ACS Chemical Neuroscience

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# Serotonin in Space: Understanding Single Fibers

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**ABSTRACT:** All brain regions contain fibers that store and release 5-hydroxytryptamine (5-HT, serotonin). Since these fibers often do not have well-defined trajectories, most studies have focused on their overall densities, a measure that can be associated with local 5-HT tone in heathy and diseased brains. However, the observed fiber densities are the consequence of the behavior of single fibers. Evidence is presented as to why understanding single-fiber trajectories is important for basic and clinical neuroscience. In particular, serotonergic fibers can be viewed as natural, readily detectable stochastic probes that sample the invisible microarchitecture of brain tissue.

**KEYWORDS:** *5-hydroxytryptamine, fibers, density, stochastic methods, random walk*

## FROM SEROTONERGIC FIBER DENSITIES TO SINGLE FIBERS

Nearly all neural processes of the vertebrate brain are spatially embedded in a dense meshwork of fibers that store and release 5-hydroxytryptamine (5-HT, serotonin). The development and gross anatomy of serotonergic fibers is well understood; they are axons of neurons located in the raphe nuclei of the brainstem. During development, these axons grow rostrally, forming a well-defined ventral projection in the mammalian brain, and eventually invade the forebrain. Their dispersal in forebrain regions also proceeds in an orderly fashion. For example, in the developing cerebral cortex, serotonergic fibers first form two layers, one in the marginal zone (future layer I) and the other in the intermediate zone (future white matter). Later in development, however, these trajectories appear to lose clear anatomical direction. They meander, change their orientation within apparently homogeneous brain areas, and eventually fill large brain regions with a serotonergic "matrix." Despite this behavior, each brain region establishes and maintains a density of serotonergic fibers that is specific to that region. This is particularly striking in the cerebral cortex, where adjacent layers often have distinctly different fiber densities (Figure 1).

Since serotonergic fibers rarely if ever branch, their high density in many brain regions is likely due to increases in the lengths of the fibers rather than new collaterals. In a serotonergic fiber, 5-HT is concentrated in specialized swellings, or varicosities. It has been estimated in an early study that the rat cortex contains around $6 \times 10^6$ varicosities/mm$^3$, and that each cortical neuron may receive around 200 varicosities.[1]

The serotonin system is currently conceptualized as "diffuse," partly due to the perceived lack of functional utility in the description of individual fibers. By taking a "macroscopic" approach, analyses typically focus on fiber density, with no attention paid to individual trajectories. This thermodynamics-like approach is undoubtedly useful and has produced interesting findings. For example, the density of serotonergic fibers has been found to be unusually high in the cerebral cortex of individuals diagnosed with autism spectrum disorders.[2]

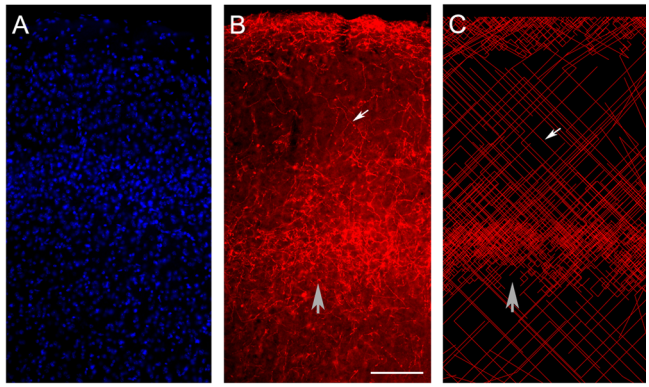


**Figure 1.** (A,B) Epifluorescence image of the insular cortex of an adult C57BL/6J mouse. (A) Cell nuclei visualized with DAPI. (B) Serotonergic fibers visualized with anti-5-HT IgG (ImmunoStar). (C) The result of random walks of 150 fibers that have been randomly seeded in 2D-space (the simulation was performed in Wolfram Mathematica 11). The height of the image was set to 1 arbitrary unit and the step size was set to different values in the five simulated layers (laminae). The heights of the layers (from the top down) are 0.10, 0.26, 0.18, 0.19, and 0.27, and their step sizes are 0.01, 0.20, 0.05, 0.01, and 0.20, respectively (in the same units). In all fibers, the random walk was performed for 70 steps and the fibers were not allowed to escape the area. The small (white) arrows point to fibers that move large distances with no turns, and the large (gray) arrows point to spontaneously rarefied pockets in otherwise dense regions. Scale bar = 100 $\mu$m.

Growing collaboration across various science fields presents new opportunities. Brain development and plasticity are traditionally viewed as orderly and globally guided processes, but they are more accurately conceptualized as self-organizing interactions among many relatively simple elements, each responding largely to its local environment. In this approach, the particular density of a serotonergic matrix is the direct

**Special Issue:** Serotonin Research 2016

**Received:** December 2, 2016
**Accepted:** December 12, 2016
**Published:** December 21, 2016





DOI: 10.1021/acschemneuro.6b00417
ACS Chem. Neurosci. 2017, 8, 893−896

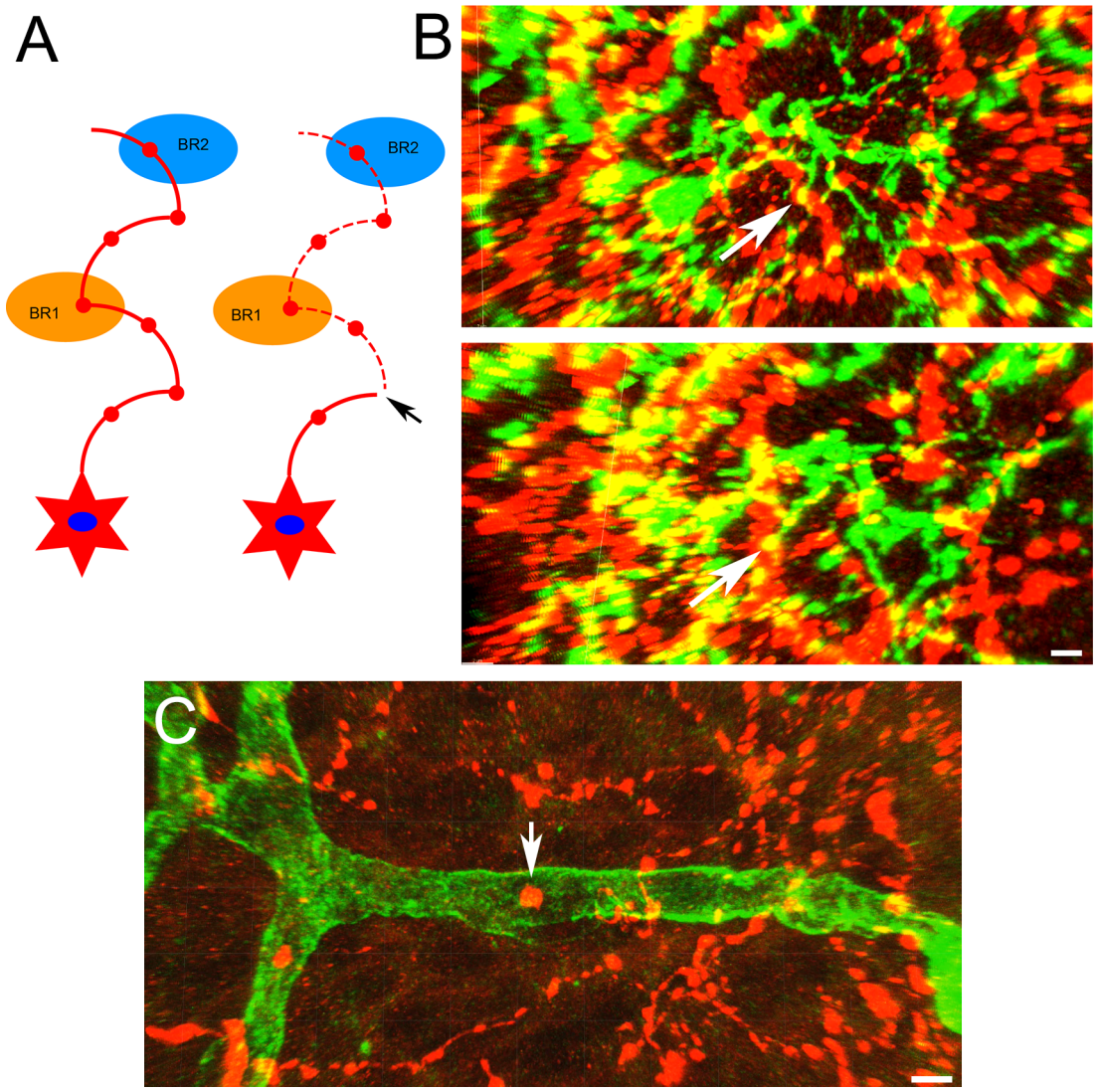


**Figure 2.** (A) Individual serotonergic fibers are likely to be routinely interrupted by physical stress, microglial activity, and other processes, which may result in degeneration of the distal fiber segment in multiple brain regions (BR1 and BR2 in the figure). (B) Two 2D-projections of the same confocal *z*-stack through the primary somatosensory cortex of an adult C57BL/6J mouse. Serotonergic fibers are visualized with anti-5-HT IgG (ImmunoStar), and microglia are visualized with anti-Iba1 IgG (Wako Chemicals USA). Microglial processes contact a serotonergic varicosity (arrows). Scale bar = 2 $\mu$m. (C) Confocal image of serotonergic fibers and a blood platelet (arrow) below the cortical plate of a C57BL/6J mouse at embryonic day 17.5. Scale bar = 5 $\mu$m.

consequence of the behavior of its individual fibers. In other words, the logic of the winding trajectory of a single fiber may determine the cumulative serotonergic tone in a given brain region and can therefore affect brain function in health and disease. This Viewpoint presents supporting evidence for this hypothesis, and briefly discusses some challenges.

## MODELING SINGLE FIBERS

To date, no one has traced a single serotonergic fiber in its entirety in the adult brain. We do not know whether a fiber can return to the same region (e.g., a thalamic nucleus) after having visited another region a long distance away (e.g., the visual cortex), or whether it eventually gets trapped in one anatomical region from which it cannot escape. We likewise know little about the behavior of single fibers when they get severed, probably a routine occurrence in the healthy brain (considering their enormous length and lack of fasciculation). Do the distal segments degenerate, producing scattered deletions in multiple brain areas? Do the regenerating proximal segments retrace the same pathway, or can they choose drastically different trajectories? Two mechanisms are immediately apparent, both leading to new insights.

A single serotonergic fiber may follow chemical cues in its environment. It is a strong possibility in development, but such cues may also be present later in life. In the adult brain, these cues then might be expected to have spatial distributions that are essentially random. Otherwise, serotonergic fibers in the same small region would have the same orientation, but they typically do not. A minor spatial shift of one cue-point may result in a major change in fiber direction, consistent with the actual observations (Figure 1B) and the general properties of complex systems. However, in such a "force field," fibers would likely get trapped in dynamical attractors (e.g., closed loops, whorls, and other inescapable space regions). Can it be that such phenomena do exist and have functional consequences but have not been looked for?



DOI: 10.1021/acschemneuro.6b00417
*ACS Chem. Neurosci.* 2017, 8, 893−896

Alternatively, a single serotonergic fiber may not follow chemical cues and be essentially a random walk, at least within a specific brain region. In the simplest (and likely simplistic) scenario, a serotonergic fiber can be assumed to grow in discrete steps, making truly random turns at every step. If step lengths are allowed to vary across brain regions, it is sufficient to reproduce the required serotonergic densities in these regions (Figure 1C). A promising aspect of this model is that it is very simple, assumes no global supervision, and in a single framework captures development, plasticity, and regeneration. For example, the relative densities would be restored if either an entire single fiber or all segments of fibers in a defined brain region were destroyed, provided fibers continue to grow. Notably, the trajectories of individual fibers would be different from the original ones, due to the random nature of the process.

The step length may be a function of the spatial distribution and density of physical obstacles. While cell somata and microcapillaries can force fibers to turn, most obstacles are subcellular (e.g., dendrites, axons, glial processes, elements of the extracellular matrix). Currently the entire spatial geometry of these elements cannot be visualized in a living brain, even though they can be studied separately. However, individual serotonergic fibers can be viewed as 1D-like probes that sample the microarchitecture of 3D-brain tissue. The 3D-properties of tissue then can be theoretically inferred from the statistics of individual fiber trajectories, by taking advantage of the sophisticated mathematical machinery of random heterogeneous materials, random walks with obstacles, and self-avoiding random walks.[3]

This approach raises an interesting question: is a specific fiber density functionally necessary, or is it merely an emergent phenomenon the surrounding cells are forced to adapt to? This suggestion may be unexpected if the brain is conceptualized as a machine, with each part serving or having served a functional purpose. However, it is perfectly logical if the brain is viewed as a cellular ecosystem whose elements can cooperate or behave "selfishly." It is well known that, in the adult brain, axons can invade neighboring territories vacated by other axons. While these processes are often associated with the brain's attempts to compensate for the loss, they may also lead to maladaptive outcomes, such as phantom limbs. With regard to serotonergic fibers, their necessity has been challenged by several mouse models that have virtually no serotonergic neurons or cannot synthesize 5-HT in the central nervous system. The massive serotonergic matrix must be energetically expensive to maintain, yet these 5-HT-free mice show minor or undetectable changes in gross neuroanatomy and behavior.[4] If the observed densities of serotonergic fibers are not functionally necessary but emerge as a mathematical consequence of random walks, a change in fiber density may be indicative of altered spatial microarchitecture. This may be a crucial piece of information, but it may have little to do with serotonergic signaling itself. For example, the reported increase in the serotonergic matrix density in autistic brains[2] might be caused by an increase in subcellular obstacles in the tissue, whereas the predicted alterations of 5-HT levels may be less important or even irrelevant.

It should be noted that Figure 1 provides only a proof-of-concept. Lévy flight models may more accurately capture the statistics of single fibers, and a recent, biologically inspired mathematical approach may provide further insights.[3] Building on our expertise in the anatomy and development of the serotonin system, we are currently developing an integrated approach aimed to facilitate interactions among experimental neuroscientists, image analysis experts, and mathematicians specializing in stochastic processes. This has revealed some workflow bottlenecks, which are discussed next.

## FROM IMAGES TO DIGITAL TRAJECTORIES

It is relatively easy to obtain high-resolution, 3D-images of serotonergic fiber segments contained in one 40−50 $\mu$m-thick section (Figure 2). However, efforts to automatically trace their trajectories reveal a problem: while serotonergic varicosities produce signal that is strong and unambiguous, signal is often weak or nearly absent from segments that connect two adjacent varicosities. For sparsely distributed fibers, the human brain may outperform the computer because its Bayesian inference can rely on experience-based properties of physical objects in space. For example, it is easy to identify a necklace in dim light, even if only its beads are visible. In this case, the brain can rely on the fact that randomly distributed beads would be unlikely to lie along such a smooth curve. Encoding this intuition in a precise algorithm may be more difficult, as the likelihood of a necklace also depends on the physical distance between adjacent beads (which should be "reasonable" for a necklace, thus implying a probability distribution). As the number of necklaces in the same spatial region increases, both the computer and the human brain run into major difficulties. At a certain necklace density, quantification can be only probabilistic, but it is still valuable, as long as it is consistent.

Our attempts to automatically trace single serotonergic fibers have revealed a surprising dearth of information about even their most basic properties. To our knowledge, only one study has quantified the size distribution of their varicosities,[5] an important piece of information for Bayesian algorithms. Another piece of missing information is how often serotonergic fibers break (Figure 2A). These events appear to be likely because individual fibers intermingle with various dynamic elements, such as mobile cells, vasculature undergoing remodeling, plastic synaptic contacts, and others, all of which are likely to produce local physical stresses. Also, a number of recent studies have shown that "resting" microglia continuously monitor presynaptic and postsynaptic elements and can remove them. Our analysis shows that microglial processes contact individual serotonergic varicosities (Figure 2B). While it can be argued that serotonergic varicosities do not form classic synapses (thus supporting the image of a "diffuse" system), this view has been challenged by some electron microscopy analyses. Whatever the causes, breaks in serotonergic fibers are likely, which poses an interesting question of what happens to the (often extremely long) distal segment, now disconnected from its cell body. Does it continue to fragment? Could intervaricosity segments degenerate first, thus releasing 5-HT-filled varicosities into the parenchyma?

The problem of single, varicosity-sized packets is actually more complex and beyond the scope of this Viewpoint. However, a brief comment is appropriate. The mammalian brain is filled with microcapillaries that contain blood platelets (Figure 2C). In brain sections not stained for microcapillaries, 5-HT-filled platelets can be nearly indistinguishable from serotonergic varicosities. This visual similarity is not superficial and extends to other shared properties, at the molecular level.[1] Platelets also can grow processes and form chains in development. Importantly, recent studies have shown that, in neuroinflammation, platelets can exit microcapillaries, enter the



DOI: 10.1021/acschemneuro.6b00417
ACS Chem. Neurosci. 2017, 8, 893−896

brain parenchyma, and interact with neural and glial cells through lipid-raft embedded gangliosides and their receptors.[6] Currently, no evidence exists that such transmigration occurs in the healthy brain, but this possibility cannot be easily dismissed because of the regional variability of the blood-brain barrier. Therefore, the presence of a single, varicosity-sized 5-HT packet presents an interpretation problem. It can be a connected varicosity whose intersegments fall below the available resolution, a remnant of a degenerating segment, or a platelet.

In a dense serotonergic meshwork, automated tracing of individual fibers may require knowledge-based algorithms. Once fiber trajectories become digitized, powerful stochastic methods become available for their further analysis. They can be used at various levels of sophistication: from simple discrete walks to self-avoiding walks in heterogeneous materials containing obstacles.

## CONCLUSIONS

The behavior of single serotonergic fibers is important because their trajectories eventually produce well-defined densities of the serotonergic matrix, an emergent structure. A regionally specific density may support the functionally important 5-HT tone, but it may also reflect the local microarchitecture of the tissue (and both can be true at the same time). Experimental and theoretical results suggest that individual fibers can be viewed as stochastic probes of brain tissue, a heterogeneous biomaterial, the function of which may depend not only on its chemical constituents, but also on the integrity of its physical microarchitecture.

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### Notes

The author declares no competing financial interest.

## ACKNOWLEDGMENTS

The author thanks Marcus Vicari and Savannah Starr (members of the laboratory) for assistance with immunohistochemistry and Giovanni Finney-Long (UCSB Department of Computer Science) for piloting image-analysis algorithms for single serotonergic fibers.

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DOI: 10.1021/acschemneuro.6b00417
*ACS Chem. Neurosci.* 2017, 8, 893−896