Journal of Neuroscience Methods 96 (2000) 119–131

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Statistical discrimination of natural modes of motion in rat exploratory behavior

Dan Drai [a,*], Yoav Benjamini [b], Ilan Golani [a]

[a] *Department of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel Aviv, Israel*
[b] *Department of Statistics, OR Tel-Aviv University, Tel Aviv, Israel*

Received 24 September 1999; received in revised form 11 November 1999; accepted 12 December 1999

Abstract

We analyze the locomotor behavior of the rat during exploration, and show that digitally collected data (time series of positions) provide a sufftcient basis for establishing that the rat uses several distinct modes of motion (first, second, third, and sometimes fourth gear). The distinction between these modes is obtained by first segmenting the time series into sequences of data points occurring between arrests (as ascertained within the resolution of the data acquisition system). The statistical distribution of the maximal amount of motion occurring within each of these episodes is then analyzed and shown to be multi modal. This enables us to decompose motion into distinct modes. In one application of this decomposition we show that the ethological ad hoc notion of stopping behavior corresponds to progression without leaving first gear. We do so by showing that the spatial spread of such progressions is confined to a small 20–50 cm range in a 6.5 m diameter arena. This provides a justification for a construct of 'staying in place'. This construct is not defined in terms of position in objective space, but purely in terms of the rat's own behavior. We test the generality of our method by applying it to mouse exploratory behavior. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Locomotor behavior; Stopping; Place fields; Spatial memory; Cognitive map; Locomotion; Computerized tracking; Saltatory search; Pathway analysis; Phenotyping mouse behavior

1. General introduction

This paper consists of three parts. In the first we show that given a time series of the coordinates of an exploring rat, one can decompose the behavior into several distinct modes of motion (first, second, third, and sometimes fourth gear). In the second part we illustrate the usefulness of the method, by showing that what has previously been labeled as stopping in rats, really corresponds to progression without leaving first gear. Finally, we test the generality of our method by analyzing locomotor behavior in mice, and discuss the implications of this methodology for the isolation of animal-defined places and for high throughput phenotyping of rodent behavior.

We begin by describing the statistical tools used throughout all parts of the paper so that the reader may refer back to them as the need arises.

2. Statistical methods

2.1. Density estimators

Density estimators (Silverman, 1980) are smoothed versions of histograms. They use moving bin location to obtain a more precise estimate of the concentration of observations at a given value. In this way the discontinuities displayed in the histogram, which are an artificial result of the non-overlapping bins, can be avoided, and better estimates are obtained. The curves obtained through a density estimator involve a choice of degree of smoothing. We choose the minimal degree of smoothing for which small random fluctuations are smoothed, while genuine features are not ironed away (Silverman, 1980).

---

* Corresponding author. Tel.: +972-3-6409391; fax: +972-3-6409403.
E-mail addresses: ddrai@post.tau.ac.il (D. Drai), ilan99@post.tau.ac.il (I. Golani)

PII: S0165-0270(99)00194-6

2.2. The gaussian mixture model

This model is used for recognizing distinct components within a population. When subjected to electrophoresis, for example, a mixture of distinct proteins ideally yields a perfect separation of the mixture into its components, i.e. all the molecules of each component lie precisely at a distance determined by their specific mass. In practice, however, the distance a particular molecule travels is affected by, e.g. convection. As a result, the distances traveled by each type of molecule form a gaussian distribution entered at the specific distance. When plotting concentration against distance from origin, one gets a single curve showing peaks corresponding to the medians of each gaussian. Given a protein mixture, the number of peaks in the curve corresponds to the number of components. The actual proportion of each component can be estimated by fitting a gaussian mixture model to the empirical curve. This model consists of a sum of distinct gaussians weighted by their corresponding proportions. Note that the model is applicable regardless of whether the individual gaussians partially overlap. We shall use the gaussian mixture model to analyze the rat's modes of motion.

2.3. Estimation of the parameters in the mixture model

The parameters of the model are estimated by using the Expectation–Maximization (EM) algorithm. The algorithm estimates the maximum likelihood parameters (proportions, means, and SDs) of a mixture with a given number of components. EM is an iterative algorithm that starts with user-given initial values, and incrementally improves the likelihood function until further iterations yield only a negligible improvement. The actual number of components of the model is determined by comparing the maximum likelihood value of a n-components mixture with that of a $(n-1)$-components mixture until the increased number of components increases the likelihood only marginally. More precisely, the spurious character of the n-th component is manifested through the fact that the log of the ratio of the likelihood of n components over that of $n-1$ components is distributed like χ^2 with two degrees of freedom (henceforth χ^2_2). For an easy exposition see Everitt (1981).

Based on this approach we use the following procedure for estimating the number of components: for each possible value, n, of the number of components (starting with one, i.e. a pure gaussian model) compute with EM the maximal likelihood value $p(n)$ for a model with n components. Then compute $\log[p(n+1)/p(n)]$. Initially, each additional component improves the likelihood by several orders of magnitude. For a certain number of components, call it k, this improvement ceases to be statistically significant [at the 0.01 level] and we can then set k as the estimated number of components. We then adopt as our model for the data the gaussian mixture with k components and with parameters (proportion, mean, and SD, for each component) yielding maximum likelihood among models with k components.

3. Part I: the decomposition of locomotor behavior into distinct modes

When placed in a large novel arena, rats alternate between full arrests, partial arrests involving scanning and stepping, walking, and running. We do not know a priori whether these categories represent convenient ad hoc landmarks within a continuum, or whether they stand for distinct and perhaps also discrete natural building blocks of behavior.

3.1. Materials and methods

3.1.1. Experimental animals

Subjects were eight juvenile Long Evans hooded rats (Department of Animal Breeding, Weizmann Institute of Science, Rehovot, Israel). Rats (four males and four females) were 43 days old at the onset of the observations, and 52 days old at their end. From the age of 14 days and on they were kept in two 35 × 25 × 15 cm cages connected by a 15 cm diameter black plastic pipe which was used as a shelter. Each rat was handled daily for 10 min and exposed to a variety of environments for another 10 min. This protocol was followed until the observation period.

3.1.2. Testing environment

Observations were performed in a 6.5 m diameter arena with a concrete floor and 40 cm high walls. Several large objects (distal landmarks) were placed outside. A pipe, similar to the one used by the rats in their cage and the only object in the arena that could be used as a hiding place, was inserted through the wall, just above floor level, and sealed. Fifteen flat small objects (proximal landmarks) were randomly placed at a 20–150 cm distance from the walls. The circle's center was, therefore, empty. The environment was thus polarized by creating a distance gradient from the familiar pipe, and a gradient between the wall and the empty central area (Fig. 1).

3.1.3. Session planning and recording procedure

Each rat was exposed to the arena for ten successive 30 min daily sessions. Observations were performed at night under artificial lights. The rat was placed near the pipe, and its behavior was recorded throughout the session by a stationary video camera whose lens covered the whole arena. Immediately afterwards the rat was returned to its cage.

3.1.4. Data acquisition and processing

The rat's path was recorded by an automated tracking system including a Telcom T/800/900 time-coding system, and software developed in our lab. The recorded *X*, *Y* coordinates were transformed to the real coordinates of the rat's location. These coordinates and timing were recorded as soon as movement was detected by the system. The spatial sensitivity of the system was set to 6 cm, with a temporal resolution of up to 20 frames per second.

The size of the arena acts as an indirect filter of small movements, inasmuch as the number of pixels occupied by the image of the rat is small relative to the size of the image of the arena. The objective size of a motion that is undetectable by the tracking system is therefore much bigger than it would be in, say, a 60 × 60 cm box.

3.2. Results

Our data consist of coordinates of the moving animal (considered as a point) sampled at 10 Hz. The distinction between the different modes of motion is obtained by first segmenting the time series into sequences of data points occurring between arrests (as ascertained by establishing the noise level of the data acquisition system). Intervals between two arrests constitute the building blocks for further analysis; we call them episodes of motion Even a cursory examination of these episodes reveals that some of them evolve into full-blown motion episodes whereas others succumb into arrest before acquiring significant velocity. In the next stage of analysis we therefore classify these episodes according to the maximal amount of motion reached within each of them (in much the same way as one would characterize mountains by peaks rather than by mean heights).

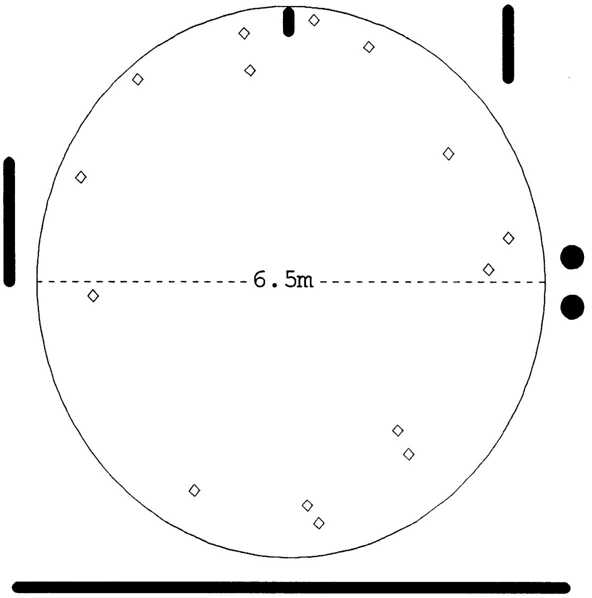

Fig. 1. The Testing Environment. Bars, small circles and rectangles represent the various objects: vertical dark bar at 12 o'clock — pipe, other dark bars and circles-large distal objects, empty rectangles — proximal small flat objects.

Analysis of the distribution of the maximal amounts of motion within episodes reveals that they are naturally clustered around three, and sometimes four distinct values. This yields, through the fitting of a Gaussian mixture model, a computable decomposition of the episodes into three or sometimes four modes.

3.2.1. Identifying points of arrest

In order to identify points of arrest we need to estimate the amount of motion within a temporal window and determine a threshold value under which a data point will be counted as arrest. This task is constrained by the fact that in hooded rats, the shortest arrests have a duration of 0.4 s (established by direct measurement of videotaped behavior). The danger of failing to reveal these stops precludes the use of wider temporal windows, such as would be necessary for the computation of velocity (Cleveland, 1977). We therefore compute the SD of the distances of the data points to their mean, within a sliding 0.4 s window.

This procedure attributes to the data point at the center of the window a numerical value that summarizes the spatial spread of the motion in the window. We denote it for each data point, pt, by SD (pt). When moving at constant velocity the SD is a linear function of velocity. The arrests are found by studying the statistical distribution of SD and determining a threshold value. We cross-check the results of two methods in order to determine the threshold that corresponds to the noise level of the tracking system. First we exploit the fact that in more than half of the sessions, at some time the rat 'goes to sleep' at the home base for several minutes. This allows us to select a long period of arrest and examine directly the angular and radial components of the rat's positions (we convert the cartesian positions to polar coordinates since the rat tends to stay close to the walls). We thus identify a long period at which the angular position is nearly constant in the time series graph of the position (Fig. 2).

We check the radial component during this interval, to eliminate the possibility that the angular constancy corresponds to a period of motion along a radius.

The noise level of the tracking system is then directly visible in the graph as the range of variation of SD during the arrest period. Quantifying the noise level is achieved either by plotting the SD values themselves (Fig. 3), or by plotting the estimated density function of SD during arrest and looking at its range (Fig. 4).

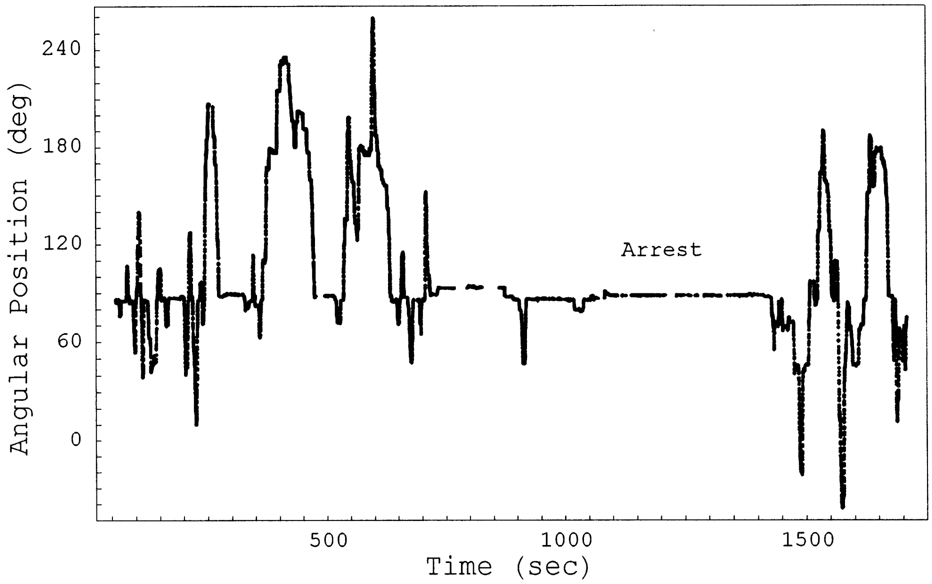

Fig. 2. A time series of angular positions of rat #2 in session 4. The X-axis represents time (in s, from the beginning of the session), and the Y-axis represents the corresponding angular position (in degrees). A period of arrest is indicated.

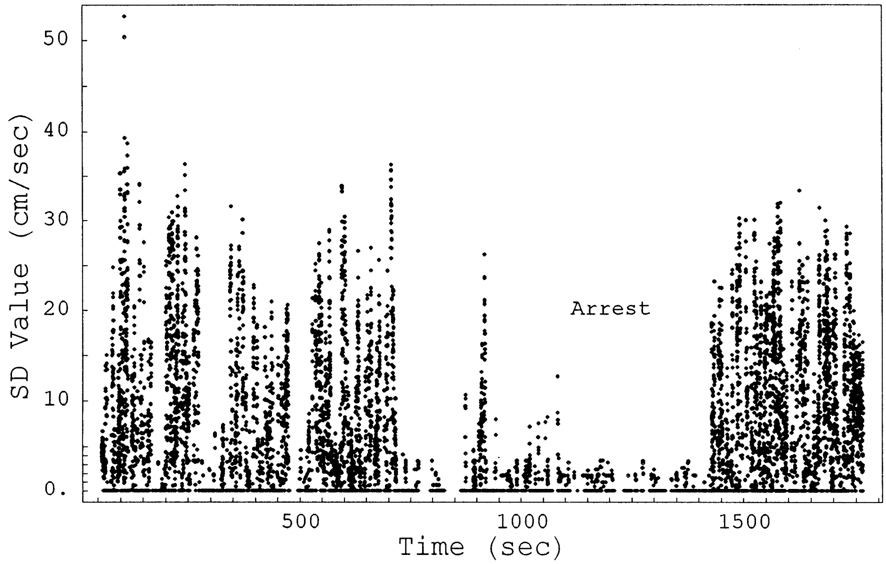

Fig. 3. A time series of SD values of rat #2 in session 4. X-axis: time (in seconds), Y-axis: the spatial spread of the points within a temporal window of half-width 0.2 s, centered at the corresponding instant. Y-axis units are in cm s^{-1}. The period of arrest corresponding to the one in Fig. 2 is indicated.

The second method uses the estimated density function of the values of SD for the whole session (see Section 2).

The curve in Fig. 5 has a clear inflexion point at a certain value of the SD. Such point is present in all curves of all sessions. We interpret it as indicating the noise threshold value.

The two methods (direct observation and inflexion point) give very close values when both are applicable. We therefore use the second when a 'going to sleep' interval is not available during that session. Once the threshold is determined we classify the points which have a SD value smaller than threshold, as arrests. Episodes of motion are now defined as intervals between two successive arrests.

3.2.2. Attributing a numerical value to episodes of motion

There appear to be episodes of motion that develop into full-blown progression, and episodes that do not. We therefore try to quantify the extent to which motion develops during an episode, summarizing each episode by its maximal SD value, measured within that episode. For standard statistical reasons (the existence of more than one order of magnitude within the range of values) we further apply a log transformation and take as the numerical attribute of an episode the logarithm of the maximal SD (log max SD) value obtaining during that episode. We can now examine the distribution of the values for the episodes of motion within a given session.

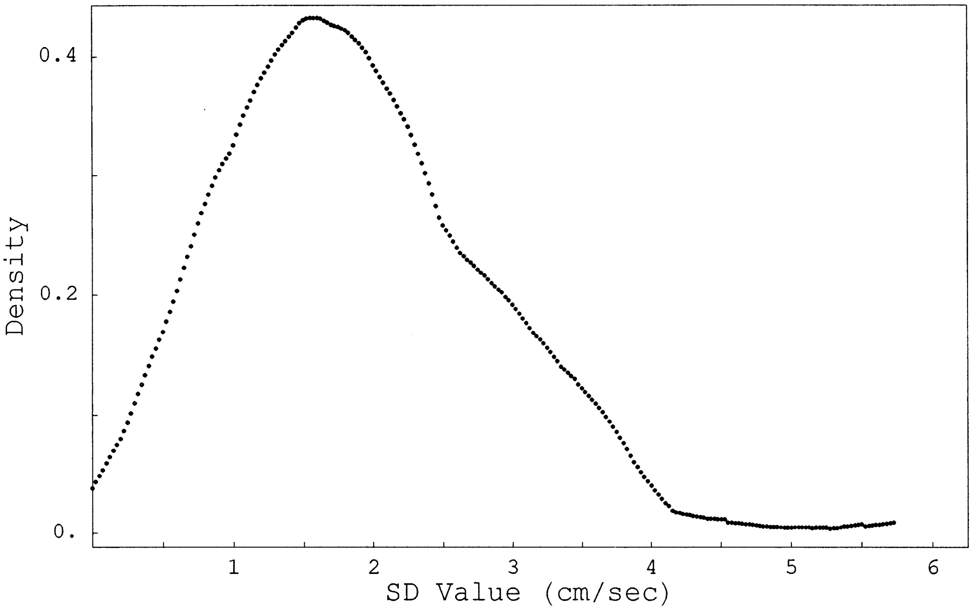

Fig. 4. Density estimation of SD during the periods of arrest in session 4 of rat #2. The X-axis represents the values of the SD at the time of arrest, and the Y-axis value is an estimate of the value of the density function for the corresponding SD value. In a density curve the values on the Y-axis are such that the area enclosed between the curve and the lines $x = x_0$, $x = x_1$ is equal to the probability that a randomly picked point has a value lying between x_0 and x_1, e.g. the total area under the curve is 1. The distribution indeed has the typical form of a distribution of noise, and the rightmost value (5.5) corroborates the value obtained as an inflexion point of the density function in Fig. 5.

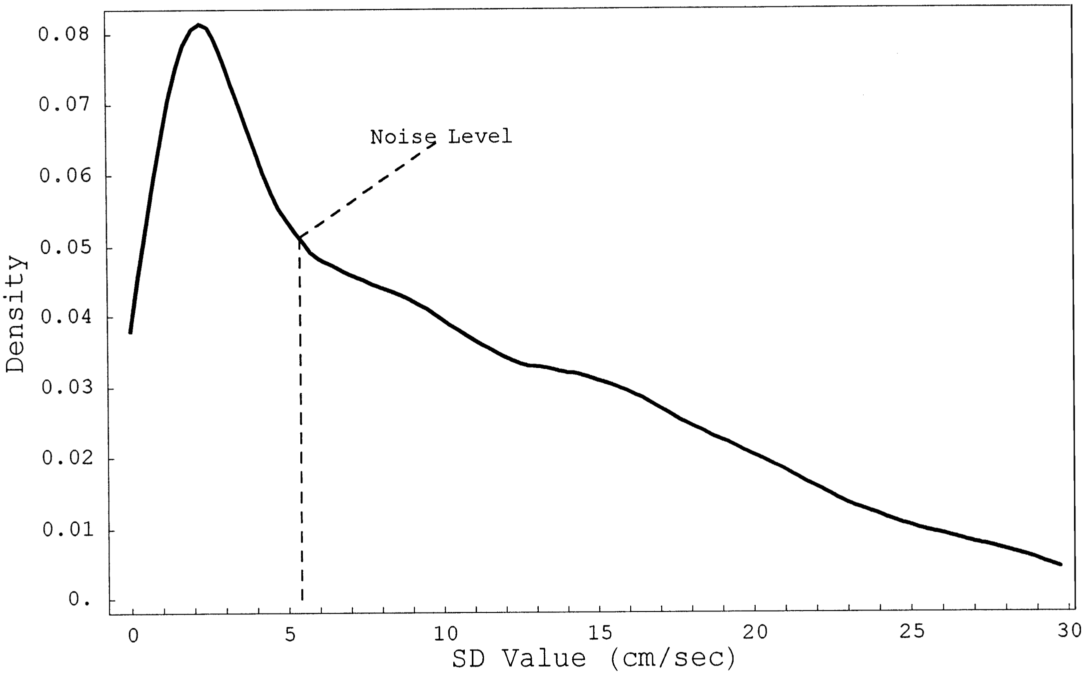

Fig. 5. The X-axis gives the values of the SD measure, and the Y-axis value is an estimate of the value of the density function for the corresponding SD value during session 4 of rat #2. Here we would set the noise level at 5.5.

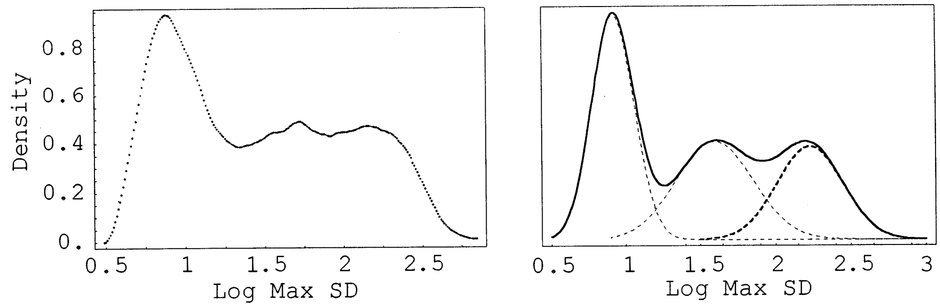

Fig. 6. Left: an estimate of the density function for log max SD values during episodes of motion (session 4 of rat # 2). Right: the maximum likelihood Gaussian mixture model of the data whose empirical density estimation is shown on the left. The *X*- and *Y*-axes are as in 6 left, the dashed lines show the individual gaussian components. The solid line shows the pondered sum of the gaussians, as estimated by the EM algorithm.

3.2.3. Establishing multi modality

To estimate the distribution of the log max SD values of all the episodes in a session, we use a density estimator (see Section 2) (Fig. 6 left).

As can be seen, the curve for this session has three ‘bumps’. Given the amount of smoothing used for the density estimation (see Section 2) these bumps represent genuine underlying clustering of the data around three typical values. Examination of the curves for all rats and sessions reveals that all the curves are multi-modal; a selection of curves is presented in Fig. 7.

Curves consisting of n non-overlapping gaussians would warrant a classification of the data into n distinct categories of episodes of motion. The underlying components of a process can, however, be reconstructed even in the presence of overlap. Fitting a gaussian mixture model to the data reveals the optimal parameters: the number of components and the proportions; means; and SDs of the gaussians. We applied the EM algorithm (see Section 2) to determine the maximum likelihood parameters. We used as initial values rough estimates of the parameters, obtained by direct observation of the empirical curves. The number of components in the data corresponding to Fig. 6 left was found to be 3. For each session the number of components is checked by computing the ratio of the logarithms of the likelihood of n components over that of $n-1$. Three components is thus seen to be the right choice for all sessions ($P<0.01$), except four sessions, in which a 4-components model seems genuinely better. Fig. 6 right shows the gaussian mixture model corresponding to the empirical density plotted in Fig. 6 left.

3.2.4. Practical determination of the threshold between two components

Given the distinction between modes of motion, how should we determine the threshold under which an episode counts as, e.g. belonging to the first component (leftmost) rather than to the second? In the case illustrated in Fig. 6 there is some overlap between the first two modes. We could therefore set the threshold somewhere between 1 and 1.5. Because of the overlap, any choice of threshold value within this interval leads to a misclassification of some segments. If we wish to classify according to which component is more likely to generate the episode, we should use the equal likelihood cutoff 1.3. Alternatively, suppose we want to be certain that none of the segments classified as belonging to the first component belongs to the second. In that case we should set the threshold as small as possible (here the graph shows that the probability that an episode with log max SD value of <1 has a virtually null probability of belonging to the second component). Finally, if we want to be certain that none of the segments classified as belonging to the second component belongs to the first one, then we should set the threshold as large as possible (here the graph shows that the probability that an episode with log max SD value of more than 1.5 has a virtually null probability of having been generated by the first component). In summary, an individual episode of intermediate maximal value (in the case of Fig. 6 between 1 and 1.5) cannot be said with certainty to belong to one of the two modes. This, however, does not refute the claim that there is a distinction; rather, it suggests that there might exist a fuller description of the rat’s behavior (in terms of inter-limb articulation) under which every episode can unequivocally be classified as belonging to a given mode of motion.

In order to further establish that the components correspond to distinct modes of motion, we study the variability of the values of the medians of each component. Fig. 8 shows a boxplot that summarizes the values of medians for the components obtained in a large data set (seven daily sessions of eight rats). As shown, although there is a large amount of variability for the values of the medians in each component, the central values do not overlap across components (except for a few outliers). To use an analogy; there are fast walkers and slow runners, but even the best walkers walk slower than bad runners run.

4. Part II: application to rat stopping behavior

We can now study the correspondence between behavior patterns that were previously established ad hoc, and the various modes of progression. One such pattern is stopping. It has been previously defined in a study performed in our lab in the following way: 'When exploring a new environment a rat alternates between progressing (i.e. walking forward or running) and stopping: it progresses forward, then stops by performing so-called closing steps (in which the stepping leg lands besides the contralateral leg instead of landing ahead of it), then freezes and/or performs vertical scanning movements while staying in place. During staying in place it may perform sideways and/or backward steps or steps in place, with each of its legs, or even step forward for a few steps' (Golani et al., 1993).

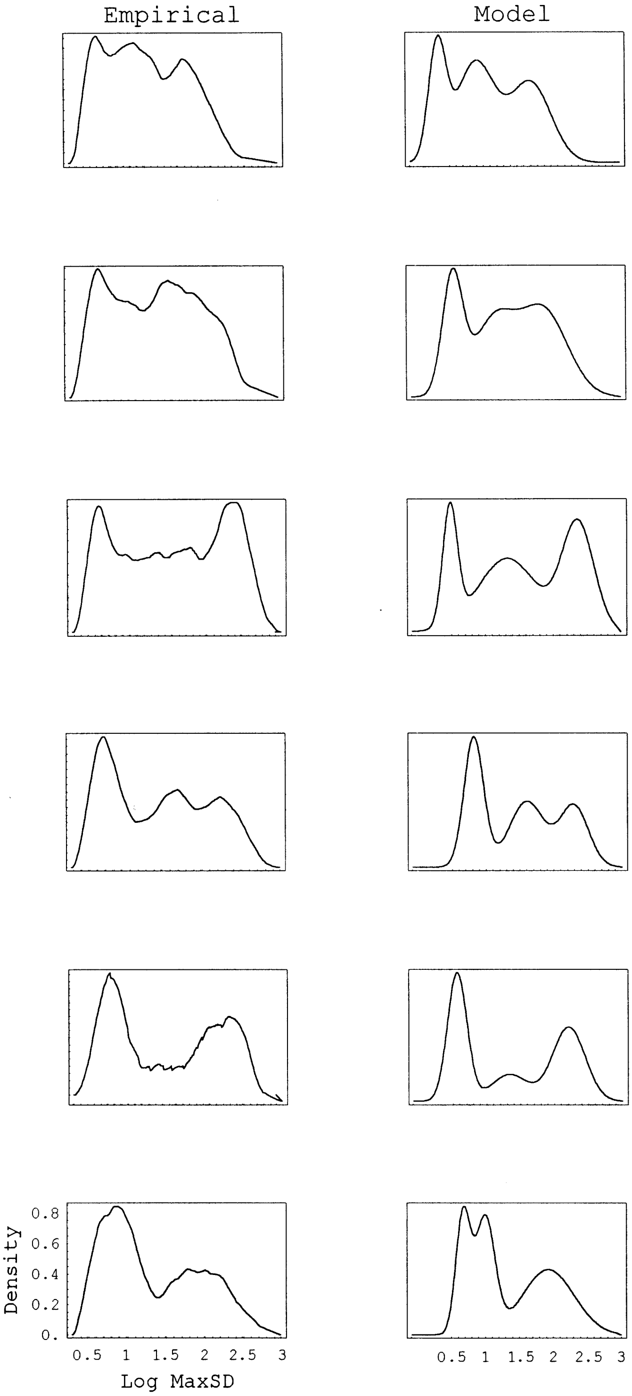

Fig. 7. Analog of Fig. 6: the curves were selected from different rat-sessions. They reflect the overall quality of the fit between empirical (left) and model estimation (right) within our data set.

What has been scored so far as stopping is thus a mode of motion distinct from both arrest and full progression. It has been defined heretofore intuitively, as a classical ethological behavior pattern or by setting an arbitrary (though reasonable) threshold value for velocity (Collins et al., 1995; Cools et al., 1997; Gingras and Cools, 1997). Parsing the rat's behavior into 'stops' and 'progressions' by setting a cut-off point between immobility and motion (such as 'if the rat does not leave a 50 × 50 cm square within half a second, call that a stop') does not warrant the distinction, however. We cannot be sure a priori that the distinction between 'stopping' and 'progressing' is categorical. Full arrest and running are clearly distinct, but where does 'stopping' end and 'progressing' begin? In other words, does ad hoc 'stopping' coincide with any of the distinct modes dictated by the statistical properties of the data?

Stopping is intuitively associated with low maximal velocities (i.e. low max SDs) and low spatial spread. The leftmost component in our graphs is similarly characterized by low MaxSD, but is it also circumscribed in space? A-priori, an animal could cover an unbounded distance while not leaving the first mode.

4.1. Lingering episodes

In our hooded rats, the leftmost component consisted of segments during which motion was detectable but never reached values much higher than noise level. To examine the hypothesis that stopping corresponds to not leaving the first mode, we lump together each bout of first mode episodes (that by definition consist of alternation of segments in the first mode and arrests) into a compound lingering episode. A progression episode is thus an interval during which there was no occurrence of either sub-noise data points or segments belonging to the first mode, and a lingering episode is defined as a segment beginning at the end of a progression and ending at the beginning of the next progression episode. A rat's trajectory is thus partitioned into progression episodes separated by simple or compound lingering episodes. A formalized version of this process of automatic segmentation is presented in Appendix A.

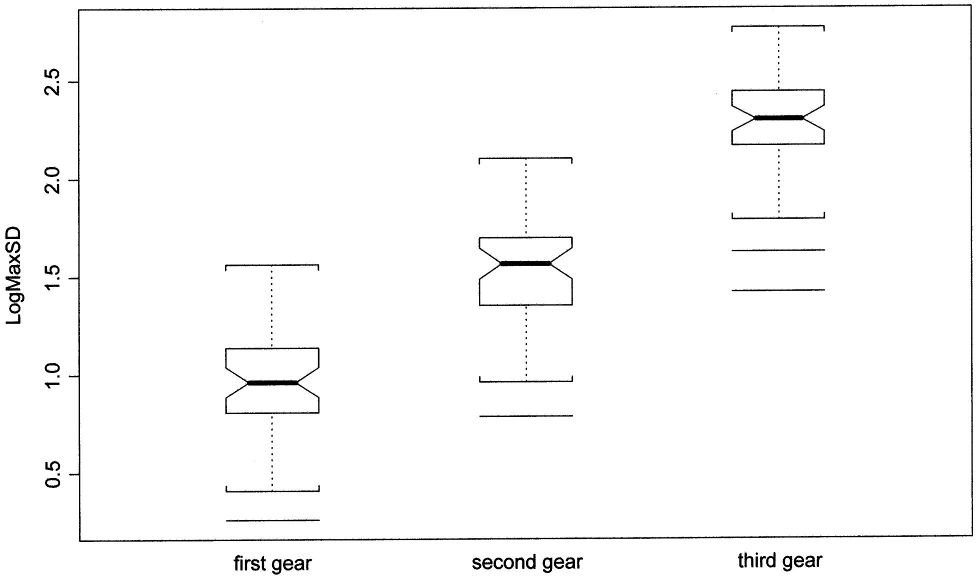

Fig. 8. Box plots of the median values of log max SD for each of the components for each session, all rats pooled. The data for the 'first gear' box, for instance, consist of the medians of the first component of the model for each of the sessions. The range between the bottom and top of each box contains the central half of the data; each box is cut by a line whose height is that of the median of the data; two whiskers extend from the top and the bottom of each box to the farthest observations that are still no more than two box lengths away from the sides of the box. Outliers are shown as individual horizontal lines.

4.2. Establishing the spatial extension of lingering episodes

Fig. 9 shows side-by-side boxplots of the quartiles of the spatial extension of lingering episodes for all rat-sessions. The median value and the upper quartiles for each session are consistently low (typically < 20 cm for the median and < 40 cm for the upper quartiles). There are, however, a few extreme outliers in the distribution of the spatial extension values. They might correspond to misclassified progression episodes (as explained in Section 3.2.4, the meaning of the threshold value is only probabilistic).

The fact that lingering episodes have a limited spatial extension is not trivial: it means that when exploring, a hooded rat does not cover large distances without leaving the first gear. We can therefore think of lingering in the open field in normal hooded rats as a 'staying in place' mode. This construct is not defined in terms of position in objective space, but purely in terms of the rat's own behavior. Having shown that it has the two properties of (i) not exceeding the lowest first gear 'velocity', and (ii) being circumscribed in space, we have established its correspondence to the previously used ad hoc stopping pattern. Like its predecessor, the new definition of stopping implies staying in the same location; unlike its predecessor it provides a precise, rat-defined algorithmic specification of 'the same location'. Furthermore, as illustrated in Fig. 8, all a rat's stopping locations during a session can be acquired by the push of a button. They can be represented as points, in terms of averaged coordinates of stopping locations without dwell time (Fig. 10a), as bubbles whose center represents stopping location, and whose size represents dwell time (Fig. 10b), and as line traces of the path followed by the rat during lingering episodes (Fig. 10c). In Fig.

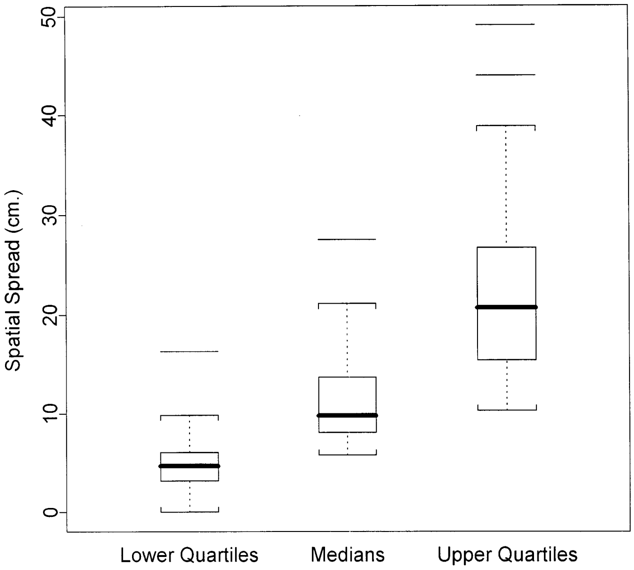

Fig. 9. Box plots of the quartiles (lower, median and upper) for the spatial spread of lingering episodes for each session, all rats pooled. The data for the 'medians' box, for instance, consists of the medians of the spatial spread values for each of the sessions. For further explanations see Fig. 8.

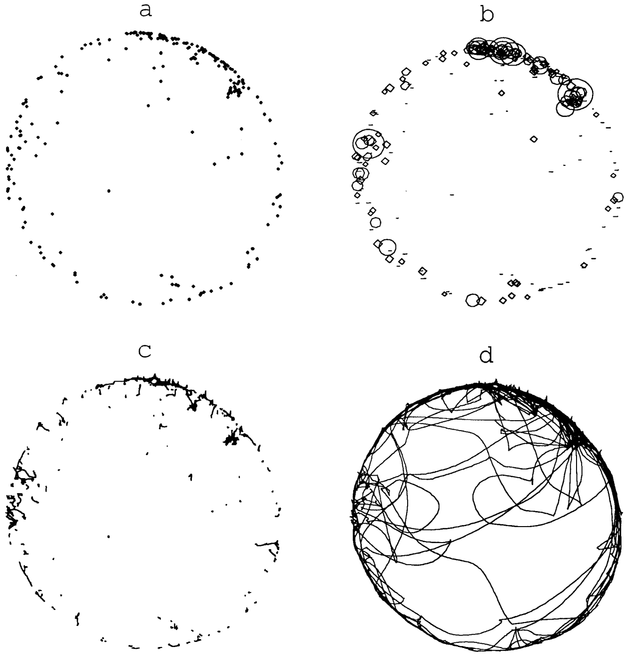

Fig. 10. (a) The stopping locations, each represented as the averaged coordinates of a stopping episode. (b) Stopping locations with dwell time: each stopping episode is represented by a circle centered at the (averaged) stopping location, with a radius proportional to the duration of the stopping episode. (c) Line traces of the path followed by the rat during lingering episodes. (d) Line traces of the path followed by the rat during progression episodes.

10d we show the trace left by progression segments, to illustrate the contrast between lingering and progressing.

5. Part 111: the generality of our method

Is our method applicable to any data set of a time series of *x*, *y* coordinates sampled at a rate of at least 10 Hz/s? Is it applicable to, e.g. mouse behavior? Can it distinguish modes of motion in this behavior, and if so, how many gears do mice use and what are the velocities that characterize the various modes? Do mice have a lingering mode, and if so, what is the spatial spread of lingering episodes? While a detailed comparison to rats is beyond the scope of the present study, preliminary results might provide a somewhat wider perspective. To tackle these questions we examined four male C57BL/6J (C57) and four male Balb/cJtau (Balb) mice, 65 days old, experimentally naive, housed in groups of four, given unlimited access to food and water. Observations were performed in a 3.30 m diameter arena Each mouse was exposed to the arena once for a 30 min session. All other videotaping conditions and procedures were similar to those used in the rat study.

Fig. 11 presents the distributions of the log-transformed SDs (left) and their decomposition (right) during one session of a male Balb mouse (top row) and one session of a male C57 mouse (bottom row). The distributions are given as estimated density functions. In both strains the decompositions show the three gaussian components of the Gaussian Mixture Model as estimated by maximum liklihood and their sum, exactly as in Section 3.2.3. It can be seen that the typical modes also exist in these mice, although their specific values are different.. This patterning applied to all eight mice.

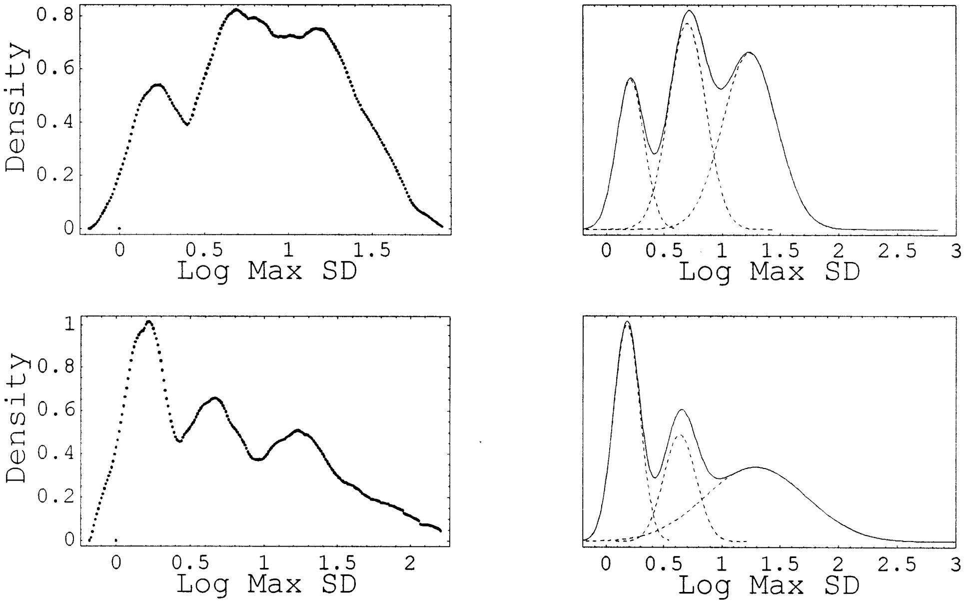

Fig. 11. Left: an estimate of the density function for log max SD values during episodes of motion of a session of a Balb mouse (top), and a C57 mouse (bottom). Right: the maximum likelihood Gaussian mixture models of the data whose empirical density estimations are, respectively shown on the two graphs on the left. The dashed lines show the individual gaussian components. The solid line shows the pondered sum of the gaussians, as estimated by the EM algorithm.

Table 1
The specific values obtained in the C57 and BALB mice

Measure	C57 mean (SD)	BALB mean (SD)
1. Path length (m)	158 (25)	136 (58)
2. Lingering max SD (cm s^{-1})	12.8 (1.09)	11.24 (1.07)
3. Second component max SD (cm s^{-1})	25.27 (1.3)	19.29 (1.2)
4. Third component max SD (cm s^{-1}) (log)	61.55 (1.25)	40.44 (1.23)
5. Proportion of first component episodes	0.19 (0.04)	0.23 (0.07)
6. Proportion of second component segments	0.22 (0.08)	0.25 (0.1)
7. Proportion of third component segments	0.53 (0.14)	0.52 (0.09)
8. Spatial spread of lingering (cm)	21 (6.8)	17 (2.2)
9. Spatial spread of second component segments (cm)	37.04 (35)	27.1 (20)
10. Spatial spread of third component segments (cm)	133 (95)	106 (82)

We summarize the results obtained for median values and proportions of the gaussian model, as well as spatial spread of lingering and progression in Table 1.

6. Discussion

Detailed studies of rat exploratory behavior done in our lab (Eilam and Golani, 1989, 1990, 1994; Eilam et al., 1989; Golani et al., 1993; Tchernichovski and Golani, 1995; Tchernichovski et al., 1996, 1998) and by others (Whishaw and Tomie, 1995; Cools et al., 1989, 1997; Gingras and Cools, 1997; Szechtman et al., 1998, 1999) revealed that in a novel environment rats establish an intricate structure of routes and places, including a home base, excursions that are performed from that home base and back to it, and principal places. Most of these findings were based on an assumption that there is, in rodents, a clear-cut distinction between progressing and stopping. The problem with that distinction was that it was based on an implicit decision (Beer, 1980), and it required labor-intensive direct observation on large amounts of data. For the data used in the present study, for example, one would have to look in slow motion at 40 h of video recording.

Increasing uneasiness with this, often arbitrary distinction, and a demand for high throughput phenotyping of rodent behavior (Nolan et al., 1997) resulted in a search for an algorithmic definition that would partition the flow of behavior into intrinsically defined units by 'the push of a button'. This search revealed that our previous work confounded three geometrical aspects of movement:

1. A distinction among several (and not necessarily two) modes of motion based on their velocity profiles (the 'gears' phenomenon).
2. The relationship between the velocity and the spatial spread characterizing each of the modes (e.g. how far does an animal typically progress without leaving first gear?).
3. The locations of successive episodes performed in the same gear and separated by visits to other locations (e.g. how compactly distributed in space are temporally distinct first gear episodes, or, to what extent does the animal tend to stay in place in the same or nearby locations).

In our arena, hooded rats indeed show a linkage between these parameters: they show three modes of motion; their intermittent first gear motion tends to be circumscribed in space and successive episodes across visits to same vicinity tend to be clustered in space (these clusters being termed principal places; Tchernichovski et al., 1996). This patterning, however, does not necessarily apply to other strains, preparations, and environments. Preliminary observations suggest the opposite: In some strains, lingering is not circumscribed in space; some strains use during particular stages of the exposure to the environment very short, and during other stages very long, movement segments; and the number of objects in the environment affects the compactness and number of places established by an animal. A comparative framework is thus provided by the dissociation into modes, each mode being characterized by a velocity profile, relative frequency, and a spatial spread. In this study we formalize only the first two aspects of movement that were confounded: that of the existence of different modes, and that of their respective spatial spread within an episode. The next obvious question, not handled yet, is that of the spatial spread of lingering across successive episodes. This would lead us into a dynamic definition of the notion of 'place'.

While the notion of place plays a central role in the neurosciences, in place learning (Morris, 1984; Whishaw and Mittleman, 1986; Silva et al., 1998; Whishaw, 1998), navigation (Thinus-Blanc, 1996; Etienne et al., 1998; Knierim et al., 1998), the study of the hippocampus (McNaughton et al., 1996; Poucet and Benhamou, 1997; O'Keefe and Burgess, 1996; Burgess et al., 1998), foraging behavior (O'Brien et al., 1989, 1990), and the behavioral study of exploratory behavior (Eilam and Golani, 1989; Golani et al., 1993; Tchernichovski and Golani, 1995; Tchernichovski et al., 1996, 1998), there is as yet no definition of the behavior that marks a place. Once we have shown that a particular mode of motion is circumscribed in space we can follow its spatial spread across episodes and obtain a dynamic description of its unfolding. In our hooded rats, staying in place appears to be clustered around a small number of spatially distinct locations. This suggests that staying-in-place-behavior defines places in this rat's operational world. Such a notion of place, obtained through the study of the clustering of staying-in-place episodes, has the advantage of being defined precisely in terms of behavior, so that we do not have to set an a priori size for a place. For instance, a place situated at the home base could be extended much more than, say, a less familiar place. The validity of a place should be further supported by its connectivity to other places via movement-segments, or the performance of incoming ballistic movement-segments from relatively long distances. It would also be interesting to examine the correspondence between behaviorally and electrophysiologically defined places. Electrophysiologically defined place-fields could monitor the animal's location regardless of whether the animal lingers or stops repeatedly in particular locations. It could also be that the two constructs partly or fully coincide.

When arena size is changed, the inter-stop distances undergo a corresponding change of scale (Golani et al., 1993). What happens to the spatial spread of individual lingering episodes? Comparison of the spatial spread of lingering episodes of rats in our arena and in a 20 times smaller arena suggests that the spatial spread of stopping episodes taken individually does not undergo scaling (unpublished results). In other words, the space covered while staying-in-place appears to be invariant under a drastic re-scaling of the environment. This suggests that while in the stopping mode, the rat's reference frame is its own body rather than the environment at large. The distinction between staying in place and progressing might thus correspond to the traditional distinction between investigating and exploring (Berlyne, 1960; Poucet et al., 1986; Thinus-Blanc, 1996).

Finally, the segmentation into stops and progressions also occurs in insects (Miller, 1979; Collins et al., 1994), nematodes, fish (O'Brien et al., 1989), lizards (Pietruska, 1986), birds (Pienkowski, 1983), and mammals (Kenagy, 1974). It would be interesting to see whether our analysis carries over to species in which similar raw data are collected.

The algorithms established so far compute the number of modes of motion used by the animal, characterizing each of them in terms of three quantities (a measure corresponding to maximal velocity, frequency distribution, and spatial spread). These algorithms also establish the foundation for the formalization of two additional algorithms that will isolate behaviorally defined places, and quantify their size and compactness. Each of these quantities characterizes an intrinsically defined behavioral endpoint that can be used for the phenotyping of rodent behavior. The main conceptual gain of this study is thus the replacement of an intuitive dichotomy based on an arbitrarily drawn line in the continuum of our perceptions with a systematically articulated framework that appears to correspond to biological reality. The practical gain is an automatic tool for phenotyping open field behavior.

Acknowledgements

Tirza Stern performed the stages of data digitation. We would also like to thank Ofer Tchernichovski for providing us with the hooded rats raw data, Neri Kafkafi for many productive discussions and suggestions, and both of them and Mark Shik for useful comments on the manuscript. This study was supported by a grant from the Adams Super Center for Brain Studies, Tel Aviv University.

Appendix A

In this appendix we outline the whole process of segmentation, starting with the raw data series and noise level of the system, and ending with an actual segmentation of the motion into episodes of distinct modes. Having been formalized once, the process of data segmentation into modes is now fully automated.

Automation

The EM algorithm requires a choice of initial values for the parameters (see 2.3). These can be fed by the user. Alternatively, let the EM algorithm run with a variety of plausible initial values generated automatically by the program, picking the result with maximum likelihood. This increases the computational toll, but liberates the whole process from further human intervention, once the data are collected and the noise level of the tracking system determined.

In summary, a schematized implementation of the whole segmentation process, given a data set DATA and a noise level ε, involves the following auxiliary functions:

Sdmove(x, Δt): computes for each data point x the SD of the positions within a temporal window of width Δt centered at x. The width of the window is set by the user according to the temporal resolution deemed reasonable. In our rat studies, for example, we set Δt at 0.4 s because it equals the duration of the rat's shortest arrests.

EM (dataset, k, initial guess): returns the parameters (i.e. proportion, mean and SD for each component) of maximum likelihood for a gaussian mixture with k components, starting the iterative algorithm with the user-provided initial guess of proportion, mean, and SD for each component. The function also returns the likelihood value. The output is, thus, a couple: [parameter, likelihood].

Em_auto (dataset, k): This function returns parameters for a gaussian mixture model of k components without being provided with an initial guess. It proceeds as follows:

- pick a random set of initial guesses for the proportions, i.e. a set of k-tuples between 0 and 1, whose sum is 1.
- Run EM for each choice of proportions with the natural guess for means and SDs [e.g. if the proportions are (0.3, 0.4, 0.3), choose as initial guess for the mean of the first component the empirical mean of the first 30% of the sorted dataset, for the mean of the second component choose the empirical mean of the portion of the data, set between the 0.3 percentile and the 0.7 percentile, etc…
- pick the result with the highest likelihood.

It is now easy to define a version of EM which does not even need a specification of the number of components:

EM_fullauto(dataset): = compute EM (k, dataset) starting with $k = 1$, and stopping when the log [likelihood (k components)/likelihood ($k - 1$) components], comes within the range of χ_2^2 with a chosen level of probability.

With these tools, given a data set DATA and a noise level ε, the segmentation process is fully automatic:

- Compute stops by picking the points in DATA, such that $\mathrm{Sdmove}(x, \Delta t) < \varepsilon$.
- Define as segments the intervals between the end of a sequence of stop points and the beginning of the next one.
- Compute for each such segment the maximum of $\mathrm{Sdmove}(x, \Delta t)$ within the segments, apply log transform and call the resulting data set log MaxSd.
- Run EM_fullauto(log MaxSd).
- Compute (from the components outputted by EM_fullauto) the values of logMaxSd which separate the first component from the second, the second from the third etc… call these $\mathrm{thresh}_{1,2}$, $\mathrm{thresh}_{2,3}$ etc.

Having obtained a segmentation, each segment is attributed a mode according to its logMaxSd value (if the value is smaller than $\mathrm{thresh}_{1,2}$ the segment is in mode one, if the value is between $\mathrm{thresh}_{1,2}$ and $\mathrm{thresh}_{2,3}$ then the segment is in mode two, etc…).

In order to check the spatial spread of motion performed without leaving the first mode, an additional processing is defined:

- define motion segments as those segments not belonging to the first mode.
- Now lingering segments are defined as the intervals between the end of a motion segment and the beginning of the next motion segment. We can thus easily compute the spatial spread of each lingering segment and study their distribution compared to either that of all the other modes or any of them taken separately.

We thus have a process which produces automatically a segmentation of the motion into modes, and information about the spatial spread of each mode. The implementation of the algorithm in the Mathematica programming language is available by e-mailing the authors.

References

Berlyne DE. Conflict, Arousal, and Curiosity. New York: Mcgraw-Hill Book Company, 1960.

Beer CG. Perspectives on animal behavior comparisons. In: Bornstein MH editor. Comparative methods in psychology, Earlbaum, 1980.

Burgess N, Donnett JG, O'Keefe J. The representation of space and the hippocampus in rats, robots and humans. Zeitschrift fur naturforchung 1998;53(7-8):504–9.

Cleveland WS. Robust locally weighted regression and smoothing scatterplots. J Am Stat Assoc 1977;74:829–36.

Collins RD, Gargesh RN, Maltby AD, Roggero RJ, Tourtellot MK, Bell WJ. Innate control of local search behavior in the house fly, *Musca domestica*. Physiol Entomol 1994;19:165–72.

Collins RD, Tourtellot MK, Bell WJ. Defining stops in search pathways. J Neurosci Meth 1995;60(1–2):95–8.

Cools AR, Brachten R, Heeren D, Willemen D, Ellenbrock B. Search after neurobiological profile of individual-specific features of wistar rats. Brain Res Bull 1989;24:49–69.

Cools AR, Ellenbroek BA, Gingras MA, Engbersen Aand, Heeren D. Differences in vulnerability and susceptibility to dexamphetamine in Nijmegen high and low responders to novelty: a dose-effect analysis of spatio-temporal programming of behavior. Psychopharmacology 1997;132:181–7.

Eilam D, Golani I. Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. Behav Brain Res 1989;34:199–211.

Eilam D, Golani I. Home base behavior in amphetamine-treated tame wild rats (*Rattus norvegicus*). Behav Br Res 1990;36:161–70.

Eilam D, Golani I. Amphetamine-induced stercotypy in rats: its morphogenesis in locale space from normal exploration. In: Cooper SJ, Hendrie CA, editors. Ethology and Psychopharmacology. New York: Wiley, 1994:241–65.

Eilam D, Golani I, Szechtman H. Agonist quinripole inducs perseveration of routes and hyperactivity but no perservation movements. Brain Res 1989;460:255–67.

Etienne AS, Maurer R, Berlie J, Reverdin B, Rowe T, Georgakopoulos J, Seguinot V. Navigation through vector addition. Nature 1998;396(6707):161–4.

Everitt BS. Finite Mixture Distributions. London: Chapman and Hall, 1981.

Gingras MA, Cools AR. Different behavioral effects of daily or intermittent dexamphetamine administration in Nijmegen high and low responders. Psychopharmacology 1997;132:188–94.

Golani I, Benjamini Y, Eilam D. Stopping behavior: constraints on exploration in rats (*Rattus norvegicus*). Behav Brain Res 1993;53:21–33.

Kenagy GJ. Daily and seasonal patterns of activity and energetics in a heteromyid rodent community. Ecology 1974;54:1201–19.

Knierim JJ, Kudrimoti HS, McNaughton BL. Interactions between idiothetic cues and external landmarks in the control of place cells and head direction cells. J Neurophysiol 1998;80(1):425–46.

McNaughton BL, Barnes CA, Gerrard JL, et al. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J Exp Biol 1996;199:173–85.

Miller PL. A possible sensory function of the stop-go patterns of running in phorids. Physiol Entomol 1979;4:367–70.

Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Meth 1984;11:47–60.

Nolan PM, Kapfhamer D, Bucan M. Random mutagenesis screen for dominant behavioral mutations in mice. Methods 1997;13:379–95.

O'Brien WJ, Evans BI, Browmans HI. Flexible search tactics and efficient foraging in saltatory searching animals. Oecologia 1989;80:100–10.

O'Brien WJ, Browmans HI, Evans BI. Search strategies of foraging animals. Am Sci 1990;78:152–60.

O'Keefe J, Burgess N. Geometric determinants of the place fields of hippocampal neurons. Nature 1996;381(6581):425–8.

Pienkowski MW. Changes in the foraging pattern of plovers in relation to environmental factors. Anim Behav 1983;31:244–64.

Pietruska RD. Search tactics of desert lizards: how polarized are they? Anim Behav 1986;34:1742–58.

Poucet B, Chapuis N, Durup M, Thinus-Blanc C. A study of exploratory behavior as an index of spatial knowledge in hamsters. Animal Learning Behav 1986;14(1):93–100.

Poucet B, Benhamou S. The neuropsychology of spatial cognition in the rat. Crit Rev Neurobiol 1997;11(2–3):101–20.

Silva AJ, Giese KP, Fedorov NB, Frankland PW, Kogan JH. Molecular, cellular, and neuroanatomical substrates of place learning. Neurobiol Learning Memory 1998;70(1–2):44.

Silverman BW. Density Estimation for Statistics and Data Analysis. London: Chapman and Hall, 1980.

Szechtman H, Culver K, Eilam D. Role of dopamine systems in obsessive-compulsive disorder (OCD): Implications from a novel psychostimulant-induced animal model. Pol J Pharmacol 1999;51(1):55–61.

Szechtman H, Sulis W, Eilam D. Quinpirole induces compulsive checking behavior in rats: A potential animal model of obsessive-compulsive disorder (OCD). Behav Neurosci 1998;112(6): 1475–85.

Tchernichovski O, Benjamini Y, Golani I. Constraints and the emergence of freedom in the ontogeny of rat exploratory behavior. Behaviour 1996;133(7-8):519–39.

Tchernichovski O, Golani I. A phase plane representation of rat exploratory behavior. J Neurosci Meth 1995;62(1-2):21–7.

Tchernichovski O, Benjamini Y, Golani I. The dynamics of long term exploratory behavior in the rat, part I. Biol Cybern 1998;78(6):423–32.

Thinus-Blanc C. Animal Spatial Cognition: Behavioural and Brain Approach. Singapore: World Scientific, 1996.

Whishaw IQ, Mittleman G. Visits to starts, routes and places by rats (*Rattus norvegicus*) in swimming and navigation tasks. J Comp Psychol 1986;100:422–31.

Whishaw IQ. Place learning in hippocampal rats and the path integration hypothesis. Neurosci Biobehav R 1998;22(2):209–20.

Whishaw IQ, Tomie JA. Of mice and mazes: Similarities between mice and rats on dry land but not water mazes. Physiol Behav 1995;60(5):1191–7.