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Mathematical analysis of locomotor behavior by mice in a radial maze

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We investigated the effects of β-estradiol on the locomotor behavior of female mice in a radial maze. Data comprising the total distance traveled during each arm entry were obtained from video records of six consecutive daily recording sessions. Distributions of these data were bimodal for both ovariectomized control and β-estradiol-treated ovariectomized subjects. Data were fit with the sum of two gamma probability distributions. Three parameters of the analytic fits were useful for quantifying the effect of β-estradiol on locomotor behavior: (*i*) the sampling distance (median of the total distance traveled during each arm entry in the short-distance peak of a bimodal distribution), (*ii*) the committed distance (median of the total per-arm-entry distance traveled in the long-distance peak), and (*iii*) the partition distance (distance represented by the minimum between the two peaks). Analysis showed that for sampling-distance arm entries β-estradiol typically had little if any significant effect on female locomotor behavior, whereas it significantly increased the total distance traveled during committed-distance arm entries on the first 2 days of exposure to the empty maze. β-Estradiol also increased the ability of females to discriminate between empty maze arms and arms that contained intact or castrated male mice and partially prevented loss of this capacity after removal of the males.

analytic fit | bimodal distribution | estrogen | gamma distribution | exploratory behavior

A long history of endocrine and behavioral research with rodents has supported the concept that estrogens foster exploratory and other locomotor responses in females with the effect of increasing contacts with reproductively competent males (reviewed in ref. 1). For example, ovariectomized female rats treated with estrogens increased their willingness to cross electrified grids and to choose appropriate arms of mazes to approach males (2, 3). Nerve cell groups supporting such behaviors have been found in rats in the lateral subdivision of the medial preoptic area (4) and in hypothalamic efferents to the midbrain central gray (5). To make use of current techniques in functional genomics, mice would be desirable for this type of experimentation. Direct approaches by female mice to males in a seminatural environment have been described (6). Genomic studies with mice have emphasized the importance of gene products coding for estrogen receptors α and β, oxytocin, the oxytocin receptor, and the vasopressin 1a receptor in the control of affiliative (as opposed to aggressive) behaviors (7–10). If estrogens heighten CNS arousal, the behavioral choice by any individual mouse in any particular situation hinges on the balance between sexual attraction and fear (11). Our mathematical analyses, which separate shorter sampling-distance entries into a maze arm from longer committed-distance entries, address this behavioral distinction.

Our analysis shows that the locomotor behavior of female mice significantly changes in the presence of estrogens. However, this effect is clearly revealed only when arm entries are partitioned into their shorter sampling-distance and longer committed-distance components.

Results

Twenty-four ovariectomized female mice were used in this experiment. Each was implanted with a silastic capsule containing only sesame oil for the control OIL group ($n = 12$) or β-estradiol (E2) in sesame oil for the treatment E2 group ($n = 12$). Females were individually introduced into the center of a four-arm radial maze on 6 consecutive days and exposed to both an intact and a castrated male on days 3 and 4. As described in *Methods*, this protocol defined three test epochs, PRE (days 1 and 2), STIM (days 3 and 4), and POST (days 5 and 6).

Initially, conventional analysis of classical measures of mouse behavior such as the number of arm entries and arm entry duration were performed by using a three-way factorial multivariate analysis of variance (MANOVA) and the Tukey–Kramer multiple-comparison post hoc test. For these analyses, data for the two empty arms were averaged together because they were not statistically different. Analysis showed that OIL females made significantly more entries (1.5-fold) than E2 females ($P < 0.05$, days 1–3 MANOVA). During the period of exposure to the stimulus males, both groups discriminated between the empty and stimulus-containing arms, as measured by average arm dwell time ($P < 0.05$ on each day of exposure).

When data were aggregated by pooling within group across the 6 days of the experiment, there was little difference in the mean latency to first arm entry (±SEM) for the OIL and E2 females (1.1 ± 0.18 s, $n = 64$ and 1.1 ± 0.12, $n = 68$, respectively). However, when the data are aggregated within group and examined by day, the mean latency typically increased in the presence of β-estradiol after the PRE epoch (Fig. 1). Because latency times to first arm entry were typically not normally distributed (days 2, 5, and 6 for OIL and days 2, 3, 4, 5, and 6 for E2 mice), we also calculated the median latency to first arm entry. This measure also increased in the presence of β-estradiol after the first day of exposure to the stimulus males (Fig. 1, day 4).

Histograms of the total distance traveled during each arm entry (the locomotor activity) for the 12 female mice within the control OIL and treatment E2 groups were found to be bimodal. The histograms obtained from data pooled within group by epoch given in Fig. 2 are representative of this locomotor activity. These data required two gamma and one Gaussian function to be well fit, whereas data for the analyses presented here were fit with the sum of two gamma probability distributions.

Quality of Analytic Fits. Table 1 summarizes the quality of the analytic fits obtained with our methodology when data for

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The authors declare no conflict of interest.

Abbreviations: MANOVA, multivariate analysis of variance; MLE, maximum likelihood estimation; KS, Kolmogorov–Smirnov goodness-of-fit test; AD, Anderson–Darling goodness-of-fit test; C.I., confidence interval.

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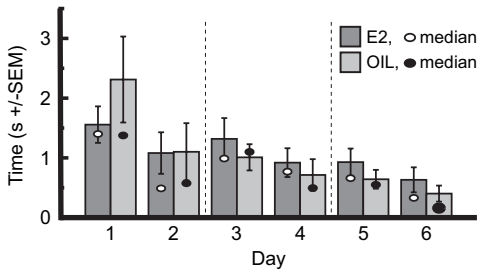

Fig. 1. Mean (±SEM) and median latency (OIL, filled ovals; E2, open ovals) to first arm entry from the center of a four-arm radial maze for control (OIL) and estrogen-treated (E2) female mice on each day of the experiment. Vertical dashed lines demarcate the 2 days of each of the PRE (*Left*), STIM (*Center*), and POST (*Right*) epochs (see *Methods* for details).

individual females were aggregated by day then pooled by arm within each of the OIL and E2 groups as described in *Methods*. Tabulated values give the average number of entries per maze arm, the average rms error of the two-gamma-function fits, and the average *P* value of these fits obtained with the Kolmogorov–Smirnov (KS) and Anderson–Darling (AD) goodness-of-fit tests. The average number of entries per arm per mouse on each day significantly increased in the presence of β-estradiol (paired two-sample *t* test: $P < 0.001$), and the rms errors of the fits were significantly smaller for OIL than for E2 data (paired two-sample *t* test: $P < 0.001$). No significant between-group differences for each maze arm were found for either the *P* values of the KS and AD goodness-of-fit tests or the average rms errors of the fit of analytic distributions to bootstrap samples (paired two-sample *t* test).

The bimodal form of the spatial distributions as exemplified in Fig. 2 precludes analysis by the usual statistical methods, such as a repeated-measures analysis of variance, because it is not possible to transform the observed bimodal distributions into the required normal form. However, as described in *Methods*, it was possible to test the effect of β-estradiol on the total distance traveled during each arm entry by using bootstrap techniques on analytic fits to the data.

Table 1. Quality of analytic fits to aggregated arm entry data (±SD, *n* = 18)

Analysis	Entries per arm*	rms error, %	KS *P* value	AD *P* value
E2 fit	20 ± 4	1.03 ± 0.29	0.50 ± 0.32	0.46 ± 0.30
OIL fit	28 ± 6	0.74 ± 0.21	0.61 ± 0.25	0.58 ± 0.29
E2 bootstrap	—	2.64 ± 0.61	—	—
OIL bootstrap	—	2.85 ± 0.81	—	—

*Mean number of entries per maze arm per animal per day.

Unpartitioned Data Pooled Within Group by Day and by Arm. Before analysis, data were aggregated within each of the OIL and E2 groups and pooled by day and by arm. The median total distance traveled during each arm entry was calculated for each bootstrap sample. The median and its $C.I._{99\%}$ and $C.I._{68\%}$ were then estimated from the bootstrap distribution of the statistic. As measured by the overlap of the $C.I._{99\%}$, during the PRE epoch, β-estradiol had little if any significant effect on female locomotor activity in either the novel maze environment or during reexposure to the now-familiar environment (Fig. 3, days 1 and 2). Similarly, β-estradiol had no significant effect on locomotor activity during the first day of exposure to the intact and castrated male mice. However, on the second day of exposure to the stimulus males, the β-estradiol-treated females distinguished between the baited arms (containing the intact and castrated males) and the empty arms, whereas control OIL females did not (Fig. 3, days 3 and 4). After stimulus removal, during the POST

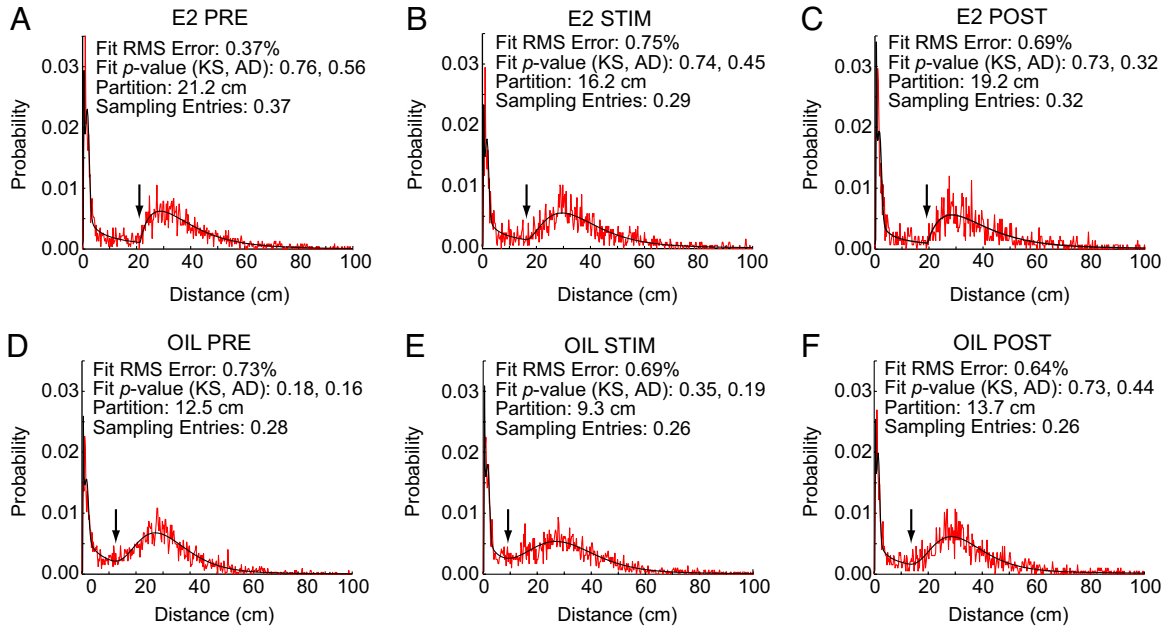

Fig. 2. Analytic fits (black lines) to observed total distances traveled during each arm entry (red lines). Before plotting, data for individual females were pooled within their respective control (OIL) and estrogen-treated (E2) groups for each of the three epochs studied. (*A* and *D*) PRE (days 1 and 2): females explore empty maze. (*B* and *E*) STIM (days 3 and 4): male stimuli present, with one intact and one castrated male each located at the extremity of a different maze arm (the same arms on the 2 days of the STIM epoch). (*C* and *F*) POST (days 5 and 6): females explore empty maze after stimulus removal. Also given are the rms errors and *P* values of the two-sample Kolmogorov–Smirnov (KS) and Anderson–Darling (AD) goodness-of-fit tests. The partition distance (Partition) is indicated by the vertical arrow and was obtained as the minimum interpeak value of the analytic fit. The proportion of the analytic distribution shorter than the partition distance (Sampling Entries) was obtained from the number of observed intervals contributing to the left peak of the fit.

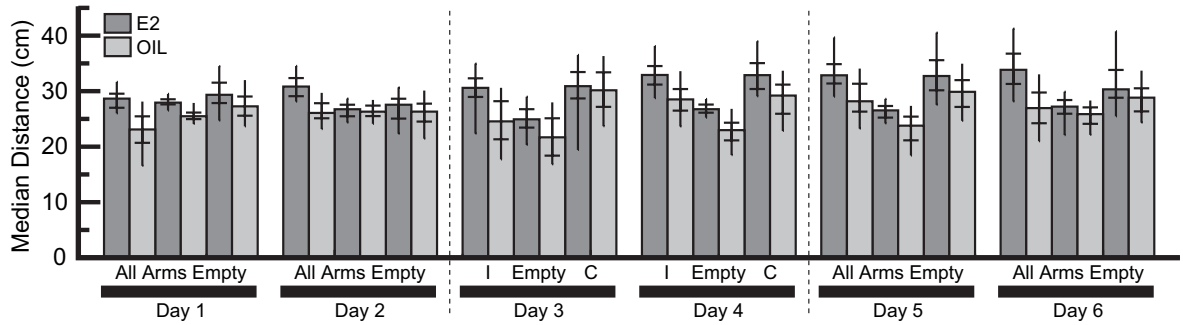

Fig. 3. Median total distance traveled by control (OIL) and estrogen-treated (E2) female mice in a four-arm radial maze during each arm entry for unpartitioned arm entry data. On each day, from left to right, pairs of bars give results for arm one, arms two and three (pooled), and arm four. Vertical dashed lines demarcate the 2 days within each of the PRE (*Left*), STIM (*Center*), and POST (*Right*) epochs (see *Methods* for details). The maze was empty during the PRE and POST epochs. During the STIM epoch (days 3 and 4), the same intact (I) and castrated (C) male mice were located at the ends of arms one and four, respectively. Long uncapped bars and inner-cross error bars give the 99% and 68% (equivalent to $\alpha = 0.05$) bootstrap C.I.s, respectively.

epoch, β-estradiol mice maintained the distinction between empty and baited arms on day five, a capacity that was lost on the final day of the experiment (Fig. 3, days 5 and 6). Similar results were obtained when the 68% C.I.s (equivalent to $\alpha = 0.05$) were considered. However, in this case it could be concluded that β-estradiol had little effect on the ability of females to discriminate between empty and baited arms, because at this level of confidence OIL females also appeared to make this discrimination (Fig. 3, days 4 and 5).

Data Partitioning. Each interval distribution obtained for the total distance traveled during each arm entry was partitioned into shorter sampling-distance and longer committed-distance arm entries. As indicated by the arrows in Fig. 1, the partition distance was defined as the total distance traveled during each arm entry with the lowest probability of occurrence between the two peaks of the analytic fit. Partition distances for interval data aggregated within group and pooled by day and by arm entered were obtained from two-gamma analytic fits made to each bootstrap sample. In the presence of β-estradiol, median partition distances (±SEM, $n = 18$) were significantly increased from a mean of 15.5 ± 0.87 (all OIL distances averaged) to 20.4 ± 0.63 cm (all E2 distances averaged). Fig. 4 summarizes the average partition distance (±SEM, $n = 3$) for maze arms on each day of the experiment. It shows that this measure was typically increased in the presence of β-estradiol and was relatively stable across the 6 days of the experiment. Notably, the shortest partition distance occurred during first exposure to the stimulus males (day 3). Also, the average partition distance of OIL females was more variable than that of E2 females from day to day of the experiment.

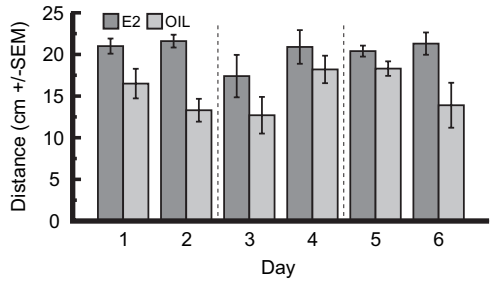

Fig. 4. Arm partition distances obtained for control (OIL) and estrogen-treated (E2) female mice in a four-arm radial maze. Data were pooled within group across arms by day. Vertical dashed lines demarcate the 2 days within each of the PRE (*Left*), STIM (*Center*), and POST (*Right*) epochs (see *Methods* for details). Error bars give the SEM ($n = 3$).

Pearson's cross-correlation coefficient r was calculated for pairwise comparisons of the median distance traveled during sampling-distance and committed-distance arm entries and the partition distance (r range 0.005–0.84). Correlations were typically larger for OIL females, and four of six significant correlations ($P < 0.05$) were between the partition-distance and committed-distance arm entries (data not shown). When data were pooled within group across days and arms, E2 sampling-distance and committed-distance arm entries were negatively correlated. OIL partition distances, sampling-distance, and committed-distance arm entries were positively correlated, as were the committed-distance arm entries of E2 and OIL females.

Partitioned Data Pooled Within Group by Day and by Arm. A more detailed picture of the effect of β-estradiol on female locomotor activity was obtained from analysis of the shorter sampling-distance and longer committed-distance arm entries. With respect to both the $C.I._{99\%}$ and the $C.I._{68\%}$, there was little if any significant effect of β-estradiol on sampling-distance arm entries (Fig. 5*A*). Similarly, there was little if any significant difference between maze arms for female locomotor activity as measured by the median sampling distance traveled during arm entries. This was not the case for committed-distance arm entries. During the PRE epoch there was little if any significant effect of β-estradiol on locomotor activity during these arm entries as judged by the $C.I._{99\%}$. However, at the $C.I._{68\%}$ level there was a significant effect of β-estradiol on the median distance of committed-distance arm entries but not on the ability of either OIL or E2 females to discriminate between the empty arms (Fig. 5*B*, PRE). During the STIM epoch, in the presence of β-estradiol, the $C.I._{99\%}$ showed that female mice distinguished between the arm that housed the castrated male mouse (C) and the empty arms but not between the empty arms and the arm that contained the intact male mouse (I). The $C.I._{68\%}$ also showed that β-estradiol had little if any significant effect on committed-distance arm entries; however, E2 females distinguished not only between empty and baited arms but also between the baited arms, whereas OIL females did not make the latter distinction (Fig. 5*B*, STIM). During the first day of the POST epoch, the $C.I._{99\%}$ showed no significant effect of β-estradiol on committed-distance arm entries; however, both OIL and E2 females distinguished the arm that had contained the castrated male from the empty arms but not between the arm that had contained the intact male and the empty arms. Considering the $C.I._{68\%}$ for the same day (day 5) did not change the result for OIL females, but showed that E2 females might discriminate between all three arms. On the final day of the experiment, the $C.I._{99\%}$ showed no significant effect of β-estradiol on female locomotor activity, and

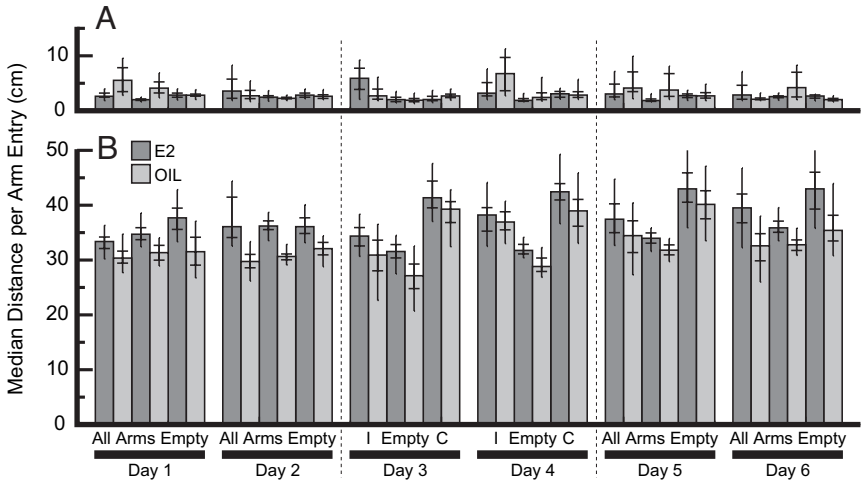

Fig. 5. Sampling-distance (*A*) and committed-distance (*B*) arm entries for control (OIL) and estrogen-treated (E2) female mice in a four-arm radial maze. On each day, from left to right, pairs of bars give results for arm one, arms two and three (pooled), and arm four. Vertical dashed lines demarcate the 2 days within each of the PRE (*Left*), STIM (*Center*), and POST (*Right*) epochs (see *Methods* for details). The maze was empty during the PRE and POST epochs. During the STIM epoch (days 3 and 4), the same intact (I) and castrated (C) male mice were located at the ends of arms one and four, respectively. Long uncapped bars and inner-cross error bars give the 99% and 68% (equivalent to $\alpha = 0.05$) bootstrap C.I.s, respectively.

neither E2 nor OIL females discriminated between the maze arms. This was not the case for committed-distance arm entries when the C.I.$_{68\%}$ was considered. In that case, β-estradiol had a significant effect on committed-distance arm entries, and in its presence, females exhibited the ability to discriminate between the arm that had contained the castrated male and empty arms, an ability lost for OIL females.

Discussion

Here we report a behavioral experiment conducted with female mice in a four-arm radial maze. The experiment was designed to assess the effects of β-estradiol on anxiety, exploratory behavior, and memory for preferences as measured by patterns of arm-entry behavior and levels of locomotor activity. That data sets comprising the total distance traveled during each maze-arm entry are not normally distributed but rather are a bimodal composite of gamma-distributed intervals has not to our knowledge been previously reported for rodent behavior in a radial maze. Such distributions greatly reduce the applicability of conventional statistical techniques. We present one approach to dealing with such data based on analyses of analytic distribution functions fit by a maximum likelihood estimation (MLE) procedure to the total distance traveled during each arm entry in a given observation period. These distributions were characteristically bimodal with two major components. Shorter arm entries were typically distributed in an exponential-like manner, whereas longer distances exhibited more gamma-like distributions. We isolated these two components by partitioning the analytic distributions. Partition distances were chosen from analytic fits to distributions of arm entries as the distances with the lowest probability of occurrence between the two flanking peaks of each bimodal distribution. This procedure revealed aspects of locomotor behavior that were inaccessible to standard statistical methods because of the bimodality of the observed distributions. Statistical testing by comparison of C.I.$_{99\%}$ and C.I.$_{68\%}$ obtained by bootstrap techniques for the sampling-distance and committed-distance arm entries of control versus treated female mice showed that the locomotor activity of committed-distance arm entries significantly increased in the presence of β-estradiol, particularly during the first 2 days of the experiment.

As noted in the Introduction, the behaviors of mice in the kind of maze used here may depend on a balance between sexual attraction and fear. Thus, when beginning to explore and enter an individual arm, the animal has essentially to calculate a risk/reward ratio. The Blanchards (12) have characterized this class of behaviors in animals as ''risk assessment'' responses. Here, the short partial (sampling-distance) arm entries measured in these results have been described by one mathematical function and the full (committed-distance) entries by another. We suggest that the intersection between the two functions, where the probability of entry distance is minimal (the partition distance), provides an excellent numerical estimate of the balance between approach responses and disinterested or fearful withdrawal responses. In this context, two sets of significant results are most interesting: (*i*) presentation of the intact stimulus male typically lowered the distance value at the intersection between the two curves; whereas (*ii*) β-estradiol encouraged the females to complete an entry, thus raising the mean committed-distance entry distance and with it, the partition distance.

Finally, we note that other kinds of analysis are possible. In particular, we previously found that an analytic methodology for quantifying the interval entropy of neuronal discharge gave considerable insight into the characteristics of patterns of neuronal discharge (13). We have recently extended this methodology to analyze the spatiotemporal and velocity components of the experiment reported here. Reanalysis of these data in terms of the Shannon redundancy (14) of the distances traveled during a set of arm entries has further clarified the effect of β-estradiol on female locomotor behavior (unpublished data).

Methods

We used the total distance traveled during each arm entry to characterize the locomotor activity of female mice in a four-arm radial maze. In summary, data analysis proceeded by fitting one or more analytic functions to a data set by MLE of parameter values such that the analytic fit was statistically indistinguishable from the distribution of intervals collected during an observation period. Parametric bootstrap (15) of the analytic fits was used to estimate 68% ($\alpha = 0.05$) and 99% ($\alpha = 5 \times 10^{-5}$) C.I.s (C.I.$_{68\%}$ and C.I.$_{99\%}$, respectively) on the median distance traveled during each arm entry. Because all analytic fits were bimodal, we found for each a partition distance, defined as the interval with the

lowest occurrence probability between the two peaks of a fit. Arm entries shorter than the partition distance are referred to as sampling-distance entries, whereas those longer than the partition distance are referred to as committed-distance entries. The partition distance of each bootstrap fit was determined, and the median distances traveled during the shorter sampling-distance and longer committed-distance arm entries were each calculated. As was the case with the unpartitioned data, the $C.I._{68\%}$ and $C.I._{99\%}$ of the sampling-distance and committed-distance arm entries were estimated from the bootstrap distributions of the respective arm entries.

Test Subjects. Twenty-four Swiss–Webster female mice and two Swiss–Webster male mice from our colony at The Rockefeller University were used in this study. All procedures were consistent with rules of the Association for Assessment and Accreditation of Laboratory Animal Care International and were approved by the university's institutional animal care and use committee. The female mice and one male mouse were gonadectomized. After surgery, mice were individually housed in standard plastic home cages (30 cm × 20 cm × 13 cm) on a 12:12 h light/dark cycle for the remainder of the study. Constant temperature (22°C) and relative humidity (32%) were maintained, and food and water were provided ad libitum. At the time of surgery, mice were 9–11 weeks old and weighed 35 ± 7 g.

All mice used in this study were sexually experienced with partners of the same strain. The original partners were excluded from the study. One week after ovariectomy, females were divided into two groups ($n = 12$ per group), and silastic capsules were s.c. implanted in the nuchal region while the animals were under pentobarbital anesthesia (Nembutal sodium solution; Abbott Laboratories, North Chicago, IL; 50 mg/kg i.p.). Capsules implanted in the control OIL group were filled with sesame oil (50 μl), whereas those implanted in the treatment E2 group contained β-estradiol [50 μg of 1,3,5(10)-estratriene-3,17β-diol in 50 μl of sesame oil]. Two weeks were allowed for recovery.

Test Apparatus. A four-arm radial maze (arm dimensions of 28.6 cm × 2.5 cm), placed on a table, was used in this study. This maze was derived from an eight-arm maze by blocking four maze arms. Spaces large enough (11 cm × 2.5 cm) to comfortably house a male mouse for a limited time were delimited at the extremity of each maze arm, separated from the proximal arm by dense mesh that allowed the passage of odor but blocked visual contact. Thus, the only contact between females and males was through olfactory cues. Two compartments were designated each to house one of the male stimuli. Visual cues were placed around the maze to promote recognition, learning, and memory by the female mice of the location of the male stimulus mice within the maze.

Experimental Protocol. The experiment was run in a soundproof room separate from the room housing the mice. Individual female mice were introduced into the maze on 6 consecutive days with exposure to males only on days 3 and 4. Data were recorded for each female during 20-min observation periods at the same time on each test day during the dark phase of the light/dark cycle. None of the mice had prior experience in a radial maze. The maze was thoroughly cleaned with Alconox (Alconox, White Plains, NY) solution and water between observation periods to eliminate any odor of the mice. Each recording session was videotaped, and recordings were analyzed offline. Data were acquired and digitized with a Polytrack video tracking system (San Diego Instruments, San Diego, CA). The sampling frequency was 6 Hz.

Days 1 and 2 provided an accommodation period, during which mice were individually introduced to the center of the empty maze and allowed freely to explore it. During days 3 and 4, females were tested for their ability to discriminate between the two male stimuli (one intact and one castrated male) and for which arm they might exhibit a preference. On each of these 2 days, the same intact male mouse was placed at the end of one maze arm, and the castrated male mouse was similarly placed at the end of a second maze arm. On days 5 and 6 of the experiment, the same female mice were tested in the empty maze for behavior that would indicate a memory of where the two male stimuli had previously been located. This protocol defined three consecutive test epochs, PRE (days 1 and 2), STIM (days 3 and 4), and POST (days 5 and 6).

Analysis. The data obtained from the video tracking system during each observation period included the total distance traveled (resolution 4.1 mm) and total time (resolution 0.167 s). From these the mean velocity of each arm entry was derived. Because both temporal and velocity distributions were unimodal, analysis was focused on the bimodal spatial distributions. In each case, the data available for analysis were the populations of spatial intervals observed for individual arms. Analysis proceeded by fitting analytic functions to different aggregations of data (see *Function Fitting*) followed by statistical comparison of the resulting measures. Two different comparisons were performed: (*i*) between-group comparisons of locomotor activity within equivalent maze arms to determine the effect of β-estradiol on female locomotor behavior, and (*ii*) within-group comparisons of locomotor activity between maze arms to determine the effect of β-estradiol on social and/or sexual preferences and memory.

Data Aggregation. Before analysis, data obtained for individual females within each of the OIL and E2 groups were pooled. Unless otherwise stated, data were pooled within group by day for the two empty arms together (arms A_2 and A_3) and separately for the two arms that contained the intact and castrated males during the 2 days of the STIM epoch (arms A_1 and A_4, respectively).

Function Fitting. Following data aggregation, the distribution of the distances traveled during each arm entry was obtained by MLE of parameters that matched an analytic function suitable to the aggregated data. Data were typically well fit with sums of two generalized gamma distributions.

The gamma distributions are of the form

$$\gamma_j(x;\, a_j,\, s_j,\, \tau_j) = \frac{(x - s_j)^{a_j - 1}}{\tau_j^{a_j}\Gamma(a_j)}\, e^{\frac{-(x-s_j)}{\tau_j}}, \quad (x \geq s_1 \geq 0;\, a_j,\, \tau_j > 0), \qquad [1]$$

where a_j, s_j, and τ_j are the shape, shift, and scale parameters of the jth gamma component, respectively (16). The gamma distribution reduces to an exponential distribution when a is set to one and gradually changes from exponential to near-Gaussian as a increases. The value of s determines how close to the origin the distribution begins to increase, and τ determines the magnitude of the variance of γ.

MLEs for parameter values were obtained by maximizing the log likelihood function

$$\ln L = \sum_{i=1}^{N} \ln\left[\sum_{j=1}^{J} f_j \gamma_j(i)\right], \qquad [2]$$

in which i runs over the N observations, f_j gives the fraction contributed to A by γ_j (Eq. **1**), and $\Sigma f_j = 1$.

To enforce the requirement that $x \geq s_1 \geq 0$ (see Eq. **1**), the shift parameter of the first gamma component (s_1) was trans-

formed to a variable β that was allowed to vary freely over positive and negative values during the MLE procedure. This transformation is given by

$$\beta = -\ln\left(\frac{x_{\min}}{s_1 - \varepsilon} - 1\right), \quad -\infty \leq \beta \leq \infty, \qquad [3]$$

where $x_{\min}$ is the smallest interval in O, and ε gives the tolerance to which $(x_{\min} - s_1)$ approaches zero (we used $\varepsilon = 1 \times 10^{-6}$). The true value of s_1 was recovered following completion of the fit by the inverse transformation

$$s_1 = \frac{x_{\min}}{1 + e^{-(\beta+\varepsilon)}}. \qquad [4]$$

Terms were added to the analytic function and refined by the MLE procedure until the analytic fit was not significantly different from the data by the KS goodness-of-fit test (17) ($P \geq 0.01$). A sampling distribution of the KS statistic was generated for each goodness-of-fit test by the parametric bootstrap method (15), using 5,000 samples of size equal to the number of observed arm entries in each case. The sensitivity of the KS test is greatest around the median value, and it detects the shift of a distribution better than a change in its spread (18). Thus, we also used the AD statistic (19). This is a powerful goodness-of-fit test for the detection of differences in the tails of a distribution (18). In the absence of appropriate tables, a sampling distribution was calculated for each AD goodness-of-fit test according to the method used for the KS goodness-of-fit test. The quality of each fit was assessed in two ways: (*i*) by *P* values calculated from the KS and AD sampling distributions (null hypothesis: OIL and E2 total distance traveled during each arm entry not drawn from different distributions), and (*ii*) by calculation of the rms error of the analytic fit to the observed data

$$E = \left\{\frac{1}{N}\sum_{i=1}^{N}[O(x_i) - A(x_i)]^2\right\}^{1/2} \times 100\%, \qquad [5]$$

where i ranges over the N observations in the observed distribution O, and A gives the value of the analytic function at O.

Statistics. The effect of β-estradiol on female locomotor activity was tested by employing bootstrap techniques to compare OIL and E2 females. In these comparisons, each bootstrap sample was obtained by drawing with replacement 12 data sets at random from the data sets obtained for the 12 females in each of the OIL and E2 groups. The number of different combinations is $12^{12}/12! = 18{,}614$. To reduce the number of replicate bootstrap samples, we drew 9,000 bootstrap samples for each analysis. For unpartitioned data sets, the median value of each bootstrap sample was calculated to give a distribution of the statistic from which the $C.I._{68\%}$ and $C.I._{99\%}$ were estimated. Partitioned data sets were generated by fitting an analytic distribution composed of two gamma components to each bootstrap sample (total number of bootstrap samples 9,000). After determination of the partition distance, the median values of the shorter sampling-distance and the longer committed-distance arm entries were calculated and the $C.I._{68\%}$ and $C.I._{99\%}$ estimated from the respective bootstrap distributions of the statistic. Pearson's product moment coefficient of correlation (r) was calculated between the median distance traveled during sampling-distance and committed-distance arm entries and between those variables and the median partition distance of individual mice pooled within groups across arms for each day and pooled across arms and days.

Software. Analysis and fitting routines were developed by the authors in MATLAB Version 7 (MathWorks, Natick, MA. www.mathworks.com) and in GNU C (The Free Software Foundation, Boston, MA). The Nelder–Mead simplex direct search algorithm (20) was implemented as a MATLAB *mex* function that incorporated suggestions by Press *et al.* (18). This implementation greatly reduced the time required to perform the fits of the analytic distributions to both the observed data and the bootstrap samples.

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