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5-HT$_{1A}$ Receptor-Mediated Autoinhibition and the Control of Serotonergic Cell Firing

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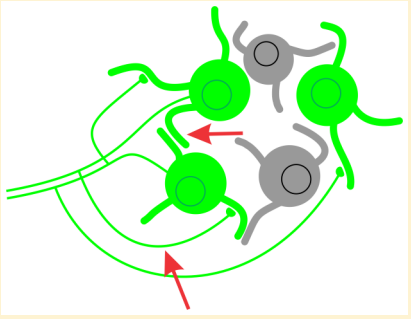
ABSTRACT: The idea that serotonergic synaptic transmission plays an essential role in the control of mood and the pharmacotherapy of anxiety and depression is one of the cornerstones of modern biological psychiatry. As a result, there is intense interest in understanding the mechanisms controlling the activity of serotonin-synthesizing (serotonergic) neurons. One of the oldest and most durable ideas emerging from this work is that serotonergic neurons are capable of autonomously regulating their own basal firing rate. Serotonergic neurons express on their surface 5-HT$_{1A}$ receptors (autoreceptors) that, when activated, induce the opening of potassium channels that hyperpolarize and thereby inhibit cell firing. Activity-dependent release of serotonin within serotonergic nuclei is thought to activate these autoreceptors, thus completing an autoinhibitory feedback loop. This concept, which was originally proposed in the 1970s, has proven to be enormously fruitful and has guided the interpretation of a broad range of clinical and preclinical work. Yet, remarkably, electrophysiological studies seeking to directly demonstrate this phenomenon, especially in *in vitro* brain slices, have produced mixed results. Here, we critically review this work with a focus on electrophysiological studies, which directly assess neuronal activity. We also highlight recent work suggesting that 5-HT$_{1A}$ receptor-mediated autoinhibition may play other roles in the control of firing besides acting as a feedback regulator for the pacemaker-like firing rate of serotonergic neurons.



KEYWORDS: *Serotonin, 5-HT$_{1A}$ autoreceptors, Raphe nuclei, autoinhibition, firing rate*

Serotonergic neurons constitute a very small fraction of the neurons in the mammalian brain and are specifically localized to a series of midline brainstem structures collectively known as the Raphe nuclei.[1] These neurons appear to be divided into two main groups, a large rostral group and a smaller caudal cell group,[2] which, in turn, can be subdivided further based on their location[3] or embryological origin.[4] The serotonergic innervation of the forebrain, including the cerebral cortex, originates predominantly from the rostral cell group and especially from the serotonergic neurons located in the dorsal raphe nucleus (DRN). These cells are derived mostly from rhombomere 1[4] and represent a large group of serotonergic neurons, features that have greatly facilitated their study. Consequently, our understanding of the cellular physiology of serotonergic neurons is based overwhelmingly on the neurons of this cell group.

CELL FIRING AND THE MACHINERY OF SEROTONERGIC AUTOINHIBITION IN THE DRN

In anesthetized rodents, serotonergic neurons of the DRN fire at relatively low frequencies, generally on the order of a 1−3 action potentials per second, in what is often described as a pacemaker-like firing pattern[5,6] (reviewed in ref 1). For the purposes of study, this firing has often been integrated and averaged over multisecond epochs[5−7] and referred to as the serotonergic cell firing rate. A similar pattern of activity is recorded from DRN serotonergic neurons in freely moving animals, although under these conditions their firing rate can be seen to depend on the sleep−wake−arousal cycle of the animal.[8−10] Recent studies have additionally shown that in freely moving animals the spontaneous firing of serotonergic neurons exhibits phasic changes in response to behaviorally relevant contingencies over a time frame much briefer than that used to determine the overall cell firing rate.[11−14]

Serotonergic neurons, in spite of their spontaneous activity seen *in vivo*, are not intrinsic pacemakers but rather depend on extrinsic inputs to drive their firing. In anesthetized animals, serotonergic cell firing requires noradrenergic inputs acting on α_1-adrenergic receptors and depends only marginally on fast excitatory glutamate-mediated synaptic inputs.[15−17] Activation of α_1-adrenergic receptors on serotonergic neurons of the DRN elicits a robust depolarization/inward current.[18] Thus, it seems likely that in anaesthetized animals, where serotonin neurons receive a reduced excitatory synaptic drive,[19] serotonin cell firing is sustained by the membrane depolarization elicited by the activation of α_1-adrenergic receptors. In contrast, in freely moving animals, serotonin cell firing appears to be mostly independent of α_1-adrenergic receptor activation.[20] In this case, multiple factors, probably including phasic synaptic inputs,[8,14,19] likely interact to determine the timing and overall frequency (firing rate) of serotonergic cell firing.

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Serotonergic neurons of the DRN express both 5-HT_{1A} and 5-HT_{1B} receptors, which are often referred to as autoreceptors. 5-HT_{1A} autoreceptor expression is restricted to the somatodendritic compartment of serotonergic neurons, whereas 5-HT_{1B} autoreceptors are strongly targeted to axonal terminals.[21,22] Administration of serotonin to serotonergic neurons of the DRN activates 5-HT_{1A} autoreceptors to elicit an outward current carried through potassium channels of the Kir3 (GIRK) family (reviewed in ref 23). Activation of this potassium current results in a membrane hyperpolarization and the inhibition of serotonergic neuron firing. Importantly, *in vivo* and *in vitro* fast-scan cyclic voltammetry studies have demonstrated stimulus-evoked serotonin release in the DRN,[24−27] whereas electrophysiological studies have shown that the stimulus-evoked release of endogenous serotonin can activate 5-HT_{1A} autoreceptors to elicit a robust membrane hyperpolarization.[28,29] All together, these results leave little doubt that serotonergic neurons can engage in 5-HT_{1A} receptor-mediated autoinhibition. However, exactly how this 5-HT_{1A} receptor-mediated autoinhibition regulates the neuronal activity of serotonergic neurons remains poorly understood.

5-HT_{1A} RECEPTOR-MEDIATED AUTOINHIBITION AND THE REGULATION OF SEROTONIN CELL FIRING IN THE DRN

The initial and most durable idea on the functional role of 5-HT_{1A} receptor-mediated autoinhibition in the DRN originates in the seminal work of Aghajanian and colleagues[30] during the 1960s and 1970s. This work, which was conducted entirely *in vivo*, showed that serotonergic neuron cell firing appeared to be homeostatically regulated *in vivo* such that manipulations that increase the synaptic availability of serotonin inhibit serotonergic neuronal firing rate, whereas manipulations that decrease the synaptic availability of serotonin accelerate firing rates. In a parallel set of experiments, the same group showed that serotonin cells were themselves inhibited by serotonin and that antidromic activation of the serotonergic neurons of the DRN resulted in a period of postactivation inhibition that appeared to be serotonin-dependent.[31,32] These results were interpreted to indicate that serotonergic neurons engaged in serotonin-mediated autoinhibition. At a mechanistic level, this autoinhibition was originally envisioned to be mediated through axonal recurrent collaterals (Figure 1A). Subsequent work using electron microscopy, however, revealed the existence of serotonin and of synaptic vesicles in the serotonergic cell dendrites.[33−35] This led to the suggestion that autoinhibition could also be mediated via dendritic release of serotonin (Figure 1A).

Thus, by the early 1980s, an extensive body of work supported the notion that serotonin neuron firing rate was homeostatically regulated. Similarly, a strong consensus had emerged that serotonin neurons could engage in serotonin-mediated self-regulation. At the time, these two phenomena were assumed to be linked, and the idea took hold that serotonergic autoinhibition was a key feedback control mechanism regulating the pacemaker-like firing rates of serotonergic neurons. In retrospect, while acknowledging the enormous insights that emerged from this early work, we can see limitations in this functional conclusion. Most notably, while serotonin autoinhibition could, in principle, act as a feedback control to homeostatically set the pacemaker-like firing of serotonergic neurons, this does not mean that it does so. Unfortunately, the experimental approaches available at the time, coupled with the lack of selective 5-HT_{1A} receptor antagonists, made it difficult to test these ideas.

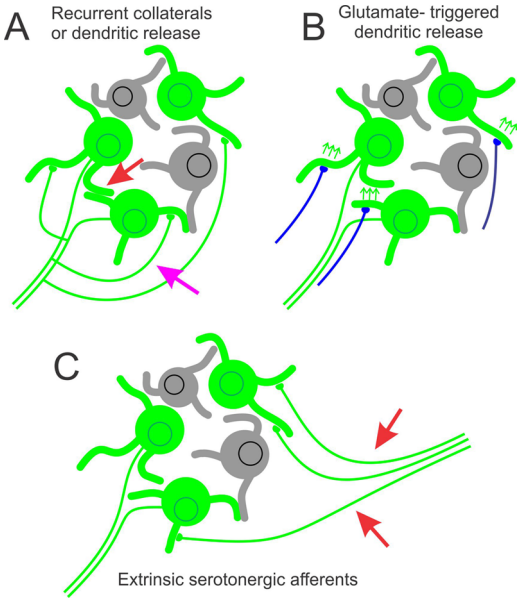

Figure 1. 5-HT_{1A} receptor-mediated autoinhibition in the DRN: some possible models. The DRN contains both serotonergic neurons (green) and nonserotonergic neurons (gray). (A) The traditional model proposes that serotonin autoinhibition is activity-dependent and is mediated through serotonin release from axonal collaterals (pink arrow) or dendrites (red arrow). (B) An alternative model proposes that serotonin is released secondary to local dendritic depolarizations elicited by glutamatergic synapses[51,52] (blue). (C) Finally, it is possible that serotonergic autoinhibition may reflect serotonergic afferents to the DRN from caudal serotonergic cell groups[54] (red arrow).

DOES 5-HT_{1A} RECEPTOR-MEDIATED AUTOINHIBITION REGULATE FIRING RATE: *IN VIVO* STUDIES

One of these limitations was removed in the 1990s, with the development of effective 5-HT_{1A} receptor antagonists.[36,37] The question of 5-HT_{1A} receptor control of firing rate was explicitly reexamined by Fornal et al. in the mid 1990s by taking advantage of the availability of the potent and selective 5-HT_{1A} receptor antagonist WAY-100635.[38] Using extracellular recordings in freely moving cats, they showed that blockade of 5-HT_{1A} receptors, which was ascertained by antagonism of exogenous 8-OHDPAT, resulted in a modest increase in firing rate in animals in a quiet waking state but surprisingly not in slow wave sleep. This remarkable state-dependent effect of WAY-100635 suggested a complex role for 5-HT_{1A} receptors in the regulation of firing rate, perhaps related to different mechanisms driving spontaneous firing during these different behavioral states (see above).

A drawback of the use of 5-HT_{1A} receptor antagonists is that they block 5-HT_{1A} autoreceptors as well as 5-HT_{1A} receptors located on postsynaptic neurons. This limitation, however, can be bypassed by using modern genetic approaches that allow for the selective targeting of 5-HT_{1A} autoreceptors in serotonergic neurons. In an elegant study, Richarson-Jones et al. used such approaches to generate two mouse strains differing in 5-HT_{1A} autoreceptor expression by approximately 30% (1A-High and 1A-Low).[39] As part of a large battery of tests, this group

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compared the firing rate of DRN neurons in these two strains using extracellular recordings in anesthetized animals.[39] If 5-HT_{1A} receptor-mediated autoinhibition homeostatically regulates firing rate, then the expectation would be that serotonin neurons in 1A-High mice should exhibit slower firing rates than cells in the 1A-low mice. Consistent with this expectation, neurons in the 1A-Low mouse exhibited higher firing rates than those seen in 1A-High mice. This would appear to provide strong support for the idea that 5-HT_{1A} autoreceptors regulate firing rate. However, it is important to note that the increase in firing rate detected in 1A-Low mice resulted not from an overall increase in firing but from the appearance of a subpopulation of fast firing neurons, whereas the remaining cells fired in the same range as those in the 1A-High mouse. This could reflect mosaicism, but it is striking that the firing rate of the fast firing cells in the 1A-Low mouse is anomalously high and falls well outside firing rates normally associated with serotonergic neurons, even after 5-HT_{1A} receptor blockade or 5-HT_{1A} receptor deletion.[38,40,41] The identification of serotonergic neurons in *in vivo* recordings is not trivial and becomes especially difficult if two of the essential diagnostic criteria, slow regular firing and sensitivity to serotonin, can no longer be relied upon for identification, as was the case in the Richardson-Jones et al. study.[39] This raises the possibility that the 1A-Low cell sample could have included faster-firing nonserotonergic neurons. Consistent with this possibility, extracellular serotonin levels measured using dialysis in the hippocampus and frontal cortex, a proxy for serotonin cell firing rate, did not differ in these two mice strains.[39] Clearly, these electrophysiological studies need to be replicated and expanded using a physiological tag to unambiguously identify serotonergic neurons.[42] Nevertheless, in our view, in aggregate these studies contain sufficient anomalies to question the idea that they provide unambiguous support for the traditional view that 5-HT_{1A} receptor-mediated autoinhibition functions simply as a negative feedback for the homeostatic regulation of the pacemaker-like firing rate of serotonergic neurons in the DRN.

DOES 5-HT_{1A} RECEPTOR-MEDIATED AUTOINHIBITION REGULATE FIRING RATE: *IN VITRO* STUDIES

An alternative approach for the study of the relationship between 5-HT_{1A} receptor-mediated autoinhibition and pacemaker-like firing rate emerged in the 1990s with the maturation of *in vitro* brain slice methodologies. The power of this approach is that, by isolating the serotonergic neurons of the DRN from their targets, it allows for the direct examination of the control of cell firing through local mechanisms, including 5-HT_{1A} receptor-mediated autoinhibition. A complication with this electrophysiological approach, however, is that serotonergic neurons in *in vitro* brain slices are electrically quiescent, the result of their deafferentation and loss of noradrenergic tone onto α_1-adrenergic receptors (see above). Therefore, initial studies relied on the addition of the α_1-adrenergic agonist phenylephrine (PE) to re-establish spontaneous pacemaker-like firing. The consensus at the time was that, upon activation, serotonergic neurons would engage in autoinhibition that should curtail their firing rate. This autoinhibition should then be detectable as an acceleration of the cell firing rates upon administration of 5-HT_{1A} receptor antagonists. This conjecture became testable with the development of 5-HT_{1A} receptor antagonists such as WAY-100635. Surprisingly, blockade of 5-HT_{1A} autoreceptors in DRN brain slices was found by multiple groups to have essentially no effect on the firing of the serotonergic neurons[37,43,44] (reviewed in ref 45). This result was clearly inconsistent with the idea that 5-HT_{1A} receptor-mediated autoinhibition is part of the intrinsic machinery controlling the pacemaker-like firing rate of serotonergic neurons.

One explanation for these unexpected results could be that they represent an artifact of *in vitro* brain slices. Three groups argued for this possibility and, more specifically, that the failure to detect 5-HT_{1A} autoreceptor-mediated feedback regulation of serotonergic cell firing reflected a loss of serotonin synthesis (and hence serotonin) in this preparation.[45−47] Support for this hypothesis came from a demonstrable time-dependent loss of serotonin in brain slices.[45−47] Importantly, this loss was palliated by adding tryptophan to the bath, a manipulation that itself results in an inhibition of firing, as expected if tryptophan rescued autoinhibition by enabling serotonin synthesis. Furthermore, tryptophan-induced inhibition was reversed by the 5-HT_{1A} receptor antagonist WAY-100635, consistent with the idea that tryptophan supplementation enabled 5-HT_{1A} receptor-mediated autoinhibition which, in turn, acts as a negative feedback on firing. While these results are highly suggestive, the need for conversion of tryptophan to serotonin appears to have never been directly tested experimentally, and electrochemical studies in *in vitro* brain slices have consistently shown serotonin release without tryptophan supplementation.[24−29] However, the main limitation with this idea, as discussed above, is the absence of the predicted robust increase in firing rate in response to WAY-100635. In our view, these findings cast doubt on the idea that loss of serotonin in slice preparations can account for the failure to observe robust 5-HT_{1A} autoreceptor-mediated regulation of firing rates.

The failure to detect the predicted robust effect of 5-HT_{1A} autoreceptors on firing rate in slices could alternatively be due to the reliance in these experiments on α-$_1$ adrenergic receptors to elicit repetitive firing in serotonergic neurons. These receptors signal through $G\alpha_{q\text{-}11}$ to activate phospholipase Cβ, leading to the breakdown of PtdIns(4,5)P_2. This phospholipid, however, is essential for the function of Kir3 (GIRK) channels downstream from the 5-HT_{1A} autoreceptors[48,49] (and references therein), and previous studies have shown that Kir3 (GIRK) channel activity is suppressed by activation of $G\alpha_{q\text{-}11}$-coupled receptors.[50] Therefore, it is possible that the reliance on PE to activate serotonergic cells resulted in an impairment in the ability of 5-HT_{1A} autoreceptors to activate Kir3 (GIRK) channels and hence enact autoinhibition. We have recently tested this possibility by determining the effect of PE on serotonin-induced (i.e., 5-HT_{1A} autoreceptor-mediated) outward currents in serotonergic neurons of the DRN (Figure 2A,B). Administration of PE elicited only a very small, albeit statistically significant, effect on the amplitude of serotonin-induced currents. This suggests to us that PtdIns(4,5)P_2 depletion is also unlikely to account for the difficulty in detecting a robust effect of 5-HT_{1A} receptor-mediated autoinhibition on firing rate.

A third possibility is that the prolonged application of PE typically used in experiments examining the regulation of firing rate may have inadvertently resulted in the depletion of releasable serotonin in the slice. In experiments examining firing rate, neurons can be expected to fire many hundreds of action potentials, which could, in principle, lead to the depletion of the pool of serotonin-containing vesicles available

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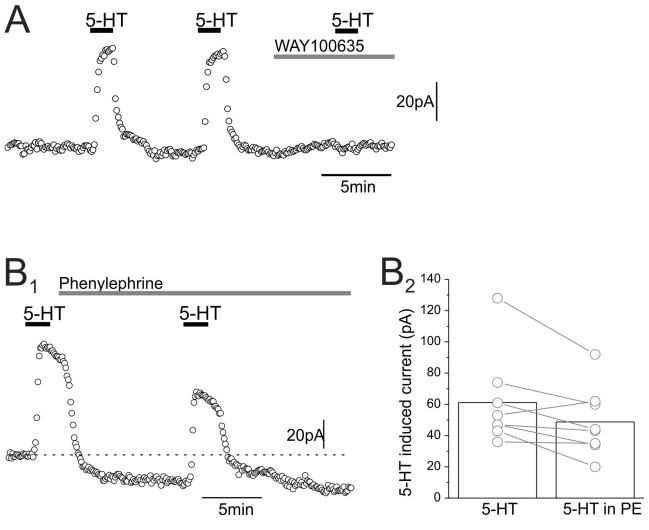

Figure 2. Effect of 5-HT and PE on serotonergic neurons of the DRN. Whole-cell recordings were obtained from serotonergic neurons of the DRN in mouse brain slices. (A) Bath application of 5-HT (10 μM) elicits an outward current that is completely blocked by WAY-100635 (1 μM), indicating that it is mediated by 5-HT$_{1A}$ autoreceptors. (B$_1$, B$_2$) Administration of PE (10 μM) induces an inward current and also a small but statistically significant (p = 0.04) reduction in the amplitude of the outward current elicited by 5-HT (30 μM).

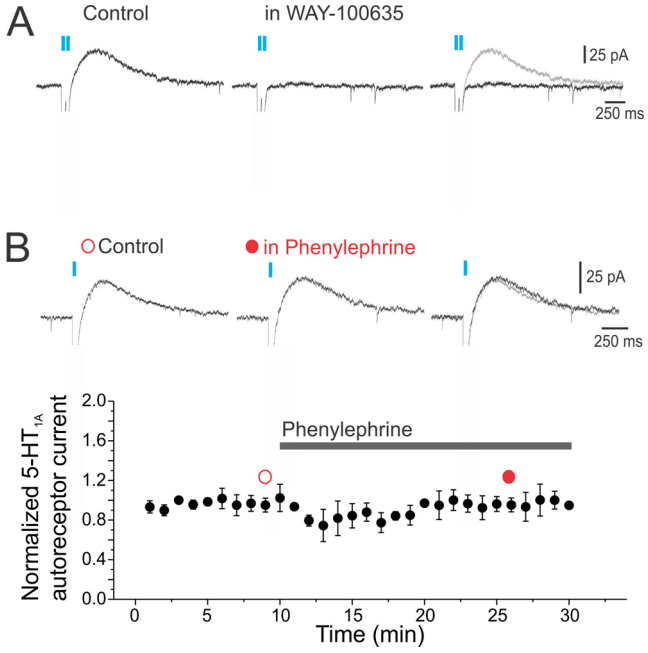

Figure 3. Effect of PE on 5-HT$_{1A}$ autoreceptor-mediated synaptic currents in serotonergic neurons of the DRN. Whole-cell recordings were obtained from brain slices derived from a mouse expressing ChR in serotonergic neurons (SERT-Cre driver [MMRRC] × Ai32 ChR reporter [Jackson Laboratories]). (A) Light stimulation (blue bars) elicits transient (ChR) inward currents that are followed by a slower transient outward current. This outward current is blocked by WAY-100635 (3 μM), indicating it is mediated by the activation of 5-HT$_{1A}$ autoreceptors. (B) Administration of PE (10 μM) has no detectable effect on these 5-HT$_{1A}$ autoreceptor-mediated synaptic currents. In separate experiments, we confirmed that PE elicits a robust inward current and serotonin cell spiking under our recording conditions (not shown).

for release. Presumably, this would not happen in experiments directly assessing serotonin release since those experiments use vastly lower stimulation frequencies. We have recently examined the stability of 5-HT$_{1A}$ receptor-mediated autoinhibition after PE administration by taking advantage of optogenetic approaches that allow for the selective excitation of serotonergic cellular elements in brain slices using light. As illustrated in Figure 3A, repeated light stimulation of DRN slices derived from mice expressing channelrhodopsin-2-(H143R)(ChR) in serotonergic neurons resulted in transient inward currents (ChR currents) followed by slower, outward currents mediated by the activation of 5-HT$_{1A}$ autoreceptors. We used these light-evoked 5-HT$_{1A}$ autoreceptor-mediated responses, which reflect synaptic release of endogenous serotonin, to assess the possible depletion of serotonin when slices are stimulated with PE. As illustrated in Figure 3B, administration of PE, which should have resulted in activation of the serotonergic neurons of the DRN, failed to elicit a significant reduction in the amplitude of the 5-HT$_{1A}$ autoreceptor currents. These experiments suggest that neither serotonin depletion nor loss of autoinhibition due to PtdIns(4,5)P$_2$ depletion likely account for the failure to observe 5-HT$_{1A}$ autoreceptor control of firing rates in brain slices stimulated with PE.

POSSIBLE ALTERNATIVE ROLES FOR 5-HT$_{1A}$ RECEPTOR-MEDIATED AUTOINHIBITION IN THE DRN

The *in vivo* and *in vitro* results reviewed above indicate that serotonergic neurons are capable of robust 5-HT$_{1A}$ receptor-mediated autoinhibition. At the same time, in our opinion, they do not support the idea that 5-HT$_{1A}$ receptor-mediated autoinhibition functions as a homeostatic feedback mechanism regulating the pacemaker-like firing rate of serotonergic neurons. Could 5-HT$_{1A}$ receptor-mediated autoinhibition have other roles in serotonergic neurons?

One possibility is that 5-HT$_{1A}$ receptor-mediated autoinhibition may regulate serotonin cell activity by regulating synaptic inputs to the DRN or perhaps even synchronizing neuronal activity within DRN networks. Of course, such actions could manifest themselves as changes in firing rate under some conditions, which could have contributed to a misinterpretation of their role, but they would not be part of a homeostatic feedback loop controlling the spontaneous pacemaker firing of these cells.

One specific implementation of these ideas emerges from the work of de Kock and colleagues,[51] who suggested that serotonin is released from dendrites following calcium influx through NMDA receptors opened in response to glutamate-mediated synaptic events (Figure 1B). A variant of this mechanism has been proposed by Colgan, Levitan, and associates,[52,53] who have argued that serotonin release from dendrites is secondary to calcium influx through L-type calcium channels that open in response to the local dendritic depolarization elicited by synaptically released glutamate. Both of these mechanisms postulate that serotonin release, and hence 5-HT$_{1A}$ receptor-mediated autoinhibition, is engaged by excitatory glutamatergic inputs to the DRN. As such, they posit that 5-HT$_{1A}$ receptor-mediated autoinhibition is engaged by glutamate synaptic inputs to the DRN, via locally triggered calcium influx, rather than by neuronal firing. This offers a parsimonious hypothesis that uncouples 5-HT$_{1A}$ receptor-mediated autoinhibition from the regulation of pacemaker

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firing and, in doing so, reconciles most previous experimental observations to date. However, one problem with this hypothesis is that electrically evoked serotonin release persists in the presence of AMPA and NMDA receptor blockers,[28,29] thus suggesting that glutamate synaptic transmission is not necessary for stimulus-evoked serotonin release in the DRN. Of course, electrical stimulation may itself directly depolarize the dendrites, thus obviating the need for glutamate receptor activation. Thus, at this point, it is difficult to fully evaluate this hypothesis on the evidence published to date.

A second alternative emerges from studies showing that serotonergic cell groups can be interconnected. The DRN in particular has been shown to receive serotonergic inputs from the caudal Raphe nuclei.[54,55] Thus, it is possible that serotonin release in the DRN may not originate from DRN serotonergic neurons but rather from extrinsic serotonergic afferents (Figure 1C).[54,55] This would explain both the presence of serotonin release in the DRN as well as the difficulty observing robust involvement of 5-HT_{1A} autoreceptors in the regulation of firing rate.

CONCLUSIONS

Historically, 5-HT_{1A} receptor-mediated autoinhibition in the DRN has been thought to function as a homeostatic feedback mechanism controlling the pacemaker-like firing, the so-called firing rate, of serotonergic neurons. However, evidence in support of this hypothesis is limited, and there is considerable evidence, especially from *in vitro* brain slice work, that is not consonant with this idea. As outlined above, we believe that accumulating evidence hints at alternative roles for 5-HT_{1A} receptor-mediated autoinhibition in the DRN beyond the homeostatic control of firing rate, although alternate theories will require further investigation. For example, it is possible that 5-HT_{1A} autoinhibition plays a role in regulating glutamate signaling to serotonin neurons or mediates inputs from distal serotonergic cell groups or yet other possible functions. Such effects would be hard to identify in brain slices, or even in anesthetized animals, which could explain the difficulties assigning a functional role to 5-HT_{1A} receptor autoinhibition. Fortunately, sophisticated tools are quickly becoming available to address synaptic organization experimentally and for manipulating the *in vivo* activity of serotonergic neurons. We anticipate that advances along these fronts will open new avenues for continuing to test the hypotheses discussed above and for uncovering new hypotheses regarding the functional role of 5-HT_{1A} receptor-mediated autoinhibition in the serotonergic system.

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Notes

The authors declare no competing financial interest.

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