removed, weighed, and refilled and reweighed. It was then replaced in the cage for another 12-h period. At the end of this time, the mice were replaced in their home cages, the hoppers were weighed and the apparatus cleaned. The amount of food eaten from each hopper was determined by deducting the weight of a hopper when it came out of the cage from its weight when it went in. For each mouse, two measures were obtained, each representing one 12-h period.

LPP is calculated by taking the difference between the amount of food consumed from each hopper as a proportion of the square root of the total food consumed [35]:

$$LPP = \frac{L - R}{\sqrt{L + R}}$$

where R is the weight of food eaten from right hopper, and L is the weight of food eaten from the left hopper.

LPP was calculated for each 12-h period in order to compute test-retest reliability. The two 12-h measures were then pooled by summing the amount eaten from each hopper over the two test periods, taking the difference between them, and dividing it by the root of the total amount eaten during the 24-h period. This was the total LPP. The degree and direction components of the LPP were also calculated. The degree component (LPP-deg) is simply the absolute value of the LPP. Mice with a negative LPP were coded as left-pawed, whereas mice with a positive LPP were coded as right-pawed. There were no mice with an LPP of 0.

Approximately two thirds of the animals (13 males and 15 females) began their trials at the beginning of the 12-h dark cycle, and had the hoppers checked and refilled at the beginning of the 12-h light cycle. Thus, for this group, the first LPP measure was taken during a completely dark cycle, and the second during a completely light cycle. This group shall be called the 'split' group. The rest of the sample (nine males and seven females) began their trials approximately 4 h into the light cycle, and had the hoppers checked approximately 4 h into the dark cycle. For this group, the first LPP was taken during a time period that was two thirds light and a third dark, and the second measure was taken during a period that was two thirds dark and a third light. This group shall be referred to as the 'mixed' group. Note that each mouse had one measure taken during a period that was mostly or completely dark, and one measure taken during a period that was mostly or completely light. All but two of the split group had the dark-cycle measure taken first, whereas all of the mixed group had the light-cycle measure taken first. The mixed and split groups are of unequal size because an effect of light/dark cycle was not anticipated, and therefore not intentionally manipulated. Waters and Denenberg [35] found that the light/dark cycle did not affect LPP; however, this observation was based on only six mice. Preliminary inspection of the data prompted an examination of the effects of the diurnal cycle. Because spatial preferences are dopamine driven, and dopamine production increases during the dark cycle when mice are most aroused [22], the light/dark cycle might be expected to affect LPP if it is driven by spatial preference. For this reason, two further variables were created: an LPP for the period that was mostly or completely dark (LPP-dark), and one for the period that was mostly or completely light (LPP-light). The degree and direction components of these variables were also examined separately.

2.4.3. Analyses

The distributions for each measure were examined to ensure that the measurements had been successful and the expected distributions obtained. Spearman–Brown test-retest reliability coefficients were calculated for MPP, SP, and LPP scores, as well as for their degree and direction components. Because each mouse had only one LPP-dark and one LPP-light score, reliability for these measures could not be calculated. However, a test-retest reliability analysis using the LPP-dark measure as the first test and the LPP-light measure as the second was conducted within the split and mixed groups.

A two-tailed within-subjects matched-pairs t-test was performed to determine whether there was a difference between the mean LPP-light and LPP-dark scores. One-way ANOVAs were calculated for all variables to determine the effect of sex. One-way ANOVAs for all LPP's by split/mixed groups were also calculated.

Log odds-ratios were calculated to determine whether the proportions of left- and right-pawed mice were different between the sexes. Log odds-ratios were also used to determine whether directional population biases were significant.

Correlations between all variables were examined for the entire sample, for the split and mixed groups separately, and for each sex separately. For continuous variables, Pearson product-moment correlations and Spearman rank-order correlations were calculated. For discrete variables (the direction components), two methods were used: the cosine-π formula for tetrachoric correlation [19], and the ϕ-coefficient. The first analysis is offered for comparison to other data (e.g. [13]), and the second is presented following Waters and Denenberg [35,36].

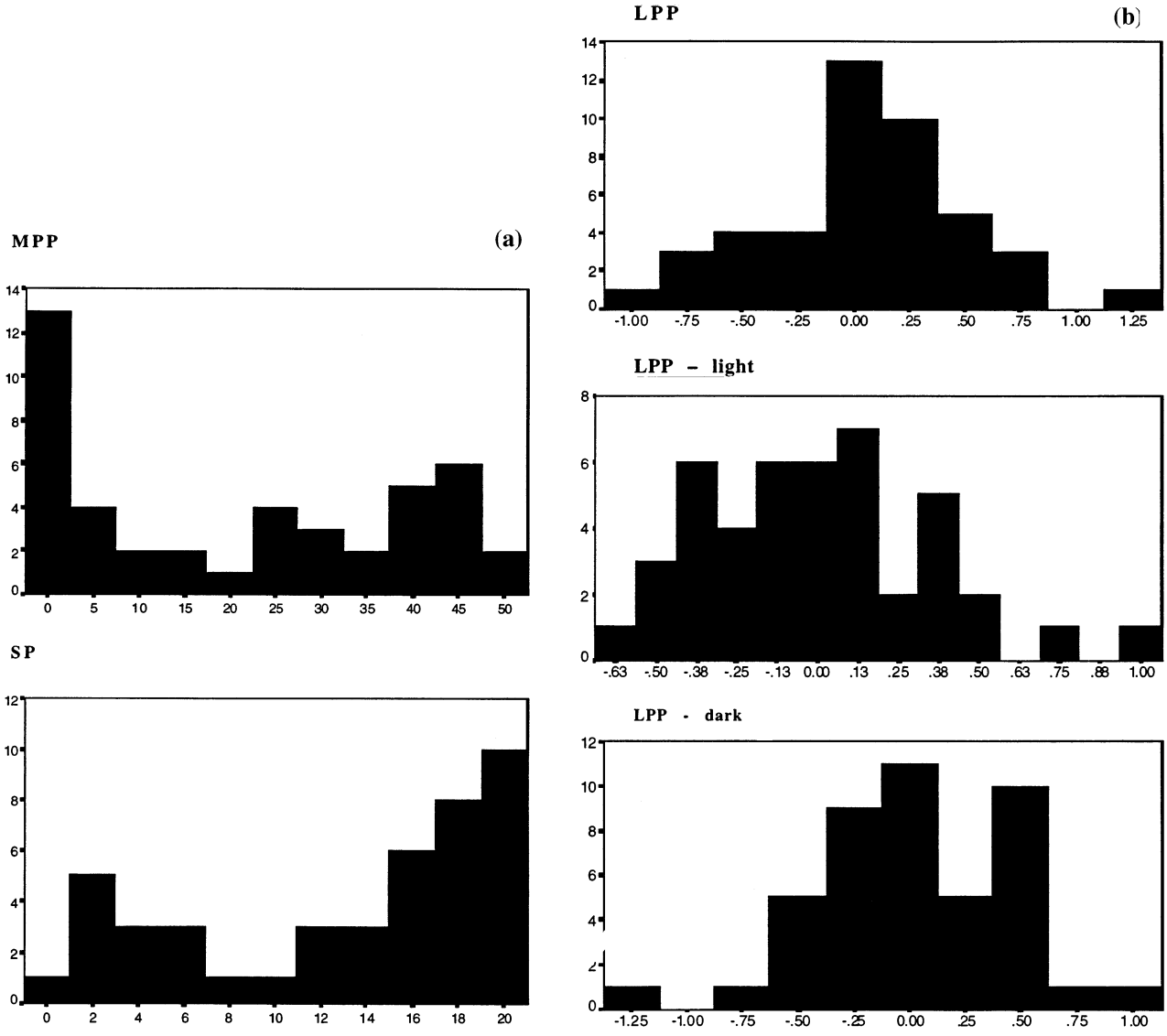

Fig. 1. a. Frequency distributions obtained from MPP, and SP measures. b. Frequency distributions obtained from LPP, LPP-light, and LPP-dark measures.

3. Results

3.1. Distributions

All distributions for the total population are presented in Fig. 1a and Fig. 1b.

As expected, the MPP distribution was bimodal, with peaks occurring at the low and high extremes of the distribution. The U-shape of the distribution was not as pronounced as in Collins [13], indicating a weaker degree of paw preference in this strain. Although the distribution appeared by inspection to be left-biased, this effect was not significant. The proportion of left-pawed mice, as measured by the MPP test, was significantly different between males and females. Seventy-three percent of males were left-pawed, whereas only 41% of females were left-pawed (LOR = 1.348; $z = 2.087$; $P < 0.05$, two-tailed).

The distribution of SP scores was also strongly bimodal, with no values in the center, indicating a strongly lateralized population. A trend toward a significant population bias was found, with more mice preferring the right side of space (LOR = -0.762; $z = -1.72$; $P < 0.09$, two-tailed). A similar population bias has been reported in the literature for rats and other strains of mice [11,18,20,23]. There was no difference between sexes in the proportions of mice with left- vs. right-side preferences.

A unimodal distribution about 0 found in this study for the LPP showed that most mice were not strongly lateralized on this measure, as they were on the other two. This distribution is comparable to that reported by Waters and Denenberg [36], which is only slightly bimodal. The distributions for the LPP-light and LPP-dark were also unimodal and distributed normally

about 0. A t-test showed no difference between the means of these distributions. There was no difference between sexes in the proportion of left-pawed mice as determined by this measure.

3.2. Reliability

Equal-length Spearman–Brown reliability estimates for the MPP and SP scores were both quite high, with coefficients of 0.96 and 0.91, respectively. MPP-deg was also highly reliable (0.87). SP-deg was less reliable (0.64), but still within acceptable bounds. The reliability estimates for the MPP scores are comparable to those reported elsewhere [13]. The SP reliability estimates presented here are somewhat higher than those obtained by Zimmerberg et al. [40]. This may be due to the use of a water T-maze instead of an electrified T-maze, or it may be due to the fact that very little time elapsed between the first and second measure in this study. Castellano et al. [12] report that degree of side preference in an electrified T-maze increases significantly with experience in rats, so this may also account for Zimmerberg's lower reliability scores.

The reliability estimates for the LPP and its components were not comparably high. The reliability coefficient for LPP was 0.32, and for LPP-deg it was 0.25. Waters and Denenberg [35] obtained reliability estimates after 2 days of testing of 0.64 for the total LPP, and 0.31 for LPP-deg. The unexpectedly low reliability estimates in the present study prompted an examination of the effect of the light/dark cycle on LPP. The reliability coefficient for the split group ($n = 28$), in which the first measure was taken completely during the dark cycle, and the second completely during the light-cycle, was 0.24. The corresponding estimate for the degree component was -0.14. The reliability coefficient for the mixed group ($n = 16$), in which the first and second measure both were taken during a period of mixed light/dark, was 0.36, somewhat higher than in the split group. The corresponding estimate for the degree component was 0.50.

3.3. ANOVAs

ANOVAs showed no effect of sex on any score, and only one effect of split/mixed groups: LPP-light differed significantly between split and mixed groups ($F = 5.22$; $P < 0.03$).

3.4. Correlations

Pearson product-moment and Spearman rank-order correlation coefficients for the full sample are shown in Table 1. Cosine-π tetrachoric correlation coefficients and ϕ-coefficients are presented for the direction components. Because of their robustness in the face of deviations from normality, only the Spearman correlations and ϕ-coefficients will be discussed. All significance tests are two-tailed.

Of the correlations between the MPP, SP and LPP measures, only the relation between SP and LPP approached significance ($r = 0.25$; $P < 0.10$). The correlation between MPP and SP was not significant. Of the degree components, no relation was significant. A reliable correlation between SP-dir and LPP-dir was found ($\phi = 0.41$; $P < 0.01$). None of the other relations between the direction components were significant.

Because of the low reliability of the total LPP, correlations involving the LPP-light and LPP-dark were examined separately. LPP-light and LPP-dark scores did not correlate significantly with each other, nor did their degree or direction components. LPP-light correlated with neither MPP nor SP; LPP-dark, on the other hand, correlated significantly with SP ($r = 0.42$; $P < 0.01$). LPP-light-deg correlated negatively with MPP-deg ($r = -.37$; $P < 0.05$), but not with SP-deg. LPP-dark-deg did not correlate significantly with either SP-deg or MPP-deg. LPP-light-dir correlated with neither MPP-dir nor with SP-dir, whereas LPP-dark-dir correlated strongly with SP-dir ($\phi = 0.52$; $P < .01$) only.

Correlations for total measures and degree components were also calculated separately for the split and mixed groups, and are shown in Table 2. For the split group, the degree components showed a negative relation between LPP-light-deg and MPP-deg ($r = -0.41$; $P < 0.03$; $n = 28$). No other relations were significant. In the mixed group, the correlation between LPP-dark and SP persisted ($r = 0.54$, $P < 0.04$; $n = 16$). However, no degree components correlated significantly in this group.

No correlation coefficient was significantly different between split and mixed groups.

The correlation coefficients among full measures and degree components for males and females are shown in Table 3. Males displayed a significant correlation between SP and LPP-dark ($r = 0.55$; $P < 0.01$; $n = 22$). SP and LPP-light did not correlate significantly. Both males and females showed a negative relation between the degree components of LPP-light and MPP, but this relation was only significant in the males ($r = -0.47$; $P < 0.05$; $n = 22$).

There were no significant correlations among the measures for the females.

4. Discussion

4.1. Independence of MPP and SP

The independence between MPP and SP found by other researchers has been confirmed in the present study by the lack of a significant relation between MPP

Table 1
Correlation coefficients for full sample

	MPP	SP	LPP	LPP-light	LPP-dark
Full measures					
MPP		$r=-0.13$	$r=0.09$	$r=0.02$	$r=0.06$
		$P<0.42$	$P<0.57$	$P<0.90$	$P<0.69$
SP	$r=-0.14$		$r=0.25$	$r=-0.8$	$r=\mathbf{0.42}$
	$P<0.34$		$P<0.10$	$P<0.58$	$P<\mathbf{0.01}$
LPP	$r=0.08$	$r=0.28$			
	$P<0.60$	$P<0.06$			
LPP-light	$r=0.03$	$r=0.01$			$r=0.16$
	$P<0.84$	$P<0.94$			$P<0.29$
LPP-dark	$r=0.08$	$r=\mathbf{0.42}$		$r=0.16$	
	$P<0.62$	$P<\mathbf{0.02}$		$P<0.30$	
Data below the diagonal are Pearson product-moment coefficients Data above the diagonal are Spearman rank-order correlation coefficients					
Direction components					
MPP		$\phi=-0.09$	$\phi=0.16$	$\phi=0.15$	$\phi=0.06$
		$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$
SP	$r=-0.16$		$\phi=\mathbf{0.41}$	$\phi=0.07$	$\phi=\mathbf{0.52}$
	$P>0.05$		$P<\mathbf{0.01}$	$P>0.05$	$P<\mathbf{0.01}$
LPP	$r=0.24$	$r=\mathbf{0.64}$			
	$P>0.05$	$P<\mathbf{0.01}$			
LPP-light	$r=0.24$	$r=0.11$			$\phi=0.08$
	$P>0.05$	$P>0.05$			$P>0.05$
LPP-dark	$r=-0.09$	$r=\mathbf{0.78}$		$r=0.38$	
	$P>0.05$	$P<\mathbf{0.01}$		$P>0.05$	
Data below the diagonal are cosine-π tetrachoric correlation coefficients Data above the diagonal are ϕ-coefficients					
Degree components					
MPP		$r=0.12$	$r=-0.09$	$r=-\mathbf{0.37}$	$r=-0.09$
		$P<0.44$	$P<0.55$	$P<\mathbf{0.05}$	$P<0.55$
SP	$r=0.12$		$r=-0.13$	$r=-0.01$	$r=-0.15$
	$P<0.42$		$P<0.38$	$P<0.96$	$P<0.32$
LPP	$r=-0.13$	$r=-0.19$			
	$P<0.40$	$P<0.22$			
LPP-light	$r=-\mathbf{0.40}$	$r=0.06$			$r=0.16$
	$P<\mathbf{0.02}$	$P<0.72$			$P<0.29$
LPP-dark	$r=-0.11$	$r=-0.25$		$r=0.12$	
	$P<0.48$	$P<0.12$		$P<0.46$	
Data below the diagonal are Pearson product-moment coefficients Data above the diagonal are Spearman rank-order correlation coefficients					

Significant relations appear in bold.
SP, spatial preference; LPP, lateral paw preference; MPP for medial paw preference. LPP-light and LPP-dark stand for measures of lateral paw preference that were taken during the light and dark portions of the diurnal cycle, respectively.

and SP measures, in both their degree and direction components.

4.2. Functional independence of MPP and LPP

Waters' and Denenberg's observations have been partially replicated. The current results show that MPP is unrelated to LPP in direction. The negative relation between MPP-deg and LPP-light-deg is in conflict with the original results, however. Waters and Denenberg do not report a relation between LPP-deg and MPP-deg [35,36].

Some measures are missing that might help to make more sense of this picture. LPP was measured once during a state of high arousal (dark-cycle), and once during a state of low arousal (day-cycle). The MPP measure took place during the daytime, but under the stress of food deprivation and a novel environment. Carlson et al. [10] have shown that food deprivation may increase the degree of dopamine-driven rotation. It is unclear, then, whether the MPP measure, relative to the other measures, was taken during a state of high or low arousal. The degree of arousal during the SP measurement is similarly unknown. The stress of being dropped into cool water was likely arousing, but it is difficult to know how arousing. Thus, SP may have been taken during a period of high, moderate, or low arousal. Furthermore, it is uncertain how increased

Table 2
Correlation coefficients for the split and mixed groups separately

	Variables	Statistic	Full measure		Degree component	
			Day	Night	Day	Night
Split group ($n=28$)	MPP/LPP	Pearson	$r=0.14$	$r=0.09$	$r=-\mathbf{0.38}$	$r=0.12$
			$P<0.49$	$P<0.67$	$P<\mathbf{0.05}$	$P<0.54$
		Spearman	$r=0.06$	$r=0.13$	$r=-\mathbf{0.41}$	$r=0.05$
			$P<0.77$	$P<0.52$	$P<\mathbf{0.03}$	$P<0.81$
	SP/LPP	Pearson	$r=0.07$	$r=0.36$	$r=-0.02$	$r=-0.34$
			$P<0.71$	$P<0.06$	$P<0.92$	$P<0.08$
		Spearman	$r=0.01$	$r=0.32$	$r=-0.04$	$r=-0.24$
			$P<0.96$	$P<0.10$	$P<0.85$	$P<0.23$
Mixed group ($n=16$)	MPP/LPP	Pearson	$r=-0.04$	$r=0.06$	$r=-0.37$	$r=-\mathbf{0.45}$
			$P<0.88$	$P<0.84$	$P<0.08$	$P<\mathbf{0.05}$
		Spearman	$r=0.03$	$r=0.01$	$r=-0.33$	$r=-0.31$
			$P<0.93$	$P<0.97$	$P<0.21$	$P<0.25$
	SP/LPP	Pearson	$r=0.02$	$r=\mathbf{0.53}$	$r=0.01$	$r=0.03$
			$P<0.94$	$P<\mathbf{0.04}$	$P<0.50$	$P<0.46$
		Spearman	$r=-0.15$	$r=\mathbf{0.54}$	$r=-0.09$	$r=0.07$
			$P<0.58$	$P<\mathbf{0.04}$	$P<0.73$	$P<0.79$

Significant relations appear in bold.
SP, spatial preference; LPP, lateral paw preference; MPP, medial paw preference.
For the split group, LPP-dark was taken during a completely dark period, and LPP-light during a completely light period. For the mixed group, LPP-dark was taken during a period that was two-thirds dark and a third light, while LPP-light was taken during a period that was two-thirds light and a third dark.

dopamine metabolism might affect lateralized behaviours. Dopamine agonists have been shown to cause rats to increase their preference for lever pressing [39], suggesting that dopamine-driven lateralized behaviours may be strengthened by increasing dopamine activity. However, dopamine receptor agonists have been shown to reduce side preference in an electrified T-maze [11,12]. Thus, it is possible that side and paw preferences may vary with arousal, but it is difficult to predict in what direction.

4.3. Relation between SP and LPP

The most interesting result is the predicted strong positive relation between SP and LPP. However, the crucial role of arousal was not anticipated. Although LPP and SP are significantly correlated overall, particularly in the direction components, this relation seems to be driven by the dark-cycle portion of the LPP. No such relation exists during the light-cycle. To a certain extent this makes sense, as dopamine metabolism—and thus SP—is at its greatest during the night. Waters and Denenberg [35] took all their measurements during the light-cycle, and still got a reliable measure. If neither MPP nor SP can explain the reliable LPP displayed during daylight hours, this suggests either that dopamine-driven SP and MPP reach some sort of stable compromise when arousal is low, or that a third behavioural lateralization really is at work.

The measure of SP in this study was taken during a period when arousal may have been high (see above). If measures of SP taken during periods of high- and low-arousal are independent, low-arousal SP might then predict the LPP-light, whereas high arousal SP may predict LPP-dark. If so, LPP can be shown to be driven at least in part by SP during all states of arousal. Few studies have directly examined the relation between measures of a behavioural laterality under different levels of arousal; at least one study, however, has found no relation between preferred directions of spontaneous rotation and amphetamine-induced rotation [37], but see also [24]. Three common tests of SP all involve potentially increasing the level of arousal: water rotation involves plunging the rodent into cool water; electrified T-maze testing involves shocking the mouse; and injection with *d*-amphetamine involves chemically inducing arousal. However, the open-field test developed by Sherman et al. [34] may be taken during low- and high-arousal conditions. Presumably this method could be used to determine whether SP varies with arousal.

If LPP depends in part on SP, it ought to vary with manipulations of SP. Previous studies have used lesion techniques to create a dopamine imbalance between hemispheres, resulting in a manufactured SP. Rats with unilateral lesions to the nigrostriatal system rotate spontaneously in the direction ipsilateral to the lesioned side [22]. Rats have also been shown to reverse their preference for bar pressing in a two-lever operant task when the hemisphere ipsilateral to the initial preference is stimulated electrically, for the duration of the stimulation [39]. These studies show that SP can be manipu-

Table 3
Correlation coefficients for males and females separately

	Variables	Statistic	Full measure		Degree component	
			Day	Night	Day	Night
Males ($n=28$)	MPP/L PP	Pearson	$r=-0.05$	$r=-0.08$	$r=-0.35$	$r=-0.22$
			$P<0.84$	$P<0.74$	$P<0.12$	$P<0.34$
		Spearman	$r=0.09$	$r=-0.02$	$r=$ **-0.47**	$r=-0.14$
			$P<0.70$	$P<0.93$	$P<$ **0.05**	$P<0.54$
	SP/LPP	Pearson	$r=0.02$	$r=$ **0.62**	$r=0.05$	$r=0.00$
			$P<0.94$	$P<$ **0.02**	$P<0.86$	$P<1.00$
		Spearman	$r=-0.17$	$r=$ **0.55**	$r=0.05$	$r=-0.01$
			$P<0.44$	$P<$ **0.01**	$P<0.83$	$P<0.97$
Females ($n=16$)	MPP/LPP	Pearson	$r=0.11$	$r=0.24$	$r=$ **-0.48**	$r=0.00$
			$P<0.64$	$P<0.28$	$P<$ **0.04**	$P<1.00$
		Spearman	$r=0.12$	$r=0.16$	$r=-0.27$	$r=-0.05$
			$P<0.59$	$P<0.47$	$P<0.22$	$P<0.82$
	SP/LPP	Pearson	$r=0.01$	$r=0.22$	$r=0.10$	$r=-0.44$
			$P<0.98$	$P<0.34$	$P<0.68$	$P<0.06$
		Spearman	$r=0.01$	$r=0.28$	$r=-0.06$	$r=-0.28$
			$P<0.96$	$P<0.21$	$P<0.77$	$P<0.21$

Significant relations appear in bold.
SP, spatial preference; LPP, lateral paw preference; MPP, medial paw preference.

lated without increasing arousal; that is, an imbalance between hemispheres can be created without increasing overall dopamine utilization. Similar methods can be used to determine whether dopamine imbalances affect LPP. Whishaw et al. [37] have shown that unilateral lesions to the nigrostriatal system, as well as unilateral DA deprivation, lead rats to prefer the ipsilateral paw in one reaching task. In that study, however, the animals were free to reach through a set of bars for food, and thus could reach either toward or away from the midline at their discretion, so it is difficult to determine whether the changes in paw preference found evidenced changes in MPP, LPP, or SP. Thus, the question of whether variations in dopamine asymmetries affect LPP is still open.

That the LPP is a measure of different phenomena, or at least a single changing phenomenon, from day to night is evident from the low correlation between day and night measures. It is possible that the measure indexes an independent behavioural laterality that is increasingly confounded with SP as arousal increases. Evidence for this view comes from comparison between the split and mixed groups. The overall level of arousal during the LPP-light and LPP-dark measures is likely more similar for mice in the mixed group than for those in the split group. The higher reliability estimate between these measures in the mixed group shows that LPP-light and LPP-dark are more similar for mice in this group. That is, the LPP for a given mouse is more reliable if taken at the same level of arousal each time; thus LPP varies with arousal. This supports the hypothesis that increasing arousal increasingly confounds the LPP.

If the LPP measures paw preference for reaches away from the midline, while MPP measures paw preference for reaches toward the midline, it is difficult to see how a measure of LPP not confounded by SP may be taken. A reach away from the midline is a reach to the side, and a reach to the side must inevitably be affected by SP. On the other hand, if independent mechanisms really do underlie these behaviours, then an examination of mice exhibiting no SP might be instructive. Such mice might still display LPP, and in them it could be measured free from the effects of SP.

5. Summary

SP is independent of MPP. However, during periods of high arousal, SP is related to LPP. The relation between SP and LPP during periods of low arousal is uncertain, as the level of arousal during the measurement of SP in the current study is not known. Thus, there are at least two possible explanations for the present pattern of results.

One explanation is that there are two independently lateralized subsystems guiding reaching behaviour in the mouse: spatial preference and paw preference. Spatial preference confounds the measurement of paw preference when animals are required to reach away from the midline, as evidenced by the relation between the measures SP and LPP during periods of high arousal. This account does not explain why neither MPP nor SP was related to LPP during periods of low arousal. However, if the animals in the current study were highly aroused by the T-maze task that indexes spatial

preference, it is possible that a measure of spatial preference taken during a state of low arousal would relate to LPP during low arousal. If so, it would be possible to conclude that spatial preference confounds the measurement of paw preference in lateral reaches during all states of arousal. This explanation would require that spatial preferences during periods of low and high arousal be independent.

The second explanation of these results posits three behavioural lateralizations: spatial preference, medial paw preference, and lateral paw preference. Medial paw preference is unrelated to lateral paw preference in direction. However, the measure of lateral paw preference is increasingly confounded by spatial preference as arousal increases, as evidenced by the correlation between SP and high-arousal LPP. During periods of low arousal, when interhemispheric dopamine differences are at their lowest, the measure of lateral paw preference is relatively unconfounded, accounting for the reliability of the measure reported in [35] and [36].

Lesioning techniques may be used to determine which of these explanations is more likely, as lesions of the nigrostriatal system can create striatal dopamine imbalances without increasing arousal. If such lesions lead to preference for the contralateral paw in lateral reaches, this would suggest that lateral paw preference is driven by spatial preference in all states of arousal.

Acknowledgements

We thank Nancy Gibson for help with the animals, Gerry Blom for building the apparatus, and Pat Wainwright for many insightful comments. We also thank two anonymous reviewers for their helpful remarks. This paper is dedicated to the memory of M.P. Bryden, a distinguished scholar, revered mentor, and good friend.

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