News Release

Title

Functional interaction between GABAergic neurons in the ventral tegmental area and serotonergic neurons in the dorsal raphe nucleus

Key Points

- **It was not clearer that how sleep-active midbrain GABAergic neurons are modulated by wake-active neurotransmitters and functional interaction with their innervated neurons.**
- **Using transgenic mice, we discovered that brainstem serotonergic (5-HT) neurons were directly innervated and inhibited by ventral tegmental area (VTA) Gad67+ neurons and in vitro recording from these neurons revealed that the cholinergic agonist carbachol activated, while dopamine, histamine and 5-HT inhibited them.**
- **This findings will contribute to understand the neural regulation of sleep/wakefulness and physiological behaviors.**

Summary

GABAergic neurons in the ventral tegmental area (VTA) have brain-wide projections and are involved in multiple behavioral and physiological functions. Here, we revealed the responsiveness of Gad67+ neurons in VTA (VTA_{Gad67+}) to various neurotransmitters involved in the regulation of sleep/wakefulness by slice patch clamp recording. Among the substances tested, a cholinergic agonist activated, but serotonin, dopamine and histamine inhibited these neurons. Dense VTA_{Gad67+} neuronal projections were observed in brain areas regulating sleep/wakefulness, including the central amygdala (CeA), dorsal raphe nucleus (DRN) and locus coeruleus (LC). Using a combination of electrophysiology and optogenetic studies, we showed that VTA_{Gad67+} neurons inhibited all neurons recorded in the DRN, but did not inhibit randomly recorded neurons in the CeA and LC. Further examination revealed that the serotonergic neurons in the DRN ($DRN_{5\text{-}HT}$) were monosynaptically innervated and inhibited by VTA_{Gad67+} neurons. All recorded $DRN_{5\text{-}HT}$ neurons received inhibitory input from VTA_{Gad67+} neurons, while only one quarter of them received inhibitory input from local GABAergic neurons. Gad67+ neurons in the DRN (DRN_{Gad67+}) also received monosynaptic inhibitory input from VTA_{Gad67+} neurons. Taken together, we found that VTA_{Gad67+} neurons were integrated in many inputs, and their output inhibits $DRN_{5\text{-}HT}$ neurons, which may regulate physiological functions including sleep/wakefulness.

Research Background

VTA is a well-characterized midbrain structure containing dopamine (DA) neurons, GABAergic and glutamatergic neurons. γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the adult mammalian brain expresses one of the two glutamic acid decarboxylase (GAD)

isoforms, Gad67. Recently, it was reported that the VTA_{Gad67+} neurons are critically involved in sleep/wakefulness regulation, but it is still unknown how these neurons are regulated by wake-promoting neurotransmitters released from their upstream regions. VTA_{Gad67+} neurons have distal projections to different areas of the brain, including to the brainstem DRN. DRN also comprises multiple cell types whereas 5-HT neurons make up around half of the total neuronal population in the raphe nucleus. However, functional connections at the neural circuit level between these two areas are still unclear.

This research group has been studying how VTA_{Gad67+} neurons are modulated by the neurotransmitter serotonin (5-HT), noradrenaline (NA), dopamine (DA), histamine (HA), carbachol (CCh) and orexin A (OX-A), which are all involved in sleep/wakefulness regulation. Functional interaction between VTA_{Gad67+} neurons and DRN 5-HT neurons has also been established using a combination of techniques, optogenetics and electrophysiological recordings. Together, they showed that VTA_{Gad67+} neurons were regulated by different neurotransmitters and the functional interaction between VTA_{Gad67+} and $DRN_{5\text{-}HT}$ neurons might contribute to the understanding of the regulatory mechanisms of sleep/wakefulness.

Research Results

Using slice patch clamp recording this group assess the activity of VTA_{Gad67+} neurons upon application of various neurotransmitters. By operating a six-channel perfusion valve controller, neurotransmitters were sequentially and locally applied for 4 s under a loose cell-attached mode recording. They found that 5-HT, DA and HA application significantly inhibited, whereas CCh activated VTA_{Gad67+} neurons but surprisingly, the wake-promoting neurotransmitters NA and OX-A had no significant effect.

Next, this group aim to reveal the downstream target neurons of VTA_{Gad67+} neurons. After confirming the expression of a blue light–gated cation channel channelrhodopsin-2 (E123T/T159C) (ChR2) into the VTA_{Gad67+} neurons, they recorded from neurons in the CeA, DRN and LC by stimulating the VTA_{Gad67+} nerve terminals. In-vitro slice current clamp recording showed that only the recorded neurons of the DRN were hyperpolarized and there was a complete cessation of

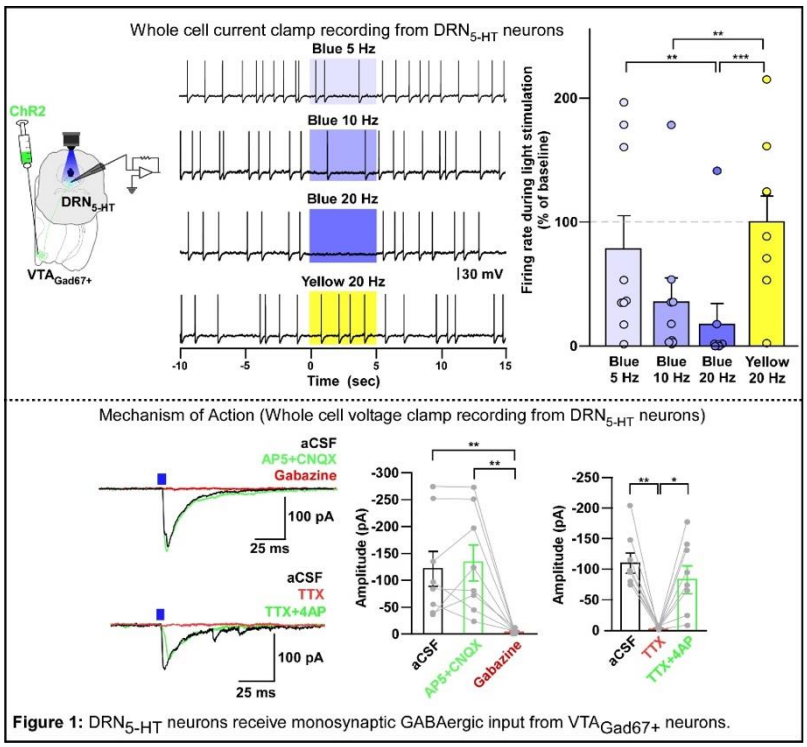

Figure 1: $DRN_{5\text{-}HT}$ neurons receive monosynaptic GABAergic input from VTA_{Gad67+} neurons.

spontaneous firing following a 20-Hz blue light stimulation, whereas activity in CeA or LC neurons was not affected. Then they narrow down their focusing into $DRN_{5\text{-}HT}$. By using trigenic mice, they recorded from the $DRN_{5\text{-}HT}$ neurons and stimulated the ChR2-positive VTA_{Gad67+} nerve terminals. In whole-cell current clamp recording, they found that a 5-, 10- and 20-Hz blue light pulse decreased the firing rate and hyperpolarized the membrane potential in a frequency-dependent manner (Figure 1). Further, by applying several glutamatergic, GABAergic and voltage gated sodium and potassium channel blockers in whole-cell voltage clamp mode, they confirmed that $DRN_{5\text{-}HT}$ neurons were directly innervated and inhibited by VTA_{Gad67+} neurons through GABA neurotransmission (Figure 1). In addition, they found that $DRN_{5\text{-}HT}$ neurons were directly innervated and inhibited by their local Gad67+ neurons. And the GABAergic population in the DRN also received monosynaptic inhibitory input from VTA_{Gad67+} neurons.

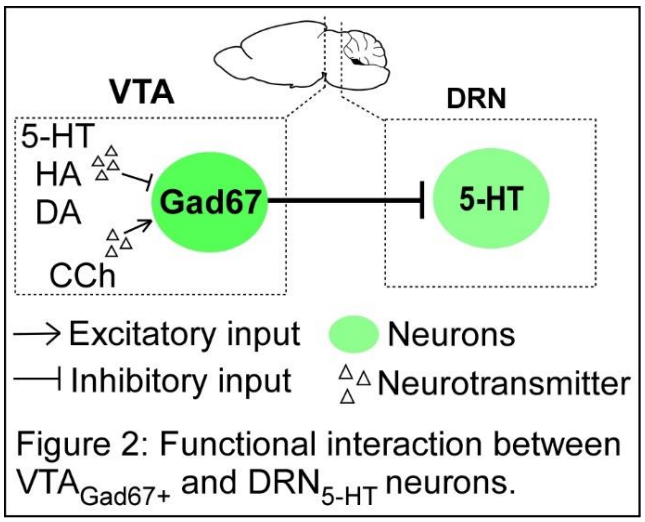

Figure 2: Functional interaction between VTA_{Gad67+} and $DRN_{5\text{-}HT}$ neurons.

From these results, it became clear that the input pathways of VTA_{Gad67+} neurons and their functional circuit with $DRN_{5\text{-}HT}$ neurons (Figure 2).

Research Summary and Future Perspective

Although many works have been done on GABAergic neurons related with sleep but local perfusion data newly elucidated that how VTA_{Gad67+} neurons were regulated by neurotransmitters involved in sleep/wakefulness regulation, such as 5-HT, DA, HA and acetylcholine, but not by OX-A or NA. VTA_{Gad67+} neurons integrated these inputs and inhibited $DRN_{5\text{-}HT}$ neurons.

This functional interaction between VTA_{Gad67+} and $DRN_{5\text{-}HT}$ neurons will contribute to the understanding of the regulatory mechanisms of sleep/wakefulness and other physiological behaviors.

Publication

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