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Serotonergic Axons as 3D-Walks

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Abstract

Experimental and theoretical research suggests that serotonergic axons (fibers) can be modeled as random walks or stochastic processes. This rigorous approach can support descriptive methods and dynamic control of the ascending reticular activating system, at the level of individual fiber trajectories.

Keywords

5-Hydroxytryptamine; fibers; random walk; von Mises-Fisher distribution; stochastic process; fractional Brownian motion

In mammalian brains, serotonergic axons (fibers) originate in the brainstem raphe complex, a nearly continuous set of nuclei with rather diffuse borders. During embryonic development, these fibers follow predictable spatial paths, but their trajectories become increasingly “random” as the brain matures. In most regions of the adult brain, individual serotonergic fibers appear to have no preferred orientation or destination, as they weave through the densely packed neural tissue. Because of their chaotic-looking trajectories and often very large numbers, they are usually described by macroscopic “density” measures. These measures can be convenient in experimental research: just like temperature in thermodynamics, they capture the global state of a system, disregarding its individual components.

Despite its usefulness, this approach is fundamentally incomplete: it is the behavior of individual serotonergic fibers that eventually determines their density in a particular brain region. Any description of this process requires an understanding of the associated dynamics and self-organization, two key ingredients of any biological system. They are essential for current models of neural activity, but their importance is often underappreciated in analyses of brain microarchitecture (a dynamic entity with a strong spatial component). In particular, self-organization underlies the plasticity, adaptation, and recovery of neural tissue.[1]

Recent progress in the resolution and diversity of microscopy techniques has set the stage for major advances in the modeling of the serotonergic system. These models will be

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Notes

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important for fundamental neuroscience and may also lead to new insights in biomedical research. For example, an increase in fiber density may be caused by more individual fibers invading the tissue volume or by a stronger tortuosity of individual fibers, with no change in the fiber number.[2,3] Also, it is not known whether individual fibers walk through large areas, freely mixing with other fibers, or whether they establish local territories that other fibers cannot invade. This level of description will play a progressively important role in the identification and characterization of mental disorders.

This Viewpoint focuses on computational tools that are currently available for the analysis and predictive modeling of individual fiber trajectories. We note at the outset that all fibers in a given brain region may be realizations of the same process that allows a rigorous mathematical description. This process may be strongly or entirely stochastic. As a consequence, we prefer to refer to serotonergic fibers as a “stochastic axon system.” This system is not unique. Other axons of the ascending reticular activating system (ARAS) fall into the same category, and some projections of the nonimage forming visual system (e.g., retinohypothalamic axons) may behave in a fundamentally similar way in their terminal fields.

Stochastic processes are a rich and vibrant area of mathematical research. However, this field rests on deep theoretical concepts (compactness, different types of continuity, filtrations, etc.) that cannot be easily replaced with intuitive shortcuts. This analytical depth is required to navigate subtle theoretical pitfalls and to achieve consistency, but it also makes stochastic models considerably less “user-friendly” than those of classical probability theory. Higher-dimensional (including three-dimensional) stochastic processes may add another level of complexity, due to nontrivial dependency structures. We hope the information provided here will facilitate the application of stochastic methods in the analysis of the brain serotonergic system, and illustrate some key points with original experimental data. For a more technical description of the possible stochastic approaches, we refer readers to our recent article.[3]

Serotonergic fibers are typically studied in fixed tissue, because in vivo live-imaging microscopy cannot achieve deep penetration and the width of single fibers falls far below the resolution of MRI imaging. The trajectories of individual fibers can often be traced with high precision in fixed preparations, but this accuracy in the spatial domain comes at the expense of losing the time dimension. It is a major loss because most stochastic analyses crucially depend on knowledge of how trajectories evolve in time. Spatial displacements and time are inextricably mixed in even basic stochastic setups (e.g., Brownian motion), and little can be said about the former without the latter. The concept of velocity may have no well-defined meaning because theoretical stochastic paths often are not differentiable. Therefore, one cannot assume (as a theoretical approximation) that, prior to fixation, a fiber moved with a constant velocity, even though it may be a meaningful proposition from the physical point of view.

We now have strong evidence that this hurdle can be overcome by using discrete random walks, where each step is assumed to be of the same length (Figure 1A). There is no natural way to assign a step-length to serotonergic fibers, but we have shown experimentally that it

can be on the order of the typical fiber width.[3] As the number of segments increases, a realistic level of smoothness can be achieved (Figure 1A). The three-dimensional turn at each step can be controlled by the von Mises–Fisher (vMF) distribution (a directional probability distribution), centered at the current direction. The spread of the possible directions is determined by the so-called concentration parameter (κ), a high value of which corresponds to a low probability of large-angle turns. Computer simulations (Figure 1B) produce trajectories that closely resemble actual serotonergic fibers, imaged at high resolution with confocal microscopy (Figure 1D, E). Importantly, this model-to-trajectories workflow can be reversed, and actual fiber trajectories can be used to estimate the numerical value of κ.[3] Efficient estimation procedures are available, the implementations of which do not involve complex calculations and are accessible to experimental neurobiologists, especially in high-level programming languages (e.g., Mathematica, Python).[4] This allows direct comparisons among the behaviors of individual serotonergic fibers in different brain areas and conditions (including brains affected by mental disorders), since the concentration parameter carries most of the information about the behavior of the fiber (here, "behavior" has only the spatial but not temporal component). For brevity, we further refer to these walks as vMF-walks.

These analyses in fixed tissue can significantly advance our knowledge, but we anticipate that time information will soon be available in more advanced, live preparations. This information will significantly expand the available stochastic methods. In particular, it will allow modeling serotonergic fibers as continuous-time random walks (CTRWs) and the fractional Brownian motion (fBm). The fBm is a mathematical generalization of the usual Brownian motion and is controlled by the so-called Hurst parameter (H), which ranges from 0 to 1 (the usual Brownian motion is represented by $H = 0.5$). At high H values, fBm trajectories become less "jittery" and may capture the behavior of serotonergic fibers (Figure 1C). Intriguingly, a recent numerical analysis has shown that, in this "superdiffusive" regime ($H > 0.5$), fBm trajectories accumulate at reflecting barriers.[5] The densities of serotonergic fibers do appear to be higher near the pia (e.g., in neocortical layer I) and in some periventricular regions, but to our knowledge this has not been investigated systematically and independently of the formal borders of anatomically defined regions.

Experimental evidence supports the potential of stochastic analyses. It can be shown that two serotonergic fiber segments can follow perpendicular orientations in the same point of space (Figure 1D). This suggests that such fibers are not guided by molecular gradients or other cues acting over larger distances. Also, individual fiber trajectories often closely resemble random walks in general and vMF-walks in particular (Figure 1E).

If serotonergic fibers are modeled as vMF-walks, several predictions can be made. We have previously estimated that the κ value of some serotonergic fibers can be around 250, assuming a step-length of approximately 0.15 μm (after moving-average smoothing of experimentally obtained traces).[3] Since longer trajectories allow better estimates of κ and many microscopes have tiling capability, it becomes important to know how large the imaging field should be, so that one can realistically capture these long trajectories (with no interruptions). A major limiting factor is the width (Z-extent) of the physical section. Even if the section were infinite in the XY-plane, any given fiber would eventually leave it by

escaping in the Z-direction. Computer simulations show that the trajectory of an average fiber with an optimally placed initial point extends only 30–50 μm in the XY-plane, before it exits a 40 μm thick section (Figure 2A). Because higher κ values give fibers more rigidity, some optimally oriented fibers may cover a larger XY-territory. However, these results suggest that tiling is not an optimal solution, and that separate images (in our case, 185 μm × 185 μm at the 63× objective magnification) are likely to provide as much information, with a less severe load on memory resources.

Another interesting question is how far an average fiber travels in the XY-plane before it becomes orthogonal to its original orientation. Computer simulations suggest this distance is less than 50 μm (Figure 2B). However, the actual probability of capturing such reorientation is lower because some fibers will escape the section in the Z-direction and, importantly, only perpendicular reorientations in the XY-plane are likely to be detected (the simulation includes reorientations in the entire three-dimensional space). Nevertheless, orthogonal reorientation should be a relatively frequent event over short distances, as supported by experimental data (Figure 1E).

If fibers are not static and continue to grow in the adult brain, it raises a natural question: What processes allow achieving a steady equilibrium of densities? We have previously suggested that in the healthy brain serotonergic fibers may be routinely interrupted and that these interruptions may be associated with microglia.[2] By using super-resolution microscopy (STED), we now have obtained evidence consistent with this hypothesis (Figure 2C). We note that this finding should not be generalized without more extensive studies.

In conclusion, stochastic methods offer a number of powerful analytical tools which, in combination with rapidly advancing imaging techniques, may revolutionize current understanding of the serotonergic system (and other stochastic axon systems). This research will require close collaboration among researchers in experimental neurobiology, automated image analysis, and stochastic spatial processes.

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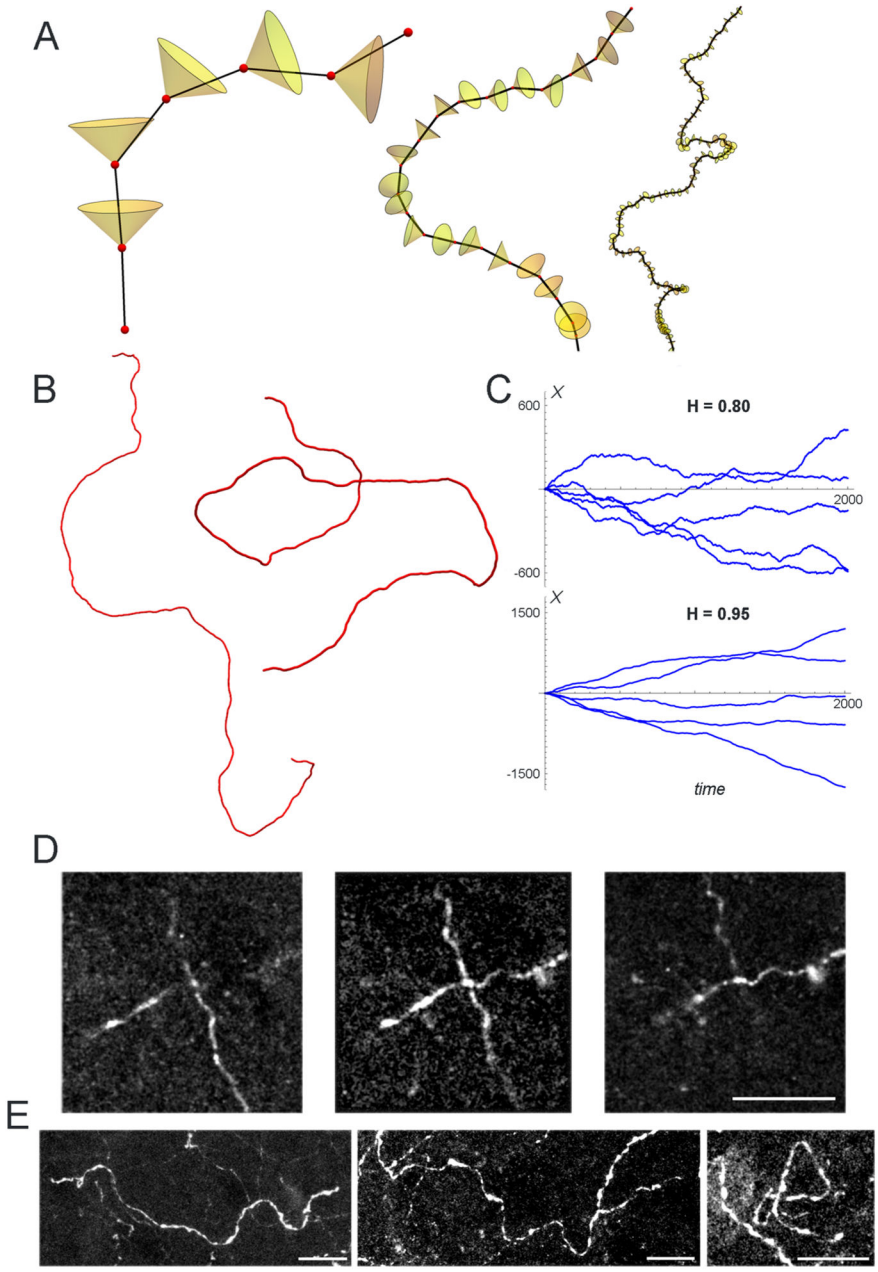

Figure 1.

(A) Serotonergic fibers can be modeled as a random walk with a constant-length step. At each step, a new direction is drawn from a directional probability distribution. For simplicity, the possible directions at each step are shown enclosed by rigid cones. Note that each cone is oriented in the current direction but allows deviations from it. By increasing the number of segments, smooth trajectories can be generated (fibers with 6, 21, and 101 segments are shown). (B) Two simulated fibers, each composed of 2000 segments. At each step, the new direction was drawn from the von Mises-Fisher distribution (centered at the current direction, with the concentration parameter $\kappa = 200$). Note that both fibers are realizations of the same exact process. For simulated fibers with different κ values, see ref 3. (C) Five

realizations of the one-dimensional fractional Brownian motion with the Hurst parameter $H = 0.80$ or $H = 0.95$. Note the different scales of the vertical X-axes showing the different spreads of the final points. This process can be extended to three dimensions. (D) Confocal images of two serotonergic fiber segments (in the mouse insular cortex) that follow perpendicular orientations in the same point of space. Each image represents a single optical section (the consecutive sections are separated by0.6 μm). Scale bar = 10 μm. (E) Serotonergic fiber trajectories in the mouse cingulate cortex (first and second images) and in the mouse primary somatosensory cortex (third image). Scale bars = 10 μm. The simulations in (B) and (C) were performed in Wolfram Mathematica 11.3. The fibers in (D) and (E) were visualized with an anti-5-HT antibody (ImmunoStar).

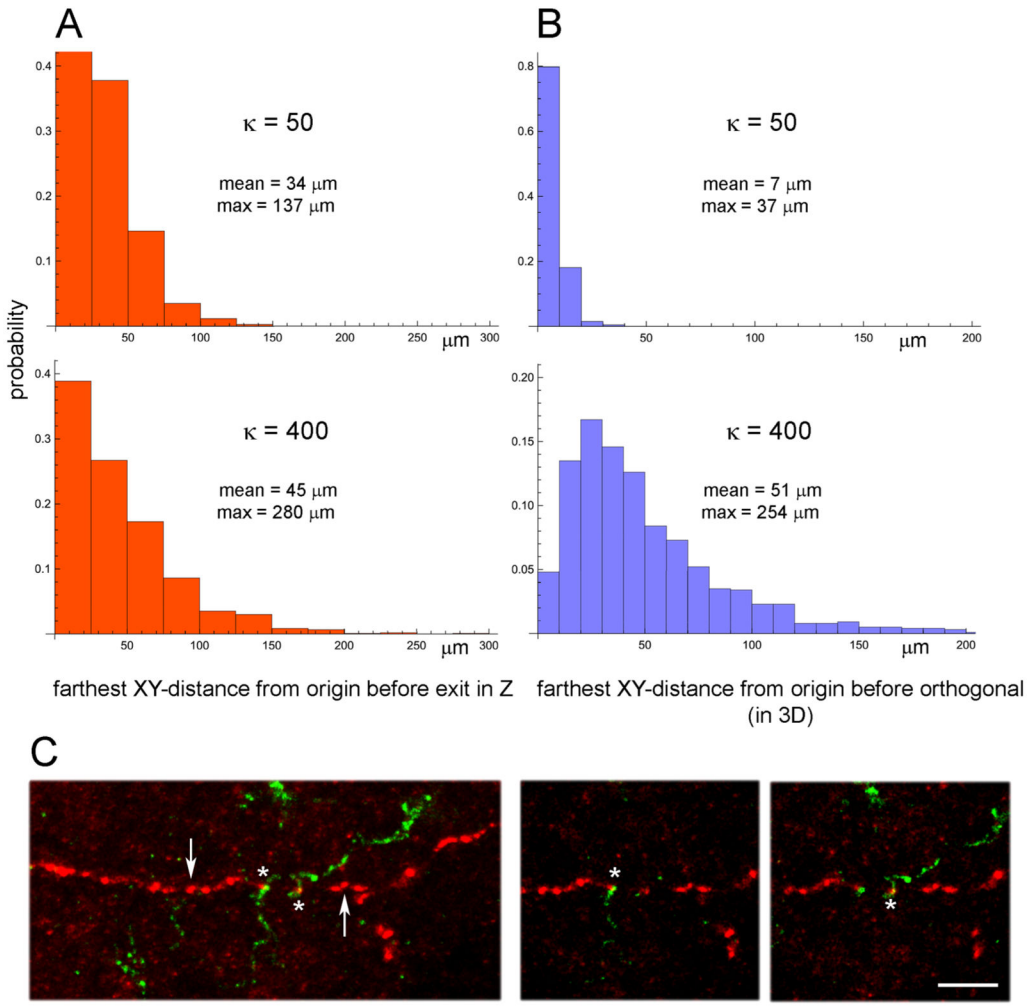

Figure 2.

(A) Distributions of the largest traveled distances (in the XY-plane) of 1000 simulated fibers, before their first exit from a 40 μm thick section. The trajectory of each fiber started equidistant from the top (+20 μm) and the bottom (−20 μm) of the simulated section (infinite in the XY-direction). At the first exit from the section, the largest XY-distance from the origin was recorded (anywhere along the trajectory). This analysis provides information about the XY-dimensions of imaging fields that are likely to contain long, uninterrupted trajectories (extending through the entire field). (B) Distributions of the largest traveled distances (in the XY-plane) of 1000 simulated fibers, before they change their original orientation to a nearly orthogonal orientation (in the three-dimensional space, with a ± 5° error). The space was infinite in all directions. In both (A) and (B), the fibers were generated as vMF-random walks (with $\kappa = 50$ and $\kappa = 400$, and each step-length equal 0.15 μm^3). The largest distance in the sample (max) may be outside the range shown. (C) Super-resolution (STED, Leica) image of contacts (asterisks) between microglial processes (green) and a serotonergic fiber (red, arrows). The contacts delimit an apparent fiber break (between the asterisks). The first image is a flattened z-stack, and the second and third images are single optical sections. The image was obtained in a section of the mouse neocortex,

immunostained with an anti-5-HT antibody (ImmunoStar) and an anti-Iba1 antibody (Wako Chemicals). Scale bar = 3 μm.