

LENTIVIRAL VECTOR DELIVERY OF OREXIN GENE TO STUDY POTENTIAL ROLE OF OREXIN AND SLEEP MODULATION IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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7.1 INTRODUCTION

Age-related aggregation of amyloid- β (A β) is an upstream pathologic event in Alzheimer's disease (AD) pathogenesis (Holtzman, Goate, Kelly & Sperling, 2011; Sperling et al., 2011). As the accumulation and aggregation of A β in the brain is known to develop ~10–15 years before the initial symptoms of AD, understanding the factors that lead to A β aggregation is important to delay the onset of the pathology and to prevent AD (Holtzman et al., 2011; Sperling et al., 2011). Diverse lines of in vitro and in vivo studies have shown that synaptic activity and specifically synaptic vesicle release is coupled with pre-synaptic A β release (Bero et al., 2011; Cirrito et al., 2008; Kamenetz et al., 2003;

Roh et al., 2012). In addition, sleep plays a role in the regulation of synaptic weight in the brain such that sleep appears to downscale the slow wave activity of the brain caused by accumulated load of synaptic potentiation during wakefulness (Vyazovskiy et al., 2009). Poor sleep quality and insufficient amounts of sleep have been noted in humans with preclinical evidence of AD (Ju & Holtzman, 2013; Ju, Lucey, & Holtzman, 2014; Roh et al., 2012). These observations suggest that understanding how integrated synaptic and network activity as measured by the sleep–wake cycle regulates A β may provide novel insights into AD pathogenesis (Musiek & Holtzman, 2016). However, how the amount and quality of sleep affects A β aggregation is not yet well-understood (Ju et al., 2014). Orexins (hypocretins) initiate and maintain wakefulness and loss of orexin-producing neurons causes narcolepsy (Chemelli et al., 1999; de Lecea et al., 1998). As has been described in Chapter 1, Gene Therapy Methods and Their Applications in Neurological Disorders, lentiviral vectors have been used to successfully enhance or inhibit signaling of neurotransmitters or function of relevant neurons.

Levels of soluble A β in the extracellular, interstitial fluid (ISF) of the hippocampus in mice are positively associated with minutes awake per hour and negatively associated with time asleep (Kang et al., 2009). In addition, the sleep–wake cycle also affects the A β pathology in the brain. Studies in mice showed that intracerebral administration of orexin can acutely increase both wakefulness and A β levels and systemic treatment with an orexin-receptor antagonist decreased A β deposition in amyloid precursor protein (APP) transgenic mouse models (Kang et al., 2009). Pharmacological experiments suggest that orexin and the sleep–wake cycle appear to be related to regulation of A β levels. In this chapter, we described whether genetic manipulation of orexin has similar effects as pharmacological manipulation and importantly, whether lentiviral vector-driven orexin signaling is influencing A β levels and A β pathology.

7.2 GENERATION OF AD MOUSE MODELS WITHOUT OREXIN SIGNALING

Female APP^{swe}/PS1 δ E9 on a B6 background (The Jackson Laboratory) mice were crossed with orexin knock-out ($-/-$) mice on a C57/Bl6 background to produce APP^{swe}/PS1 δ E9 hemizygous/orexin $+/-$ mice. Then, APP^{swe}/PS1 δ E9; orexin $+/-$ mice were crossed with orexin $-/-$ mice to obtain APP^{swe}/PS1 δ E9 hemizygous orexin $-/-$ mice (APP^{swe}/PS1 δ E9/OR $^{-/-}$). APP/PS1-21/OR $^{-/-}$ mice were obtained using the same methods beginning from crossing the APP/PS1-21 mice (C57/Bl6) with orexin $-/-$ mice (C57/Bl6). APP/PS1-21 mice (C57/Bl6) were generously provided from Dr. Mathias Jucker at the University of Tübingen and orexin knock-out mice were generously provided from Dr. Masashi Yanagisawa at the University of Texas Southwestern. All studies were approved by the Animal Studies Committee at Washington University.

7.3 MARKED REDUCTION OF A β PATHOLOGY IN THE APP/PS1/OREXIN KNOCK-OUT (OR $^{-/-}$) MICE

To more specifically address the role of orexin in regulating A β levels and pathology, we utilized APP/PS1-21 transgenic mice (Radde et al., 2006), which begin to develop A β deposition at $\sim$ 2 months of age and bred them to orexin knock-out (OR $^{-/-}$) mice (Chemelli et al., 1999; de Lecea et al., 1998). When orexin was knocked out in APP/PS1-21 mice (APP/PS1-21/OR $^{-/-}$ mice), there

was a marked reduction of A β plaque pathology at 3.5 months as well as at 8.5 months of age compared to APP/PS1-21 mice (Fig. 7.1A–F). Similar patterns of reduction in A β deposition were also seen when orexin was knocked out in another mouse model that develops A β deposition, APP/PS1 δ E9 mice (Jankowsky et al., 2004), which begin to develop A β deposition at 4 months of age. APP/PS1 δ E9/OR^{-/-} mice had a marked reduction in A β deposition at 6 months of age compared to APP/PS1 δ E9 mice expressing orexin (Fig. 7.1G–I). APP/PS1 δ E9/OR^{-/-} mice demonstrated significantly decreased wakefulness by 12.2% during the 12 h dark phase at 3 months of age compared to mice expressing orexin, prior to the onset of A β pathology in the brain (Fig. 7.1J, K). Fibrillar A β deposition assessed by X-34 staining was also markedly reduced in the brains of APP/PS1-21/OR^{-/-} mice and APP/PS1 δ E9/OR^{-/-} mice compared to mice expressing orexin (Fig. 7.2). We also assessed A β levels biochemically. The total amount of both A β 40 and A β 42 solubilized by 5 M Guanidine was also significantly reduced in the hippocampus as well as in the cortex in both lines of APP/PS1/OR^{-/-} mice compared to APP/PS1 mice in the hippocampus as well as in the cortex. Similar patterns of pathological A β reduction were also noted in APP/PS1 δ E9/OR^{-/-} mice compared to APP/PS1 δ E9 mice (Fig. 7.3).

7.4 PRODUCTION OF LENTIVIRAL VECTORS AND OVEREXPRESSION OF OREXIN BY LENTIVIRAL VECTOR

Lentiviral vectors have been widely used to model brain diseases, to increase or decrease functions of neurotransmitters, and to deliver therapeutic materials in animal models. The Hope Center Viral Vectors Core of Washington University in St. Louis has made successfully third generation HIV-1-based lentiviral vectors and supported diverse experiments (https://hopecenter.wustl.edu/?page_id=99). For focal overexpression of orexin, orexin or green fluorescent protein (GFP) driven by an ubiquitin promoter was overexpressed via lentivirus in the hippocampus bilaterally in APPswe/PS1 δ E9 mice (B6C3) starting at 5 months of age and the amount of sleep and A β pathology from both groups were compared at 9 months. To investigate the effect of focal overexpression of orexin before A β plaque pathology was formed in the hippocampus, the same lentiviral injection experiments were performed in APPswe/PS1 δ E9 mice (B6C3) with viral injection at 3 months and pathologic assessment at 6 months. For rescue of orexinergic neurons, orexin or GFP driven by an hypocretin/orexin promoter was expressed via lentivirus in the bilateral hypothalamus in APPswe/PS1-21/Orexin knock-out mice (C57/Bl6) starting at 1.5 months of age and the amount of sleep and A β pathology from both groups were compared at 3 months. The mouse hypocretin/orexin promoter was generously provided from Dr. De Lecea.

7.5 RESCUE OF FOCAL OREXIN SIGNALING VIA LENTIVIRAL VECTORS WITHOUT CHANGES IN A β PATHOLOGY

Orexin receptors are present on hippocampal neurons, so we focally overexpressed orexin by stereotaxic injection of a lentiviral vector expressing orexin into the hippocampus of APP/PS1 δ E9 mice to determine whether local orexin signaling was directly leading to the effects observed on

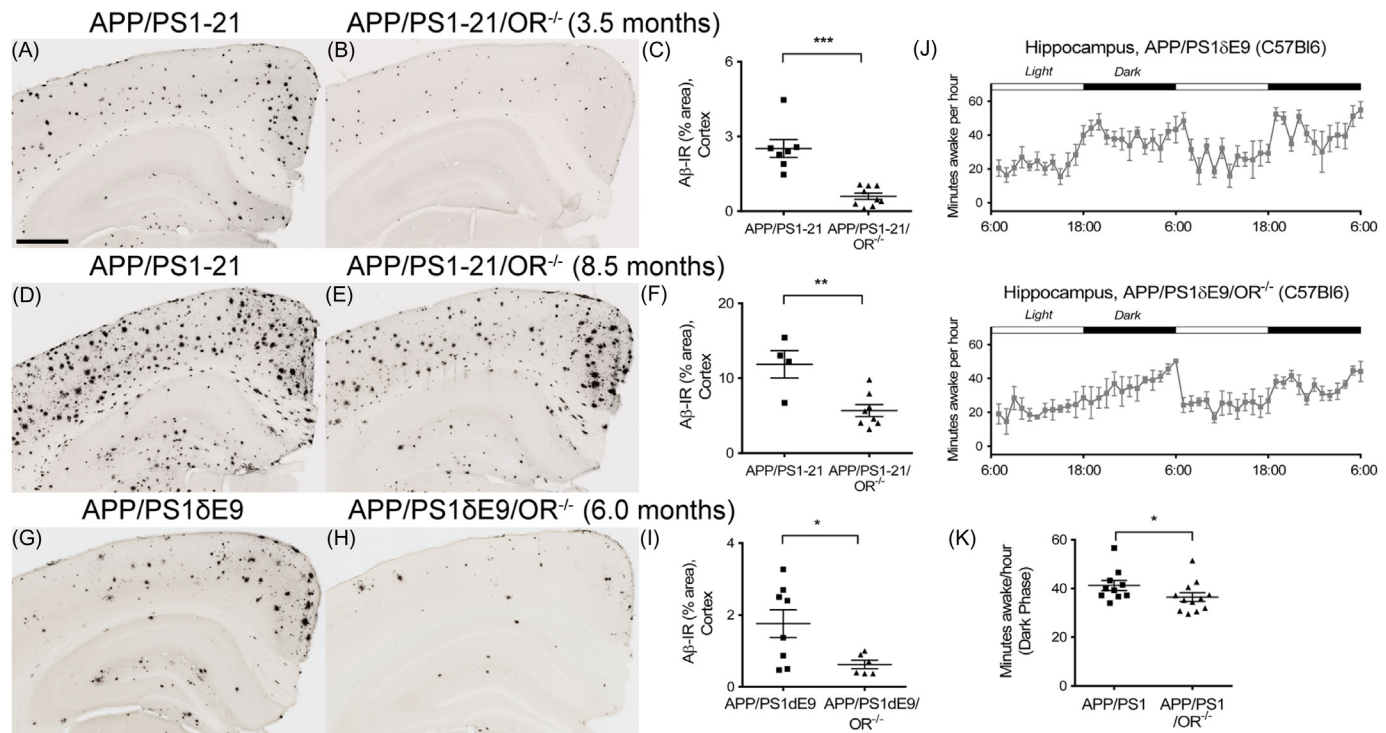


FIGURE 7.1

Marked reduction of Aβ pathology in the APP/PS1/Orexin knock-out (OR^{-/-}) mice compared to APP/PS1 mice. The amount of Aβ pathology was noted at 3.5 months (A–C) and at 8.5 months (D–F) in APP/PS1-21 mice line at 6 months in APP/PS1δE9 mice line (G–I). Amount of wakefulness at 3 months of age prior to the onset of Aβ pathology in the brain was compared between APP/PS1δE9 and APP/PS1δE9/OR^{-/-} mice (J, K) ($n = 5–9$ in each group, two-tailed t -test). Each mouse was investigated independently one time. Data are presented as mean $\pm$ s.e. m. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Scale bar in (a), 500 μ m. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211(13):2487–96.

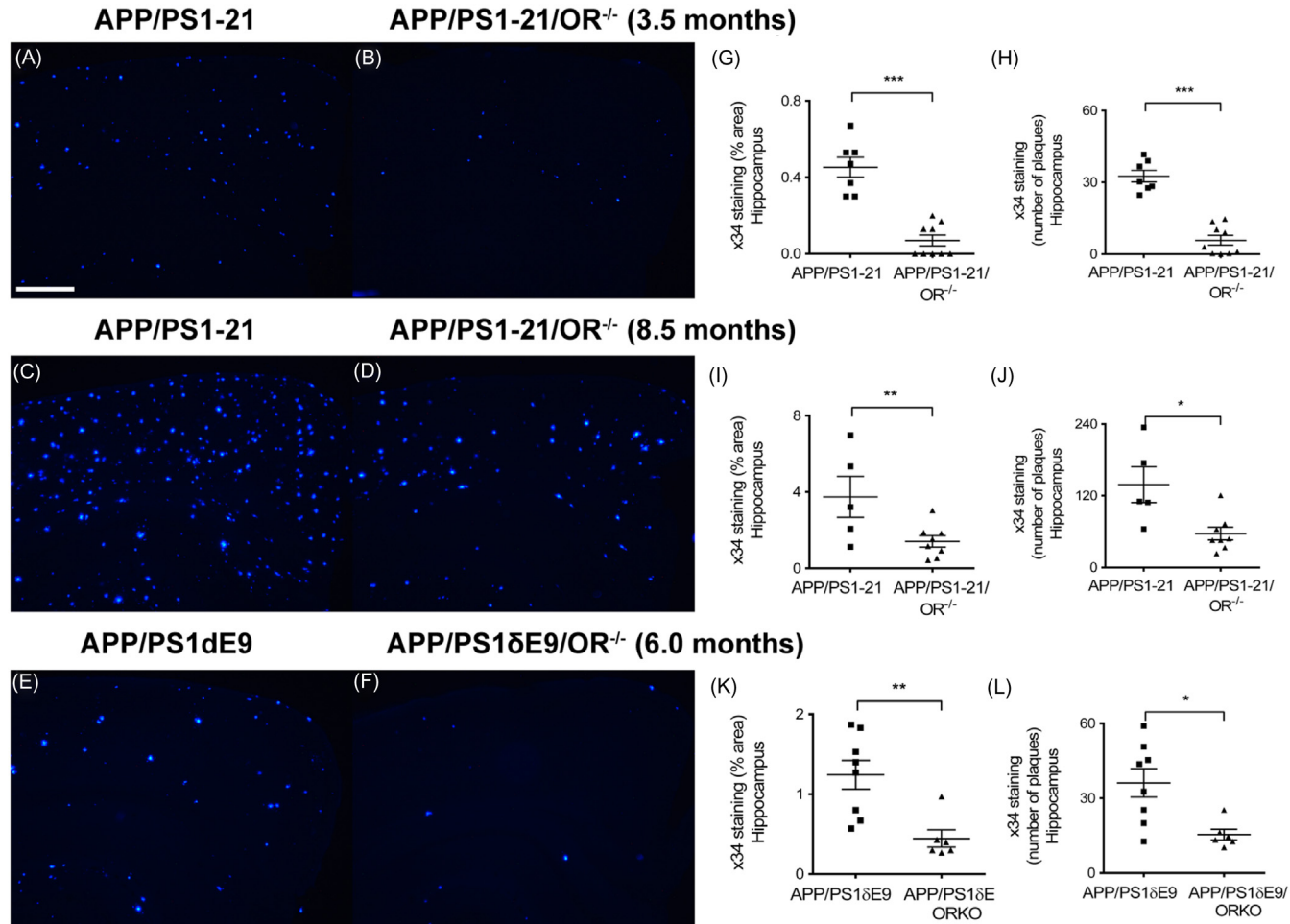


FIGURE 7.2

Marked reduction of amyloid pathology in the APP/PS1/Orexin knock-out (OR^{-/-}) mice compared to APP/PS1 mice. Amyloid pathology measured by Aβ immunoreactivity and number of amyloid plaques after X-34 staining is noted at 3.5 months (A, B, G, H) and at 8.5 months (C, D, I, J) in the APP/PS1-21 mice line and at 6 months in the APP/PS1ΔE9 mice line (E, F, K, L) ($n = 5-9$ in each group, two-tailed t -test). Each mouse was investigated independently one time. Data are presented as mean $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Scale bar in (a), 100 μm. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211(13):2487–96.

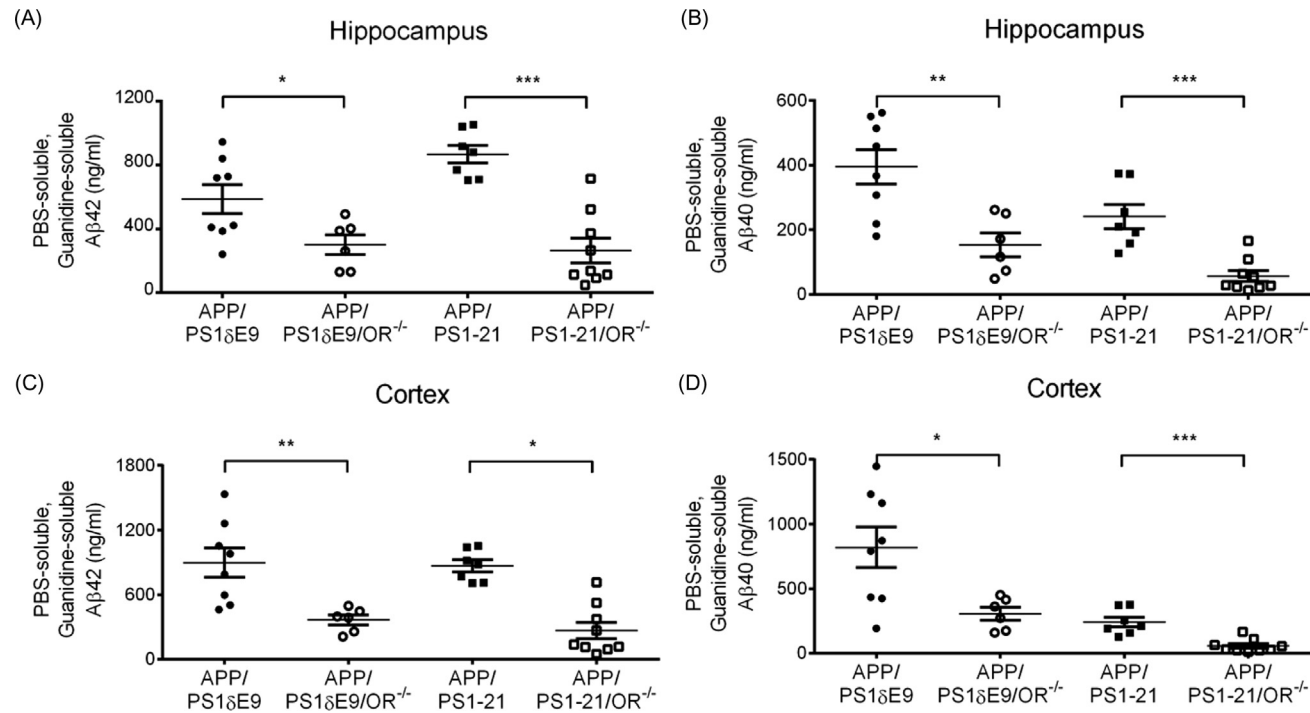


FIGURE 7.3

Strong reduction in Aβ species in APP/PS1 mice lacking orexin. The amount of PBS-soluble and guanidine-soluble forms of Aβ40 and 42 were compared in APP/PS1δE9/OR^{-/-} mice vs APP/PS1δE9 mice (left 2 columns of subfigures) and in APP/PS1-21/OR^{-/-} mice vs APP/PS1-21 mice (right 2 columns of subfigures; A–D). Results are obtained from the hippocampus (A, B) and from the cortex (C, D) of each group of mice ($n = 5–9$ in each group, two-tailed t -test). Each mouse was investigated independently one time. All samples were measured in triplicate. Data are presented as mean $\pm$ s.e.m. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211 (13):2487–96.

A β deposition. This treatment resulted in no changes in levels of A β deposition in the brain nor changes in sleep time, despite focal increases in the level of orexin in the hippocampus (Fig. 7.4). Amount of A β pathology was compared after focal injection of ubiquitin-driven orexin lentiviral vector vs ubiquitin-driven GFP lentiviral vector in the hippocampus from 3 to 6 months (A, B, E) or 5 to 9 months (C, D, F) in APP/PS1 δ E9 mice. The amount of wakefulness in APP/PS1 δ E9 mice was compared after injection of ubiquitin-driven orexin lentiviral vector vs ubiquitin-driven GFP lentiviral vector. Levels of orexin in the hippocampus and CSF were compared after injection of orexin or GFP lentiviral vector driven by ubiquitin promoter (G).

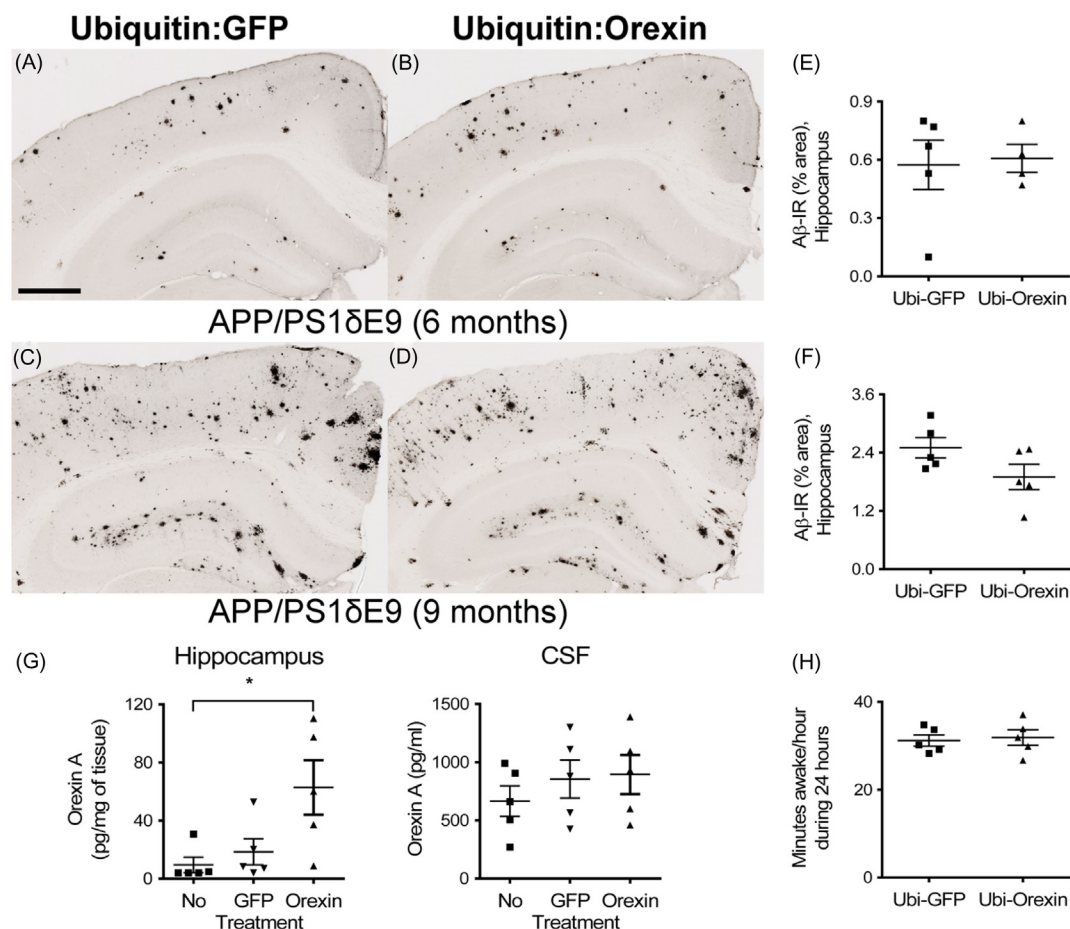
7.6 RESCUE OF SYSTEMIC OREXIN SIGNALING VIA LENTIVIRAL VECTORS WITH ACCUMULATION OF A β PATHOLOGY

To further investigate whether the reduction in A β pathology in orexin knock-out mice is mainly due to the effects of orexin signaling on sleep, expression of orexin specifically in orexinergic neurons was instituted in 1.5 month old APP/PS1-21/OR $^{-/-}$ mice. We utilized an orexin lentiviral vector in which orexin is driven by the hypocretin/orexin promoter. Following injection of this vector bilaterally in the hypothalamus, expression of orexin in orexinergic neurons was restored (Fig. 7.5F). Six weeks following injection with the hypocretin promoter-driven orexin lentiviral vector, APP/PS1-21/OR $^{-/-}$ mice had a significantly increased amount of wakefulness as well as increased amount of A β deposition in the hippocampus and cortex compared to mice injected with a control vector (Figs 7.5E and 7.6). Taken together, results from focal and generalized orexin expression in different brain regions using different promoters, strongly support the idea that the reduction in A β pathology noted in APP/PS1/OR $^{-/-}$ mouse models is due to secondary changes in sleep time induced by hypothalamic expression of orexin rather than by alteration in orexin signaling in hippocampus and other brain regions.

We additionally analyzed the functional effect of APP/PS1/OR $^{-/-}$ mice in which orexin expression was rescued in orexinergic neurons by measuring the diurnal fluctuation of A β in the extracellular space using in vivo microdialysis. In APP/PS1-21/OR $^{-/-}$ mice injected with a lentiviral vector in which the hypocretin/orexin promoter is driving orexin expression bilaterally in the hypothalamus, restoration of diurnal fluctuation of ISF A β _{x-40} was found, suggesting possible functional rescue of orexinergic neurons in the hypothalamus bilaterally (Fig. 7.5A–D). There was, however, no difference in the amplitude of circadian rhythms assessed by cosinor analysis between the groups (Fig. 7.5C, D). This is likely due to the number of mice used in this experiment (N = 6 per group).

7.7 MINIMUM AMOUNT OF SLEEP CHANGES USING LENTIVIRAL VECTORS TO MARKEDLY REDUCE A β PATHOLOGY IN AD BRAINS

Increasing evidence suggests that neuronal activity in many brain regions is physiologically regulated by the sleep–wake cycle (Bero et al., 2011). It has been noted in animals that decreases in sleep time and increases in amount of A β levels and deposition have a reciprocal relationship

**FIGURE 7.4**

No changes in Aβ deposition and amount of wakefulness by focal overexpression of orexin. Amount of Aβ pathology was compared after focal injection of ubiquitin-driven orexin lentiviral vector vs ubiquitin-driven GFP lentiviral vector in the hippocampus from 3 to 6 months (A, B, E) or 5 to 9 months (C, D, F) in APP/PS1δE9 mice. The amount of wakefulness in APP/PS1δE9 mice was compared after injection of ubiquitin-driven orexin lentiviral vector vs ubiquitin-driven GFP lentiviral vector. Levels of orexin in the hippocampus and CSF were compared after injection of orexin or GFP lentiviral vector driven by ubiquitin promoter (G). ($n = 4-8$ in each group, two-tailed t -test in E, F, H, one-way analysis of variance, followed by Tukey's post hoc test in G). Each mouse was investigated independently one time. Data are presented as mean $\pm$ s.e.m. * $P < 0.05$. Scale bar in (A), 500 μ m. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211(13):2487–96.

Attenuated diurnal fluctuation of ISF amyloid beta in OR^{-/-} mice

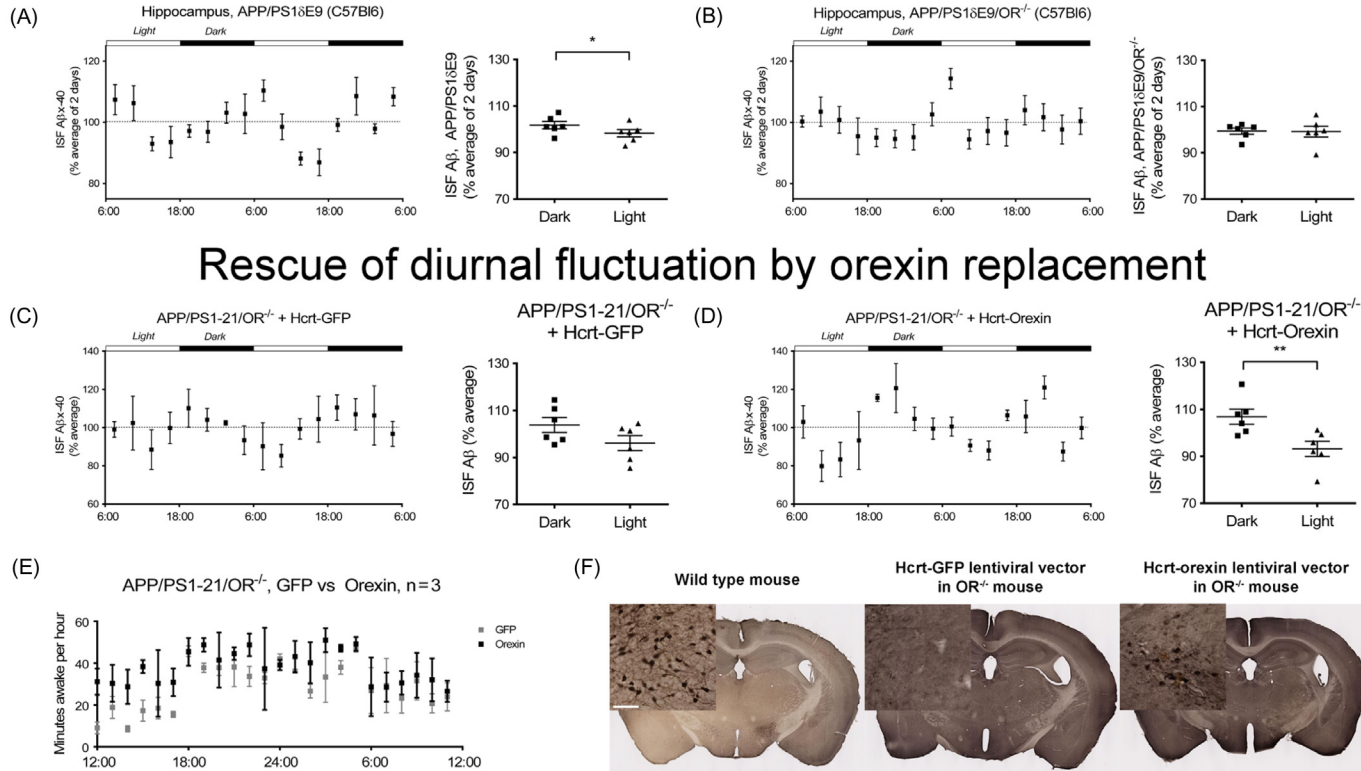
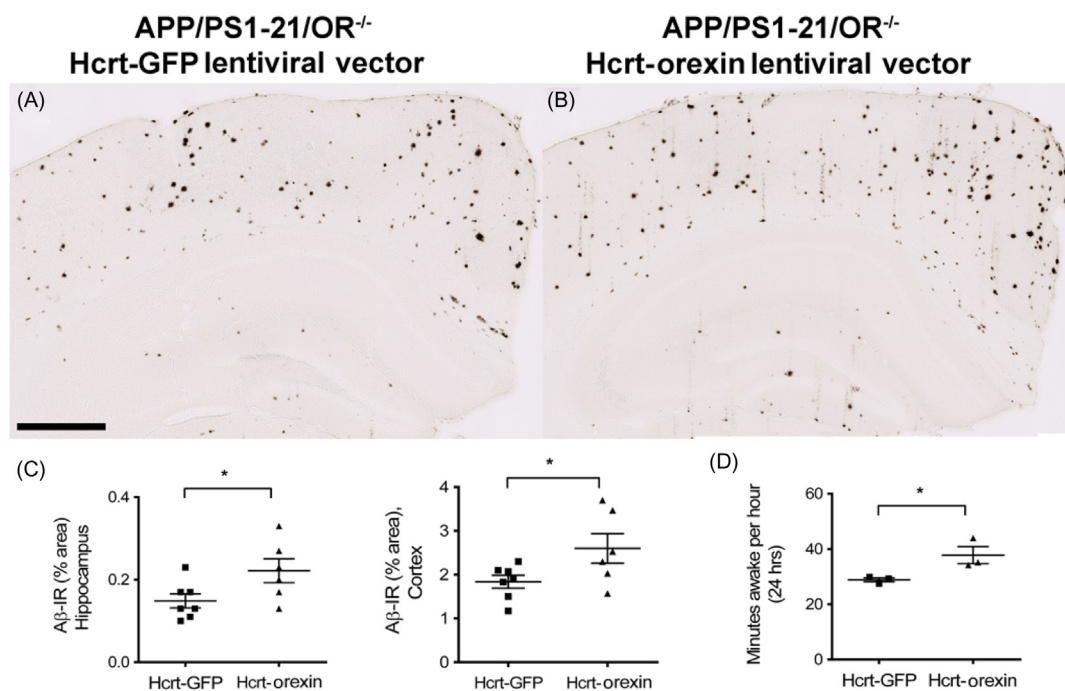


FIGURE 7.5

Restoration of diurnal fluctuation of ISF Aβ₄₀ via rescue of orexin expression in APP/PS1/OR^{-/-} mice. Diurnal fluctuation of ISF Aβ₄₀ in young APP/PS1δE9 mice was compared with the diurnal fluctuation in the same mice when orexin was knocked-out (APP/PS1δE9/OR^{-/-} mice; A, B). Diurnal fluctuation of ISF Aβ₄₀ in the hippocampus and minutes awake per hour of APP/PS1-21/OR^{-/-} mice treated with hypocretin/orexin-promoter driven lentiviral vector overexpressing orexin in the hypothalamus bilaterally were compared with mice treated with the same lentiviral vector expressing GFP (C, D, E). Expression of orexin by immunostaining in orexinergic neurons in the hypothalamus of APP/PS1-21/OR^{-/-} mice treated with hypocretin/orexin-promoter driven lentiviral vector expressing GFP or orexin (right 2 columns) compared to wild type mice (left column) (F). ($n = 6$ in each group, two-tailed t -test in A–D; $n = 3$ in each group, Mann–Whitney test in E). Each mouse was investigated independently one time. All ISF Aβ measurements were done in triplicate. Data are presented as mean $\pm$ s.e.m. * $P < 0.05$ and ** $P < 0.01$. Scale bar in (F), 100 μ m. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211(13):2487–96.

**FIGURE 7.6**

Increase in Aβ deposition and wakefulness by rescue of orexin expression in APP/PS1/OR^{-/-} mice via expression of orexin with lentiviral vector driven by hypocretin/orexin promoter in the bilateral hypothalamus. Amount of Aβ pathology in the hippocampus and cortex of the APP/PS1-21/OR^{-/-} mice after bilateral injection of hypocretin promoter driven orexin lentiviral vector in the hypothalamus of the APP/PS1/OR^{-/-} mice was compared with results from injection of the same lentiviral vector overexpressing GFP (A–C). The amount of wakefulness as measured by polysomnography was compared between the same groups of mice (D). ($n = 6–7$ in each group two-tailed t -test in C, $n = 3$ in each group, Mann–Whitney test in D). Each mouse was investigated independently one time. Data are presented as mean $\pm$ s.e.m. * $P < 0.05$. Scale bar in (A), 500 μ m. Contents described in this figure were originally published in the *Journal of Experimental Medicine* in 2014; 211 (13):2487–96.

(Ju et al., 2014; Kang et al., 2009; Roh et al., 2012). Intriguingly, recent results from humans also indicate that the quality or amount of sleep is inversely associated with the amount of Aβ measured in the CSF (Ju & Holtzman, 2013; Ooms et al., 2014) and that the amount of sleep inversely correlates with fibrillar forms of Aβ quantified by amyloid PET scan (Spira et al., 2013). Treatment with active vaccination with Aβ₄₂ prevented development of Aβ pathology and abnormalities of the sleep–wake cycle in APP/PS1E9 mice (Roh et al., 2012). This demonstrated that Aβ aggregation and accumulation caused a sleep abnormality. The current study indicates the opposite is also true, i.e., an increase in sleep time caused by orexin deficiency resulted in a decrease in the amount of Aβ pathology that developed in the brain. Collectively, those findings

suggest that increasing certain types of sleep (e.g., non-rapid eye movement (NREM) sleep) should be considered as therapeutic target to decrease A β -associated AD pathogenesis. Possible side effects of sleep induction such as excessive daytime sleepiness or cataplexy driven by knocking out or decreasing orexin function need to be considered in the process of designing new therapeutic interventions targeting sleep.

It is notable that a 12% increase in the sleep time during the dark phase was associated with more than 50% reduction in the development of A β pathology in the brain. This matches well with the previous findings that an $\sim$ 15% decrease in the amount of soluble forms of A β in the extracellular space resulted in an up to 50% reduction of A β plaque growth and formation in the brain (Yan et al., 2009). It suggests that a small increase in NREM sleep or small improvement in sleep quality and the resulting decrease in A β release into the extracellular space could be beneficial for the delay or attenuation of emergence of A β -associated pathologies in AD. While our data support a mechanism, whereby decreased neuronal activity during sleep results in a decrease in ISF A β , we cannot yet rule out the contribution of increased A β clearance from the ISF as proposed by Nedergaard and colleagues (Xie et al., 2013). It will be important in future experiments to determine if different methods of sleep or orexin modulation will trigger new methods of intervention targeting A β pathophysiology in the brain. Given the association between neuronal activity and release of tau in the brain, further studies to determine if the sleep–wake cycle also influences this key molecule in the pathogenesis of AD and other neurodegenerative diseases will be critical (Yamada et al., 2014). It is important to note that other systems involved in the sleep–wake cycle are also likely to affect the pathophysiology of AD. Thus, modulation of other molecules which increase or decrease wakefulness such as noradrenergic and GABAergic modulation are also likely to influence AD pathophysiology (Roh et al., 2014).

The location of A β deposition in the human brain overlaps very closely with a brain network called the “default mode” network, which is made up of brain regions that appear to have the highest measures of aerobic glycolysis when individuals are at rest (Raichle et al., 2001; Vlassenko et al., 2010). In addition to the global reduction in neuronal activity resulting from an increase in sleep time as shown here, focal alteration of brain activity, connectivity, or even focal sleep modulation through pharmacologic or other methods may also merit consideration as potential therapies in AD and other neurodegenerative disorders (Nir et al., 2011; Vyazovskiy et al., 2011). In this chapter, we demonstrated modulation of orexin via lentiviral vectors and its potential effects on sleep appear to modulate A β pathology in the brain. Such manipulation may be a powerful way to modulate A β levels and pathology by taking advantage of normal brain physiology.

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FURTHER READING

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