Int. J. Devl Neuroscience 39 (2014) 9–15

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Serotonin dynamics in and around the central nervous system: Is autism solvable without fundamental insights?

Skirmantas Janušonis*

Department of Psychological and Brain Sciences, University of California, Santa Barbara, CA 93106-9660, USA

ARTICLE INFO

Article history:
Received 20 May 2014
Accepted 20 May 2014

Keywords:
Autism
Serotonin (5-hydroxytryptamine, 5-HT)
Brain
Platelets
Hyperserotonemia
Vasculature

ABSTRACT

Altered serotonin (5-hydroxytryptamine, 5-HT) signaling has been implicated in some developmental abnormalities of autism spectrum disorder (ASD). However, the presumed role of 5-HT in ASD raises new questions in fundamental neuroscience. Specifically, it is not clear if the current piecemeal approach to 5-HT signaling in the mammalian body is effective and whether new conceptual approaches may be required. This review briefly discusses 5-HT production and circulation in the central nervous system and outside of it, especially with regard to ASD, and proposes a more encompassing approach that questions the utility of the "neurotransmitter" concept. It then introduces the idea of a generalized 5-HT packet that may offer insights into possible links between serotonergic varicosities and blood platelets. These approaches have theoretical significance, but they are also well positioned to advance our understanding of some long-standing problems in autism research.

1. Introduction: Autism and fundamental neuroscience

The problem of autism is a proverbial rabbit hole that leads to problems in fundamental neuroscience. Over the past decade, autism spectrum disorder (ASD) has become an ultimate Rorschach test in neurobiology, and the pathologies that have been associated with ASD now cover a vast array of neurobiological processes and brain structures (Rodier, 2002; Courchesne et al., 2007; Amaral et al., 2008; Fatemi et al., 2012; Gadad et al., 2013; Gesundheit et al., 2013; Parellada et al., 2014; Banerjee et al., 2014). Likewise, massive genetic studies have yielded enormous lists of potentially relevant but weakly associated genes (Anney et al., 2010; Betancur, 2011; Lee et al., 2013), the fundamental or practical value of which has yet to be demonstrated. If ASD is defined as a specific applied problem, fundamental neurobiology thus far has contributed surprisingly little to its solution.

The current situation calls for an honest reassessment of where we truly are. A contemporary scientist is an odd cross between an objective observer unbound by the here-and-now and a merchant who needs to sell his/her goods to the public. This can and does work in many science fields, but autism research has been heavily biased toward the latter. Efforts to serve families with autistic children have a noble goal, but they are best left to applied fields seeking to improve interventions, therapies, and treatments. Beyond that, ASD may be simply unsolvable without a much deeper understanding of the key building elements that make this condition possible. This may require major improvements in fundamental neuroscience and may contradict the public's intuition of what is important.

At present an unsettling possibility remains that ASD may be a kind of "limping syndrome" that can be easily observed and approached therapeutically, but that cannot be rigorously studied as a single neurobiological entity. A more optimistic view is that ASD is a solvable problem, but fundamental neuroscience is not ready for it (to paraphrase Paul Erdős' comments on the $3n+1$ conjecture). ASD is likely to be the tip of a large iceberg, but focusing on this visible tip may be a shortsighted approach. Instead, a few fundamental problems have to be addressed directly and immediately. The most obvious one is how normal brains align their neural activities in social interactions. Another one is the interplay between the cognitive and motor systems in the brain (which may lead to major revisions in our current understanding of the basal ganglia and the cerebellum). This brief review focuses on yet another deep problem that thus far has attracted too little attention: the integrated dynamics of serotonin (5-hydroxytryptamine, 5-HT) in the mammalian body.

2. Serotonin in autism

A large number of studies have shown that ethnically diverse groups of autistic individuals have elevated mean 5-HT levels in

* Tel.: +1 805 893 6032; fax: +1 805 893 4303.
E-mail address: skirmantas.janusonis@psych.ucsb.edu

http://dx.doi.org/10.1016/j.ijdevneu.2014.05.009
0736-5748/

blood platelets (Schain and Freedman, 1961; Hanley et al., 1977; Anderson et al., 1987b, 1990, 2002b; Cook, 1996; McBride et al., 1998; Coutinho et al., 2004, 2007; Mulder et al., 2004; Hranilovic et al., 2007, 2008; Melke et al., 2008; Tordjman et al., 2013). This phenomenon, known as the blood or platelet hyperserotonemia of autism, is considered to be one of the most well-replicated findings in biological psychiatry (Anderson, 2002).

The biological causes of the platelet hyperserotonemia remain unknown (Janušonis, 2008), despite the fact that this observation is over half a century old. This surprisingly long-lasting ignorance can be explained, in part, by the fact that the blood is a relatively foreign system to most neuroscientists. Even though the brain has a very dense capillary network (Duvernoy et al., 1981), the anatomical basis for functional MRI imaging, the blood is typically assumed to be on the other side of the blood-brain barrier (BBB) and therefore not immediately relevant to most neurobiological experimentation. This conveniently ignores the fact that the BBB is built after the brain comes into existence during development, and that it is actually a shorthand for a number of barriers that emerge at different developmental times (Wenzel and Felgenhauer, 1976; Virgintino et al., 2004; Ge et al., 2005; Daneman et al., 2010; Liddelow et al., 2013). In the human brain, tight junctions develop prenatally (Mollgard and Saunders, 1986; Bell et al., 1991; Virgintino et al., 2004). In the adult brain, the BBB remains a flexible entity and is better comparable to a country's immigration policies than to the Great Wall of China. The BBB can be dynamically modulated by various environmental factors, such as drugs, stress, and others (Sharma, 2004a). Importantly, the BBB is likely to be affected in ASD (Theoharides and Zhang, 2011; Noriega and Savelkoul, 2014).

Rodent models of hyperserotonemia have found associations between blood 5-HT levels and autistic-like behaviors (Kahne et al., 2002). Further studies have shown related alterations in the oxytocin system (Whitaker-Azmitia, 2005; McNamara et al., 2008; Madden and Zup, 2014). One caveat in these models is that they use 5-methoxytryptamine (5-MT), a non-selective agonist on several 5-HT receptors. While this non-selectivity correctly mimics the action of 5-HT itself, it is not clear if 5-MT is taken up by blood platelets. Currently, there is no evidence that in autism 5-HT levels are also elevated in platelet-free plasma, and some studies have reported the opposite effect (Spivak et al., 2004).

We have shown that a commonly used inbred mouse strain, C57BL/6, shows transiently accelerated brain growth with respect to another inbred mouse strain, BALB/c (Flood et al., 2012). A key difference between these strains is that a polymorphism in the *Tph2* gene leads to a relatively higher 5-HT synthesis rate in the brain of the C57BL/6 strain (Zhang et al., 2004). This transiently accelerated growth is similar in magnitude to that observed in autistic brains (Courchesne et al., 2003). Interestingly, the C57BL/6 strain also shows blood hyperserotonemia with respect to the BALB/c strain, which persists into adulthood (Flood et al., 2012).

Considerably less is known about 5-HT alterations in the brains of autistic individuals. One study has found an altered developmental dynamic of 5-HT synthesis capacity in autistic brains (Chugani et al., 1999), but these results have yet to be independently replicated. A recent study has shown that in autism the density of serotonergic fibers in the cortex is significantly higher than in normally developing brains (Azmitia et al., 2011). Again, a replication of these results in a larger set of brains is important, because the immunohistochemical 5-HT signal is often sensitive to the intensity and length of fixation, a factor that is difficult to control in autopsied human brains. If this finding holds up, it will become one of the most important discoveries in autism research.

In summary, there is little doubt that 5-HT signaling is affected in ASD, perhaps very early in development, but the specifics (or even directions) of these alterations in the central nervous system (CNS) remain elusive. In this regard, the platelet hyperserotonemia of autism is a remarkably robust finding.

3. A traditionalist view: Two 5-HT systems

In order to understand the dynamics of 5-HT in the mammalian body, one can start with an observation that there are two principal sites of 5-HT production: the gastrointestinal system and the brain. Both systems have a tubular organization, which may or may not be coincidental (Veeman et al., 2010). In both systems, 5-HT is synthesized from L-tryptophan, an amino acid. In both systems, 5-HT is typically removed by converting it to 5-hydroxyindoleacetic acid (5-HIAA). Tryptophan can cross the BBB, but its entry into the CNS is limited by the competition among several neutral amino acids (tryptophan is one of them). In contrast, the BBB is generally thought to be impermeable to 5-HT, which implies two virtually independent 5-HT pools in the body: one inside and the other outside the CNS. It is important to note that the non-CNS 5-HT, when released into the general blood circulation, flows through the CNS in the immediate vicinity of neurons, but it is assumed to be unable to escape into the brain parenchyma.

In the CNS, 5-HT is synthesized by serotonergic neurons, most of which are located in the raphe nuclei of the brainstem. In this system, the enzyme that converts tryptophan into 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, is tryptophan hydroxylase 2 (Tph2) (Walther et al., 2003). In the CNS, 5-HT can be detected by (perhaps all) neurons and glial cells (astrocytes, oligodendrocytes, and microglia) (Cohen et al., 1999; Whitaker-Azmitia, 2001; Millan et al., 2008), as well as by endothelial cells, as described later. Serotonergic fibers (axons), originating in the raphe nuclei, spread throughout the brain, which becomes virtually embedded in their meshwork. Even though it is typically assumed that 5-HT is released from serotonergic fibers "diffusely," 5-HT signaling among neurons may occur through conventional synapses (Papadopoulos et al., 1987; Parnavelas and Papadopoulos, 1989). Extracellular 5-HT is pumped by the 5-HT transporter (SERT) back into serotonergic fibers, where the 5-HT may be recycled. The extracellular 5-HT concentration in the brain is low and in the rat rostral raphe nuclei may not usually exceed 2–8 nM. This concentration may not be high enough to activate 5-HT_{1A} autoreceptors unless 5-HT levels become excessive (Adell et al., 2002). It has been reported that in development some neurons may express tryptophan hydroxylase 1 (Tph1) (Nakamura et al., 2006; Manjarrez-Gutierrez et al., 2012), but these observations may need further validation (Hale et al., 2011).

Developing thalamocortical neurons (and some other non-serotonergic neurons) can transiently express SERT and take up 5-HT, even though they themselves do not synthesize 5-HT (Lebrand et al., 1996, 1998). The exact pattern of the transient SERT expression shows species-specific variation (Lebrand et al., 2006).

In the CNS, 5-HT is converted to 5-HIAA that enters the cerebrospinal fluid (CSF) and can be measured in lumbar puncture samples. The concentration of 5-HIAA in the human CSF has been estimated to be around 122 nM (Narayan et al., 1993). These 5-HIAA levels can be used to indirectly assess 5-HT function in the normal and autistic CNS (Anderson et al., 1988; Narayan et al., 1993). It has been suggested that CSF 5-HT levels, when analyzed with great care to minimize blood contamination, may provide a more direct and accurate measure of extracellular 5-HT in the CNS (Anderson et al., 2002a). The concentration of 5-HT in the CSF of *Macaca mulatta*, a non-human primate, has been estimated to be around 87 ng/L (Anderson et al., 2002a).

Outside the CNS, most 5-HT is synthesized by gut enterochromaffin (EC) cells, with some contribution from neurons in the enteric nervous system (ENS). Over 95% of the body 5-HT is in the