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Endocannabinoids blunt the augmentation of synaptic transmission by serotonin 2A receptors in the nucleus tractus solitarii (nTS)

James R. Austgen and David D. Kline

Department of Biomedical Sciences & Dalton Cardiovascular Research Center, University of Missouri, 134 Research Park Dr., Columbia, MO, 65211

Abstract

Serotonin (5-Hydroxytryptamine, 5-HT) and the 5-HT₂ receptor modulate cardiovascular and autonomic function in part through actions in the nTS, the primary termination and integration point for cardiorespiratory afferents in the brainstem. In other brain regions, 5-HT₂ receptors (5-HT_{2R}) modify synaptic transmission directly, as well as through 5-HT_{2AR}-induced endocannabinoid release. This study examined the role of 5-HT_{2AR} as well as their interaction with endocannabinoids on neurotransmission in the nucleus tractus solitarii (nTS). Excitatory postsynaptic currents (EPSCs) in monosynaptic nTS neurons were recorded in the horizontal brainstem slice during activation and blockade of 5-HT_{2AR}s. 5-HT_{2AR} activation augmented solitary tract (TS) evoked EPSC amplitude whereas 5-HT_{2AR} blockade depressed TS-EPSC amplitude at low and high TS stimulation rates. The 5-HT_{2AR}-induced increase in neurotransmission was reduced by endocannabinoid receptor block and increased endogenous endocannabinoids in the synaptic cleft during high frequency, but not low, TS stimulation. Endocannabinoids did not tonically modify EPSCs. These data suggest 5-HT acting through the 5-HT_{2AR} is an excitatory neuromodulator in the nTS and its effects are modulated by the endocannabinoid system.

Keywords

autonomic nervous system; neuromodulator crosstalk; serotonin receptors

1. INTRODUCTION

The nucleus tractus solitarii (nTS) in the dorsomedial brainstem serves as the central termination site for multiple visceral afferents. While glutamate has been established as the primary neurotransmitter at the sensory afferent-nTS cell synapse, other neuromodulators influence its release and postsynaptic conductance. Serotonin (5-hydroxytryptamine, 5-HT)

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Corresponding Author: David D. Kline, PhD, Dalton Cardiovascular Research Center, 134 Research Park Dr., University of Missouri, Columbia, MO, 65211, Phone: (573) 884-0505, Fax: (573) 884-4232, klinedd@missouri.edu.

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is a neuromodulator in the nTS and adjacent dorsal motor nucleus of the vagus that alters presynaptic glutamate release or postsynaptic activity (Browning and Travagli, 1999; Cui et. al. 2012; Feldman, 1994; Feldman, 1995).

Several 5-HT receptors, including the 5-HT₂ subtype (Raul, 2003), have shown to play a physiological role in the dorsal vagal complex, which contains the nTS and dorsal motor nucleus of the vagus. We have recently demonstrated that the 5-HT_{2C} receptor (5-HT_{2C}R) is produced in nTS, tonically augments sensory afferent neurotransmitter release, and depolarizes neurons through inhibition of postsynaptic potassium currents (Austgen et. al. 2012). In addition to 5-HT_{2C}R, the mRNA and protein for the 5-HT_{2A}R subtype has been identified in the nTS with its protein co-localizing, in part, with tyrosine hydroxylase (Nosjean et. al. 2002). While a facilitatory role for 5-HT_{2A}R on glutamatergic transmission and action potential discharge has been shown in other brain regions (Beique et. al. 2007; Hasuo et. al. 2002), inhibition has also been demonstrated (Marcoli et. al. 2001). Such diverse responses may be due to the synaptic localization of the receptor (i.e., axon versus dendrite) or the brain region studied. The role of 5-HT_{2A}R in nTS synaptic activity is unknown. When activated *in vivo*, 5-HT_{2A}R in the nTS produce hypotension and bradycardia, suggestive of an excitatory role in the baroreflex pathway (Comet et. al. 2007). Extracellular *in vivo* recording of putative monosynaptic nTS cells further suggests 5-HT_{2A}R are facilitatory (Sevoz-Couche et. al. 2000), although inhibition by 5-HT₂R has also been reported in nTS (Jordan, 2005). Such discrepancies for 5-HT_{2A}R's action may be due to its location, role in modulating glutamate release or receptor efficacy directly, or the influence of other neuromodulators.

Endocannabinoids are neuromodulatory lipids that generally decrease glutamatergic and GABAergic synaptic and neuronal activity (Kano et. al. 2009), including within the nTS (Accorsi-Mendonca et. al. 2008; Chen et. al. 2010; Derbenev et. al. 2004).

Endocannabinoids in the nTS enhance the baroreflex through disinhibition (Brozoski et. al. 2005; Seagard et. al. 2005). Interestingly, activation of 5-HT_{2A}R induces endocannabinoid release to modify synaptic transmission in other central nuclei or assays (Best and Regehr, 2008; Parrish and Nichols, 2006). While this 5-HT_{2A}R-endocannabinoid system crosstalk is becoming increasingly appreciated (Aso et. al. 2009; Mato et. al. 2007), including a role for glutamate receptors in this crosstalk (Gorzalka et. al. 2005), whether it occurs in the nTS to modify synaptic transmission is unknown.

This study sought to define the role of 5-HT_{2A}R in modulating synaptic function in nTS. We also evaluated the role of endocannabinoids in the 5-HT_{2A}R effects on synaptic transmission.

2. RESULTS

2.1 5-HT_{2A} receptors amplify sensory afferent evoked synaptic activity

Across all neurons studied ($n = 73$), TS stimulation produced EPSCs (TS-EPSCs) that were evoked at invariant latencies (i.e., jitter, $183.2 \pm 8.5 \mu\text{s}$) and low failure rate ($6.3 \pm 1.0\%$), suggesting they were second-order nTS cells. Latency was $4.8 \pm 0.2 \text{ ms}$.

The role of 5-HT_{2A}R activation in TS-evoked synaptic transmission was examined by brief or acute (5 min) application of the 5-HT_{2A}R agonist TCB-2. We tested TCB-2 at several concentrations [in μM : 1 ($n=9$), 10 ($n=7$), and 50 ($n=6$)] and compared its effects to each cell's individual baseline aCSF control. As demonstrated in the representative traces from one cell in Figure 1A, acute 5-HT_{2A}R activation by TCB-2 (1 μM) increased the amplitude of TS-EPSCs evoked at 0.5 Hz from its individual baseline aCSF control. Quantitatively, 1 μM TCB-2 significantly increased the group TS-EPSC amplitude from control ($p = 0.045$,

Fig 1B). Following five minutes of washout, TS-EPSC amplitude remained elevated and not different from TCB-2 (Fig 1B). Ten and fifty μM TCB-2 also significantly enhanced TS-EPSC amplitude from their individual aCSF controls [$p = 0.019$ and $p = 0.031$, respectively, Fig 1C & D), with the 10 μM washout remaining elevated compared to baseline (Fig 1C). The magnitude of TS-EPSC amplitude increase relative to baseline among the three TCB-2 groups is presented in Figure 1E. As shown, all concentrations of TCB-2 significantly increased EPSC amplitude from their control, but the responses were not different from each other. TCB-2 did not alter holding currents, failure rate or input resistance. These results demonstrate acute activation of 5-HT_{2A}R increase synaptic transmission.

To further define the role of 5-HT_{2A}R activation during increases in afferent activity to nTS neurons, TS stimulation was increased to 20 Hz for 20 events to mimic sustained increases in *in vivo* sensory afferent activity. As is typical of the first synapse of the nTS, repeated TS stimulation during aCSF control progressively reduced synaptic current amplitude following the first event (i.e., use-dependent depression, Fig 2A, left). Acute bath application of 1 μM TCB-2 (5 minutes, $n=9$, representative example for 1 μM TCB-2 shown in Fig 2A, right) increased the overall amplitude of the TS-EPSCs from aCSF control, and TS-EPSC amplitude declined with repeated TS-stimulation. Quantitative data indicate the increase in TS-EPSC amplitude during 5-HT_{2A}R activation (1 μM TCB-2, Fig 2B) was sustained across the stimulus train. Ten ($n=7$) and fifty ($n=5$) micromolar TCB-2 also increased the initial EPSCs within the stimulus train (2 way RM ANOVA). The mechanism by which 5-HT_{2A}R activation augments TS-EPSCs may derive from an increase in presynaptic glutamate release, an augmentation in postsynaptic glutamate receptor conductance, or a combination of both. To investigate the pre- vs. postsynaptic mechanism for TCB-2's elevation of TS-EPSCs, we examined the paired pulse ratio (PPR) in the 20 Hz stimulus trains in which the amplitude of the second EPSC is compared with the first EPSC (TS-EPSC₂/TS-EPSC₁, Austgen et al., 2012). A change in the current amplitude ratio by TCB-2 is thought to reflect a presynaptic mechanism, whereas a lack of change is expected to be due to postsynaptic mechanisms. Across the three TCB-2 concentrations, the PPR did not change from their aCSF control in response to 5-HT_{2A}R activation (Fig 2C). These results suggest the 5-HT_{2A}R increase in synaptic transmission is likely due to a postsynaptic mechanism.

Miniature (m)EPSCs, spontaneous excitatory currents recorded in the presence of TTX to eliminate network-driven action potentials, were examined during brief 5-HT_{2A}R activation to further evaluate potential pre- or postsynaptic sites of action. Alterations in mEPSC frequency denote potential changes in presynaptic neurotransmitter release, whereas changes in mEPSC amplitude reflect alterations in postsynaptic receptor properties. Compared to their aCSF control, acute TCB-2 (1 μM , 5 minutes, $n=6$) did not alter mEPSC amplitude (aCSF control, 16.4 ± 1.5 pA vs. TCB-2, 14.1 ± 1.7 pA, $p = 0.10$) or instantaneous frequency (aCSF control, 9.0 ± 1.7 Hz vs. TCB-2, 9.0 ± 1.1 Hz, $p = 0.98$). Thus, acute 5-HT_{2A}R activation with TCB-2 does not alter mEPSCs, suggesting network activity is required for 5-HT_{2A}R actions.

2.2 Blockade of 5-HT_{2A}Rs reduce TS-EPSCs

5-HT₂ receptors *in vivo* and *in vitro* exhibit constitutive (tonic) activation (Aloyo et. al. 2009; Roth et. al. 1998), including the 5-HT_{2C} receptor (Austgen et al., 2012). We examined the degree to which tonic activation of 5-HT_{2A}Rs may modify TS-EPSCs by applying the 5-HT_{2A}R antagonists MDL 11,939 (100 μM , 5 minutes) or 4F 4PP (100 μM , 5 minutes). As shown in the representative traces from a single cell (Fig 3A), 5-HT_{2A}R blockade with MDL 11,939 reduced TS-EPSC amplitude evoked at 0.5 Hz (30 sweeps, averaged) from aCSF baseline control. The mean decrease in TS-EPSC amplitude with MDL 11,939 from aCSF control is shown in Fig. 3B ($n=9$, $p=0.012$). When stimulation frequency was increased to 20 Hz, 5-HT_{2A}R blockade with MDL 11,939 reduced the overall TS-EPSC amplitude (Fig 3C,

n=9) and use-dependent depression was again not altered. The depression in TS-EPSC amplitude was not accompanied by a change in the PPR (Fig 3D). In addition, in another group of cells 5-HT_{2A}R block with a separate 5-HT_{2A}R antagonist, 4F 4PP, also significantly reduced TS-EPSC current amplitude from aCSF baseline control (Fig. 3E, n=5, p=0.018). The relative reduction in TS-EPSC amplitude from their individual aCSF controls was comparable between MDL 11,939 and 4F 4PP (Fig 3F). Neither MDL 11,939 nor 4F 4PP altered holding currents, failure rate, or input resistance from control. These results suggest 5-HT_{2A}R is tonically active in the nTS.

We also examined whether MDL 11,939 could attenuate or eliminate TS-EPSC enhancement by TCB-2. Compared to its aCSF control, prior application of the 5-HT_{2A}R antagonist (~3 min, 100 μ M) prevented the increase in TS-EPSCs by TCB-2 (10 μ M, 5 min with MDL) evoked at 0.5 Hz (Fig 3G, p = 0.495) or 20 Hz (2 way RM ANOVA). These results further suggest TCB-2 actions occur through the 5-HT_{2A} receptor.

mEPSCs were again recorded to evaluate the pre- or postsynaptic site of action for the decrease in EPSC amplitude by 5-HT_{2A}R blockade. MDL 11,939 (100 μ M, 5 min) did not alter the amplitude (aCSF control, 17.8 ± 3.3 pA vs. MDL 11,939, 16.3 ± 3.0 pA, n=8, p=0.273) nor instantaneous frequency (aCSF control, 8.8 ± 1.6 Hz vs. MDL 11,939, 10.2 ± 1.5 Hz, n=8, p=0.121) of mEPSCs compared to aCSF control. Thus, 5-HT_{2A}R blockade with MDL 11,939 does not alter mEPSCs, suggesting network activity is required for 5-HT_{2A}R actions.

2.3 Endocannabinoids contribute to 5-HT_{2A}R modulation of TS evoked activity

Activation of 5-HT_{2A}Rs induce release of endocannabinoids in other central systems and *in vitro* assays (Best and Regehr, 2008; Parrish and Nichols, 2006). Endocannabinoids then may alter glutamatergic signaling via their binding to pre- or postsynaptic cannabinoid receptors (e.g., CB-1Rs). To examine the possibility that 5-HT_{2A}R modulation of glutamate signaling is influenced by endocannabinoids in nTS, we examined TS- and mEPSCs during 5-HT_{2A}R manipulation while antagonizing the CB-1R or increasing endocannabinoids in the synaptic cleft. The endocannabinoid CB1 receptor antagonist AM251 (Best and Regehr, 2008; Derbenev et. al. 2004; Haj-Dahmane and Shen, 2009) or anandamide transport/fatty acid amide hydrolase (FAAH) inhibitor AM404 (Brozoski et. al. 2005; Kim and Alger, 2010; Parrish and Nichols, 2006) was applied alone, or prior to and concurrent with the 5-HT_{2A}R agonist (TCB-2) or antagonist (MDL 11,939), while monitoring EPSCs.

Individually, neither blocking the CB-1R (AM251, 5 μ M, 5 min) nor increasing synaptic endocannabinoids (AM404, 5 μ M, 5 min) alone altered TS-EPSC amplitude evoked at 0.5 Hz from their own aCSF baseline (Fig 4A, left). As shown in previous protocols, acute 5-HT_{2A}R activation with 1 μ M TCB-2 alone increased TS-EPSC amplitude evoked at 0.5 Hz from aCSF baseline. The 5-HT_{2A}R-mediated increase in TS-EPSCs by TCB-2 was not affected by prior and concurrent CB-1R block (TCB-2 + AM251, Fig 4A, middle). However, increasing endocannabinoids in the synaptic cleft with AM404 abolished the TCB-2 increase in TS-EPSC amplitude from aCSF (TCB-2 + AM404, Fig 4A, middle). As also discussed above, blocking tonically active 5-HT_{2A}R with MDL 11,939 significantly decreased TS-EPSC amplitude evoked at 0.5 Hz relative to its own aCSF baseline (Fig 4A, right). The decrease in TS-EPSCs by 5-HT_{2A}R antagonism was not altered by prior and concurrent CB-1R blockade (MDL + AM251, Fig 4A, right). However, increasing synaptic endocannabinoids abolished the MDL decrease in EPSCs from aCSF control (MDL + AM404, Fig 4A, right).

We also examined the 5-HT_{2A}R and endocannabinoid interaction at high TS-stimulation frequencies. Increasing TS stimulation frequency to 20 Hz did not alter total current

amplitude (i.e., synaptic throughput; sum of all EPSCs) from aCSF baseline during individual CB-1R blockade with AM251 or elevated synaptic endocannabinoids with AM404 (Fig 4B, left). As shown in previous protocols, 5-HT_{2A}R activation (1 μ M TCB-2) increased synaptic throughput from aCSF. Interestingly, both CB-1R blockade with AM251 and increased synaptic endocannabinoids with AM404 abolished the 5-HT_{2A}R (TCB-2) increase in synaptic throughput, relative to their individual aCSF control (TCB + AM251 and TCB + AM404, respectively, Fig 4B, middle). Conversely, 5-HT_{2A}R blockade significantly decreased total synaptic throughput compared to its own aCSF baseline (Fig 4B, right). The decrease of synaptic throughput from 5-HT_{2A}R block persisted during prior and concurrent CB-1R blockade (MDL + AM251, Fig 4B). Increasing synaptic endocannabinoids concurrent with 5-HT_{2A}R blockade ablated the decrease in synaptic throughput relative to its own aCSF baseline (MDL + AM404, Fig 4B, right).

We also examined the potential contribution of endocannabinoids on 5-HT_{2A}R modulation of mEPSCs. Neither CB-1R blockade (AM251, 5 μ M, 5 minutes, n=6) nor increases in synaptic endocannabinoids (AM404, 5 μ M, 5 minutes, n=6) alone altered mEPSC amplitude or frequency from their individual aCSF controls. As above, neither 5-HT_{2A}R activation (TCB-2, 1 μ M, n=6) nor blockade (MDL 11, 939, 100 μ M, n=6) individually altered mEPSC amplitude or frequency, and prior and concurrent blocking the CB-1R (AM251) or augmenting synaptic endocannabinoids (AM404) did not further alter mEPSCs (n=6 for each group).

3. DISCUSSION

In the present study, we demonstrate 5-HT_{2A}Rs are functional at the sensory afferent-nTS synapse. Specifically, activation of 5-HT_{2A}Rs augments afferent-driven excitatory synaptic transmission, whereas blockade of 5-HT_{2A}Rs decreases such synaptic currents. Modulation occurs primarily through postsynaptic mechanisms and is tonic at rest. The influence of endocannabinoids on 5-HT_{2A}R-EPSC enhancement is related to TS-stimulation frequency.

Numerous serotonin receptors have been identified in the nTS, including the 5-HT₂R. We have previously established an important role of 5-HT_{2C}R, one member of the 5-HT₂R family, in synaptic transmission in the nTS. Here, we extend our understanding of the 5-HT₂R family by showing that acute activation of the 5-HT_{2A} receptor with TCB-2 augments afferent (TS) evoked EPSCs under both low and high frequency stimulation (0.5 and 20 Hz, respectively). 5-HT_{2A}R activation did not, however, alter the PPR or failure rate, indicative that presynaptic alterations do not contribute to EPSC changes. These data together suggest 5-HT_{2A}Rs primarily enhance the conductance or number of postsynaptic glutamate receptors, or somatodendritic signaling pathways, rather than the magnitude of presynaptic glutamate release. Similar postsynaptic actions of 5-HT_{2A}R have been observed in other central nuclei (Browning and Travagli, 1999; Hasuo et. al. 2002; Huang et. al. 2009; Zhang and Marek, 2007). Our results are also consistent with previous nTS studies illustrating postsynaptic localization of 5-HT_{2A}R (Cornea-Hebert et. al. 1999; Cornea-Herbert et. al. 2002), including within somas and dendrites (Huang and Pickel, 2002) and catecholaminergic cells (Nosjean et. al. 2002). It should be noted that 5-HT_{2A}Rs have also been localized to the TS fiber tract, unmyelinated axons and glial cells (Huang and Pickel, 2003), thus there may also be additional components to 5-HT_{2A}Rs action in the nTS that require further study.

Blockade of 5-HT_{2A}Rs reduced TS-EPSC amplitude without altering the PPR or failure rate. Such data are in agreement with changes in postsynaptic activity, similar to the effects of activation. These data further suggest a basal or tonic activation of post-synaptic 5-HT_{2A}R in the nTS. Such results are consistent with other studies (Aloyo et. al. 2009; Austgen et. al.

2012; Raul, 2003; Roth et. al. 1998). While our data suggest primarily a postsynaptic site of action, it is conceivable that this tonic effect may also mask potential presynaptic actions. Tonic post-synaptic activation of 5-HT_{2A}R signaling by either receptor occupation and/or its triggered intracellular second messengers may underlie the absence of effect of 5-HT_{2A}R activation with mEPSC amplitude. The lack of effect of 5-HT_{2A}R on mEPSCs may also suggest that 5-HT_{2A}Rs do not modify signaling within the synapse per se, but rather at extrasynaptic sites (Huang and Pickel, 2003) where 5-HT volume transmission may amplify glutamatergic signaling through converging and possibly synergistic biochemical mechanisms (Ciranna, 2006).

5-HT_{2A}Rs are coupled to G_q and G₁₁ and activate phospholipase C (PLC) to induce neuronal excitability (Hoyer et. al. 2002). In other central systems and assays, PLC activation and augmented cellular activity, including that derived from 5-HT_{2A}R, induces the release of endocannabinoids that reduce synaptic transmission and action potential discharge, and modify behavior (Aso et. al. 2009; Best and Regehr, 2008; Connelly and Baggott, 2009; Galante and Diana, 2004; Haj-Dahmane and Shen, 2011; Kano et. al. 2009; Parrish and Nichols, 2006). While the magnitude of 5-HT_{2A}R-endocannabinoid crosstalk in nTS is unknown, the endocannabinoid system is expressed and functional in the dorsal vagal complex. Cannabinoid receptors (CB-1R) are located on nTS somas and synaptic terminals (Derbenev et. al. 2004; Partosoedarso et. al. 2003), including vagal afferents (Rogers and Hermann, 2012). Exogenous endocannabinoids in the dorsal vagal complex inhibit presynaptic release of GABA and glutamate (Accorsi-Mendonca et. al. 2008; Derbenev et. al. 2004) and reduce postsynaptic potassium currents (Derbenev et. al. 2004). nTS somal depolarization also results in endocannabinoid-mediated retrograde inhibition of presynaptic GABA release (Chen et. al. 2010). The evidence from other preparations that 5-HT₂R enhances endocannabinoid release, which decreases glutamatergic transmission, prompted us to explore this possibility in the nTS. Thus, we examined the degree to which the excitatory synaptic effects of the 5-HT_{2A}R may be opposed by endocannabinoid inhibition.

Our data demonstrated that blockade of the CB-1R or augmentation of endocannabinoids in the synaptic cleft did not individually alter TS-evoked EPSCs or mEPSCs. This would suggest that under our *in vitro* recording conditions, endocannabinoids are not tonically released in the nTS spontaneously or by brief TS stimulation. This is consistent with reports that AM 251 did not alter EPSCs and IPSCs in the majority of dorsal vagal complex cells (Derbenev et. al. 2004) and that depolarization to at least -40 mV is required for endocannabinoid modulation of neurotransmitter release in nTS (Chen et. al. 2010).

We had reasoned that if 5-HT_{2A}R's, either due to acute TCB-2 - induced activation or its tonic activity, prompt endocannabinoid release to oppose TS-EPSCs then block of CB-1Rs with AM251 would augment EPSC amplitude. However, during low frequency (0.5 Hz) TS stimulation, CB-1R block did not potentiate the increase in TS-EPSCs by 5-HT_{2A}R activation (TCB-2), nor attenuate the decrease in TS-EPSCs by 5-HT_{2A}R blockade (MDL). Yet, increasing endogenous endocannabinoids in the synaptic cleft with AM404 did prevent 5-HT_{2A}R modulation of EPSCs. These effects were activity-dependent as mEPSCs were not altered by either endocannabinoid or 5-HT_{2A}R modulation. Taken together, these data would suggest that during minimal afferent stimulation, or in the absence of activity (e.g., during TTX), the 5-HT_{2A}R-endocannabinoid-CB-1R system does not contribute to EPSC modulation. However, endocannabinoids in the nTS are capable of modulating EPSCs when given exogenously (Accorsi-Mendonca et. al. 2008; Derbenev et. al. 2004), or elevated by blocking their re-uptake or breakdown (current study). Our results suggest brief stimulation does not induce sufficient endocannabinoid release to overcome its re-uptake or breakdown.

On the contrary, block of CB-1R prevented the acute TCB-2-induced augmentation of EPSC total throughput when evoked at 20 Hz. This CB-1R modulation of 20 Hz synaptic throughput is absent when 5-HT_{2A}Rs are blocked with MDL 11,939. These results were contrary to our expected increase in EPSCs with CB-1R block, and could be due to one or more mechanisms. For instance, endocannabinoids in nTS may, in a subset of cells, also augment glutamatergic synaptic transmission (Accorsi-Mendonca et. al. 2008) and neuronal discharge (Himmi et. al. 1998), perhaps through interneurons or activation of sensory afferent TRPV1 receptors (Peters et. al. 2010; Tognetto et. al. 2001). However, this is less likely as the majority of studies demonstrate endocannabinoids reduce glutamatergic transmission (Derbenev et. al. 2004; Kano et. al. 2009). Further, our data showing TCB-2 activation of 5-HT_{2A}R augment 0.5 Hz TS-EPSCs during CB-1R block suggest 5-HT_{2A}Rs themselves influence neurotransmission and do not simply function as an excitatory endocannabinoid-releasing agent. Another possibility is that CB-1Rs are not the only endocannabinoid receptors in the nTS, and that during CB-1R antagonism or synaptic endocannabinoid increases, activation of these other CB receptors inhibit the 5-HT_{2A}R augmentation of EPSCs (Kano et. al. 2009). However, CB-1R block did not alter total synaptic throughput during 5-HT_{2A}R antagonism via MDL 11,939, suggesting acute activation of the 5-HTR and perhaps, specific intracellular downstream pathways, is required for CB-1R effects. Last, endocannabinoids and CB-1R may alter one or more ion channels in the pre- and postsynaptic neuron prior to and during its synaptic release (van der Stelt and Di Marzo, 2005). For instance, exogenous endocannabinoids decrease somal potassium currents in the dorsal vagal complex (Derbenev et. al. 2004), which may enhance neuronal/network activity and induce synaptic inhibition through one of more neuromodulator. Endocannabinoids are also capable of behaving as intracellular signaling messengers to alter postsynaptic signaling (van der Stelt and Di Marzo, 2005). While additional work is required to elucidate these potential mechanisms, our results suggest the need for greater depolarization concurrent with G-protein activation for endocannabinoid release and synaptic effects (Best and Regehr, 2008; Chen et. al. 2010; Kano et. al. 2009).

Interestingly, increases in endogenous endocannabinoids by AM404 ablated TS-EPSC changes in 5-HT_{2A}R activation (i.e., TCB-2) and block (MDL 11,939) during low and high TS frequency. They do not, however, alter EPSCs alone (i.e., AM404). While the mechanisms may be multiple, one possibility as to why AM404 eliminates changes in synaptic transmission when 5-HT_{2A}R is manipulated, but not alone, may be due to increased endocannabinoids at multiple locations in the available neuroaxis. Elevation of endocannabinoids within the nTS may result in a mutual decrease in GABAergic and glutamatergic neurotransmission, as well as altered channel activity and signaling pathways outside of the neurons recorded. As such, this may result in a period of low(er) nTS activity similar to TTX, in which we do not see effects of 5-HT_{2A}R activation or blockade. Future studies will be needed to confirm this notion.

Our experimental model mimics physiological activation of the nTS through afferent stimulation. Results demonstrate 5-HT_{2A}R activation tonically increases synaptic transmission through postsynaptic mechanisms. However, in other systems, 5-HT_{2A}R activation may also inhibit synaptic transmission and neuronal activity. Such inhibitory actions may be due to the neurocircuitry in their preparation or the activation of other neuromodulators by 5-HTR activation, such as endocannabinoids. The greater role for endocannabinoids during 5-HT_{2A}R activation at 20 Hz is consistent with the observation that elevated activity induces endocannabinoid release and synaptic effects (Kano et. al. 2009). Yet elevated activity alone is not sufficient to alter nTS synaptic transmission, and requires co-activation of the 5-HT receptor. Admittedly, our stimulation protocol was brief compared to *in vivo* afferent activation and thus it is possible that the relationship between endocannabinoids and 5-HT_{2A}R may be stronger during chronic afferent or nTS activity, as

is observed in hypertension (Brozoski et. al. 2009). Regardless of this interaction, we demonstrate 5-HT_{2A}R alone modulates synaptic transmission in the nTS. Moreover, the interactions among the numerous neuromodulators in the nTS, such as the glutamate-serotonin-endocannabinoid systems shown in the present study, likely contribute to the important adaptations and plasticity of cardiorespiratory reflexes under physiological and pathophysiological circumstances.

4. EXPERIMENTAL PROCEDURES

4.1 Animals

All experiments were undertaken under guidelines of the NIH "Guide for the Care and Use of Laboratory Animals" and were approved by the Animal Care and Use Committee of The University of Missouri. Male Sprague-Dawley rats (3–5 weeks of age, Harlan, Indianapolis, IN) were boarded within an in-house animal facility, under a 12 hour day/night cycle, with room temperature and humidity maintained at 22°C and 40%, respectively. Food and water were available *ad libitum*.

4.2 *In vitro* brainstem slice preparation and electrophysiology

Animals were anesthetized with isoflurane (Vet-One, Meridian, IN), decapitated, and brainstems rapidly removed and submerged into ice-cold low Ca²⁺ high Mg²⁺ artificial cerebrospinal fluid (aCSF, in mM: 124 NaCl, 3 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 D-glucose, 0.4 L-Ascorbic Acid, 1 CaCl₂, and 2 MgCl₂, saturated with 95% O₂-5% CO₂, pH 7.4, 315–325 mOsm). The ventral medulla was trimmed and horizontal slices (~280 μm) were sectioned on a vibratome (Leica VT1000S). Tissue slices were placed in a superfusion chamber on an Olympus BX51WIF microscope, secured with a nylon harp and superfused at 3–4 ml/min with normal recording aCSF (in mM: 124 NaCl, 3 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 D-glucose, 0.4 L-Ascorbic Acid and 2 CaCl₂ saturated with 95% O₂-5% CO₂, pH 7.4, 315–325 mOsm) at ~33°C.

Excitatory postsynaptic currents (EPSCs) were recorded from nTS neurons within medial and commissural nTS, medial to the TS, typically ± 250 μm from obex (the opening of the central canal to the forth ventricle). Currents were recorded with glass electrodes (8250, 3.5–4.5 MΩ, Garner Glass, Claremont, CA) filled with (mM) 10 NaCl, 130 K⁺ gluconate, 11 EGTA, 1 CaCl₂, 10 HEPES, 1 MgCl₂, 2 MgATP, 0.2 NaGTP, pH 7.3, 295–300 mOsm. Evoked EPSCs were generated by placing a concentric bipolar stimulating electrode (F. Haer, Bowdoinham, ME) on the afferent containing solitary tract (TS) and stimulating for 0.1 ms with an isolated programmable stimulator (AMPI, Jerusalem, Israel). TS stimulation intensity was increased until an EPSC was evoked, after which final TS intensity was set at 1.5X threshold. Tetrodotoxin and GABAzine were added to the bath to eliminate action potential-driven and spontaneous GABAergic events for the recording of miniature (m)EPSCs. All neurons were clamped at a holding potential of –60 mV in the whole-cell configuration. A –5 mV step from –60 mV was used to determine input resistance, or changes in membrane conductance, of the cell. All data were filtered at 2 kHz and sampled at 10 kHz using pClamp10 software (Molecular Devices, Sunnyvale, CA).

4.3 Drugs

We used the high affinity agonist TCB-2 to acutely activate 5-HT_{2A}R (Bos et. al. 2013; Chang et. al. 2009; Fox et. al. 2010; McLean et. al. 2006). The 5-HT_{2A}R antagonists MDL 11,939 and 4F 4PP oxalate were used to examine whether 5-HT_{2A}Rs tonically alter synaptic events. MDL11,939 and 4F 4PP have 100 fold greater affinity for 5-HT_{2A}R than 5-HT_{2C}R (Andrade et. al. 2012; Herndon et. al. 1992). The endocannabinoid CB1 receptor antagonist AM251 (Best and Regehr, 2008; Derbenev et. al. 2004; Haj-Dahmane and Shen, 2009) and

the anandamide transport/fatty acid amide hydrolase (FAAH) inhibitor AM404 (Brozoski et. al. 2005; Kim and Alger, 2010; Parrish and Nichols, 2006) was used to examine the role of synaptically released endocannabinoids in nTS, and their contribution to 5-HT_{2A}R mediated effects. All drugs were bath applied. In protocols in which we applied a single drug (e.g., TCB-2, MDL 11,939, AM251 or AM404), perfusion was for 5 minutes. In protocols in which we applied two drugs sequentially, the first drug (e.g., AM251) was applied for ~3 minutes, and the second drug was applied concurrently with the first (e.g., AM251 + TCB-2) for 5 minutes. Such time allowed perfusion of the drug through tubing dead space, reach a steady state in the bath and diffuse to the cell(s) that are typically 1–2 cells layers deep. For recording of mEPSCs, tetrodotoxin citrate (TTX, 1 μ M) and SR 95531 HBr (GABAzine, GABA_A antagonist, 25 μ M) were continuously in the bath (Austgen et. al. 2011; Austgen et. al. 2012). The above chemicals were purchased from Tocris.

4.4 Data Analysis

All electrophysiological parameters data were collected during the last minute of aCSF control or a given drug perfusion. Data were analyzed using Clampfit 10 software (Molecular Devices). nTS cell inclusion criteria include a resting membrane potential depolarized no more than –45 mV and holding currents between 0 and –50 pA upon initial membrane rupture, input resistance greater than 100 M Ω , and invariant EPSC onset latency (jitter) less than 300 μ s (Accorsi-Mendonca et. al. 2011; Hisadome et. al. 2010; Jin and Andresen, 2011; Kline et. al. 2010a; Laaris and Weinreich, 2007; Miles, 1986). Jitter was calculated as the standard deviation of the time from shock artifact to TS-EPSC initiation. The paired pulse ratio (PPR) of two consecutive TS-EPSCs was determined from the amplitude ratio of the second TS-EPSC to the first TS-EPSC (TS-EPSC2/TS-EPSC1). Total synaptic throughput was expressed as the sum of 20 synaptic currents evoked at 20 Hz (Kline et. al. 2010b). TS-EPSC failure rate was the percentage of TS stimulations that failed to evoke an EPSC response during 30 current sweeps. mEPSCs were discriminated from background noise by removing events that were less than 2.5 X the root mean square value of noise. The magnitudes of EPSC change during a given pharmacological intervention are reported as the change from their individual aCSF baseline controls (depicted as “1”). Statistical analyses were performed with SigmaPlot 12 (Systat Software) or GraphPad Prism 6. Synaptic and membrane parameters between baseline control and drug periods were compared by a paired t-test or Wilcoxon signed-rank test. Comparisons among groups were via ANOVA, when appropriate. P values $\leq$ 0.05 were considered significant. Values are reported as mean $\pm$ SEM.

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References

- Accorsi-Mendonca D, Almado CE, Dagostin AL, Machado BH, Leao RM. Inhibition of spontaneous neurotransmission in the nucleus of solitary tract of the rat by the cannabinoid agonist WIN 55212-2 is not via CB1 or CB2 receptors. *Brain Res.* 2008; 1200:1–9. [PubMed: 18308297]
- Accorsi-Mendonca D, Castania JA, Bonagamba LG, Machado BH, Leao RM. Synaptic profile of nucleus tractus solitarius neurons involved with the peripheral chemoreflex pathways. *Neuroscience.* 2011; 197:107–120. [PubMed: 21963868]
- Aloyo VJ, Berg KA, Spampinato U, Clarke WP, Harvey JA. Current status of inverse agonism at serotonin_{2A} (5-HT_{2A}) and 5-HT_{2C} receptors. *Pharmacology & Therapeutics.* 2009; 121:160–173. [PubMed: 19109993]

- Andrade R, Barnes NM, Baxter G, Bockaert J, Branchek T, Cohen ML, Bumuis A, Eglen RM, Gothert M, Hamblin M, Hamon M, Hartig PR, Hen R, Herrick-Davis K, Hills R, Hoyer D, Humphrey PPA, Latte KP, Maroteaux L, Martin GT, Middlemiss DN, Mylecharane E, Peroutka SJ, Saxena PR, Sleight A, Villalon CM, Yocca F. 5-Hydroxytryptamine receptors: 5-HT_{2A} receptor. 2012. IUPHAR database (IUPHAR-DB). Jan 29.2013
- Aso E, Renoir T, Mengod G, Ledent C, Hamon M, Maldonado R, Lanfumey L, Valverde O. Lack of CB1 receptor activity impairs serotonergic negative feedback. *J Neurochem*. 2009; 109:935–944. [PubMed: 19302195]
- Austgen JR, Dantzler HA, Barger BK, Kline DD. 5-hydroxytryptamine 2C receptors tonically augment synaptic currents in the nucleus tractus solitarius. *J Neurophysiol*. 2012; 108:2292–2305. [PubMed: 22855775]
- Austgen JR, Hermann G, Dantzler HA, Rogers RC, Kline DD. Hydrogen sulfide (H₂S) augments synaptic neurotransmission in the nucleus of the solitary (NTS). *J Neurophysiol*. 2011; 106:1822–32. [PubMed: 21734104]
- Beique JC, Imad M, Mladenovic L, Gingrich JA, Andrade R. Mechanism of the 5-hydroxytryptamine 2A receptor-mediated facilitation of synaptic activity in prefrontal cortex. *Proc Natl Acad Sci U S A*. 2007; 104:9870–9875. [PubMed: 17535909]
- Best AR, Regehr WG. Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. *J Neurosci*. 2008; 28:6508–6515. [PubMed: 18562622]
- Bos R, Sadlaoud K, Boulenguez P, Buttigieg D, Liabeuf S, Brocard C, Haase G, Bras H, Vinay L. Activation of 5-HT_{2A} receptors upregulates the function of the neuronal K-Cl cotransporter KCC2. *Proc Natl Acad Sci U S A*. 2013; 110:348–353. [PubMed: 23248270]
- Browning KN, Travagli RA. Characterization of the in vitro effects of 5-hydroxytryptamine (5-HT) on identified neurones of the rat dorsal motor nucleus of the vagus (DMV). *Br J Pharmacol*. 1999; 128:1307–1315. [PubMed: 10578146]
- Brozoski DT, Dean C, Hopp FA, Hillard CJ, Seagard JL. Differential endocannabinoid regulation of baroreflex-evoked sympathoinhibition in normotensive versus hypertensive rats. *Auton Neurosci*. 2009; 150:82–93. [PubMed: 19464961]
- Brozoski DT, Dean C, Hopp FA, Seagard JL. Uptake blockade of endocannabinoids in the NTS modulates baroreflex-evoked sympathoinhibition. *Brain Res*. 2005; 1059:197–202. [PubMed: 16154548]
- Chang CW, Poteet E, Schetz JA, Gumus ZH, Weinstein H. Towards a quantitative representation of the cell signaling mechanisms of hallucinogens: measurement and mathematical modeling of 5-HT_{1A} and 5-HT_{2A} receptor-mediated ERK1/2 activation. *Neuropharmacology*. 2009; 56(Suppl 1):213–225. [PubMed: 18762202]
- Chen CY, Bonham AC, Dean C, Hopp FA, Hillard CJ, Seagard JL. Retrograde release of endocannabinoids inhibits presynaptic GABA release to second-order baroreceptive neurons in NTS. *Auton Neurosci*. 2010; 158:44–50. [PubMed: 20580326]
- Ciranna L. Serotonin as a modulator of glutamate- and GABA-mediated neurotransmission: implications in physiological functions and in pathology. *Curr Neuropharmacol*. 2006; 4:101–114. [PubMed: 18615128]
- Comet MA, Vernard JF, Hamon M, Laguzzi R, Sevoz-Couche C. Activation of nucleus tractus solitarius 5-HT_{2A} but not other 5-HT₂ receptor subtypes inhibits the sympathetic activity in rats. *Euro J Neurosci*. 2007; 26:345–354.
- Connelly WM, Baggott MJ. Role of endocannabinoids in 5-HT₂ receptor-mediated effects. *J Neurophysiol*. 2009; 101:5–7. [PubMed: 19004992]
- Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L. Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J Comp Neurol*. 1999; 409:187–209. [PubMed: 10379914]
- Cornea-Herbert B, Watkins K, Roth B, Kroeze W, Gaudreau P, Leclerc N, Descarries L. Similar ultrastructural distribution of the 5-HT_{2A} serotonin receptor and microtubule-associated protein MAP1A in cortical dendrites of adult rat. *Neuroscience*. 2002; 113:23–35. [PubMed: 12123681]

- Cui RJ, Roberts BL, Zhao H, Zhu M, Appleyard SM. Serotonin activates catecholamine neurons in the solitary tract nucleus by increasing spontaneous glutamate inputs. *J Neurosci*. 2012; 32:16530–16538. [PubMed: 23152635]
- Derbenev AV, Stuart TC, Smith BN. Cannabinoids suppress synaptic input to neurones of the rat dorsal motor nucleus of the vagus nerve. *J Physiol*. 2004; 559:923–938. [PubMed: 15272041]
- Feldman PD. Electrophysiological effects of serotonin in the solitary tract nucleus of the rat. *Naunyn Schmiedebergs Arch Pharmacol*. 1994; 349:447–454. [PubMed: 8065458]
- Feldman PD. Effects of serotonin-1 and serotonin-2 receptor agonists on neuronal activity in the nucleus tractus solitarius. *J Auton Nerv Syst*. 1995; 56:119–124. [PubMed: 8786274]
- Fox MA, French HT, LaPorte JL, Blackler AR, Murphy DL. The serotonin 5-HT(2A) receptor agonist TCB-2: a behavioral and neurophysiological analysis. *Psychopharmacology (Berl)*. 2010; 212:13–23. [PubMed: 19823806]
- Galante M, Diana MA. Group I metabotropic glutamate receptors inhibit GABA release at interneuron-Purkinje cell synapses through endocannabinoid production. *J Neurosci*. 2004; 24:4865–4874. [PubMed: 15152047]
- Gorzalka BB, Hill MN, Sun JC. Functional role of the endocannabinoid system and AMPA/kainate receptors in 5-HT2A receptor-mediated wet dog shakes. *Eur J Pharmacol*. 2005; 516:28–33. [PubMed: 15913602]
- Haj-Dahmane S, Shen RY. Endocannabinoids suppress excitatory synaptic transmission to dorsal raphe serotonin neurons through the activation of presynaptic CB1 receptors. *J Pharmacol Exp Ther*. 2009; 331:186–196. [PubMed: 19592666]
- Haj-Dahmane S, Shen RY. Modulation of the serotonin system by endocannabinoid signaling. *Neuropharmacology*. 2011; 61:414–420. [PubMed: 21354188]
- Hasuo H, Matsuoka T, Akasu T. Activation of presynaptic 5-hydroxytryptamine 2A receptors facilitates excitatory synaptic transmission via protein kinase C in the dorsolateral septal nucleus. *J Neurosci*. 2002; 22:7509–7517. [PubMed: 12196574]
- Herndon JL, Ismaiel A, Ingher SP, Teitler M, Glennon RA. Ketanserin analogues: structure-affinity relationships for 5-HT2 and 5-HT1C serotonin receptor binding. *J Med Chem*. 1992; 35:4903–4910. [PubMed: 1479590]
- Himmi T, Perrin J, El OT, Orsini JC. Neuronal responses to cannabinoid receptor ligands in the solitary tract nucleus. *Eur J Pharmacol*. 1998; 359:49–54. [PubMed: 9831292]
- Hisadome K, Reimann F, Gribble FM, Trapp S. Leptin directly depolarizes preproglucagon neurons in the nucleus tractus solitarius: electrical properties of glucagon-like Peptide 1 neurons. *Diabetes*. 2010; 59:1890–1898. [PubMed: 20522593]
- Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav*. 2002; 71:533–554. [PubMed: 11888546]
- Huang CC, Liang YC, Lee CC, Wu MY, Hsu KS. Repeated cocaine administration decreases 5-HT(2A) receptor-mediated serotonergic enhancement of synaptic activity in rat medial prefrontal cortex. *Neuropsychopharmacology*. 2009; 34:1979–1992. [PubMed: 19212317]
- Huang J, Pickel VM. Differential distribution of 5HT2A and NMDA receptors in single cells within the rat medial nucleus of the solitary tract. *Synapse*. 2002; 44:64–75. [PubMed: 11891878]
- Huang J, Pickel VM. Ultrastructural localization of serotonin 2A and N-methyl-D-aspartate receptors in somata and dendrites of single neurons within rat dorsal motor nucleus of the vagus. *J Comp Neurol*. 2003; 455:270–280. [PubMed: 12454991]
- Jin YH, Andresen MC. GABA(B) restrains release from singly-evoked GABA terminals. *Neuroscience*. 2011; 193:54–62. [PubMed: 21820490]
- Jordan D. Vagal control of the heart: central serotonergic (5-HT) mechanisms. *Exp Physiol*. 2005; 90:175–181. [PubMed: 15604109]
- Kano M, Ohno-Shosaku T, Hashimoto-dani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev*. 2009; 89:309–380. [PubMed: 19126760]
- Kim J, Alger BE. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. *Nat Neurosci*. 2010; 13:592–600. [PubMed: 20348918]

- Kline DD, King TL, Austgen JR, Heesch CM, Hasser EM. Sensory afferent and hypoxia-mediated activation of nucleus tractus solitarius neurons that project to the rostral ventrolateral medulla. *Neuroscience*. 2010a; 167:510–527. [PubMed: 20153814]
- Kline DD, Ogier M, Kunze DL, Katz DM. Exogenous Brain Derived Neurotrophic Factor Rescues Synaptic Dysfunction in Mecp2 Null Mice. *Journal of Neuroscience*. 2010b; 30:5303–5310. [PubMed: 20392952]
- Laaris N, Weinreich D. Prostaglandin E2 depresses solitary tract-mediated synaptic transmission in the nucleus tractus solitarius. *Neuroscience*. 2007; 146:792–801. [PubMed: 17367942]
- Marcoli M, Rosu C, Bonfanti A, Raiteri M, Maura G. Inhibitory presynaptic 5-hydroxytryptamine(2A) receptors regulate evoked glutamate release from rat cerebellar mossy fibers. *J Pharmacol Exp Ther*. 2001; 299:1106–1111. [PubMed: 11714900]
- Mato S, Aso E, Castro E, Martin M, Valverde O, Maldonado R, Pazos A. CB1 knockout mice display impaired functionality of 5-HT1A and 5-HT2A/C receptors. *J Neurochem*. 2007; 103:2111–2120. [PubMed: 17944876]
- McLean TH, Parrish JC, Braden MR, Marona-Lewicka D, Gallardo-Godoy A, Nichols DE. 1-Aminomethylbenzocycloalkanes: conformationally restricted hallucinogenic phenethylamine analogues as functionally selective 5-HT2A receptor agonists. *J Med Chem*. 2006; 49:5794–5803. [PubMed: 16970404]
- Miles R. Frequency dependence of synaptic transmission in nucleus of the solitary tract in vitro. *J Neurophysiol*. 1986; 55:1076–1090. [PubMed: 3012009]
- Nosjean A, Hamon M, Darmon M. 5-HT2A receptors are expressed by catecholaminergic neurons in the rat nucleus tractus solitarius. *Neuroreport*. 2002; 13:2365–2369. [PubMed: 12488828]
- Parrish JC, Nichols DE. Serotonin 5-HT(2A) receptor activation induces 2-arachidonoylglycerol release through a phospholipase c-dependent mechanism. *J Neurochem*. 2006; 99:1164–1175. [PubMed: 17010161]
- Partosoedarso ER, Abrahams TP, Scullion RT, Moerschbaecher JM, Hornby PJ. Cannabinoid1 receptor in the dorsal vagal complex modulates lower oesophageal sphincter relaxation in ferrets. *J Physiol*. 2003; 550:149–158. [PubMed: 12879865]
- Peters J, McDougall S, Fawley J, Smith S, Andresen M. Primary afferent activation of thermosensitive glutamate release at central neurons. *Neuron*. 2010; 65:657–669. [PubMed: 20223201]
- Raul L. Serotonin2 receptors in the nucleus tractus solitarius: characterization and role in the baroreceptor reflex arc. *Cell Mol Neurobiol*. 2003; 23:709–726. [PubMed: 14514026]
- Rogers RC, Hermann GE. Tumor necrosis factor activation of vagal afferent terminal calcium is blocked by cannabinoids. *J Neurosci*. 2012; 32:5237–5241. [PubMed: 22496569]
- Roth BL, Willins DL, Kristiansen K, Kroeze WK. 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): where structure meets function. *Pharmacol Ther*. 1998; 79:231–257. [PubMed: 9776378]
- Seagard JL, Hopp FA, Hillard CJ, Dean C. Effects of endocannabinoids on discharge of baroreceptive NTS neurons. *Neurosci Lett*. 2005; 381:334–339. [PubMed: 15896495]
- Sevoz-Couche C, Spyer KM, Jordan D. In vivo modulation of vagal-identified dorsal medullary neurones by activation of different 5-Hydroxytryptamine(2) receptors in rats. *Br J Pharmacol*. 2000; 131:1445–1453. [PubMed: 11090119]
- Tognetto M, Amadesi S, Harrison S, Creminon C, Trevisani M, Carreras M, Matera M, Geppetti P, Bianchi A. Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. *J Neurosci*. 2001; 21:1104–1109. [PubMed: 11160380]
- van der Stelt M, Di Marzo V. Anandamide as an intracellular messenger regulating ion channel activity. *Prostaglandins Other Lipid Mediat*. 2005; 77:111–122. [PubMed: 16099396]
- Zhang C, Marek GJ. Group III metabotropic glutamate receptor agonists selectively suppress excitatory synaptic currents in the rat prefrontal cortex induced by 5-hydroxytryptamine2A receptor activation. *J Pharmacol Exp Ther*. 2007; 320:437–447. [PubMed: 17021259]

HIGHLIGHTS

- Activation of nTS 5-HT_{2A}R's augment the amplitude of afferent (TS)-driven EPSCs
- Block of 5-HT_{2A}Rs reduced TS-EPSC amplitude, demonstrating tonic activation
- Endocannabinoids modulate the 5-HT_{2A}R-induced effects of TS-EPSCs

Figure 1. 5-HT_{2A}R activation augments TS-EPSCs

A. Example of TS-EPSCs evoked at 0.5 Hz in the absence and presence of the 5-HT_{2A}R agonist TCB-2 (1 μ M, 5 min). Note the increase in current amplitude with TCB-2. **B–D.** TS-EPSC amplitude during aCSF control, three concentrations of TCB-2 and washout (**B**, 1 μ M, $n=9$; **C**, 10 μ M, $n=7$; **D**, 50 μ M, $n=6$). *, $p < 0.05$ vs. aCSF baseline control, paired t -test. During washout of 50 μ M TCB-2, EPSCs were no longer statistically higher, likely due to the increase in current variation. **E.** Comparison of augmentation of TS-EPSC amplitude from their respective aCSF control in response to 1, 10 and 50 μ M TCB-2. Each cell's individual baseline is denoted as "1". * $p < 0.05$, ANOVA vs. aCSF baseline.

Figure 2. 5-HT_{2A}R activation increases TS-evoked EPSCs during prolonged stimulation

A. Representative example of 20 TS-EPSCs during a 20 Hz stimulus train during control and TCB-2 (1 μ M, 5 min). Note the increase in current amplitude. In the example, stimulus artifacts were digitally removed to improve visualization of EPSCs. **B.** Group data showing amplitude of TS-EPSCs during 1 μ M TCB-2 illustrating TCB-2 increased overall EPSC amplitude from aCSF baseline control [as illustrated by the right upward arrow]. *, $p < 0.05$, main effect of TCB-2, two way RM-ANOVA. **C.** PPR (amplitude ratio of TS-EPSC-2/TS-EPSC-1) during control and TCB-2 (1, 10 and 50 μ M). The PPR did not differ among the treatments groups or within a group (i.e., TCB-2 did not alter the PPR).

Figure 3. Blockade of 5-HT_{2A}R reduces TS-evoked EPSCs

A. Example of the reduction in TS-EPSCs evoked at 0.5 Hz in the presence of the 5-HT_{2A}R antagonist MDL 11,939 (100 μ M). Example is an average of 30 current sweeps. **B.** TS-EPSC amplitude during aCSF control, MDL 11,939 and wash during 0.5 Hz stimulation. $n=9$, * $p < 0.05$ vs. baseline control, paired- t -test. **C.** TS-EPSCs are reduced during a 20 Hz stimulus train in the presence of MDL 11,939 ($n=9$, 2 way RM-ANOVA). **D.** PPR of the first two TS-EPSCs (ratio of TS-EPSC-2/TS-EPSC-1) in the 20 Hz train under control and MDL. **E.** In the presence of 4F 4PP oxalate, another 5-HT_{2A}R antagonist, TS-EPSCs are also reduced ($n=5$, *, $p < 0.05$ vs. baseline control, paired t -test). **F.** Comparison of changes in TS-EPSC amplitude from baseline in the presence of MDL 11,939 and 4F 4PP oxalate. Each cell's individual aCSF baseline control is denoted as "1" (dashed line). * $p < 0.05$, ANOVA vs. baseline. **G.** MDL 11,939 pretreatment prevented the TCB-2 (10 μ M) induced increase in current amplitude from aCSF during 0.5 Hz TS stimulation (**G.**).

Figure 4. Endocannabinoids influence 5-HT_{2A}R-modulation of afferent evoked EPSCs
 Group data demonstrating the change in TS-EPSC amplitude following pharmacological manipulation of 5-HT_{2A}R and/or the endocannabinoid system. Data are plotted as change from each group's aCSF baseline control (denoted as "1", dashed line). **A.** Change in TS-EPSC amplitude evoked at 0.5 Hz. **B.** Change in total throughput of TS-EPSCs during a 20 Hz stimulus train (sum of 20 events). The number of cells for each group in A and B are as follows: AM251 (n=4), AM 404 (n=4), TCB-2 (n=9), TCB-2 + AM 251 (n=7), TCB-2 + AM 404 (n=7), MDL 11, 939 (n=9), MDL + AM 251 (n=5), MDL + AM404 (n=6). *, p < 0.05 vs. individual group's own aCSF baseline control (paired t-test).