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Review Article

Emotional Modification of the Cardiorespiratory Regulation System

Tomoyuki Kuwaki, Wei Zhang*

Department of Molecular and Integrative Physiology, Autonomic Physiology, Chiba University, Graduate School of Medicine, Chiba, Japan

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Abstract

Our daily life is not solely composed of a calm resting state but is rather full of perturbations that induce active states such as moving, eating, communicating, and so on. During such active conditions, cardiorespiratory regulation should be adjusted according to the body's demand, which differs from that during a resting state, by modulating or resetting the operating point. To explore the neural mechanisms of state-dependent adjustment of central autonomic regulation, we focused on the stress-induced defense (fight-or-flight) response because stressors induce not only cognitive, emotional and behavioral changes but also autonomic changes. In this mini-review, we summarize our recent discovery using orexin knockout mice and orexin neuron-ablated mice of the possible contribution of orexin, a hypothalamic neuropeptide, in the state-dependent adjustment of central autonomic regulation. The diversity of synaptic control of the cardiovascular and respiratory neurons seems necessary for animals to adapt themselves toward ever-changing life circumstances and behavioral states. The orexin system is likely to work as one of the essential modulators for coordinating circuits controlling autonomic functions and behavior. [*Tzu Chi Med J* 2008;20(2):82–90]

*Corresponding author. Department of Molecular and Integrative Physiology, Chiba University, Graduate School of Medicine, Inohana 1-8-1, Chuo-ku, Chiba 260-8670, Japan.
E-mail address: kuwaki@faculty.chiba-u.jp

1. Introduction

Fundamental neural substrates participating in the central autonomic regulation of circulation and respiration have been established. For example, rostral ventrolateral medulla and pre-Bötzinger complex have been revealed as the sympathetic and respiratory centers, respectively, that are indispensable for maintenance of our life [1,2]. We have abundant, though not complete, knowledge of the homeostatic mechanisms that stabilize circulation and respiration around an operating point at a resting state.

Our daily life, however, is not solely composed of a calm resting state. It is rather full of perturbations that induce active states such as moving, eating, communicating, and so on. During such active conditions, cardiorespiratory regulation should be adjusted according to the body's demand that differs from that

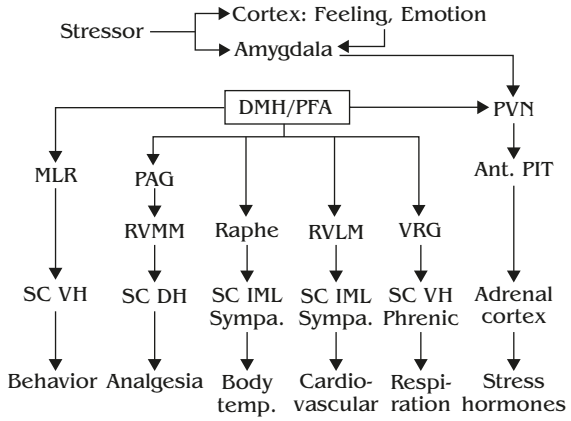


Fig. 1 — Hypothetical neural circuit for the defense response against stressors. Note that what are depicted here are only the circuits relevant to this review. See also Fig. 2 and Section 3 on 'Emotional behavior'. Ant. PIT=anterior pituitary; Temp.=temperature; DH=dorsal horn; DMH=dorsomedial hypothalamus; IML=intermediolateral cell column; MLR=medullary locomotor region; PAG=periaqueductal gray; PFA=perifornical area; PVN=paraventricular nucleus; RVLM=rostral ventrolateral medulla; RVMM=rostral ventromedial medulla; SC=spinal cord; Sympa.=sympathetic nerve; VH=ventral horn; VRG=ventral respiratory group.

during a resting state by modulating or resetting the operating point [3]. Research on the adjustment mechanism has been relatively sparse, although it is important from the quality-of-life point of view.

To explore the neural mechanisms of state-dependent adjustment of central autonomic regulation, we have recently focused on the stress-induced defense (fight-or-flight) response because stressors induce not only cognitive, emotional and behavioral changes but also autonomic changes. These changes include increases in blood pressure, heart rate, muscle blood flow, respiratory frequency, tidal volume, and body temperature and suppression of the baroreceptor reflex and pain sensitivity. Although research on the neural circuits underlying these changes has implicated the hypothalamus as the key structure in the defense response (Fig. 1), the neurotransmitters involved in this multifaceted and coordinated response have not been revealed.

2. Orexin

Orexins (orexin-A and orexin-B), also known as hypocretins (hypocretin-1 and hypocretin-2, respectively), were identified as a ligand for a G-protein coupled orphan receptor in 1998. They are cleaved from a common precursor molecule, prepro-orexin (130 residues), forming orexin-A (33 amino acids) and orexin-B (28 amino acids) [4,5]. Orexin-containing cell bodies are restrictedly located in the lateral hypothalamus, the perifornical area (PFA), and the dorsomedial hypothalamus (DMH). On the other hand, orexin-containing fibers and terminals are widely distributed in the hypothalamus, thalamus, cerebral cortex, circumventricular organs, brain stem and spinal cord, suggesting that the orexinergic neurons have widespread connections with other brain regions [6,7]. This anatomic feature establishes the basis for contributions by orexin to the control of multiple physiological functions, including feeding behavior, energy homeostasis, sleep-wake cycle, and regulation of the autonomic and neuroendocrine systems [5,8–10].

3. Emotional behavior

Animals cope with stressors by two strategies. An active coping strategy (fight or flight) is evoked if the stress is predictable, controllable, or escapable. A passive coping strategy (immobility or decreased responsiveness to the environment) is evoked if the stress is inescapable. The active strategy is associated with sympathoexcitation (hypertension, tachycardia), whereas the passive strategy is associated with sympathoinhibition and/or parasympathetic activation (hypotension, bradycardia). The passive strategy also helps to facilitate recovery and healing. The active strategy is also called the *fight or flight response* from a behavioral point of view or *defense response* from an autonomic point of view. The passive strategy is sometimes called *playing dead* or *paradoxical fear*. Distinct neural substrates mediating active versus passive emotional coping have been identified within the brainstem [11,12]. In the posterior hypothalamus, the PFA and the DMH are known as the center for defense response and is sometimes called the "defense area" [13].

Pioneering work by Hess [14] showed that electrical stimulation of the posterior hypothalamus in cats elicited behavioral *rage* along with the specific autonomic responses that was termed the *defense response*. Although some reports using chemical stimulation with excitatory amino acids (e.g., glutamate) had shown no effect or even a depressor effect [15,16], it was later shown that the negative results might have been caused by the stimulation of specific subregions in the posterior hypothalamus and/or dosage of drugs [17]. In fact, the PFA was the most reliable region to elicit cardiovascular defense response [13] and overdose of excitatory amino acids sometimes inhibited neuronal activity, an effect known as excitation block phenomenon [18]. Disinhibition with a $GABA_A$ receptor antagonist, bicuculline methiodide, of the PFA reliably and dose-dependently induced the defense response [19]. Moreover, injection of a GABA agonist

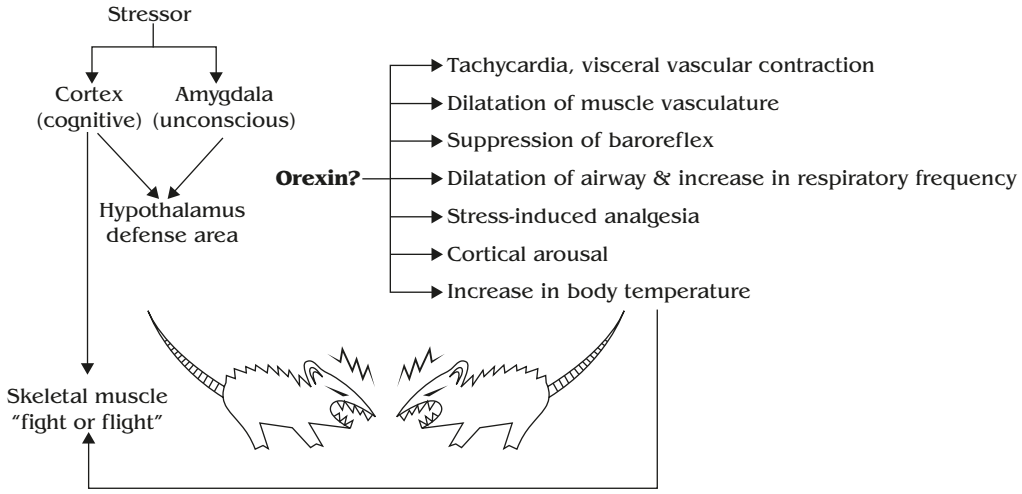


Fig. 2 — Multifaceted nature of the defense response. Simultaneous and coordinated changes in the cardiorespiratory, sensory and thermoregulatory systems support the efficiency of the behavioral fight-or-flight response. Orexin plays a role as a master switch of these orchestrated responses. (Adapted from Reference 10.)

to the defense area inhibited stress-induced rise in arterial pressure (AP) and heart rate (HR) [20].

Sympathoexcitatory cardiovascular response is not the sole characteristic of the defense response. The defense response is characterized by a coordinated rise in AP, HR, respiratory frequency, and resistance in the visceral vascular beds along with a fall in resistances in airway and blood vessels in the skeletal muscles when an animal encounters stressors. Baroreceptor reflex is suppressed or reset to a higher-pressure range to allow higher AP than that in resting condition. Moreover, body temperature increases [21], cortical arousal is promoted, and the sensation of pain is actively suppressed during the defense response. All these changes support the efficiency of the behavioral response of fight or flight (Fig. 2) [11,13,22].

Some neurotransmitters have been proposed to be involved in the efferent pathways of the defense response. For example, activation of serotonin (5HT)-1A receptors in the medullary raphe reduced cardiovascular changes [23] and inhibition of 5HT-3 receptors in the nucleus tractus solitarius prevented the baroreflex bradycardia inhibition during the defense response [24]. Microinjections of adenosine into the rostral ventrolateral medulla augmented the increase in AP evoked by electrical stimulation of the hypothalamic defense area [25]. Pros and cons were reported on participation of glutamate in the cardiovascular component of the defense response [26,27]. To date, however, there are no reports on the molecular basis of the defense response underlying its multifaceted nature of simultaneous and coordinated changes in cardiovascular, respiratory, sensory, and behavioral parameters.

We hypothesized that intrinsic orexin, synthesized in the PFA/DMH, regulates the multifaceted features of the defense response. In fact, stress activated orexinergic neurons [28–32]. Pharmacological and anatomical evidence supports our hypothesis. Exogenous administration of orexin induced analgesia [33] and cardiovascular [34–39] and respiratory [39,40] activation. Orexinergic neurons projected to the cardiovascular centers [34,41–44], respiratory centers [40,45], and sympathetic premotor neurons controlling brown adipose tissue [46,47] in the medulla oblongata and the spinal cord. About 50% of the hypothalamic neurons that innervate both sympathetic efferent and motor cortex or medial prefrontal cortex, which is implicated in mental stress, showed orexin-like immunoreactivity [48,49]. Numerous neurons in the amygdala, a putative center for biological value judgment [50], were retrogradely labeled by trans-synaptic transport of tetanus toxin expressed by the orexin promoter [51].

4. Evidence for participation of orexin in the defense response

To test our hypothesis that orexin contributes to the multiple efferent pathways of the defense response, we performed several lines of experiments using genetically engineered orexin-deficient mice. At present, there are two genetically engineered mice models of orexin deficiency to study the possible roles of intrinsic orexin in physiological functions. One is the prepro-orexin knockout mouse (ORX-KO) that was developed by a conventional knockout technique [52] and another is the orexin neuron-ablated mouse [53]. The latter was developed using a transgenic technique by introducing a truncated Machado-Joseph disease gene product (ataxin-3) with an expanded polyglutamine

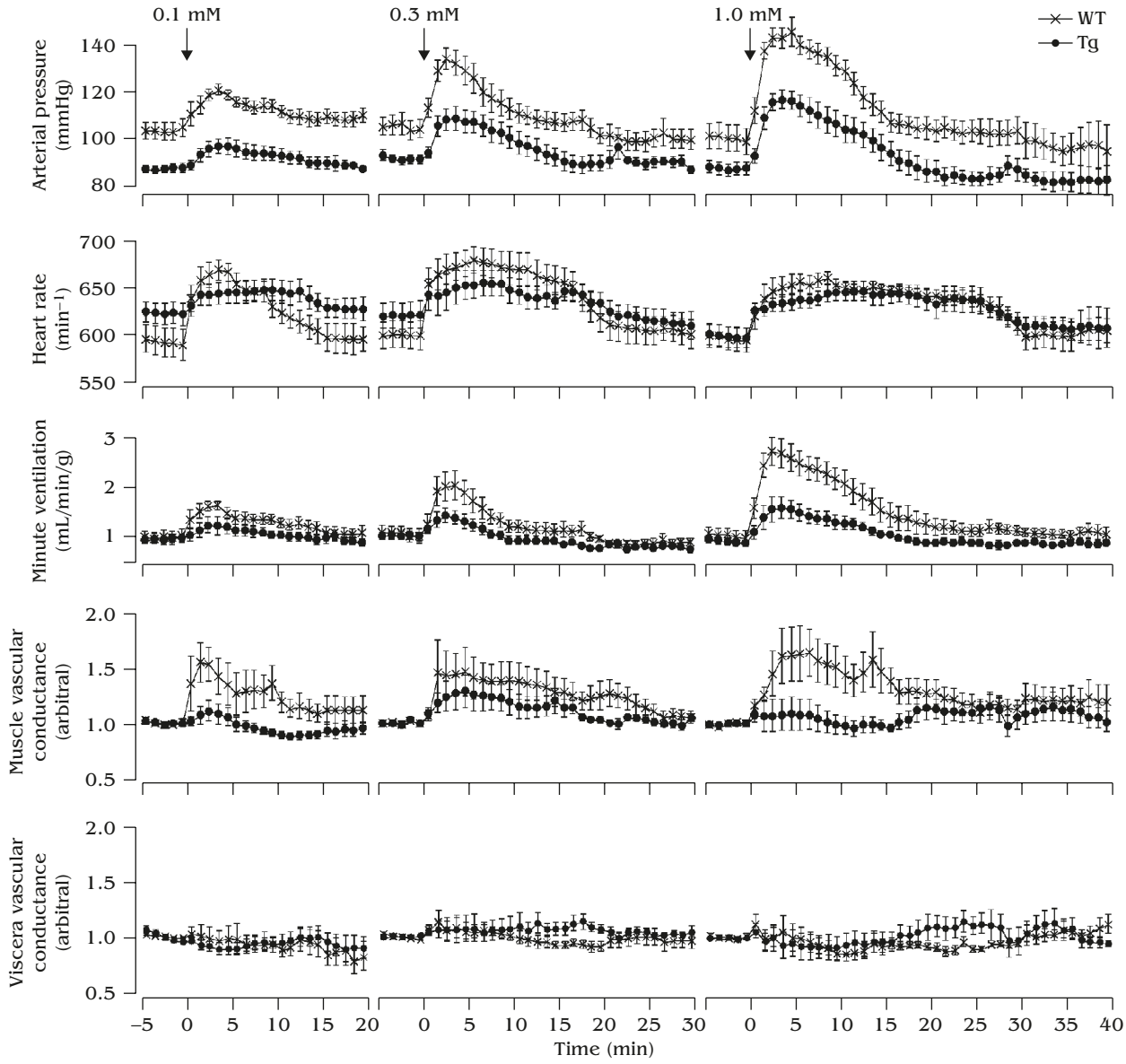


Fig. 3 — Effects of microinjection of bicuculline methiodide to the perifornical area in wild-type (WT) mice and orexin/ataxin-3 transgenic mice (Tg) on arterial blood pressure, heart rate, respiratory minute ventilation, and the muscular and visceral vascular conductance. Arrowheads indicate timing of microinjection of bicuculline (20 nL). Data are presented as mean±standard error of the mean of 8 WT mice and 9 Tg mice. (Adapted from Reference 56.)

stretch under the control of the orexin promoter. In these orexin/ataxin-3 transgenic mice (ORX/ATX-Tg), orexinergic neurons are selectively and postnatally degenerated and reaches >99% loss at 4 months of age (53). In these mice, not only orexin but also other neurotransmitter candidates contained in the orexinergic neurons, such as dynorphin, galanin, and glutamate, are considered deficient (54). Both ORX-KO and ORX/ATX-Tg showed a phenotype strikingly similar to human narcolepsy (52,53). The data described below were obtained from offspring of the originally described ORX-KO (52) and ORX/ATX-Tg (53) in which the genotypes and orexin deficiency were confirmed.

4.1. Stimulation to the PFA resulted in an attenuated defense response in ORX-KO and ORX/ATX-Tg

As stated above, the defense response is characterized not only by increases in AP and HR but also by increases in ventilation and cortical arousal and by shift of blood flow from visceral to skeletal vasculature. To test our hypothesis that orexin contributes to expression of all the features of the defense response, we compared the effects of chemical stimulation of the PFA by bicuculline on the above-mentioned parameters among ORX-KO, ORX/ATX-Tg and wild-type (WT)

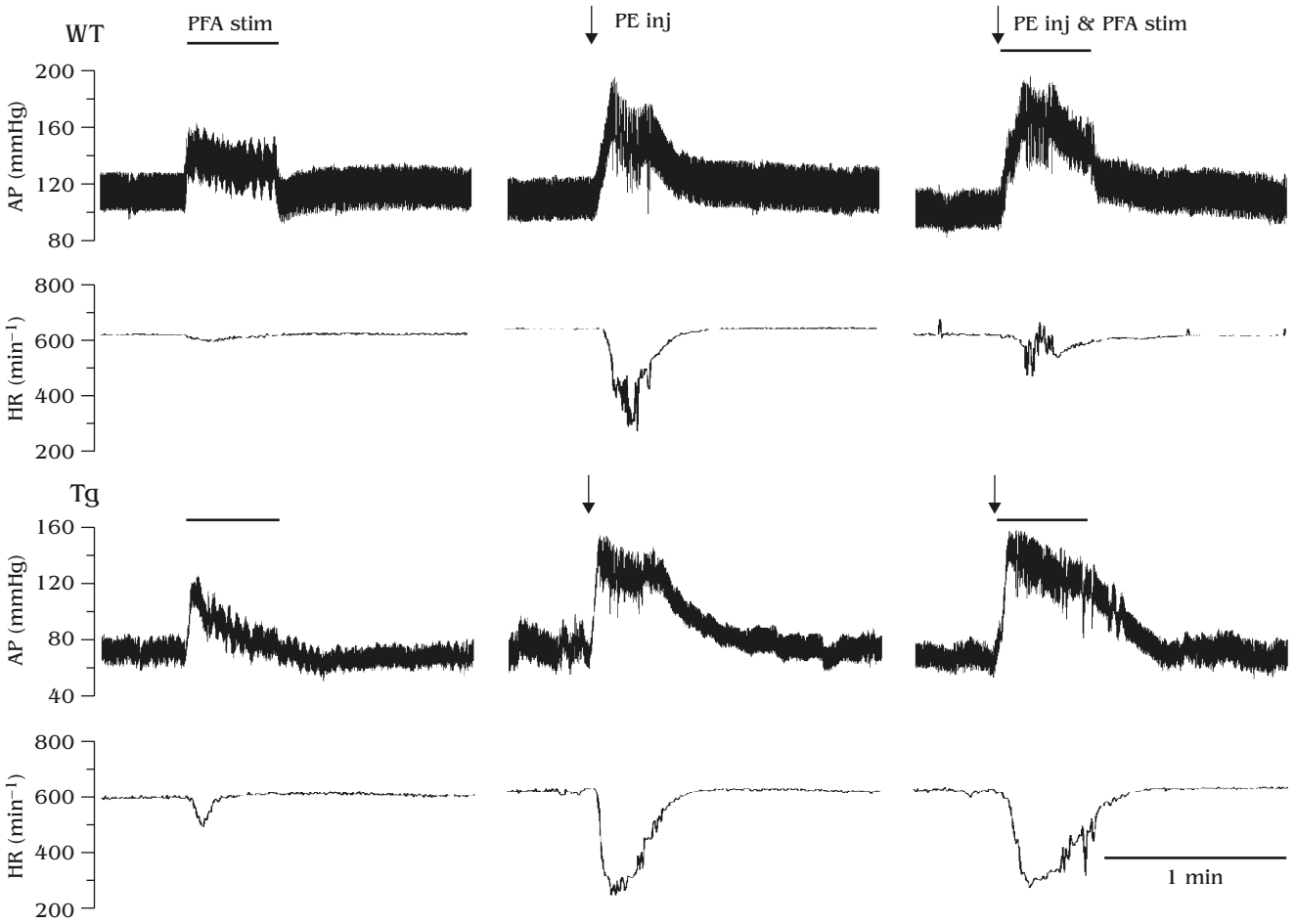


Fig. 4 — Typical example of the inhibitory effect of perifornical area (PFA) stimulation on baroreflex bradycardia in wild-type (WT) mice and orexin/ataxin-3 transgenic mice (Tg). (LEFT) PFA was electrically stimulated for 30 seconds (PFA stim, horizontal bar) after pretreatment with atenolol (5 mg/kg, i.p.). (MIDDLE) Baroreflex bradycardia was induced by an intravenous injection of phenylephrine (50 μg/kg, PE inj, arrow). (RIGHT) PE-induced bradycardia was reduced by simultaneous PFA stimulation in WT but not in Tg. AP=arterial blood pressure; HR=heart rate (in beats/min). (Adapted from Reference 56.)

mice. As expected, increases in AP, HR, respiratory frequency, and β-band power of electroencephalogram (an index of cortical arousal) were smaller and/or shorter-lasting in ORX-KO than in WT littermates (55). In a similar manner, increases in AP, HR, and respiratory minute volume and vascular dilatation in skeletal muscle were attenuated in ORX/ATX-Tg mice (Fig. 3) (56). Therefore, we concluded that orexin-containing neurons in the PFA play a role as a master switch to activate multiple efferent pathways of the defense response. Orexin but not cotransmitters in the neurons seemed to be important at least for the changes in AP, HR and respiration.

4.2. *Suppression of the baroreceptor reflex during the defense response was also attenuated in ORX/ATX-Tg mice*

During the defense response, baroreceptor reflex is suppressed or reset to a higher pressure range to allow higher AP than that in resting condition. This is another important feature of the defense response to ensure effective behavior of fight or flight. To test whether the suppression of the baroreceptor reflex was normal or not in ORX-deficient mice, we performed two lines of experiments.

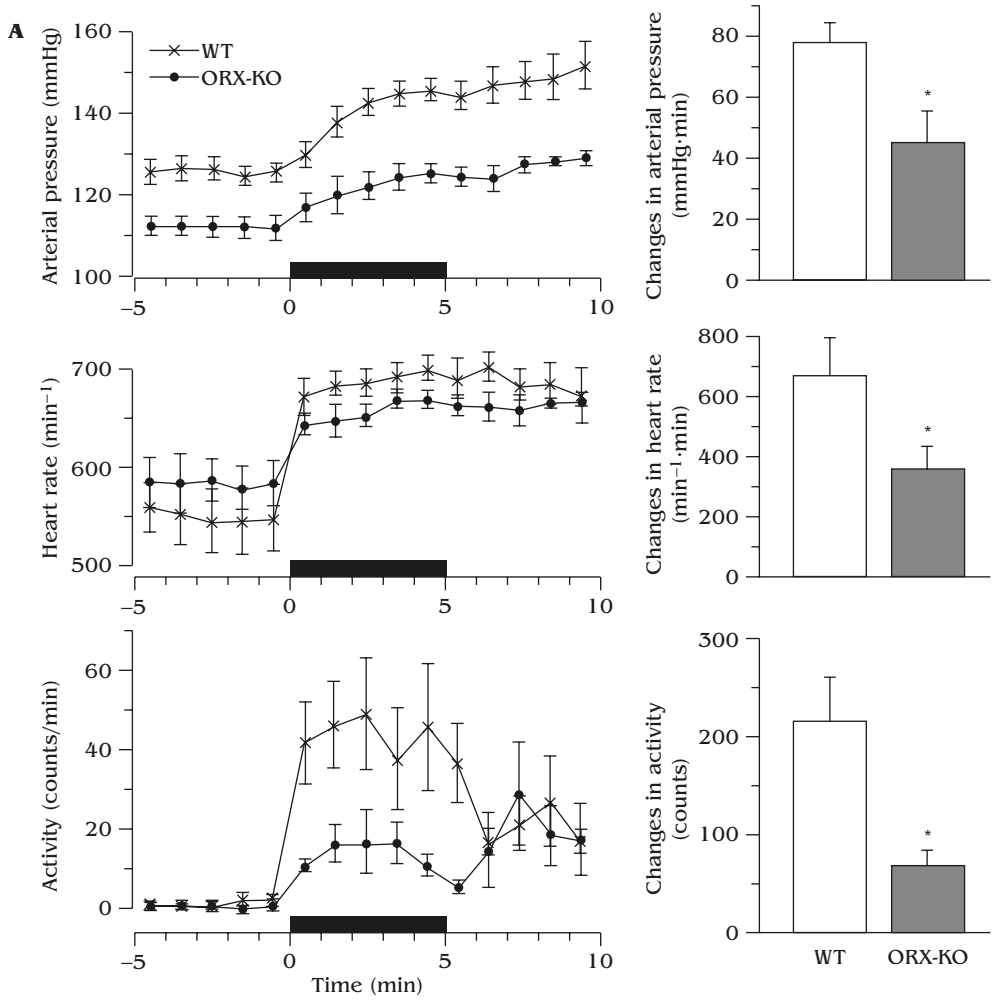
First, the baroreceptor reflex under the resting condition (i.e., without stimulation to elicit the defense response) was compared between ORX/ATX-Tg and WT littermates. To do so, a *naturally occurring sequence method* (57) was employed. In brief, recordings of AP from unanesthetized animals were scanned by a home-made computer program to identify the spontaneous sequences of three or more consecutive beats in which systolic AP (SAP) progressively increased or decreased by more than 1 mmHg per beat. Of these SAP sequences, those that were associated with baroreflex-driven lengthening or shortening in the RR intervals of electrocardiographic signals were selected and defined as baroreflex sequences. A linear regression analysis between SAP and RR interval

was applied to each baroreflex sequence, and the slope of the regression line was calculated. An average value of the slopes in a mouse was taken as the gain of baroreflex in the animal. We also calculated the baroreflex effectiveness index, defined as the ratio between the number of baroreflex sequences and the total number of SAP ramps regardless of the possible occurrence of concomitant reflex change in RR intervals (58). There was no statistically significant difference between ORX/ATX-Tg and WT littermates in the slope of the regression line, i.e., gain of the baroreceptor reflex, and the baroreflex effectiveness index. In addition, the number of SAP ramps was comparable between the mutant and WT mice, showing that overall fluctuations in AP were similar between both genotypes.

In the second series of experiments, we examined the baroreceptor reflex during activation of the PFA by either electrical or chemical stimulus. We used anesthetized mice because stimulation to the PFA was difficult in awake animals. To observe vagally-mediated reflex bradycardia, the animals were pretreated with intraperitoneal injection of a β-blocker, atenolol. Baroreflex bradycardia was induced by a rise in AP with an intravenous injection of phenylephrine. In WT mice, the combination of PFA stimulation and phenylephrine injection elicited only a small decrease in HR, although injection of phenylephrine alone induced a large decrease in HR. In ORX/ATX-Tg mice, on the other hand, phenylephrine induced a large decrease in HR irrespective of PFA stimulation (Fig. 4) (56). Therefore, orexin seemed to contribute to baroreflex suppression during the defense response but not during the resting condition.

4.3. *Attenuation of the defense response in ORX-KO and ORX/ATX-Tg mice was observed with natural stimulation*

To exclude the possibility that the observed difference between orexin-deficient mice and WT littermates were due to the difference in their susceptibility to an anesthetic, we tested the defense response in



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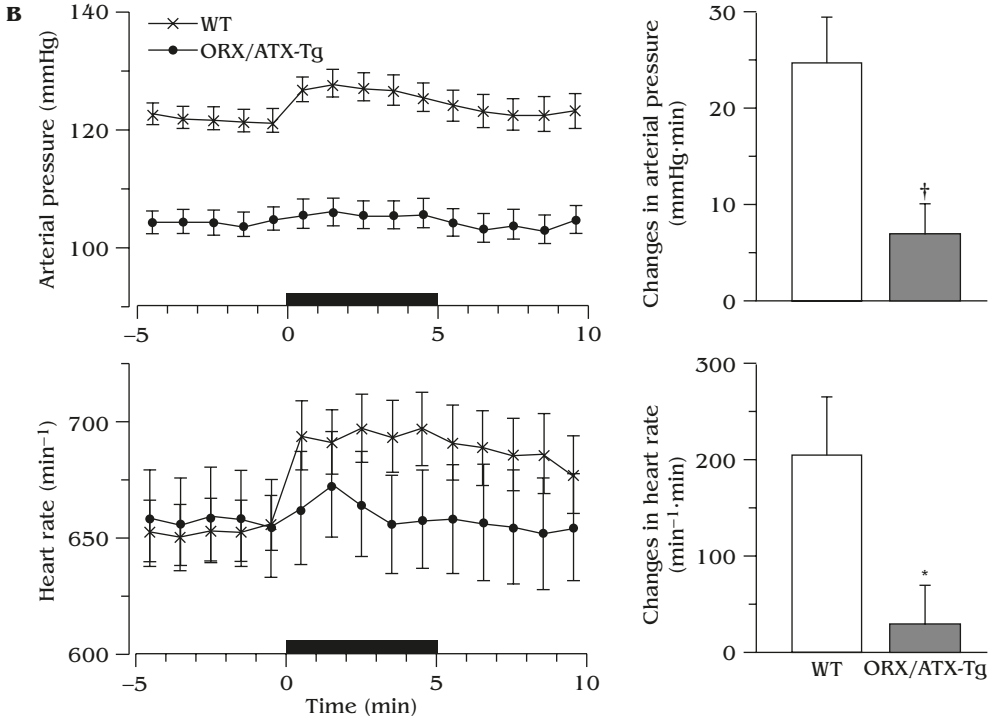

Fig. 5 — Cardiovascular and behavioral responses during natural stressful stimulation in conscious orexin-deficient mice and their wild-type (WT) littermates. (A) Resident-intruder test was performed in radio telemeter-indwelled freely moving WT and orexin knockout (ORX-KO) mice. The presence of an intruder is indicated by the horizontal solid bar. Right side panels are the changes in blood pressure, heart rate, and activity expressed as area under the curve (AUC) during 5 minutes when an intruder was present in the same cage. (B) Air jet stress was applied to catheter-indwelling lightly restrained WT and orexin/ataxin-3 transgenic (ORX/ATX-Tg) mice. Duration of air jet stress is indicated by the horizontal solid bar. Right side panels are the changes in arterial pressure and heart rate expressed as AUC during 5 minutes of the stress. Data are presented as mean±standard error of 6–8 mice in each genotype. $^{*}p<0.05$ and $^{\dagger}p<0.01$ *vs.* WT mice. (Adapted from References 55 and 56.)

conscious animals. In this experiment, we used natural stimulation rather than artificial stimulation to the PFA. For this purpose, we used a resident-intruder test or air-jet stress paradigm with telemeter-implanted animals. As expected, emotional stressor-induced increases in AP, HR, and locomotor activity were smaller in orexin-deficient mice (ORX-KO and ORX/ATX-Tg) than in WT littermates (Fig. 5) (55,56).

4.4. Attenuated stress-induced analgesia in ORX-KO mice

In the last series of experiments, we examined foot shock stress-induced analgesia in the mutant mice. In the WT mice, foot shock induced long-lasting analgesia as evidenced by increases in tail flick latency from noxious hot water. Although the ORX-KO mice showed moderate analgesia, it was significantly smaller than in WT littermates (30). In line with this result, we observed numerous expression of c-fos, a marker for cellular activation, in neurons with orexin-like immunoreactivity after the foot shock.

5. Conclusions

Here, we summarized evidence showing the possible contribution of orexin in the defense response. Attenuation of all features of the defense response so far tested in orexin-deficient mice points to the notion that orexin acts as a master switch to elicit orchestrated changes in the defense response against stressors. This notion is not yet conclusive since our results were obtained from a mixture of experiments using different animal models and compensation cannot be denied. However, phenotypes of ORX-KO and ORX/ATX-Tg mice were very similar so far tested (i.e., narcolepsy, decreased food intake, and some features of the defense response). Possible developmental compensation *in utero* can be denied since neuronal degeneration in ORX/ATX-Tg begins 1–2 weeks after birth (53). Moreover, supplementation of orexin rescued respiratory abnormality in ORX-KO (59). Therefore, we believe that the above notion would need very slight revision, if any.

Another feature of the defense response that has not yet been examined in orexin-deficient mice is the

increase in body temperature. Rise in body temperature accelerates nerve conduction velocity and thus supports fight-or-flight behavior. There is only one paper that deals with the possible role of orexin in temperature regulation (60). Mochizuki et al reported that temperature drop during sleep was attenuated in ORX-KO mice. Therefore, orexin may serve to decrease rather than increase body temperature, at least during sleep. But how about during the defense response? We think this theme should be examined in the near future.

The diversity of synaptic control of the cardiovascular and respiratory neurons seems necessary for animals to adapt themselves toward ever-changing life circumstances and behavioral states. The orexin system is likely to work as one of the essential modulators for coordinating circuits controlling autonomic functions and behavior.

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