Why narcoleptic mice exhibit faster recovery from sickness behavior?

Hypocretin/ataxin-3 mice (which are postnatally depleted of hypothalamic hypocretin neurons) were administered LPS. As a result, hypocretin/ataxin-3 mice were increased in sleep compared with wild-type littermates and showed faster recovery from sickness behaviour. We examined changes in the hypothalamic vigilance system and in the hypothalamic inflammatory factors in response to LPS in hypocretin/ataxin-3 mice. Peripheral immune challenge affected the hypothalamic immune response and vigilance states. This response was altered by the loss of hypocretin.

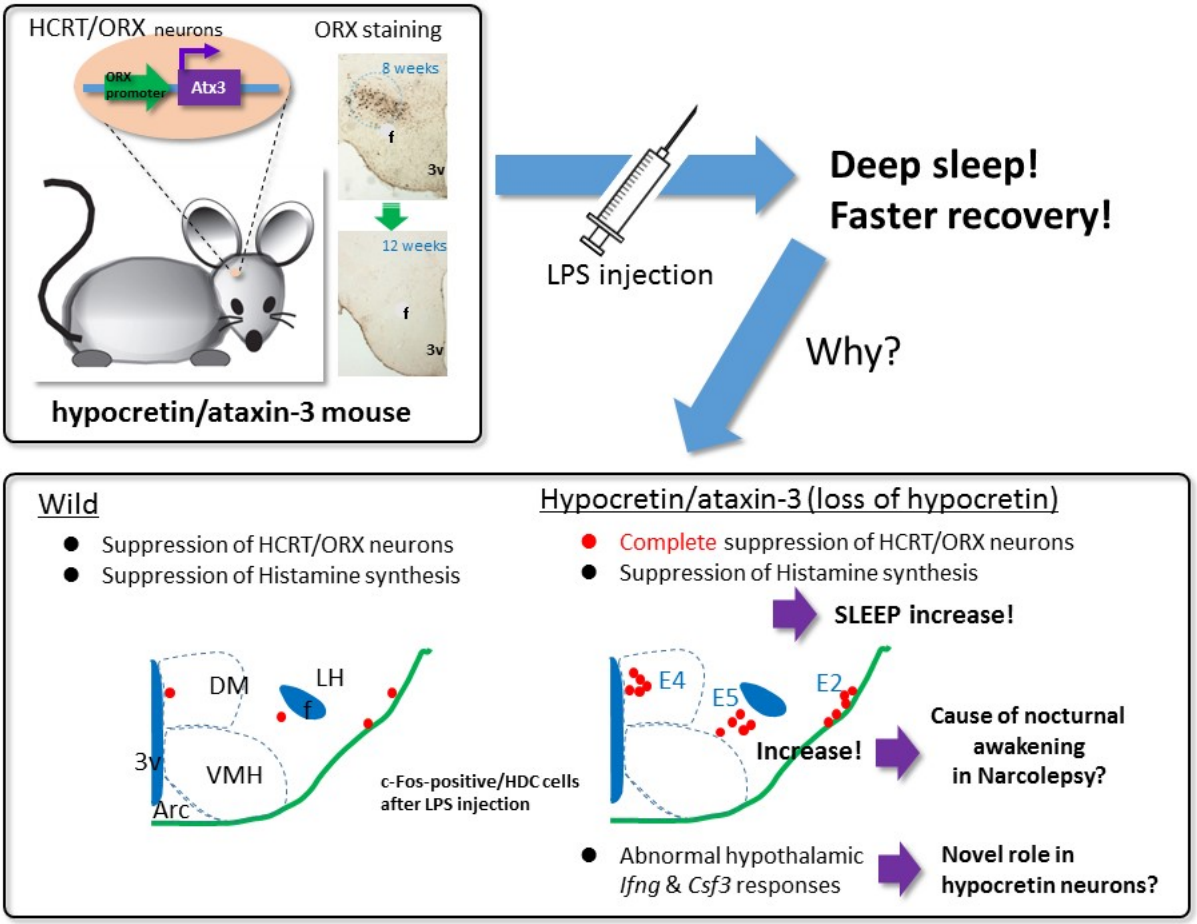

Fig. 1. c-Fos-positive/HDC cells are shown as red dots in illustration of wild-type and hypocretin/ataxin-3 hypothalamus. HCRT: hypocretin, ORX: orexin, Atx3: ataxin-3 f: fornix, 3v: third ventricle, DM: dorsomedial hypothalamic nucleus, VMH: ventromedial hypothalamic nucleus, Arc: arcuate hypothalamic nucleus, LPS: lipopolysaccharide. The E1–E5 cluster classification system was used for the different histaminergic neuronal subgroups in the tuberomammillary nucleus, as described in previous reports (Inagaki et al, Exp Brain Res. 1990; Moriwaki et al, J Chem

Neuroanat. 2015).

Hypocretin, also known as orexin, maintains the vigilance state and, therefore, is involved with the regulation of various physiological processes, such as arousal, sleep, food intake, energy expenditure, and reward. Hypocretin neurons, which show exclusive localization of the perifornical area of the lateral hypothalamus, have been identified as one of the neural mediators against sickness behaviour. The loss of hypothalamic hypocretin neurons is also thought to cause narcolepsy. The mice with the expression of the ataxin-3 transgene under regulation of the hypocretin promoter (hypocretin/ataxin-3 mice), over 90% of whose hypothalamic hypocretin neurons were destructed by approximately twelve weeks of age, showed a considerably *narcolepsy-like phenotype.* We previously found that the administration of lipopolysaccharide (LPS) against hypocretin/ataxin-3 mice affected significantly in the degree of increase in sleep periods and in recovery from sickness behaviour (Tanaka S et al., Biomed Rep. 2015). It was *suggested that* the loss of hypocretin neurons affected the hypothalamic immune response and consequently impacted the vigilance state instability observed in narcolepsy.

We therefore examined responses in the hypothalamic vigilance system and inflammatory factors to LPS in hypocretin/ataxin-3 mice. Since *narcolepsy has non-canonical cytokine profiles in their periphery and cerebrospinal fluid, this study* would provide insight into the pathophysiology of narcolepsy.

Peripheral LPS injection affected the hypothalamic wakefulness system and immune response. The loss of hypocretin induced the alteration of these responses. Whereas LPS induced approximately 80% reduction in hypocretin expression, it nearly completely abolished that expression in hypocretin/ataxin-3 mice, suggesting that *some factors in addition to hypocretin underlie the increase in sleep periods with LPS.*

Histaminergic neurons in the hypothalamic tuberomammillary nucleus are necessary for keeping highly vigilant state during wakefulness in concert with hypocretin neurons. Histamine is synthesized by histidine decarboxylase (HDC) and can be metabolized by two enzymes, histamine N-methyltransferase (HNMT) and amine oxidase, copper containing 1 (AOC1). Hypocretin/ataxin-3 mice shows *Hdc* elevation and the elevation decreased to the levels comparable to their wild-type littermates following LPS. Therefore, the increase in sleep periods with LPS in hypocretin/ataxin-3 mice may also be mediated by the suppression of histaminergic neurons. There was no significant difference in *Hnmt*, however *Aoc1* was increased in hypocretin/ataxin-3 mice. *Aoc1* was suppressed by LPS in all animals. It is necessary to observe not only on HNMT but also on AOC1 in narcolepsy.

Hypocretin loss did not change in reactivity of hypothalamic inflammatory factors against LPS*, except for* interferon gamma *(IFNG) and* colony stimulating factor 3 (CSF3)*.* Identification of

induced IFNG and its receptors in the hypothalamus by using a histological approaches is necessary in the future. Also, a reduction of response to peripheral immune stimulation in *Csf3*, which has a neuroprotective effect, might exacerbate the hypocretin loss in narcolepsy onset.

Even during the rest period, the number of c-Fos-positive/HDC-positive cells in *hypocretin/ataxin-3* mice with LPS was elevated in all areas, suggesting the activity increase of histaminergic neurons in hypocretin/ataxin-3 mice with LPS. Furthermore, chronic immune challenge might induce abnormal firing in these cells, resulting in nocturnal awakening, as observed in narcolepsy.

We showed a novel role for hypocretin in the hypothalamic response to peripheral immune challenge. Our findings contribute to the understanding of the pathophysiology of narcolepsy.

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