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Behavioral and Physiological Consequences of Adult Brain 5-HT Depletion in Mice

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Review of Whitney et al.

Serotonergic neurons from the dorsal raphe innervate the amygdala, the mPFC, and the hippocampus, suggesting they have a substantial role in the modulation of emotional states (Lowry et al., 2005). Consistent with this hypothesis, serotonin (5-HT) dysfunction is a key feature of several psychiatric disorders. Therefore, expanding our understanding of the serotonergic system may provide insight into the mechanisms underlying these diseases.

Animal models are useful in revealing how circuits, physiology, and behavior are affected by 5-HT depletion. Several of these models involve pharmacological or genetic manipulation of two rate-limiting enzymes involved in 5-HT biosynthesis: tryptophan hydroxylase (Tph) and aromatic amino acid decarboxylase (AADC). Tph converts the amino acid tryptophan to 5-HT, which is then converted to 5-HT by AADC. Although such models have provided valuable insight into 5-HT functions, most of the methods used to date have been difficult to interpret because 5-HT was depleted in the periphery, as well as in the brain tissue, because other

monoamine signaling pathways were also affected or because 5-HT was depleted throughout development rather than selectively in adult neurons.

To avoid these problems, Whitney et al. (2016) developed a new strategy for depleting 5-HT selectively in the serotonergic ascending pathway of adult mice. Briefly, they injected adeno-associated virus-expressing Cre recombinase (AAV-Cre) into the serotonergic anterior raphe nuclei of mice in which the brain-specific Tph isoform, Tph2, was flanked by loxP sites (*Tph2^{fl/fl}* and *Tph2^{fl/-}*). This allowed deletion of Tph2 selectively in the serotonergic nuclei. This method almost completely eliminated Tph2 and 5-HT immunoreactivity in the dorsal raphe nucleus. Importantly, AADC, norepinephrine, and dopamine levels were unaffected, at least at 2 weeks after injection.

The disruption of the 5-HT machinery selectively in serotonergic neurons not only confirmed previous findings regarding its role in sleep–wake cycles and locomotor activity (Alenina et al., 2009), but also revealed novel physiological and behavioral consequences. Although classical measures of anxiety-like behavior (i.e., time spent in bright/unprotected vs dark/protected areas) were not affected by 5-HT depletion, a marked increase in locomotor activity in the open field was found. Moreover, compared with control, 5-HT-depleted mice spent less time in inactive behaviors in the home cage and

more time in active behaviors during both the active (dark) and inactive (light) phase of the day. These data rule out the novelty-induced hyperlocomotion and indicate a hyperactive phenotype. Furthermore, 5-HT-depleted mice showed also an advanced onset and a delayed offset of their daily activity, suggesting alteration in their circadian activity pattern. Average activity time across the day showed that, although overall activity of 5-HT-depleted mice was increased, it was significantly higher only at specific times, confirming that the increase in activity was not simply the result of an extended active phase. Most notably, the authors found that the brief period of null activity during the active phase of the day, known as siesta, was absent in 5-HT-depleted mice. These data strongly support the role of brain 5-HT in the regulation of activity and maintaining the normal circadian behavior.

These results are interesting given that a link between reduced extracellular 5-HT levels and susceptibility for attention-deficit hyperactivity disorder (ADHD) has been proposed (Banerjee and Nandagopal, 2015). Indeed, patients carrying a polymorphism of the Tph2 gene show higher susceptibility to develop ADHD (Sheehan et al., 2005). Accordingly, mice lacking the 5-HT transporter show higher 5-HT extracellular levels (Kim et al., 2005) and lower locomotor activity than controls (Kalueff et al., 2007). The hyperactive phenotype described by Whitney et al.

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(2016) is consistent with these previous results and add strong support for a role of 5-HT in the regulation of locomotor activity and for serotonergic involvement in ADHD.

The hyperactive phenotype reported by Whitney et al. (2016) in 5-HT-depleted mice was not accompanied by changes in anxiety-like behavior in the open field, elevated plus maze, and light/dark box tests. This finding is surprising, considering that genetic and pharmacological mouse models targeting the 5-HT transporter (Kalueff et al., 2007), specific 5-HT receptors (Ramboz et al., 1998), or 5-HT depletion (Näslund et al., 2013), have consistently shown alterations in anxiety-like behavior. Moreover, dense serotonergic projections from the dorsal raphe reach the amygdala, where 5-HT modulates the activity of GABAergic interneurons (Woodruff and Sah, 2007), suggesting that 5-HT neurons modulate amygdala activity. It has been shown that glutamate and 5-HT corelease by dorsal raphe neurons signal reward (Liu et al., 2014). Investigating whether a similar mechanism is involved in the modulation of amygdala activity by 5-HT neurons would help explain the unaltered anxiety-like behavior of adult 5-HT-depleted mice. It is worth mentioning that, in the study by Whitney et al. (2016), not all 5-HT nuclei were targeted. However, the near absence of forebrain 5-HT in these mice makes it unlikely that spared nuclei contributed to the observed behavior in anxiety tests. Nonetheless, the modulatory role of 5-HT in adult anxiety-like behavior seems to be more relevant during development than in the adult brain (Yu et al., 2014). In this sense, the effectiveness of SSRI in depression and anxiety seen in adult subjects may be explained by neuroplastic changes accompanying the increase in 5-HT synaptic availability.

The extension of the active period in 5-HT-depleted mice described by Whitney et al. (2016) confirms the role of 5-HT in maintaining the normal circadian rhythm (Ciarleglio et al., 2011). Interestingly, Whitney et al. (2016) also found that 5-HT depletion suppressed breaks during nocturnal activity (siestas). The latter adds strong support for a role of 5-HT in the regulation of the sleep–wake cycle, particularly in the promotion and maintenance of sleep. These data led Whitney et al. (2016) to suggest that 5-HT dysfunction is an important factor for circadian/sleep disturbances seen in neuropsychiatric disorders (Jagannath et al., 2013). However, the effects of 5-HT defi-

ciency in sleep architecture and in sleep-related neural oscillatory activity remain to be known.

Complementing the behavioral results obtained by Whitney et al. (2016) with *in vivo* electrophysiological recordings would help to address open questions. First, it would be interesting to test whether circuit level alterations take place following 5-HT depletion, which may account for the observed phenotype. Neurophysiological changes within the nucleus accumbens have been shown in *Clock-Δ19* mice (Dzirasa et al., 2010), which may account for their hyperactive phenotype. The same group reported abnormal functional interactions in the mPFC-amygdala circuit in a mouse model of 5-HT deficiency with depressive-like behavior (Dzirasa et al., 2013). Analogously, it would be interesting to test whether 5-HT depletion is accompanied by modifications in specific neural circuits. In addition, given that mechanisms of sleep disturbances accompanying neuropsychiatric disorders related to 5-HT hypofunction are largely unknown, chronic *in vivo* recordings during sleep will be useful to test whether sleep-related neural oscillations or sleep architecture are modified in 5-HT-depleted mice, as has been described for other models of 5-HT dysfunction (Wisor et al., 2003).

Subsequent studies using the approach described by Whitney et al. (2016) in other behaviors will surely reveal valuable data regarding the role of 5-HT in the adult brain. For example, cognitive inflexibility (Clarke et al., 2004) was observed after pharmacological 5-HT depletion, whereas improved reversal learning was observed in SERT knock-out mice (Brigman et al., 2010), suggesting a modulatory role of 5-HT on cognitive performance. On the other hand, mechanisms of psychiatric disorders involving 5-HT deficiency would be better investigated by partial, rather than severe, 5-HT depletion. For example, mice with a less efficient *Tph2* knockin show reductions in extracellular 5-HT levels ~60%–80%; they exhibit increased latency to cross to lit area in the light–dark box test and increased marble burying, both indicative of anxiety-like behavior (Jacobsen et al., 2012). Therefore, reduced but preserved 5-HT signaling following adult brain 5-HT partial depletion would avoid the effects of 5-HT absence during development while keeping within the pathophysiological frame of depression and anxiety-related disorders.

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