

*Review article*

## **Cerebellar Control of Defense Reactions under Orexin-mediated Neuromodulation as a Model of Cerebellohypothalamic Interaction**

Masao Ito <sup>1,\*</sup> and Naoko Nisimaru <sup>1,2</sup>

<sup>1</sup> RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama, 315-0198, Japan

<sup>2</sup> Department of Physiology, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama, Yufu, Oita 879-5593, Japan

\* **Correspondence:** Email: masao@brain.riken.jp; Tel: +81-48467-6984; Fax: +81-48467-6975.

**Abstract:** Recent evidence has indicated that, when an animal is exposed to harmful stimuli, hypothalamic orexinergic neurons are activated via the amygdala and in turn tune the neuronal circuits in the spinal cord, brainstem, and an area of the cerebellum (folium-p of the flocculus) by neuromodulation. The animal would then initiate “defense reactions” composed of complex movements and associated cardiovascular responses. To investigate neuronal mechanisms of the defense reactions, Nisimaru et al. (2013) analyzed cardiovascular responses induced by an electric foot shock stimulus to a rabbit and found two major effects. One is redistribution of arterial blood flow from visceral organs to active muscles, and the other is a modest increase in blood pressure. Kainate-induced lesions of folium-p impaired these two effects. Moreover, folium-p Purkinje cells were shown to project to the parabrachial nucleus, one of the major cardiovascular centers in the brainstem. These data indicate that folium-p Purkinje cells regulate cardiovascular defense reactions via parabrachial nucleus under orexin-mediated neuromodulation. In this article, we review these data from the viewpoint that the defense reactions are expressions of “anger and anxiety”, which respectively lead to “fight and flight” behaviors. The present orexin case may provide a model of cerebellohypothalamic interactions via neuropeptides or amines of hypothalamic origin, which may underlie various types of emotion and behavior.

**Keywords:** arterial redistribution; defense; flocculus; neuromodulation; orexins

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### **1. Introduction**

The basis for assuming roles of the cerebellum in emotion is threefold: A) the presence of anatomical connections between the cerebellum and the hypothalamus [1–3]; B) disturbances of

emotion regulation in cerebellar patients, such as “cerebellar cognitive-affective syndrome” [4,5]; and C) decreased activities in the cerebellum as revealed by brain imaging of schizophrenic patients [6–8]. However, little has been known about the mechanisms by which cerebellar dysfunctions lead to impaired regulation of emotion and behavior.

We have paid special attention to connections between the cerebellum and the hypothalamus via beaded fibers containing neuropeptides or amines. In particular, orexin-containing (assumed to be orexinergic) fibers originate solely from the hypothalamus and distribute broadly in the spinal cord, brainstem, and also the flocculus region of the cerebellum [9]. Recently, Nisimaru et al. [10] showed in rabbits that orexinergic fibers mediate the cardiovascular component of defense reactions to harmful stimuli. The cardiovascular defense reactions evoked by applying electric foot shock stimuli to a rabbit were twofold. First, arterial blood flow is redistributed from visceral organs to active muscles, conforming to a high demand for arterial blood in active muscles, and second, a modest increase in blood pressure maintaining cardiovascular homeostasis. Kainate-induced lesions of folium-p impaired these effects. Moreover, folium-p Purkinje cells were shown to project to the parabrachial nucleus, one of the major cardiovascular centers in the brainstem. Folium-p Purkinje cells may regulate cardiovascular defense reactions via parabrachial nucleus under orexin-mediated neuromodulation.

In this article, we review and interpret Nisimaru et al.’s [10] observations from the viewpoint that the cardiovascular defense reactions are part of the expressions of anger and anxiety against harmful stimuli, which would respectively lead to fight and flight behaviors. Extending the present case of cerebellohypothalamic interactions via orexins, we may hypothesize that there are a number of neuropeptides or amines of hypothalamic origin, which form a selection mechanism for different types of emotion and behavior.

## **2. Neuromodulation that the hypothalamus exerts on the cerebellum**

The cerebellar cortex receives two well-identified types of afferent, mossy fibers and climbing fibers, arising from various sources. In addition, there is a third type of afferent, as first described by Haines and Dietrichs [1]. This type of afferent consists of characteristically beaded fibers, which contain amines or neuropeptides (see [11]). For example, histamine-containing fibers originate from the tuberomammillary nucleus of the hypothalamus and broadly extend in the cerebellum [12]. In contrast, orexin-containing fibers originate solely from the perifornical regions of the hypothalamus and innervate almost exclusively the flocculus, that is, the phylogenetically old part of the cerebellum [9]. In contrast, beaded fibers containing another neuropeptide, angiotensin II, arise from the paraventricular and supraoptic nuclei of the hypothalamus [13] and impinge globally upon the cerebellum. Among twenty-four different neuropeptides so far located in the cerebellum [14], some others may also be expressed in beaded fibers.

Beaded fibers distribute in a target area in a characteristically sparse and diffuse manner. For example, through the granular layers, Purkinje cell layers, and molecular layers of the rabbit flocculus, the spatial density of orexinergic fibers is at the maximum 3.33 pieces/mm<sup>2</sup> coronal cross-sectional area of the flocculus ([10], Table S1). It appears that, whereas mossy fibers and climbing fibers form neuron-to-neuron-specific connections, beaded fibers convey information diffusely to determine the general activity or the mode of operation of their target neurons. This is the manner of innervation defined as neuromodulation by Marder and Thirumalai [15].

### 3. Orexinergic neurons mediate cardiovascular defense reactions

When a few selected brain areas are stimulated electrically or chemically, animals exhibit defense reactions, which are composed of complex motor activities for fight or flight behavior and associated cardiovascular responses [16–18]. The major defense area is located over the perifornical region of the hypothalamus, which is the sole source of orexinergic neurons. The close relationship between the cardiovascular defense reactions and orexinergic neurons has been revealed in the following ways. When the fight or flight movements were suppressed under general anesthesia, a large transient increase in blood pressure is evoked by electric stimulation of the defense area. This blood pressure response attenuates after intravenous administration of orexinergic antagonists [10]. The blood pressure response also attenuated in prepro-orexin–null knockout mice [19] and orexinergic neuron-ablated transgenic mice [20]. Hence, the blood pressure rise evoked from the defense area under anesthesia should be mediated by orexinergic neurons.

Under natural behavioral conditions, defense reactions can be evoked by harmful stimuli to an animal, which would activate orexinergic neurons via the amygdala (for review, see [21]). Typically, electric foot shock stimuli to an awake rabbit induce defense reactions [10], in which complex movements are associated with certain cardiovascular responses. Namely, arterial blood flow is redistributed from visceral organs or resting muscles to active muscles [16] by the action of the sympathetic nervous system [22]. In spite of this drastic change in arterial blood flow, blood pressure only slightly increases. These observations indicate that a subtle control of arterial blood flow is exerted to maintain cardiovascular homeostasis while conforming to a high demand for arterial blood supply to those muscles actively involved in defense reactions. Because intravenous administration of orexin antagonists attenuates the foot-shock-evoked redistribution of arterial blood flow [10], such subtle cardiovascular control must be mediated by orexinergic neurons. On the other hand, there is no evidence for involvement of orexinergic neurons in the motor part of defense reactions.

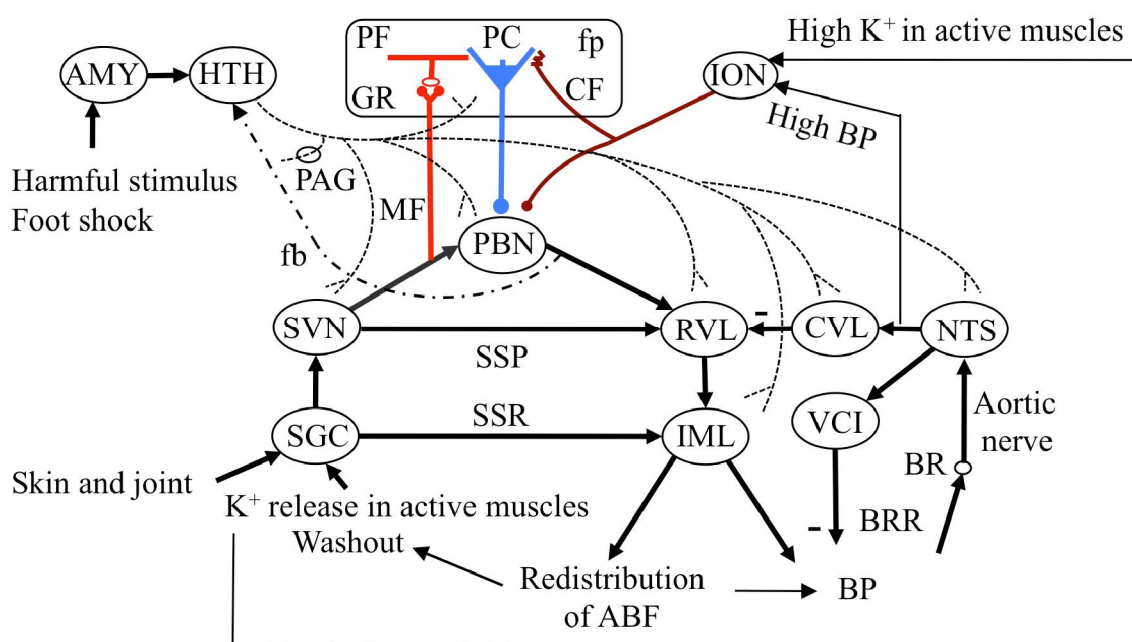
### 4. Neuronal circuit for cardiovascular defense reactions

Nisimaru et al. [10] determined the neuronal circuit of the cardiovascular defense reactions by referring to numerous reports including their own ones (Figure 1). Cardiovascular defense reactions are evoked by harmful stimuli, such as electric foot shock, to the skin, muscles, and joints, and are mediated by segmental pathways to evoke somatosympathetic reflexes (SSR) [23]. SSRs act on sympathetic nerves in arterial blood vessels via the intermediolateral nucleus (IML). The SSR pathway is superposed by a supraspinal pathway (SSP) connecting spinal and trigeminal sensory nuclei (SVN) to the rostroventrolateral nucleus (RVL). SSP is further superposed by a higher-order connection mediated by the parabrachial nucleus (PBN), which receives input from SVN and projects to RVL. These connections constitute the cardiovascular defense reaction circuit, as it may be called. Orexinergic fibers innervate the entire cardiovascular defense reaction circuit. The excitatory synaptic action of orexinergic fibers has been confirmed by recording from their target neurons, namely, folium-p Purkinje cells [10], histaminergic neurons of the tuberomammillary nucleus [24], locus coeruleus neurons [25], and interpositus nuclear neurons [26]. Under anesthesia, electric stimulation of orexinergic fibers may cause synchronized activation of neurons in the cardiovascular defense reaction circuit and may cause the sharp rise in blood pressure via MIL. In awake behaving animals, natural harmful stimuli may activate orexinergic neurons in more organized manner to induce purposeful redistribution of arterial blood flow.

The projection of folium-p Purkinje cells to the ipsilateral PBN suggests that these Purkinje cells adaptively control cardiovascular defense reactions via PBN. Nisimaru et al [10] confirmed that after bilateral lesioning of folium-p, electric foot shock stimuli induced an abnormally large increase in the arterial flow in active muscles, whereas the foot-shock-induced decrease in the arterial blood flow to visceral organs was abnormally small [10]. The foot-shock-induced modest increase of blood pressure turned to be smaller or even reverse to a decrease [10]. Folium-p Purkinje cells appear to be controlling cardiovascular defense reaction circuit via PBN under orexinergic neuromodulation.

## 5. Return connections from cerebellar nuclei to the hypothalamus

As indicated in Figure 1 by a dashed line labeled fb, there is a return projection from PBN to the hypothalamic region where orexinergic neurons distribute [3]. This projection may feed back orexin-evoked neuronal activities in the brainstem and cerebellum to hypothalamic orexinergic neurons. Hence, a lesion of folium-p of the flocculus might impair the activities of orexinergic neurons via PBN and thus could be another cause of the disturbed expression of anger or anxiety.



**Figure 1. Neuronal circuit diagram for cerebellar control of defense reactions. AMY, amygdala; BR, baroreceptor; CF, climbing fiber; fb, feedback pathway; GR, granule cell; HTH, hypothalamus; MF, mossy fiber; PF, parallel fiber. Other abbreviations are defined in the text. (reproduced from Figure 9 [10])**

## 6. Roles of the cerebellum in emotion

It has been reported that the pairing of acoustic and nociceptive stimuli induces animals to express fear responses to otherwise neutral acoustic stimuli. In cerebellar slices obtained from fear-conditioned rats, both excitatory and inhibitory transmissions to Purkinje cells were potentiated for up to 24 hours after conditioning [27]. How these changes are induced is presently unknown, but one possibility is that the neuromodulation is mediated by a yet-unidentified hypothalamic

neuropeptide. Orexins are unlikely, because Scelfo et al. [27] apparently sampled Purkinje cells widely outside of the flocculus that exclusively receives orexinergic fibers. Orexinergic fibers, nevertheless, are relayed by the locus coeruleus neurons whose noradrenergic axons reach the cerebellar cortex broadly [25]. One possibility is that the fear conditioning affects Purkinje cells throughout the cerebellum via orexin-noradrenalin relay.

It is now apparent that hypothalamic orexinergic neurons activate the cardiovascular defense reaction circuit in the manner of neuromodulation, and thereby contribute, at least in part, to the expressions of anger and anxiety that respectively lead to emergence of fight and flight behaviors. The present study of orexinergic innervation may provide a prototype of the mechanism for selecting emotional behavioral repertoires via neuropeptidergic or aminergic neuromodulation. Each peptide or amine may ensemble a unique set of neuronal circuits through the spinal cord, brainstem, and cerebellum, which jointly express a specific emotion and behavior. It will be important to study more peptides or amines, which, by bridging the cerebellum and hypothalamus, play a role in the selection of emotional and behavioral repertoires.

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### Conflict of Interest

All authors declare no conflicts of interest in this paper.

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