

The dynamics of spatial behavior: how can robust smoothing techniques help?

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Abstract

A variety of setups and paradigms are used in the neurosciences for automatically tracking the location of an animal in an experiment and for extracting features of interest out of it. Many of these features, however, are critically sensitive to the unavoidable noise and artifacts of tracking. Here, we examine the relevant properties of several smoothing methods and suggest a combination of methods for retrieving locations and velocities and recognizing arrests from time series of coordinates of an animal's center of gravity. We accomplish these by using robust nonparametric methods, such as Running Median (RM) and locally weighted regression methods. The smoothed data may, subsequently, be segmented to obtain discrete behavioral units with proven ethological relevance. New parameters such as the length, duration, maximal speed, and acceleration of these units provide a wealth of measures for, e.g., mouse behavioral phenotyping, studies on spatial orientation in vertebrates and invertebrates, and studies on rodent hippocampal function. This methodology may have implications for many tests of spatial behavior. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

In the neurosciences, data on locomotor behavior, spatial orientation, navigation, spatial memory, and even social behavior often consist of a time series of coordinates representing the organism's location. Common experimental setups collecting such data include the Open Field Test, the Photobeam Cage, the Morris Swim Task, the Elevated Plus Maze, the Holeboard, and a variety of other spatial mazes. Most of the studies performed in these setups focus on the animal's location, ignoring velocity and acceleration (see, however, Kafkafi et al., 2001; Pierce-Shimomura et al., 1999; Tchernichovski and Golani, 1995; Tchernichovski et al., 1998; Wallace et al., 2002; Whishaw et al., 2001).

The benefits of moment-to-moment record of velocity and acceleration cannot, however, be overestimated. Within a dynamic framework, the acceleration and velocity of the animal are the outcome of all the concurrent endogenous and exogenous "forces" acting upon it. Conversely, the attraction or repulsion exerted by a wall, a cliff, a familiar place, a partner or a chemical gradient is revealed by the momentary values of these parameters. In rodent open field studies, for example, the forces exerted by the animal's home base (Eilam and Golani, 1989), or any other familiar place (Tchernichovski et al., 1996), are reflected in the animal's velocity and acceleration. The momentary velocity of an animal can tell us whether it "thinks" it is running away or toward its home base, or how familiar the immediate environment is (Tchernichovski and Golani, 1995; Tchernichovski et al., 1998), or what method of navigation it uses (Wallace et al., 2002; Whishaw et al., 2001).

One ethologically-relevant point regarding velocities is that involving zero or close-to-zero velocities, i.e., stops. An organism's locomotor behavior often consists of an al-

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ternation between progression and stopping, be it a nematode (Pierce-Shimomura et al., 1999), an insect (Collins et al., 1994, 1995; Miller, 1979), a fish (Nilsson et al., 1993; O'Brien et al., 1989; Winberg et al., 1993), a lizard (Pietruska, 1986), a bird (Pienkowski, 1983), or a mammal (Golani et al., 1993; Kenagy, 1974). The movements it performs during a stop, be it foraging movements, scanning or movements related to any other form of information gathering, are reflected indirectly in the spatiotemporal properties of the stop, (e.g., Draï et al., 2000). Mouse inbred strains, for example, may differ substantially in the rate, type, rhythm, and number of scans they perform during a stop. These differences in the manner and intensity of information gathering are indirectly reflected in the duration, spatial spread, and average velocity of movement during stopping behavior (Draï and Golani, 2001). Characterizing the stop-and-go behavior should therefore be both ethologically meaningful and results-wise fruitful.

As elaborated in this study, however, the data acquired by the above listed mazes and setups suffer from noise and artifact problems, which are inherent to all tracking systems and critically affect the results. Even a straightforward measure such as the overall distance traveled by the animal is highly sensitive to these problems, but they have a particularly devastating effect on the derivation of velocities and accelerations. Smoothing the raw data is required to obtain a smooth path, correct computation of velocities when the animal is moving, and an isolation of arrests (zero velocity) when stopping. As we show, however, the sometimes-erratic nature of animal movement requires the correct application of the appropriate smoothing methods. Furthermore, those methods appropriate when the animal is on the go become inappropriate when it stops. Therefore, a combination of methods must be used. An automated high-throughput analysis of moment-to-moment velocities becomes proper only after the data have been carefully smoothed by such combination of methods.

2. Methods of testing

As a test case for investigation of the noise sources and of performances of smoothing methods we used the Open Field test (Hall, 1934) with mice of several common inbred strains, tracked with Noldus EthoVision® video tracking system (Noldus et al., 2001; Spink et al., 2001) at a rate of 25 records (frames) per second. The diameter of the arena was 250 cm and the spatial resolution about 1.3 cm per video pixel (for detailed description of methods and analysis see Kafkafi et al., 2003a). In the tracking system, the arena was specified as slightly larger than its actual boundaries, in order to prevent any change in the spatial distribution of the noise when the animal is at the very edge of the arena. The image from the same video camera that was used for the tracking was recorded, in parallel, on time-coded videotapes. The output data files containing the records of locations at

time t , each involving the values at two perpendicular coordinates ($X(t)$, $Y(t)$), were exported from the tracking system into SEE (Software for the Exploration of Exploration, see Draï and Golani, 2001), which enables a large repertoire of visualizations and calculations. Path plots and the location and velocity series, processed with the optional smoothing methods with different parameter values, were compared to each other and to the video record of the same behavior, using controls that enabled the observer to run the videotape frame-by-frame or in any required speed.

While our approach is implemented in the present study on data borrowed from rodent locomotor and exploratory behavior, using a video tracking system, our findings clearly pertain to any study of an organism's behavior making use of spatial data, using many types of tracking.

3. Sources of noise in tracking spatial behavior

Most current tracking systems (either photobeam, photocell or video systems) are constrained by the resolution of a recording system using pixels or "tiles". The recorded location is therefore of discrete nature—two records cannot be closer than the resolution level unless they are at exactly the same location. Furthermore, since the typical pixel length is smaller than the animal, the system actually records the location of the animal's "center of gravity".

Whatever the noise level of the location measurement, it will be even higher for estimating the velocity (the first derivative of location) in the same time resolution. This is clearly demonstrated in Fig. 2 (compare noise levels between top and bottom graphs in raw data and within each smoothing method). For example, when estimating velocity by differencing the locations measurements at successive time points, the variance of the noise level of a difference of independent measurements is the *sum* of the individual noise level variances. Estimating the acceleration (the derivative of velocity or the second derivative of location) will, for the same reason, increase the noise level even further. In general, as measures of behavior become increasingly complex and depend on more coordinates, they accumulate more noise terms, and it is very easy to reach a situation where the signal-to-noise ratio is lower than 1, even when the original noise appears to be small.

The proper smoothing method should be suitable to the type of noise in the system. By comparing tracking results with the videotape of the actual movement, we found that the sources of tracking noise can be categorized into three main groups: precision level noise, tracking system erratic noise (mostly in the form of outliers) and body wobble. In the following subsections we describe and illustrate these three sources of noise.

3.1. Precision level noise

As noted above, tracking systems of most kinds actually see the arena as a paved area of "tiles" or (with video

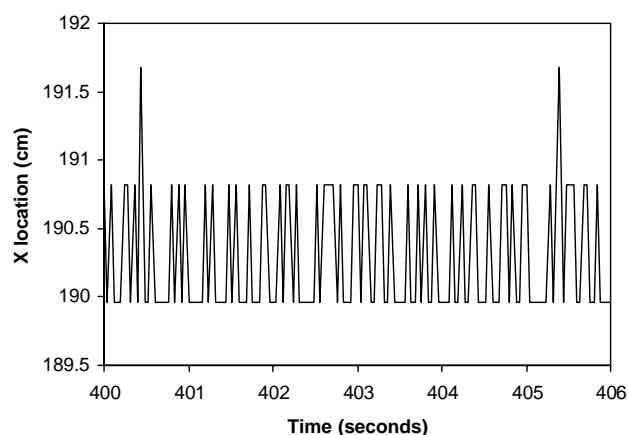


Fig. 1. A typical 6 s sample out of the X coordinates of an anesthetized mouse as recorded by the tracking system at a rate of 25 frames/s.

tracking) pixels, typically smaller than the animal's size, so that a "center of gravity" of the animal's image is given as the location. If the boundaries of the tracked animal do not fall exactly on one pixel, they might vacillate between two neighboring pixels, so that the "center of gravity" (by whatever method it is calculated) will waver between discrete values even if the animal is stationary. To demonstrate this problem we placed an anesthetized mouse in the arena and tracked it (Fig. 1).

For a pixel corresponding to 0.5–2 cm distance in the arena, the motionless anesthetized mouse accumulates a considerable total distance traveled. In 15 min (Fig. 4), the anesthetized mouse, according to the raw data, "traveled" 94 m and had an average velocity of more than 10 cm/s! It is important to note that this discretization of the measurement process affects not only a stationary mouse, but also a moving mouse. Even in the absence of any other sources of noise, a smooth, slow progression of the mouse (as confirmed by a human observer) will be erroneously recorded as a step-like series of arrests. Precision level noise is likely to increase in direct relation to the tracking rate, but we found that the high tracking rate we used is necessary to capture many properties of rodent behavior, especially in the fast-moving mouse.

3.2. Tracking system erratic noise: system outliers

Outliers are observations 'far' from other neighboring (in time) observations (e.g., Fig. 3), most likely due to some erratic behavior of the tracking system. Intuitively speaking, the difference between noise and outliers is that outliers are something one would have immediately corrected with no scruples whatsoever, if one just happened to notice them. When the data are automatically tracked and analyzed, however, this observer-based definition becomes impractical.

Outliers might have, of course, a devastating effect on many measures of behavior such as the total distance traveled, progression segment length, etc. The considerable

difference between the outlier and the adjacent points is likely to yield erroneously high velocities (e.g., Fig. 3, top). Moreover, outliers have a devastating effect on many smoothing methods that are required in order to deal with the previous problem of precision level noise (Fig. 3).

3.3. Body wobble

This source of "noise" is due to the animal's own movements. By "body wobble" we refer to all movements of the animal that are not part of its whole-body progression, e.g., head movements or incipient shifts of weight, which affect the "center of gravity" measured by the tracking system. Body wobble may, depending on the goal of the study, be the very subject of investigation, but for the purpose of obtaining the animal's path or its velocity it is an undesired side effect and thus should be treated as noise. As in our case, the researcher may want to filter out body wobble during the animal's progression but retain it during stops, where it is indicative of rearing, scanning movements and other ethologically-relevant behaviors (the word "stop" is used in this study in the sense of "lingering", see [Drai et al. \(2000\)](#), i.e., staying in place while possibly executing non-locomotor movements, while the word "arrest" is reserved for complete immobility or zero velocity). This again calls for a combination of different smoothing methods for progression and stopping. Note that although, as in our situation, the same smoothing procedures may be used in order to remove both precision level noise and body wobble, these two types of noise have different sources, and might be of different magnitudes depending on the animal size, animal speed, spatial resolution and other factors. In such situations, the parameters of the smoothing method need to be adjusted differently to remove the two different sources of noise.

4. Common smoothing techniques and their properties

Most current photobeam and photo-cell systems and some video tracking systems do not employ any form of smoothing, and are therefore exposed to the noise sources described in Section 3. We are not aware of any serious attempt to evaluate the results of such systems against the actual behavior. Some video systems try to cope with the problem by reducing the sampling rate (also known as down sampling), for example, by using only every other recorded location. The purpose of down-sampling is to separate body wobble from true location. However, it is not a true smoothing method, in its statistical meaning, as the level of the other sources of variation that are described in Section 3 is not being reduced. The down-sampling also affects the analysis of short arrests.

Some video tracking systems do employ different methods of smoothing. Not all smoothing methods, however, were created equal. The current section gives a general

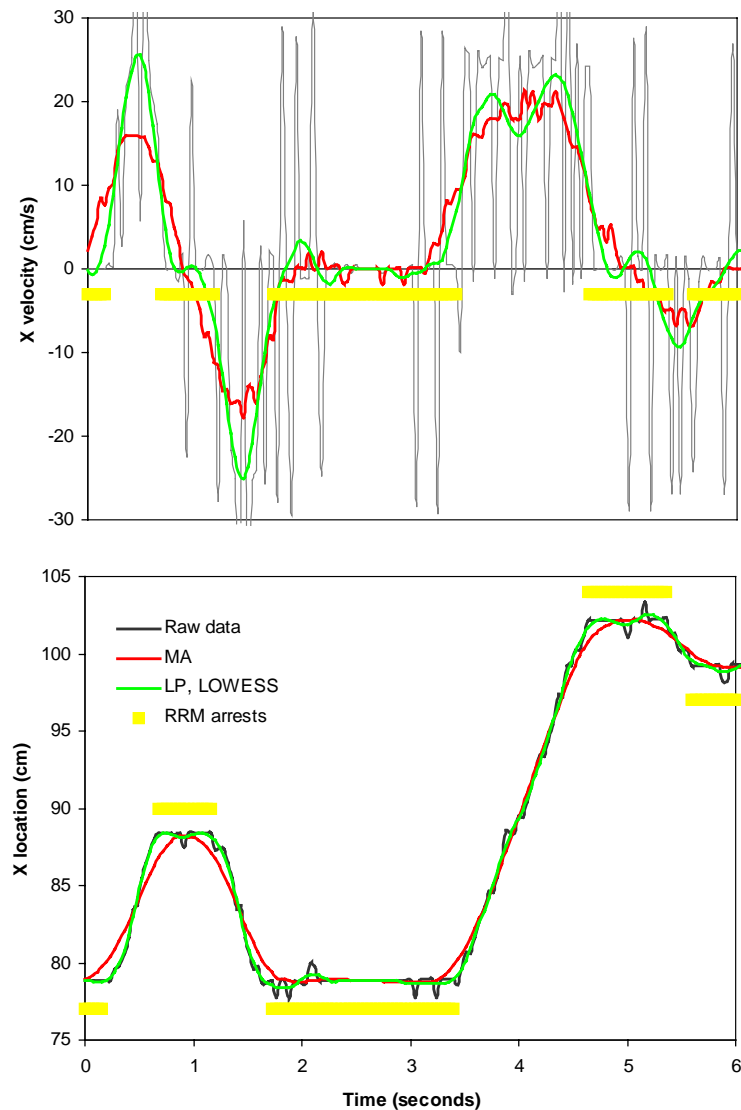


Fig. 2. Location (bottom graph) and velocity (top graph) out of 6 s of a mouse's movement, smoothed with several methods. "Raw" velocity (gray, top graph) is calculated as the differences between consecutive raw locations. MA velocity (top, red) is calculated as the differences between consecutive MA smoothed locations. LP velocity (top, green) is calculated directly by the LP. LOWESS-smoothed locations and velocities were almost identical to those calculated by LP since there were no outliers. Arrests (time range denoted by yellow stripes) were computed as zero velocity in the RRM smoothed series. MA is applied with a window width of 15 frames. LP is applied with a window width of 15 frames and a degree of 2. RRM is applied with four iterations using half windows of 3, 2, 1, and 1.

overview of some of the most commonly used smoothing techniques and their properties in view of the research goals.

The most common smoothing technique is the Moving Average (MA, see Box and Jenkins, 1970; Velleman and Hoaglin, 1981). In MA, the smoothed location at time t is the average over a time "window" centered on this point and $2h + 1$ points wide, (h denotes the "half window width"). By increasing window width a 'stronger' smoothing is achieved, reducing the noise variability at the cost of reduced time resolution. MA can be further improved by using a Moving Weighted Average (MWA), which assigns larger weights to data points in the center of the time window. For the same window width, MWA follows the original data more closely than MA. Numerical differentiation of the MA or MWA

smoothed series usually leads to much more realistic velocities when compared to numerical differentiation of raw locations (Fig. 2, top).

For the purpose of dynamic analysis of behavior, however, both MA and MWA have a disadvantage: they tend to lower speed peaks while increasing the speed during slowing down or arrest (Fig. 2, top). The latter especially has grave consequences, as it shortens the duration of arrests and even eliminates abrupt arrests entirely, erroneously joining two movement segments into a single longer one (Table 1). MA and MWA are thus unsuitable for the analysis of arrests and progression segments.

Another commonly used smoothing technique is the Local Polynomials method (LP, see Fan and Gijbels, 1996). As

Table 1

Identification of 4 frames arrest embedded within 20 frames artificially produced series, as performed by Moving Average (MA) method (fourth and fifth columns) and Running Median (RM) method (sixth and seventh columns)

Time (t) (frames)	Raw $X(t)$ location (cm)	True move/arrest	MA smoothed location	MA move/arrest conclusion	RM smoothed location	RM move/arrest conclusion
1	36	Move	–	–	–	–
2	31	Move	–	–	–	–
3	27	Move	28.2	–	27	–
4	24	Move	26.4	Move	27	Arrest
5	23	Move	23.8	Move	24	Move
6	27	Move	21.4	Move	23	Move
7	18	Move	19.2	Move	18	Move
8	15	Move	17	Move	15	Move
9	13	Move	13.6	Move	13	Move
10	12	Move	12	Move	12	Move
11	10	Arrest	11	Move	10	Arrest
12	10	Arrest	10.4	Move	10	Arrest
13	10	Arrest	10.2	Move	10	Arrest
14	10	Arrest	11	Move	10	Arrest
15	11	Move	12.2	Move	11	Move
16	14	Move	14	Move	14	Move
17	16	Move	16	Move	16	Move
18	19	Move	18	Move	19	Move
19	20	Move	–	–	–	–
20	21	Move	–	–	–	–

Both MA and RM were applied with a half-window of $h = 2$, and an “arrest” was considered as no change in location from the previous record. The “arrest” conclusion of the RM in frame no. 4 is a boundary effect and can therefore be disregarded.

in MA, LP uses a time window centered on each time point, but instead of using the average location over the window, it fits a low-order polynomial (usually a straight line or a parabola) of location in time to the data at the window. Once such a polynomial is fitted (using simple or weighted least squares) the smoothed location at time t is the value of the polynomial at that point. LP can also provide an estimation of the velocity by using the derivative of the fitted polynomial at time t . The strength of LP smoothing is controlled mainly by choosing the window’s width (as in MA) but also by the degree of the polynomial. With a proper choice of these parameters, the LP method is more flexible than the MA or MWA, in the sense that it can accommodate better to the pattern of the data within a window (Fig. 2, bottom) while still producing a smooth series. Another advantage of LP over MA is as follows: if an animal moves along the (circular) edge of the arena, its path will generally curve towards the arena’s center. The linear MA will “pull” such a path away from the edge towards the center. LP, in contrast, will better capture the curvature of the path and reduce the effect of this artifact. LP somewhat reduces the problem of eliminating arrests or shortening them, although it does not solve the problem completely (Fig. 2, top).

MA, MWA and LP all share, however, another crucial disadvantage: they are not robust in handling system outliers. They typically form a ‘dent’ in the direction of the outlier (Fig. 3, bottom) and a wave-like form in the velocity time series (Fig. 3, top). These artifacts stretch over a range of data that is wider than the original outlier (as wide as the window width, in fact). More dangerously, these artifacts (as

opposed to the original outliers in the raw data) are often visually indistinguishable from the natural movement, so that the researcher might fail to identify them as artifacts when examining the smoothed series.

A seemingly very different set of smoothing tools is the Smoothing Splines (SS, see Härdle, 1991). The location versus time, as filtered by SS, balances between the following two contradicting tasks: minimizing the sum of squares of deviations between the data and the filtered function on the one hand, and minimizing a penalty which is proportional to the sum of squares of accelerations, on the other hand. Obviously, the less smooth the filtered function is as a function of time, the larger the squared acceleration is. However, SS is very similar in its outcome to LP, where the constant of proportion in the penalty in SS plays the role of the window width in the LP. A disadvantage of the SS, however, is that it does not handle outliers.

Finally, the Fast Fourier Transform (FFT) approach to smoothing takes a different direction (Efromovich, 1999). The raw data are represented as a weighted sum of periodic functions. The terms involving higher frequencies are then dropped, and the result is back transformed to the original scale, producing a smoother series. The FFT is excellent for smoothing periodic functions and remains very useful for other smooth functions. It nevertheless fails to cope with inhomogeneous functions of differing smoothness levels at different parts of the time series (Ramsey and Silverman, 1997) as is the case with the path of the mouse, although sometimes this failure can be overcome (Tchernichovski et al., 2001). It is also not robust enough to cope with outliers.

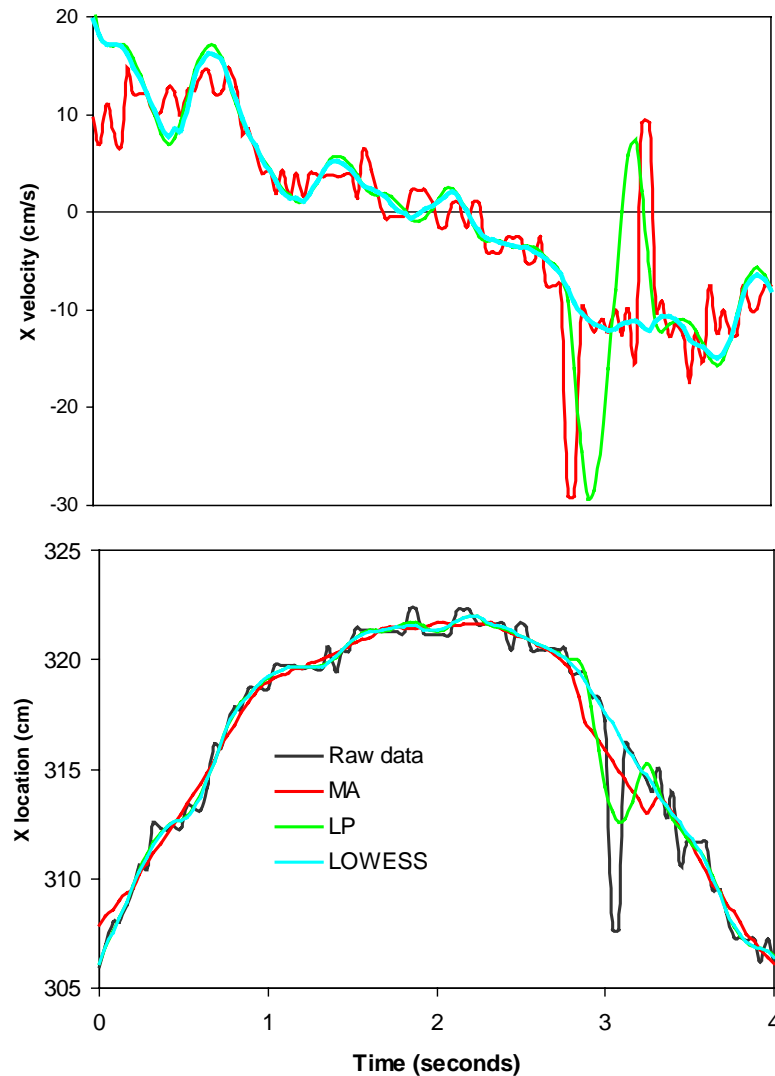


Fig. 3. Location (bottom graph) and velocity (top graph) out of 4 s of a mouse's movement, containing an outlier observation at about 3 s, smoothed with several methods. MA velocity (top, red) is calculated by the difference of consecutive MA smoothed locations. LP velocity (top, green) and LOWESS velocity (top, blue) are calculated directly by LP or LOWESS, respectively. Parameters are as in Fig. 2.

5. LOWESS: a robust smoothing technique

In order to solve both the precision noise and outliers problems we have incorporated the method of Locally Weighted Scatter Plot Smoothing (LOWESS, see Cleveland, 1977) into our smoothing algorithm. This is an iterative procedure combining the ideas of LP smoothing with robustness to outliers (see Appendix A for the detailed algorithm and choice of parameters). As in the weighted LP, the first iteration of LOWESS fits a polynomial to the data in a time-window centered at t . The resulting polynomial, however, is used only as a first estimation. Each original data point is then assigned a weight according to its difference from its first estimation (residual). A larger residual (indicating a poorer fit) results in a smaller weight for the corresponding data point, implying it will be less relevant for computing the next fitted polynomial. At the extreme,

a very large residual indicates that the point is an outlier, and it is assigned a zero weight, implying it will have no effect at all on the next iteration. In the second iteration of LOWESS the raw data in the window is fitted again with weighted LP, but this time using also the weights according to the residuals. In the original algorithm these iterations continue as above until no further change occurs, but practically we found that two iterations suffice. At this stage the fitted polynomial is used to derive the LOWESS smoothed location and velocity at time t . As with LP, LOWESS can be also employed for estimating the velocity, by using the derivative of the fitted polynomial at each time point. As Fig. 3 demonstrates, LOWESS is robust to outliers, but like LP (Fig. 2, top) it still tends to eliminate very short arrests and shorten longer arrests. Hence, LOWESS is inappropriate for the task of identifying arrests. To this end it would be preferable to complement it with an even more robust

method that can cope with the abrupt changes in the location as a function of time exhibited near the arrests. Such a method is discussed in the following section.

6. Identifying arrests with Repeated Running Median

The simplest robust smoother is the Running Median (RM, see Tukey, 1977). The RM procedure is similar to the MA, but instead of replacing an observation with the average of its neighboring observations, one uses their median. This seemingly small change has enormous effect on the performance, as the median is a robust function of the data. In simple words, in any window containing more than two observations a single outlier, however wild, will have no effect on the median. Generally speaking, the disadvantage of the RM is that if there is a sequence of values repeating for more than h times (at least half the data points in the window), then the median would be fixed at that value. Visually, the resulting estimator is not that smooth and thus is not recommended for evaluating velocities in general. This disadvantage, however, becomes an advantage for the purpose of identifying arrests (see the example in Table 1). RM is usually used in an iterated manner called the Repeated Running Median (RRM, see Tukey, 1977): first smooth the measured data with a RM, next smooth the resultant smoothed series with another RM, possibly with a different window width, and so forth. Such repetitions have an effect of giving more weight to locations closer in time to the center of the window. A proper choice of parameters (the window widths of the Repeated Running Medians, see Appendix A) was found to yield identification of arrests that coincides very well with the identification by several experienced observers that examined each arrest several times in the videotape (and see Fig. 2). The choice of the window sizes in the sequence was done by a trial-and-error but also followed Tukey's guidelines, who recommended repeating the RM steps starting from wider window width to smaller or same size window widths (Tukey, 1977). Once obtaining a final smoothed set of locations, a run of at least l locations that are not different by more than a small distance ε are marked as arrests. We found that $l = 5$ (equivalent to 0.2 s at a rate of 25 frames/s) and a very small ε (practically 0) yield an identification of arrests almost identical to that done by an experienced human observer (see Section 8 and Fig. 2). For more details see Appendix A.

7. SEE Path Smoother: a combined smoothing algorithm

The smoothing algorithm we constructed combines the advantages of LOWESS for robust smoothing and velocity estimation during progression with the advantages of RRM for robust identification of arrests. The algorithm smooths the raw data with both methods in parallel. For locations the LOWESS-smoothed results are used during movement, but

during arrests (as identified by the RRM) the locations are set to a linear interpolation between the (LOWESS-smoothed) start coordinate and end coordinate of the arrest. For velocity estimation the LOWESS-computed velocities are used during movement, but in data points that were identified as arrests by the RRM the velocity is reduced to 0. The values of the LOWESS and RRM parameters, and the arrests defining parameters l and ε are all user-defined (see Appendix A for detailed definitions and parameter values). The algorithm is implemented as an executable program called "SEE Path Smoother" (SPSM) which is available from the authors. A typical output of the velocity is seen in Fig. 7.

When used in the framework of SEE, SPSM is employed as the first stage of analysis, and is followed by separation of non-arrest segments into two intrinsically distinct populations: progression segments and "local" movements (Drai et al., 2000). Series of arrests separated by only local movement are then joined into stops ("lingering episodes", see Fig. 7). SPSM is therefore essential for treating spatial behavior as a string of discrete, ethologically relevant behavioral units, each having reliable dynamic properties.

8. Examples and experimental evaluation

In this section, we evaluate the previously described smoothing methods side-by-side, using typical examples and samples out of the behavior of mice from several inbred strains.

Fig. 2 displays 6 s (150 frames) from the movement of a DBA/2 mouse. For the sake of simplicity we consider only the movement along the X dimension (the same analysis is also performed in the Y dimension). It is not hard to identify from the raw data (or from watching the video) that during this period the mouse stopped five times. Note the (most probably) precision level noise effect in the form of small upward and downward bumps. Retrieving velocities from the raw data without any smoothing (i.e., by numerical differentiation of successive raw data locations) yields very erratic and unrealistic velocities (Fig. 2, top). Attempting to identify arrests in the figure by searching for zero velocity segments would miss the second arrest, and the fourth arrest is shortened to a length that is hardly discernible.

Clearly, using MA or LP reduces the noise substantially. LOWESS smoothing is, in the absence of outliers, nearly identical to the LP smoothing. The velocities smoothed by both methods present a much more realistic picture than the non-smoothed velocity, but still cause arrests to look shorter than what they really are (Note especially the second and fourth arrests.) Note that if we would have chosen to define arrests by introducing a cutoff on the smoothed velocity, this cutoff would have to be almost ± 10 cm/s in order to avoid shortening the arrests, and this means that a large part of the behavior, mainly slow progression and small movements inside "lingering" (Drai et al., 2000), would have been masked

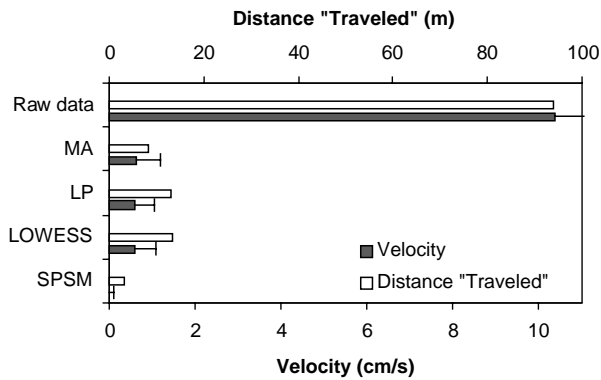


Fig. 4. The overall distance traveled and the velocity (mean and standard deviation) measured for an anesthetized mouse during a 30 min session, using the raw data and smoothing with MA, LP, LOWESS and SPSM. The standard deviation for the raw data velocity was 10.81 (error bar is truncated in order to focus on the relevant range). Velocity was computed using derivatives of the fitted polynomial for LOWESS and LP, and by numerical differentiation for the raw data, and numerical differentiation of smoothed locations for the MA. For the SPSM velocities are either 0 (in arrests identified by RRM) or the derivative of LOWESS (different from 0).

out. Arrests defined by the RRM, on the other hand, fit much better the location raw data.

Fig. 3 displays another example out of a 4 s (100 frames) movement sequence of a DBA/2 mouse. This example contains a typical outlier coordinate. The non-robust methods MA and LP (with same parameters as in the previous example) yield smoothed locations that are being “pulled” downward by the outlier. The smoothed velocities are also distorted. The robust LOWESS again performs very similar to the LP most of the time, but it is not affected by the outlier in either the location or the velocity.

In order to estimate the effect of precision level noise (Section 3.1) we placed an anesthetized mouse in the arena and tracked it for 30 min, smoothing the results with several methods (Fig. 4). Using the raw data (no smoothing), the overall distance “traveled” by the anesthetized mouse was almost 94 m. Smoothing generally reduced this noise by about an order of magnitude. The SPSM method, however, measured only 3.28 m, considerably less than the second best smoothing method (MA, 8.38 m). This difference was even more pronounced using the average velocity (0.01 cm/s with SPSM in comparison to 0.59 cm/s with LP, second best of the smoothing methods).

Theoretically, MA performs better than LP when the location is either constant or linear in time (i.e., constant velocity). This is reflected in the lower distance traveled of the anesthetized mouse found by MA, compared to LP and LOWESS. SPSM, which is specially designed for identifying arrests, worked even better than MA for the anesthetized mouse. A moving mouse, however, frequently accelerates and decelerates. LP, LOWESS and SPSM are able to adapt better to the variable velocity, while MA is likely to over-smooth the path and produce *too* short a distance.

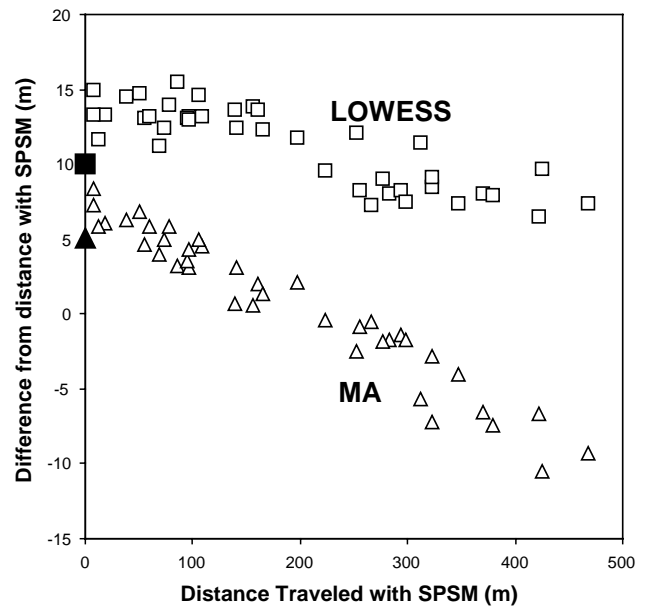


Fig. 5. The difference between the distance traveled using MA smoothing and SPSM smoothing (triangles), and the difference between the distance traveled using LOWESS smoothing and SPSM smoothing (squares) as a function of the distance traveled using SPSM smoothing, in 40 mice from different strains. The filled triangle represents the difference between the MA distance and SPSM distance in the anesthetized mouse, as seen in Fig. 4. The filled square represents the difference between the LOWESS distance and SPSM distance in the anesthetized mouse, again as seen in Fig. 4.

This is indeed suggested by comparing the distance traveled by moving mice, as smoothed with MA, LOWESS and SPSM, in 40 mice of 5 common inbred strains: BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J and FVB/NJ. The true distance traveled by a moving mouse is unknown, but one can still measure differences between the methods. Fig. 5 displays the differences of distances between MA and SPSM and between LOWESS and SPSM as a function of the distance measured by SPSM. For inactive mice MA produced a longer distance traveled than SPSM, as reflected by positive values in Fig. 5, but this relation is reversed for active mice. In fact, using regression, we find the following linear relationship between the two methods: $MA = 7.6 + 0.96 \text{ SPSM}$ ($r^2 > 0.999$), meaning that MA generally reduces the movement distance by about 4% relative to SPSM. Even the most active mice, however, are still part of this linear relationship which intersects 0 distance at a difference close to that found with the anesthetized mouse. This suggests that SPSM properly takes account of arrests in active mice as well. We further compared the SPSM to the LOWESS (Fig. 5), and found that $LOWESS = 14.7 + 0.98 \text{ SPSM}$ ($r^2 > 0.999$). That is, LOWESS generally reduces movement distance by only 2% relative to SPSM. Note in Fig. 5 that the difference in distance traveled between the LOWESS and MA is larger in moving mice than in the anesthetized mouse and, furthermore, this difference increases with the increase in activity.

To assess the extent of the outliers problem we analyzed 30 min sessions of mice from three strains of different colors: FVB/N, C3H/He and DBA/2, and checked how many outliers were recorded. For this purpose we defined an outlier as a time point at which the residual (i.e., distance of raw data from the smoothed result) is larger than six times the median of all the absolute values of the residuals in the window (corresponding to 4 standard deviations in normally distributed noise). The fraction of outliers out of the overall data points was slightly more than 4% in all three strains.

We estimated the ability of the methods to recognize stops by comparing the number of stops recorded in a 5 min session of a FVB/N mouse. While MA, LP and LOWESS recognized only 40, 25 and 29 stops respectively, SPSM recognized 97 stops. An experienced observer (blind to these results) went over the video record of the same sequence three different times, in which she recognized 89, 96 and 102 stops.

9. Potential applications

As demonstrated in the previous sections, a combination of the appropriate smoothing methods must be employed for correct analysis of spatial behavior. This is true even for simple measures such as the distance traveled, but is especially critical for dynamic analysis involving higher derivatives of locations and the identification of stops. Automatic high-throughput recording of undistorted velocities and accelerations has wide implications for ethology and the various branches of behavioral neuroscience.

One such application is in behavioral phenotyping. Rapid advances in bioinformatics have created a demand for tests of mouse behavior having high discriminatory power across strains and preparations, high replicability across laboratories, and high-throughput for mass screening (Wahlsten et al., 2001). In response to this demand, behavior geneticists now employ a variety of measures including a richer array of behavioral tests (Lederhendler and Schulkin, 2000), standardization of these tests (Crabbe et al., 1999), and establishment of a Mouse Phenome Database (Paigen and Eppig, 2000). A complementary measure, suggested by our group, is automated recognition and measurement of a large number of ethologically relevant patterns reflecting motor, motivational and cognitive functions, all derived from open field behavior, using SEE (Drai and Golani, 2001; Kafkafi et al., 2003a,b). This automation process is based on an algorithmic recognition of patterns, and must be preceded by smoothing the data.

To appreciate the effect of the smoothing compare the two graphs in Fig. 6, which represent the path and velocity-trajectory traced by a DBA/2 mouse in the course of half a minute. The path and the velocity-trajectory on the top graph are based on the measured location. From this type of noisy data one can only get the distance traveled, the proportions of that distance traveled along and

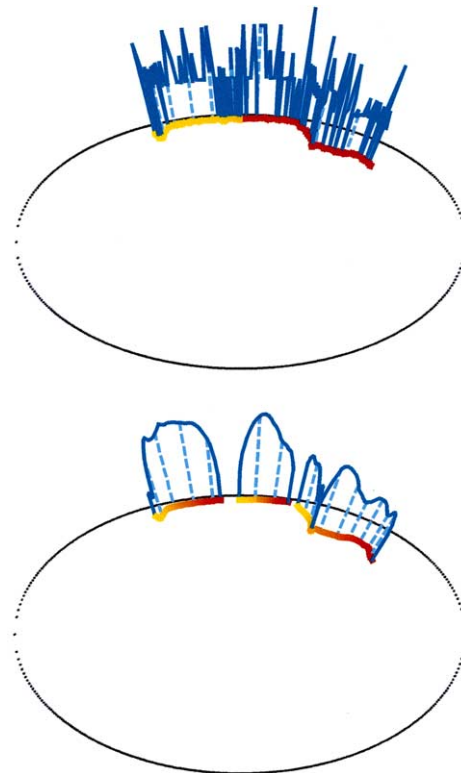


Fig. 6. A three-dimensional representation of the same movement sequence of a DBA/2 mouse in a circular arena, using either the un-smoothed path and velocity trajectory (top) or the SPSM smoothed and SEE segmented data (bottom). The yellow-to-red lines represent location and direction of progression. Azure lines designate velocity trajectories on the vertical axis, generated by simple differencing from the raw data (top) or smoothed by the SPSM (bottom).

away from the wall, and the amount of winding of the path (all contaminated with noise). In contrast, the path and velocity-trajectory at the bottom were obtained by SPSM. This plot uncovers a sequence consisting of an alternation between progression and lingering segments—discrete behavioral units with proven ethological relevance for rodents (Drai et al., 2000). These units can be characterized by simple measures such as their length, duration, maximal speed, acceleration and other measures derived from these (see Fig. 7). Treating the path as a string of discrete building blocks rather than a continuous series of coordinates allows a more straightforward analysis of complex structures (Drai and Golani, 2001; Kafkafi et al., 2001, 2003a,b). We presently have some 32 carefully designed endpoints that characterize these building blocks. The endpoints are computed by SEE Endpoint Calculator, a publicly available software. This framework can also be applied to other spatial mazes, such as the Morris water maze (Morris, 1984) and the elevated plus maze, if they are conducted with video tracking.

Since velocity betrays the forces acting on the animal, it can be used to uncover attractors, such as familiar places in the environment, or repellors, such as the same

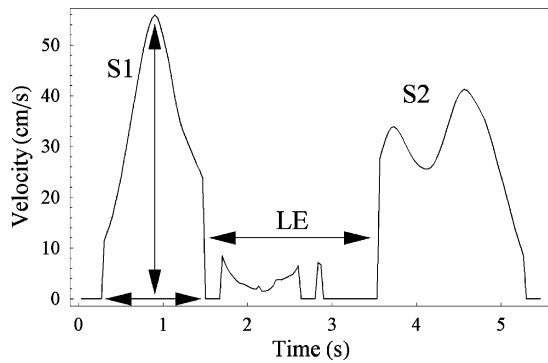


Fig. 7. A time series of velocity smoothed by SPSM out of the movement of a C57BL/6 mouse. This series includes two progression segments (S1 and S2) separated by a stop or a “lingering episode” (LE) in between. LE includes three arrests and two segments of local movements. The ratio between the maximal speed of a progression segment (e.g., vertical arrow in S1) and the segment’s duration (e.g., horizontal arrow in S1) describes a measure akin to segment acceleration (from Kafkafi et al., 2003b).

places later on, when the animal becomes disinterested in them and avoids them (Tchernichovski and Golani, 1995; Tchernichovski et al., 1998). In using this framework, Wallace et al. (2002) and Whishaw et al. (2001) compared the exploratory behavior of control rats and rats with fimbria-fornix lesions. They have shown that the higher velocity of the homeward portion of trips performed from a rat’s home base (Eilam and Golani, 1989) requires an intact hippocampal formation. In using the distinction between progression segments and lingering episodes they have shown that while control and fimbria-fornix rats had similar outward segments, the last progression segment on the way home was significantly faster (and straighter) in the controls, when compared with that of the lesioned rats. This result was independent of testing in light or dark conditions, suggesting that rats employ dead reckoning navigational strategies to conclude the homeward portion of exploratory movements. Using the methodology outlined in this study, it is now possible to extend the analysis to whole-session velocity trajectories as well as to other derived measures (e.g., Kafkafi et al., 2003b). This is important because the resolution and information content of these trajectories would finally match that used in electrophysiological studies of the hippocampus (e.g., Best et al., 2001), enabling a common framework for phenotyping, lesion and electrophysiological studies. In addition, the record would reflect not only the attractive properties of the home base during the first exposure to a novel environment, but also predictable changes (such as from attraction to repulsion) embodying spatial memory and habituation during later exposures (Tchernichovski et al., 1998).

Could the hippocampal place-cells and their corresponding place-fields, (e.g., Best et al., 2001; Mittelstaedt, 2000; Wood et al., 2001) define operational places in the environment rather than locations? There is now a substantial amount of literature on how place cell firing corresponds

to different behavioral contexts such as direction of movement relative to a start box and goal (Redish et al., 2000), progressive changes in the shape of places (Ekstrom et al., 2001), regions of particular behavioral significance even when these regions are completely unmarked (Hollup et al., 2001), and the relationship between multi-sensory processing, head direction cells and place cell firing (Wiener et al., 2002). Place cell firing thus appears to represent constructs that are more complex than location. Given the new tools for moment-to-moment computation of velocities, the identification of arrests and the separation of lingering movements from progression it should now be possible to study the correspondence between place and direction cell firing and behaviorally defined places of freely moving rats.

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Appendix A

We outline the algorithms of LOWESS smoothing for finding locations and velocities and the RRM, which is used for recognizing arrests. Location is composed of two coordinates ($X(t)$, $Y(t)$), and the two algorithms are being applied separately for $X(t)$ and $Y(t)$. We then explain how to combine the two sets of smoothed locations to obtain velocity and identify arrests.

A.1. The LOWESS algorithm

The LOWESS algorithm requires several parameters:

- d Polynomial degree. The same degree is used in each window.
- h “half window”, the number of records in the window to the side of the center data point, so that the total number of data points in the window is $2h + 1$.
- r The number of iterations for the robust smoothing.

For a given choice of these parameters and a given data set, $\{X_t\}_{t=1}^n$:

Step A.

- (i) For each $1 \leq t \leq n$ define a neighborhood of size h , as follows:

$$N_h(t) = \{s : |s - t| \leq h\}$$

- (ii) For each $1 \leq t \leq n$, define weights $w_t(s)$ for all $1 \leq s \leq n$:

$$w_t(s) = \begin{cases} \left(1 - \left|\frac{t-s}{h}\right|\right)^3, & s \in N_h(t) \\ 0, & s \notin N_h(t) \end{cases}$$

Step B. For each t , fit a d th degree polynomial using weighted least squares with the weights defined in Step A(ii). The fitted value at each t is denoted by \hat{X}_t and is the value of the polynomial at that t (Note that a separate regression is fitted for each t .)

Step C.

- (i) Define the residuals to be $u_s = X_s - \hat{X}_s$. Let m be the median of $|u_s|$ for $s \in N_h(t)$.
- (ii) Assign another set of weights, δ_s as follows:

$$\delta_s = \begin{cases} \left(1 - \left|\frac{u_s}{6m}\right|\right)^2, & |u_s| \leq 6m \\ 0, & |u_s| > 6m \end{cases} \quad \text{for } s \in N_h(t)$$

(i.e., Large residuals result in small weights and small residuals result in large weights).

Step D. Iterate Steps B and C r times but with $\delta_s w_t(s)$ replacing $w_t(s)$.

The entire procedure including the initial computation and the iterations is referred to as robust locally weighted regression or LOWESS.

On the choice of smoothing parameters:

Number of iterations (r): the LOWESS can be repeated until convergence, or by specifying a number of iterations. We are using two iterations ($r = 2$), which for most cases is sufficient. This saves computation time.

Half window width (h): the wider the window is, the smoother the output will be. There are statistical procedures for automatically choosing the width of the window (e.g., cross-validation, Fan and Gijbels, 1996). In our data, however, we are using $h = 10$. This choice came from trial-and-error where the output data looked relatively smooth on the one hand, and on the other hand, it still kept the important features of the data. This was done by trying different window widths and comparing the output to the actual video.

Polynomial degree (d): the higher the degree of the polynomial is, the smoother the output is. Since each window is very short in time (less than a second), it is reasonable to assume that a mouse behavior is pretty smooth, reflecting a low degree polynomial. We are using a second degree polynomial, as this allows us to estimate the acceleration as well

as the velocity. Note that although the degree of the polynomial affects the amount of smoothing, the window width has a larger effect.

A.2. Estimating velocities

In Step B of LOWESS, estimation of the Local Polynomial means estimating the $d + 1$ coefficients of the polynomial. The derivative of the polynomial at each time, which is the estimated coefficient of the linear term, gives an estimate of the velocity. If V_x and V_y are the estimates of the velocities in directions X and Y , respectively, at time t , we estimate the velocity at time t by $\sqrt{V_x^2 + V_y^2}$. This is done for all t 's to get the velocities at all times.

A.3. Repeated Running Median (RRM)

The RRM algorithm in its role of identifying arrests requires several parameters:

- $h_1 \geq h_2 \geq \dots \geq h_r$ "half windows" of the Running Median iterations.
- l minimal length of arrests.
- ε 'closeness' parameter.

The RRM algorithm is based on repeated iterations (applications) of Running Median smoothing, which requires a single parameter h , the "half" window width of the smoothing. The Running Median algorithm is as follows: given a data set $\{X_t\}_{t=1}^n$, the smoothed location at time t , \hat{X}_t , is the median of the $2h + 1$ X_s s closest in time to X_t .

For a given set of "half windows" widths, $h_1 \geq h_2 \geq \dots \geq h_r$, the RRM smooths the data using the Running Median with "half" window width h_1 , then it runs the Running Median on the smoothed sequence using h_2 , and so on r times.

The RRM result is a sequence of smoothed locations. When a rodent stops, the smoothed sequence at the relevant time has the same value. We need to decide how many successive repetitions of the same values would be considered as an arrest. This is done by the parameter, l . The last parameter needed is the 'Closeness' parameter ε (a small number), determining how far from each other data points can be, within the window of length l , in order to still count them as the same point.

This procedure is done separately on $\{X_t\}_{t=1}^n$ and on $\{Y_t\}_{t=1}^n$. We identify an arrest when the mouse does not progress on both directions at the same time.

The values of smoothing parameters we are using are:

Half window widths (h_i): the sequence of windows widths for the RRM follows Tukey's recommendations (Tukey, 1977). The algorithm was verified against the video to make sure we identify arrests correctly. We use four repetitions $h_1 = 3, h_2 = 2, h_3 = h_4 = 1$.

Minimal length of arrests (l): 5 frames.

Closeness parameter (ϵ): for mice 0 (practically 0.0001). However, for larger animals (say a rat) a larger parameter is needed.

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