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Role of histamine H1-receptor on behavioral states and wake maintenance during deficiency of a brain activating system: A study using a knockout mouse model

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ABSTRACT

Using knockout (KO) mice lacking the histamine (HA)-synthesizing enzyme (histidine decarboxylase, HDC), we have previously shown the importance of histaminergic neurons in maintaining wakefulness (W) under behavioral challenges. Since the central actions of HA are mediated by several receptor subtypes, it remains to be determined which one(s) could be responsible for such a role. We have therefore compared the cortical-EEG, sleep and W under baseline conditions or behavioral/pharmacological stimuli in littermate wild-type (WT) and H1-receptor KO (H1−/−) mice. We found that H1−/− mice shared several characteristics with HDC KO mice, i.e. 1) a decrease in W after lights-off despite its normal baseline daily amount; 2) a decreased EEG slow wave sleep (SWS)/W power ratio; 3) inability to maintain W in response to behavioral challenges demonstrated by a decreased sleep latency when facing various stimuli. These effects were mediated by central H1-receptors. Indeed, in WT mice, injection of triprolidine, a brain-penetrating H1-receptor antagonist increased SWS, whereas ciproxifan (H3-receptor antagonist/inverse agonist) elicited W; all these injections had no effect in H1−/− mice. Finally, H1−/− mice showed markedly greater changes in EEG power (notably in the 0.8–5 Hz band) and sleep-wake cycle than in WT mice after application of a cholinergic antagonist or an indirect agonist, i.e., scopolamine or physostigmine. Hence, the role of HA in wake-promotion is largely ensured by H1-receptors. An upregulated cholinergic system may account for a quasi-normal daily amount of W in HDC or H1-receptor KO mice and likely constitutes a major compensatory mechanism when the brain is facing deficiency of an activating system.

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1. Introduction

Neurons containing histamine (HA) are located in the tuberomamillary nucleus and the adjacent posterior hypothalamus. They send widespread inputs to most cerebral areas. In freely-moving cats and behaving mice, these neurons fire tonically and exclusively during wakefulness (W) (Sakai et al., 1990; Vanni-Mercier et al., 2003; Takahashi et al., 2006), a pattern of activity that is the most

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wake-selective one among the brain activating systems identified to date. This wake-selective activity enhances cellular excitability and activity of a wide range of target cells via different HA receptor subtypes (reviewed in Schwartz et al., 1991; Lin, 2000; Brown et al., 2001; Haas et al., 2008; Vu et al., 2015; Panula et al., 2015), contributing to a general cortical activation during W. Hence, impairment of the brain histaminergic transmission using pharmacological interventions or knockout (KO) mouse models causes somnolence and wake deficits, whereas enhancement of transmission promotes cortical arousal and W (Lin et al., 1986; Monti et al., 1991; Lin, 2000; Lin et al., 2011a). HA neurons make multiple interactions with other brain arousal systems such as cholinergic aminergic and orexinergic neurons (reviewed in McCormick, 1992, Lin, 2000; Haas et al., 2008; Haas and Lin, 2012). The latter ones send particularly dense inputs to HA cells and excite them by direct depolarization through orexin-2 receptors (Eriksson et al., 2001). Mice selectively lacking the orexin-2 receptors show signs of chronic sleepiness, a phenotype rescued by restoration of orexin-2 receptors on neurons in the tuberomamillary areas (Mochizuki et al., 2011). Recently, the wake-promoting effects of HA were shown to be balanced by its co-neurotransmitter GABA (Yu et al., 2015).

Using KO mice lacking the HA-synthesizing enzyme, histidine decarboxylase (HDC), we have previously shown that long-term abolition of HA impairs cortical EEG, deteriorates both sleep and waking quality, causing sleepiness and behavioral deficits. Consequently, these mice are unable to remain awake in crucial situations, such as at lights-off or when faced with an environmental change (Parmentier et al., 2002; Anaclet et al., 2009; Lin et al., 2011b).

Since the central actions of HA are mediated by several receptor subtypes, it remains to be determined which one(s) could be responsible for its wake-promoting effects. The importance of the H1-receptor has long been suggested because brain–penetrating H_1-receptor antagonists cause sedation and drowsiness in man and increase in slow wave sleep in animals (Douglas, 1985; Nicholson and Stone, 1986; Schwartz et al., 1991; Yanai et al., 1999; Lin, 2000; Panula et al., 2015). However, whether the long-term loss of H1-receptors causes similar genotypes of HDC KO mice in terms of EEG, behavioral states and how the brain remains awake during deficiency of a brain activating system remains unknown. We have therefore studied and compared the cortical EEG and sleep-wake cycle under baseline conditions and after behavioral challenges in littermate wild type and H_1-receptor knockout mice. In order to assess brain plasticity involved in wake maintenance during deficiency of an activating system, we also compared in those mice pharmacological responses to ligands of the aminergic and cholinergic systems known for their role in controlling cortical activities and the sleep-wake cycle.

2. Materials and methods

2.1. Detection of the histamine H1-receptor gene using PCR and in situ hybridization

At 3 weeks of age, tail biopsies were taken from all mice and analyzed by PCR for genotyping. The WT allele was amplified using primers located within the H1 gene and the KO allele was amplified with one primer within the neomycin resistance cassette. These primers were 5′-TGA AGT ATC TGG CTC TGA GTG G-3′ (5′-primer 5′-upstream of H1R gene) and 5′- CCA TCG ATG GCT CCC TCC CTG GGA G -3′ (H1R gene 5′-primer complementary to H1R gene), the expected product size being 1100 base pairs. The mutant allele was amplified using the same 5′-primer and a primer located within the neo^r gene, this being 5′-TCT ATC GCC TTC TTG ACG AG-3′, with an expected product size of 900 base pairs. These two sets of primers were included separately and PCR was performed using 30 cycles of 30 s at 94 °C, 30 s at 62 °C, and 1 min at 72 °C, followed by one cycle at 72 °C for 5 min. The whole reaction mix was then fractionated on a 2% agarose gel, and the PCR product visualized by ethidium bromide staining.

We also carried out *in situ* hybridization of mRNA for the H1-receptor according to the protocol modified from Lintunen et al. (1998). Briefly, the adult brains were removed after decapitation to obtain frozen and coronal sections (20 μm thickness). The H1 probe (GTG GGG AGG TAG AAG TTG TGA TGA GCG GTC TGA ATC TTG AAC CAA) is complementary to nucleotides 563–608 in the mouse H1R cDNA. The H1-receptor was labeled with 33P-dATP. All sections were hybridized with the probe at 50 °C for 15–18 h and washed at 56 °C with 1×SSC. Competitive hybridizations were performed with a 100-fold excess of unlabeled oligonucleotide compared to the labeled one to abolish the unspecific signal. The film (KodaK BioMax MR) exposure times were 3 weeks.

2.2. Animals and surgery

Fourteen pairs of littermate male wild-type (WT or H1+/+) and histamine (HA) H-receptor knockout (H1R KO or H1R−/−) mice were descendants of the mouse strain generated by Inoue et al. (1996) kept on the C57BL/6J genomic background. The present study used only H1+/+ and H1−/− littermates to ensure an identical genetic background of mice except for the H1-receptor gene. All experiments followed EEC Directive (2010/63/EU) and every effort was made to minimize the number of animals used and any pain and discomfort. The general protocol of chronic sleep-wake recordings in mice was approved by the Ethic Committee of Animal Experimentation of Claude Bernard University.

At the age of 12 weeks and with a body weight of 23–28 g, animals were chronically implanted, under deep anesthesia (sodium pentobarbital, 55–60 mg/kg, i.p.), with six cortical electrodes (gold-plated tinned copper wire, Ø = 0.4 mm, Filotex, Draveil, France) and three muscle electrodes (fluorocarbon-coated gold-plated stainless steel wire, ⌀ = 0.03 mm, Cooner Wire. Chatworth, CA) to record the electroencephalogram (EEG) and electromyogram (EMG) and to monitor the sleep-wake cycle. All electrodes were previously soldered to a multi-channel electrical connector and each was separately insulated with a covering of heat-shrinkable polyolefin/polyester tubing. The cortical electrodes were inserted into the dura through 3 pairs of holes (Ø = 0.3 mm) made in the skull, located, respectively, in the frontal (1 mm lateral and anterior to the bregma), parietal (1 mm lateral to the midline at the midpoint between the bregma and lambda), and occipital (2 mm lateral to the midline and 1 mm anterior to the lambda) cortex. The muscle electrodes were inserted into the neck muscles. Finally, the electrode assembly was anchored and fixed to the skull with Super-Bond (Sun Medical Co., Shiga, Japan) and dental cement. This implantation allowed stable polygraphic recordings to be made for more than 4 months.

2.3. Polygraphic recording, data acquisition and analysis

After surgery, animals were housed individually in transparent barrels (Ø 20 cm, height 30 cm) in an insulated sound-proofed recording room maintained at an ambient temperature of 22 ± 1 °C and on a 12 h light/dark cycle (lights-on at 7 a.m.), food and water being available ad libitum. In some animals and for some experiments, an infra video camera was set up in the

recording room to observe and score the animal's behavior during both the light and dark phases. After a 14 day recovery period, mice were habituated to the recording cable for 7 days before starting polygraphic recordings.

Cortical EEG (ipsi- and controlateral frontoparietal and frontooccipital leads) and EMG signals were amplified, digitized with a resolution of 256 and 128 Hz, respectively, and computed on a CED 1401 Plus (Cambridge, UK). Using a Spike2 script and with the assistance of spectral analysis using the fast Fourier transform, polygraphic records were visually scored by 4-sec epochs for wakefulness (W), slow wave sleep (SWS), and paradoxical sleep (PS) according to previously described criteria validated for mice (Valatx, 1971; Valatx and Bugat, 1974; Parmentier et al., 2002; Gondard et al., 2013).

To avoid any variation due to the positioning of cortical electrodes, the cortical EEG used for power spectral density analysis was captured from frontoparietal leads, set with reference to the bregma, lambda, and midline in all mice. For power spectrum analysis polygraphic records were visually scored by 4 s epochs. EEG power spectra were computed for consecutive 4 s epochs within the frequency range of 0.8–60 Hz using a fast Fourier transform routine. The data were collapsed in 0.4 Hz bins. On the basis of visual and spectral analysis, epochs containing artifacts occurring during active waking (with large movements) were visually identified and omitted from the spectral analysis when the threshold value in the 0–1 Hz band was exceeded; this represented 0.25 ± 0.09% of the total recording time. The power densities obtained for each state were summed over the frequency band of 0.8–60 Hz (total power). To standardize the data, all power spectral densities at the different frequency ranges, i.e., δ 0.8–3 Hz, slow activity 0.8–5 Hz, θ 3–9 Hz, spindle frequencies 9–15 Hz, α 15–20 Hz, β 20–30 Hz, γ 30–60 Hz and β+γ 20–60 Hz (fast rhythms), were expressed as a percentage relative to the total power (e.g., power in the δ band/power in the 0.8–60 Hz) of the same epochs. To evaluate contrast in the cortical EEG between SWS and W or PS, we used an EEG power ratio determined by the averaged cortical EEG total power density during SWS divided by that during either W or PS.

2.4. Experimental procedures

In each experiment, recordings were simultaneously made from an equal number of H1+/+ and H1−/− mice. Mice were submitted to the following experimental procedures.

2.4.1. Spontaneous cortical EEG and sleep-wake cycle

During the period of days 15–20 post-surgery, drug-naive mice (12 pairs) were subjected to two separate 24 h recording sessions, beginning at 7 p.m. During each recording session, the animals were left undisturbed. The data from the two sets of mice were then compared.

2.4.2. Cortical EEG and sleep-wake cycle following behavioral stimuli

Recordings were made from WT and H1−/− mice for 24 h after each of the three tests described below, which were performed in a random sequence. As a criterion of somnolence and drowsiness, the latencies to SWS and PS, defined as the time between the end of the stimuli and the onset of the first SWS or PS episode lasting more than 30 s, were also measured. The three tests consisted of: 1) a simulation of injection (at either 10 a.m. or 8 p.m., n = 60 from 12 pairs of animals), consisting of the handling of the animal and sham intraperitoneal injection without needle insertion; 2) a change of litter (at 2 p.m., n = 40 from 10 pairs of mice), which was a routine care performed at light phase every 5–7 days to clean the cage and which usually causes a period of waking and behavioral excitation in rodents; in this test, we compared the excitability of the two groups of mice following this routine care. 3) a new environment, the mice being transferred for 4 h from their habitual transparent barrel to an opaque rectangular box (21 × 30 cm, height 20 cm, with open field); in this test, the ability of the two genotypes to remain awake following this environmental change was tested. Each mouse (n = 11 pairs) was subjected to this test four times separated by an interval of 10–14 days, twice at 2 p.m. when the animals normally being sleeping for most of the time, and twice at 6 p.m. when they would normally be awake a majority of the time. Sleep-wake stages during their stay in the new environment were compared between the two groups and with the baseline recordings for the same group.

2.4.3. Cortical EEG and the sleep-wake cycle after drug administration

In order to compare the effects of drugs acting on the histaminergic or other systems involved in sleep-wake control, the two genotypes of mice were injected intraperitoneally (or s.c.) with the following agents with an interval of at least 7 days between injections, subsequent recordings being made for 24 h.

During the dark phase at 8 p.m.:
Saline alone (0.1 ml) or containing the following drugs.

1) Terfenadine (1 and 5 mg/kg, n = 9 for each genotype), a H_1-receptor antagonist that does not pass through the blood brain barrier.
2) Triprolidine (1 and 5 mg/kg, n = 18 for each genotype), a potent, specific and brain penetrating antagonist of histamine H_1 receptor.
3) Zolantidine (5 mg/kg, n = 8 for each genotype), a brain penetrating antagonist of histamine H_2 receptor.
4) Prazosin (1 mg/kg, n = 10 for each genotype), an α_1 adrenoreceptor antagonist.
5) SCH23390 (1 mg/kg, n = 9 for each genotype), a D_1 dopamine receptor antagonist.
6) Haloperidol (1 mg/kg, n = 9 for each genotype), a D_2 dopamine receptor antagonist.
7) Scopolamine (0.25 and 0.5 mg/kg, n = 9 and 18 for each genotype), an antagonist of cholinergic muscarinic receptor.

During the light phase at10 a.m:
Saline alone (0.1 ml) or containing the following drugs.

1) Cyclopropyl-(4-(3-(1H-imidazol-4-yl) propyloxy) phenyl) ketone (ciproxifan, 1 mg/kg; Bioprojet, Paris, France) (n = 8 for each genotype), a potent inverse agoniste/antagonist of histamine H3-receptor (Schwartz et al., 1991) which controls histamine release and synthesis by autoinhibition.
2) Physostigmine (0.3 and 0.5 mg/kg, n = 9 and 6 for each genotype), a pharmacological agent that enhances the endogenous cholinergic tone by inhibiting cholinesterase, the enzyme of the acetylcholine degradation. To facilitate data description, physostigmine is defined as indirect cholinergic agonist in the following text.

All drugs (Sigma, except specific mention), expressed as salt weight, were dissolved immediately before use. Results obtained in the saline- and drug-injected animals were compared.

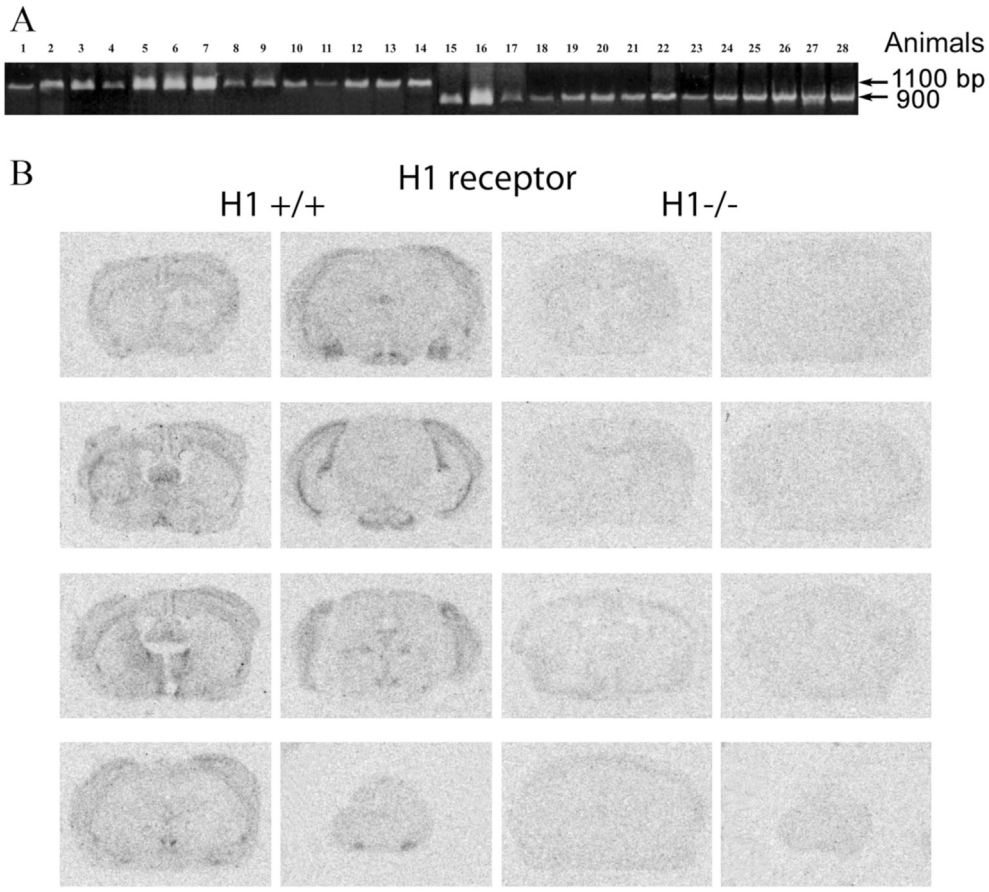

Fig. 1. Confirmation of genotypes by PCR and *in situ* hybridization. A, PCR genotyping. Lanes 1–14, H1+/+ mice; lanes 15–28, H1−/− mice. Note that all H1+/+ mice displayed a 1100 bp band corresponding to the H1 gene fragment, whereas all H1−/− mice showed a 900 bp band corresponding to the mutant gene fragment. B, Expression of H1-receptor mRNA in H1+/+ and H1−/− mice, note positive mRNA signaling in H1+/+ but not in H1−/− mouse brains.

2.5. Evaluation of the cholinergic muscarinic receptor (M1-M5) mRNA using quantitative real time-PCR

In order to assess whether H1-receptor abolition results in any differential gene expression of cholinergic muscarinic receptors also known for their role in cortical activation, we carried out quantitative PCR analysis on the muscarinic receptor genes (M1-M5) using the protocol modified from that reported in Roche et al. (2015). 5 male adult H1+/+ and H1−/− mice (age of 7 ± 2 months) were used in this study. Briefly, total RNA from mouse frontal cortex cell lines was extracted using miRNeasy Microkit with DNase treatment (Qiagen).Total RNA yield was measured by OD260 and the purity confirmed by reaching an A260/A280 ratio of 1.9:2.1 on a Nanodrop ND-1000. Reverse transcription was then performed using 500 ng of total RNA, with Bio-Rad Laboratories' iScript cDNA Synthesis Kit in a total volume of 20 µl. The reaction was incubated at 25 °C for 5 min followed by incubation at 42 °C for 30 min and 85 °C for 5 min. Quantitative real-time PCR was performed as follows: 95 °C for 20s then 40 cycles of denaturation at 95 °C for 1 s and hybridization and elongation at 60 °C for 20 s on a 7900HT Fast Real-Time PCR System using FAST SYBR Green Master Mix (AppliedBiosystems). The levels of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) transcript were used to normalize the potential amount variation of sample cDNAs added to each reaction. For each gene, the relative expression ratio was then determined using the 2ΔΔCt calculation method resulting in the relative gene expression levels presented in Table 4. Muscarinic receptors and reference primers were designed with Primer-Blast software (National Centre for Biotechnology Information/NCBI, Bethesda, USA) and purchased from Eurogentec. They are listed below:

M1	
F primer	GCGTTTAGGCAGGAAGTCAG
R primer	AGGGCCTACCTGGATGAGTT
M2	
F primer	GAATGGGGATGAAAAGCAGA
R primer	GCAGGGTGCACAGAAGGTAT
M3	
F primer	ACCTGATTTTGGTCCAATGC
R primer	GGCAGACCAATTTCTGAGGA
M4	
F primer	TCCTCACCTGGACACCCTAC
R primer	TTGAAAGTGGCATTGCAGAG
M5	
F primer	TCAGCCATCAAATGACCAAA
R primer	AGTAACCCAAGTGCCACAGG
GRADH	
F primer	GCTAGGACTGGATAAGCAGGG
R primer	TGAGGTCAATGAAGGGGTCG

2.6. Statistical analysis

ANOVA and the *post hoc* Student's *t* test (two tailed) were used to evaluate differences between H1 +/+ and H1 −/− mice in the cortical EEG and sleep-wake parameters under normal conditions

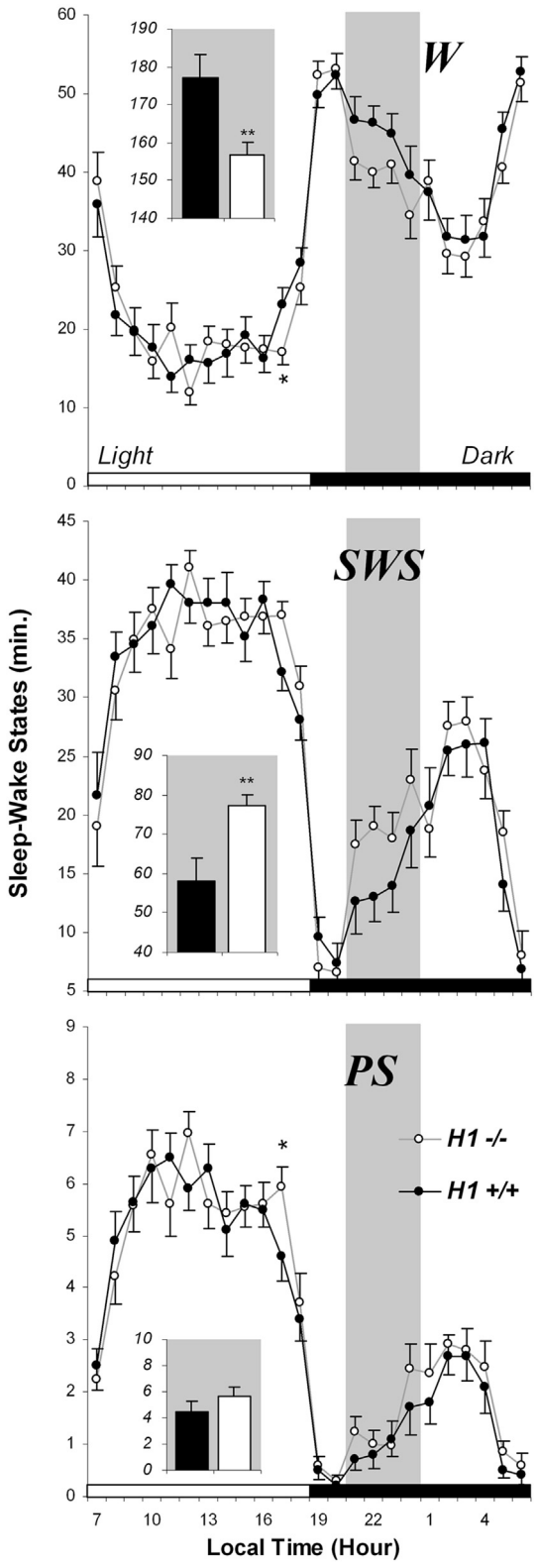

Fig. 2. Quantitative comparison of spontaneous sleep-wake states in H1+/+ and H1−/− mice. Data are expressed as mean hourly values ± SEM (min), H1+/+ mice: filled symbols and columns; H1−/− mice: unfilled symbols and columns. The grey area corresponds to the period between 9 p.m. and 01 a.m. and the total sleep-wake values for each state during this period are indicated in the histogram. Note that, compared to H1 +/+ mice, H1 −/− mice exhibit a deficit of wakefulness (W) after lights-off without major change in the daily amount of all sleep-wake stages; n = 24, corresponding to 2 × 24 h recordings from 12 animals of each genotype. SWS, slow wave sleep; PS, paradoxical sleep; *, **, $p < 0.05$, 0.01, respectively (two-tailed *t*-test after significance of a two-way ANOVA for repeated measures).

or after treatment and differences in these parameters between control data (baseline recordings or saline injection) and data following treatment in the same group of animals; in the latter case, individual animals served as their own control. Scheffe post hoc test after significance in ANOVA was used to examine the difference in muscarinic gene expression between genotypes.

3. Results

3.1. Genotyping by PCR and in situ hybridization

We performed PCR on genomic DNA from tail biopsies from all mice. As shown in Fig. 1A, a strong H_1 receptor signal, corresponding to a 1100 base pair band, was detected in the H1+/+ mice (animals 1–14 in Fig. 1), and a strong Neo^r signal, corresponding to a 900 base pair band, was detected in the H1−/− mice (animals 15–28), proof that, in the KO animals, the H1-receptor gene had been disrupted and the Neo^r gene inserted.

The deletion of H1-receptor gene was further confirmed by *in situ* hybridization using an H1-receptor probe. Indeed, well contrasted cerebral autoradiograms were obtained in H1+/+ mice, but H1-receptor mRNA expression was not detectable in the H1−/− mouse brains (Fig. 1B).

3.2. General observations

As wild type (WT or H1+/+) littermates, H_1-receptor gene disrupted (H1−/− or H1R KO) mice appeared to develop normally. No apparent troubles were noted in terms of fertility, general morphology, movement or usual behaviors under basal conditions. At the age of approximately 12 weeks, their body weight was similar (25.7 ± 0.6 g for H1+/+ mice and 25.4 ± 0.5 for H1−/− mice at the age of 87 ± 3 days, n = 14 for each genotype, $p > 0.05$; Student's *t* test).

3.3. Spontaneous sleep-wake cycle in H1−/−mice

Under basal conditions during which the animals were left undisturbed, both sets of mice exhibited a circadian sleep-waking rhythm characteristic of the C57Bl6/J strain (Franken et al., 1999; Anaclet et al., 2009; Gondard et al., 2013), i.e., with larger amounts of W during the dark period than during the light one (Fig. 2). No significant difference was observed in the total amount of W and SWS during either the light or dark phase or over 24 h. No sleep fragmentation was noted as the number of W, SWS or PS episodes as well as episode duration remained unchanged.

Despite this lack of a major difference in the daily amount of spontaneous W, hourly analysis of sleep-wake states (Fig. 2) revealed a significantly smaller amount of W in H1−/− mice during the periods after lights-off between 7 p.m–1 a.m. Thus, as expected for a nocturnal rodent, H1+/+ mice anticipated and responded to lights-off with a significant increase in W, accompanied by a high level of behavioral activity whereas this feature was less manifest in the H1−/−mice. Concomitant to their deficit of W (157 ± 3 vs 177 ± 6 min in H1+/+ mice, $p < 0.01$), there was an increase in both SWS and PS between 9 p.m. and 1 a.m. (Fig. 2, boxed areas). The W deficit during this period was compensated over 24 h, since there was no major change in the daily total W.

3.4. Characteristics of cortical EEG in H1−/− mice

From the frontoparietal and frontooccipital leads, the cortical EEG of both genotypes manifested marked and specific changes across the behavioral states and signs characteristic of mice, i.e., a dominant presence of θ frequencies, notably during PS and W

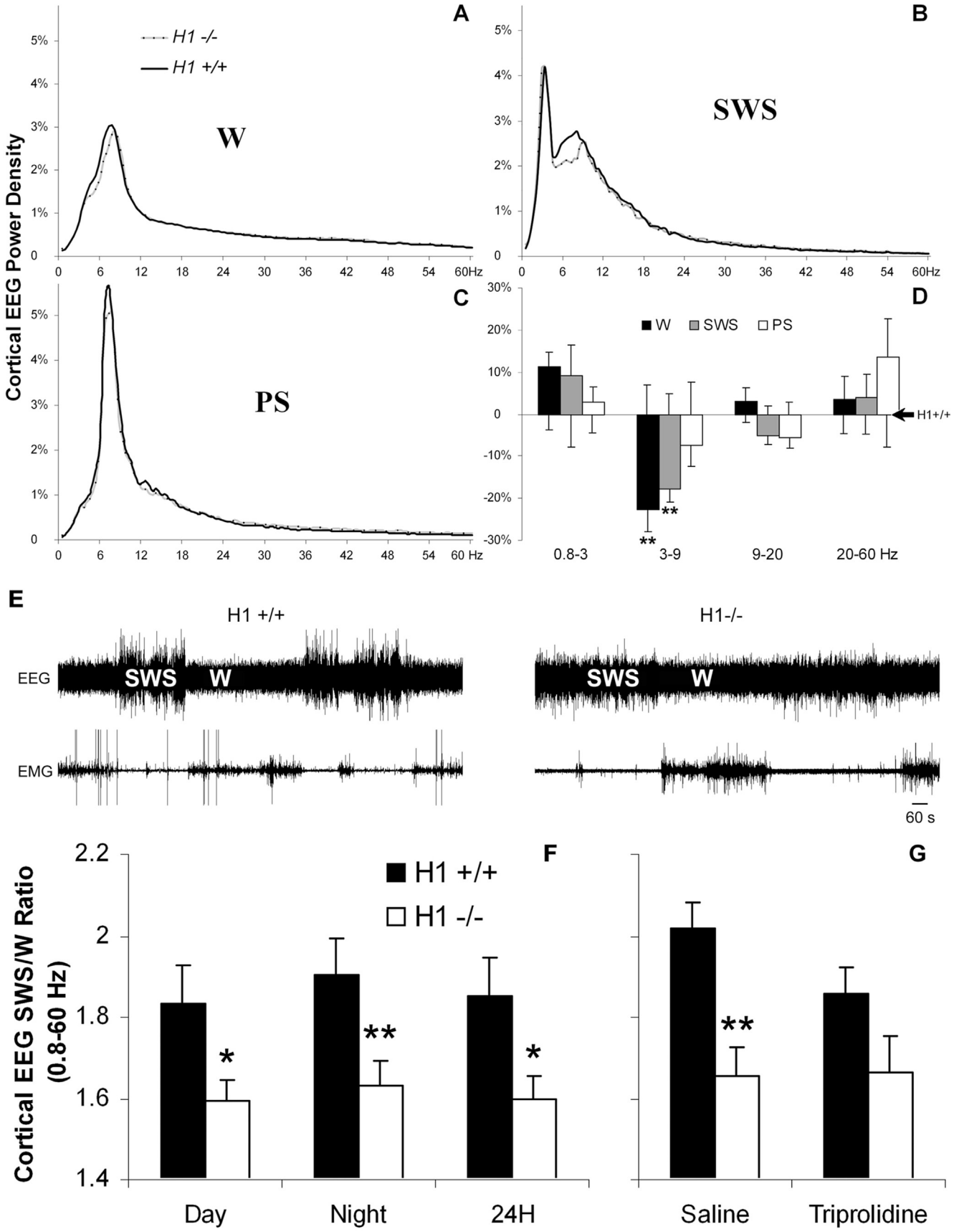

Fig. 3. Spectral analysis of cortical EEG) in H1+/+ and H1−/− mice. A–D, Mean spectral distribution of cortical EEG power density in spontaneous sleep-wake states in H1+/+ and H1−/− mice. The data were obtained from 14 pairs of animals by pooling consecutive 4 s epochs during the period of 7–10 p.m. using the fast Fourier transform routine within the frequency range of 0.8–60 Hz. A–C, mean percentage power density calculated as the mean power (in μV^2) in each 0.4 Hz frequency bin divided by the total power (0.8–60 Hz) in

Table 1
Mean latencies (±SE) to slow wave sleep (SWS) and paradoxical sleep (PS) in H1+/+ and H1−/−mice after behavioral challenges and ciproxifan injection. Note a significant decrease in the latencies to SWS in H1−/− compared to H1+/+ mice after all behavioral challenges except after cage change at 6 p.m. and after ciproxifan injection (n = number of experiments performed on 10 pairs of animals.*, **, ***p < 0.05, 0.01, 0.001 compared with values of H1+/+ mice (two-tailed t-test after significance in two-way ANOVA for repeated measures).

Genotype		Latencies to SWS (min.)		Latencies to PS (min.)	
		H1 +/+	H1 −/−	H1 +/+	H1 −/−
Simulation of injection	(n = 60)	41 ± 3	31 ± 3*	99 ± 7	82 ± 5
Litter change	(n = 40)	73 ± 4	58 ± 3**	87 ± 3	78 ± 3**
Environmental changeat 2 p.m.	(n = 18)	71 ± 6	54 ± 6*	99 ± 6	82 ± 5*
Environmental changeat 6 p.m.	(n = 22)	71 ± 13	69 ± 14	154 ± 18	160 ± 5
Ciproxifan 1 mg/kg i.p.	(n = 8)	68 ± 6	29 ± 6***	89 ± 6	47 ± 5***

Table 2
Quantitative variations of sleep-wake states in H1+/+ and H1−/− mice after an environmental change. Mean time (min) spent in each behavioral state ± SEM are shown. In parentheses, percentages (in italics) as compared to each group's own control values under baseline recordings. 100 means no change (control level), >100% = increase, while < 100% = decrease. Note, in two genotypes, a significant increase in W and a concomitant decrease in SWS and PS for the 4 h test both at 2 and 6 p.m. as compared to baseline value (control). No significant difference between the two genotypes was found.*, **p < 0.01, 0.001, (two tailed *t*-test after significance in a two-way ANOVA for repeated measures).

Genotype		H1 +/+			H1 −/−		
Behavioral state		W	SWS	PS	W	SWS	PS
Environmental change at 2 p.m.	(n = 22)	135 (*173*) ± 5**	93 (*66*) ± 5**	12 (*59*) ± 0.7**	127 (*180*) ± 5**	100 (*68*) ± 5**	12 (*54*) ± 0.8**
Environmental change at 8 p.m.	(n = 22)	208 (*117*) ± 5*	30 (*53*) ± 5*	2 (*26*) ± 0.3**	204 (*122*) ± 6**	35 (*67*) ± 5**	2 (*31*) ± 0.4**

(Fig. 3). Nevertheless, compared to H1+/+ littermates, H1−/− mice showed the following changes:

- A remarkably lower averaged EEG SWS/W power ratio (0.8–60 Hz), during either light phase or over 24 h (Fig. 3E). This decreased ratio was seen during all recorded baseline periods (days 15–45 post-surgery). Interestingly, the cortical EEG SWS/PS power ratio was unchanged in H1−/−mice (not shown), indicating that this qualitative change occurs only specifically between SWS and W.
- A decrease in the power density of cortical slow θ rhythm (3–9 Hz). This was most marked during W, less prominent during SWS, and not significant during PS (Fig. 3D).
- A decrease in the peak power and amplitude of the cortical EEG at θ frequencies associated with the deficit of θ power during SWS (Fig. 3B). Changes in other frequency bands were not statistically significant.

3.5. Effects of behavioral challenges on sleep latencies and the sleep-wake cycle

In the light of less reactivity observed in HDC KO mice when exposed to behavioral stimuli (Parmentier et al., 2002), the same tests were performed in H1−/−mice. A shorter latency to SWS and PS in H1−/− mice than in H1+/+ mice was identified following a routine litter change or a simulation of injection (Table 1).

Similarly, in the new environment test performed during the light phase, which consisted of transferring the mice from their habitual home cage to a new and different one, a decreased latency to SWS and PS was seen in H1−/− mice compared to H1+/+ mice (Table 1). This decreased latency did not occur when the animals were placed back into their home cages after the 4 h stay (20 and 42 min to SWS and PS for the KO vs 22 and 42 min for the WT), suggesting that novelty plays an important role in the new environment-elicited awakening in the H1+/+ mice and that this is impaired in H1−/− mice. No significantly decreased latency was seen, however, when the test was performed during the dark phase. There was no difference between the two genotypes in terms of sleep-wake amounts during their 4 h stay in the new environment (Table 2).

3.6. Effects of pharmacological administration on the cortical EEG and sleep-wake cycle

3.6.1. Triprolidine & terfenadine, H1-receptor antagonists

In H1+/+ mice, intraperitoneal injection of the brain-penetrating H1-receptor antagonist, triprolidine (1 and 5 mg/kg) enhanced cortical slow activity and SWS as compared to saline injection in the same animals. These effects were accompanied by a decrease in W, and interestingly, an increase in PS (Table 3;

the same epoch. D, EEG power spectra in H1−/− mice (columns, n = 14) expressed as a mean percentage change (±SEM) relative to those (±SEM) in H1+/+ mice (baseline 0, n = 14). Note that the H1−/− mice show a deficit of power density of θ rhythm (3–9 Hz) during W and SWS. No significant difference was found between genotypes on δ 0.8–3 Hz, α + spindle frequencies 9–20 Hz, β+γ 20–60 Hz (fast rhythms). E, EEG samples form a H1+/+ or H1−/−mouse showing several sleep-wake transitions. Note a decreased cortical EEG ratio between slow wave sleep (SWS) and wakefulness (W) in the H1−/− mouse compared to the H+/+ mouse. F and G, Cortical EEG SWS/W power ratio (0.8–60 Hz) in H1+/+ and H1−/− mice during the night or day periods or over 24 h of spontaneous recordings or during 4 h after injection of saline or Triprolidine (5 mg/kg, i.p. n = 12). Filled columns, H1+/+ mice; unfilled columns, H1−/− mice. Note the significantly reduced ratio in H1−/− mice during baseline conditions (F) or after saline injection (G), but not after Triprolidine injection (*, **p < 0.05, 0.01; two-tailed *t* test). Other abbreviations: PS, paradoxical sleep; EMG, electromyogram.

Table 3
Mean values of each sleep-wake stage during the 4h after pharmacological dosing of aminergic and cholinergic ligands in WT and H1−/− mice. n = number of drug injection experiments performed on 8–10 pairs of animals. In parentheses, percentages (in italics) as compared to each group's own control values obtained with saline injections. 100 means no change (control level), >100% = increase, while < 100% = decrease. Note 1) no significant difference between saline injection and injection of terfenadine or zolantidine in both genotypes; 2) an increase in slow wave sleep (SWS) and paradoxical sleep (PS) after injection of triprolidine in H1+/+ but not in H1−/− mice; 3) a decrease in wakefulness (W) and increase in SWS after scopolamine, prazosin, haloperidol, and SCH23390 dosing.*, **, ***, ****p < 0.05, 0.01, 0.001, 0.0001, compared with values of saline injection within genotype (two-tailed *t*-test after significance in two-way ANOVA for repeated measures). °, °°, °°°, °°°° p < 0.05, 0.01, 0.001, 0.0001 between genotypes (ANOVA).

	Dose (mg/kg)	Route		H1 +/+			H1 −/−		
				W	SWS	PS	W	SWS	PS
Triprolidine	1	i.p.	(n = 18)	56 (*85*) ±3**	40 (*126*) ±2**	4 (*183*) ±0.4***	60 (*100*) ±2°	38 (*99*) ±2°	2 (*107*) ±0.3°
	5	i.p.	(n = 18)	53 (*77*) ±3****	44 (*147*) ±2***	4 (*222*) ±0.4****	65 (*105*) ±3°°°°	33 (*92*) ±3°°°°	2.0 (*96*) ±0.4°°°
Terfenadine	1	s.c.	(n = 9)	64 (*99*) ±4	33 (*101*) ±3	3 (*105*) ±1	63 (*94*) ±6	36 (*113*) ±5	2 (*102*) ±1
	5	s.c.	(n = 9)	55 (*92*) ±3	42 (*111*) ±4	3 (*119*) ±1	56 (*99.0*) ±5	42 (*103*) ±4	2.2 (*79.1*) ±1
Zolantidine	5	i.p.	(n = 8)	63 (*94*) ±4	35 (*112*) ±3	2 (*123*) ±1	63 (*96*) ±5	36 (*106*) ±4	2 (*141*) ±1
Ciproxifan	1	i.p.	(n = 8)	113 (*186*) ±6****	110 (*70*) ±4****	17 (*75*) ±2**	70 (*103*)±8°°	143 (*98*) ±7°°	21 (*103*) ±2°
Prazosin	1	i.p.	(n = 10)	43 (*67*) ±5***	55 (*174*) ±5***	2 (*49*) ±1	42 (*70*) ±5**	56 (*146*) ±2**	2 (*119*) ±1
Scheme 23390	5	i.p.	(n = 9)	47 (*80*) ±3**	49 (*128*) ±3*	4 (*140*) ±1	52 (*84*) ±3*	45 (*126*) ±3*	3 (*129*) ±1
Haloperidol	1	i.p.	(n = 9)	56 (*85*) ±3*	41 (*126*) ±2*	3 (*166*) ±1	50 (*85*) ±1*	47 (*124*) ±3*	3 (*99*) ±1
Scopolamine	0.25	i.p.	(n = 9)	49 (*75*) ±6*	49 (*153*) ±5**	2 (*92*) ±1	44 (*66*) ±4**	54 (*174*) ±2***	2 (*91*) ±1
	0.5	i.p.	(n = 18)	55 (*77*) ±4***	44 (*163*) ±3****	1 (*78*) ±0.2	49 (*76*) ±4***	50 (*151*) ±1***	1 (*43*) ±0.2*
Physostigmine	0.3	i.p.	(n = 9)	64 (*156*) ±19**	52 (*75*) ±15**	4 (*38*) ±4***	69 (*143*) ±15**	47 (*77*) ±13*	4 (*37*) ±3****
	0.5	i.p.	(n = 6)	81 (*181*) ±17*	38 (*59*) ±15**	1 (*11*) ±4***	84 (*151*) ±14*	35 (*63*) ±14**	1 (*11*) ±3***

Figs. 3–4). Because H1-antagonists are well known to reduce PS in the cat, rat and other animals especially with high doses (reviewed in Monti et al., 1991; Lin, 2000) and because this effect is usually thought to result from their unspecific antagonism of the muscarinic receptor (reviewed in Schwartz et al., 1991), the increase in PS seen here with triprolidine suggests that the doses we used in mice are specific for the H1-receptor.

In H1−/− mice, the above effects of triprolidine were totally absent (Table 3; Figs. 3–4), results that also reinforce our assertion that triprolidine at the doses used is selective for the H1-receptor.

Unlike triprolidine in the WT mice, injection of terfenadine (1 and 5 mg/kg, s.c.), a H1-receptor antagonist that does not pass through the blood brain barrier, resulted in no changes in the sleep-wake parameters during the 4 h post-injection either in WT or H1−/− mice (Table 3), indicating the noninvolvement of peripheral H1-receptors in the observed sleep-wake effects using triprolidine.

Importantly, application of triprolidine (5 mg/kg, i.p.) to the WT mice reduced their cortical EEG power ratio between SWS/W. Thus, when this ratio is compared with that of the H1−/−mice, there was no longer any significant difference between genotypes as that was seen during baseline recordings or after saline injection (Fig. 3E, F). Here, we showed that acute pharmacological inactivation of the H1-receptor can produce a phenotype similar to that seen in H1−/− mice.

3.6.2. Zolantidine, H2-receptor antagonist

Injection of the brain penetrating H2-receptor antagonist, zolantidine (5 mg/kg, i.p) (Young et al., 1988; Calcutt et al., 1988) did not affect sleep-wake cycle parameters in both genotypes (Table 3) even with larger doses (non-shown), suggesting that the loss of H1-receptor in the KO mice does not provoke either upregulation or hypersensitivity of the H2-receptor.

3.7. Ciproxifan, H3 receptor inverse agonist/antagonist

In H1+/+ mice, intraperitoneal injection of ciproxifan (1 mg/kg), a potent and specific HAH3 receptor inverse agonist/antagonist (Ligneau et al., 1998) caused suppression of cortical slow activity (0.8–5 Hz) and α frequencies (15–20 Hz) during the light phase, resulting in a marked cortical activation (Fig. 5), i.e., low voltage electrical activity with dominant activities in the β and γ bands (20–60 Hz). Furthermore, ciproxifan injection increased the power density of these neocortical fast rhythms (Fig. 5). The effects on the cortical EEG were manifested on polygraphic scoring as an almost total waking state, characterized by a significantly delayed sleep latency (Table 1) to, and suppression of, SWS and PS, during the 4 h recordings (Table 3).

In H1−/− mice, the same injection of ciproxifan had no effect on either the cortical EEG or the sleep-wake states compared to saline injection in the same animals (Table 3; Fig. 5), indicating that the effects seen with the H3-receptor inverse agonist also depend on the postsynaptic H1-receptor.

3.7.1. Prazosin, α1-adrenoreceptor antagonist

In both genotypes, injection of prazosin (1 mg/kg, i.p) increased markedly SWS at the expense of waking, while PS was not significantly modified. The effects are similar between genotypes without statistical difference (Table 3).

3.7.2. Haloperidol & SCH23390, dopamine D1-or D2-receptor antagonist

Like the adrenergic antagonist prazosin, SCH23390 (0.25 mg/kg, i.p) or haloperidol (1 mg/kg, i.p.), respectively caused, in both genotypes, an increase in SWS and a decrease in W without significant effects on PS. There was no statistical difference between genotypes (Table 3).

3.7.3. Scopolamine & physostigmine, cholinergic antagonist or indirect agonist

When the muscarinic antagonist scopolamine was injected intraperitoneally (0.25 or 0.5 mg/kg), the two mouse genotypes all exhibited an enhancement of cortical EEG slow activities. This marked effect prompted us to perform power spectral density analysis. We found that the effect was more pronounced in H1−/− than in WT mice (Fig. 6A) characterized by a greater increase in the whole power density (0.8–60 Hz) during W and in slow activities (0.8–5 Hz) during SWS (Fig. 6A,B). These effects were longer lasting in the KO mice with the EEG returning to control level at 197 ± 7 min vs 168 ± 6 min WT) (Fig. 6A,C). Changes at other EEG frequencies were not significantly different.

This EEG qualitative effect was manifested on the polygraphic recordings as a decrease in W and increase in SWS in both genotypes. At 0.25 mg/kg, scopolamine resulted in a decrease in W and

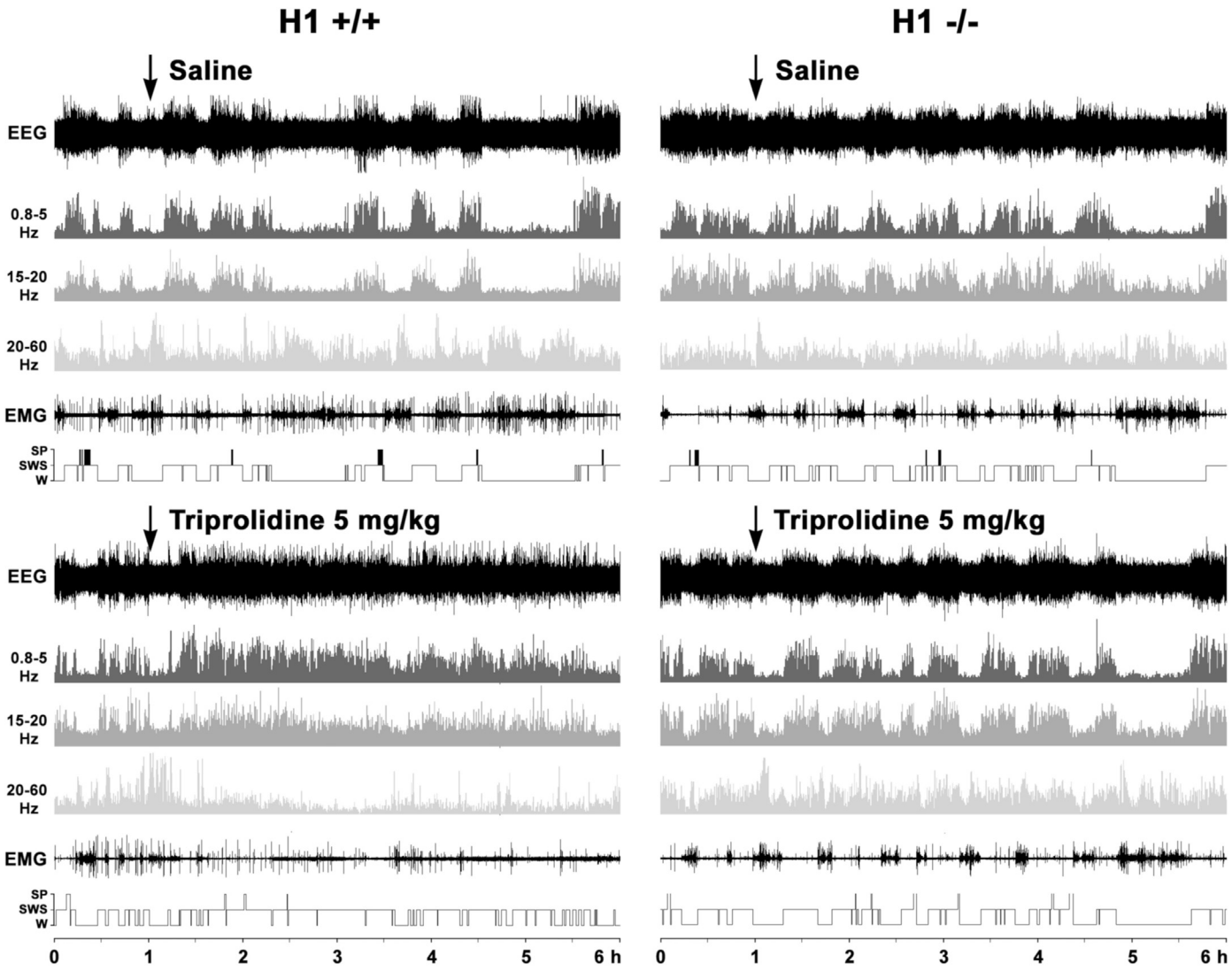

Fig. 4. Effects of Triprolidine on the cortical EEG and sleep-wake states in H1+/+ and H1−/− mice. Examples of polygraphic recordings, cortical EEG power density (μV^2) in different frequency bands, and the corresponding hypnograms illustrating enhancement of cortical EEG power at 0.8–5 Hz (slow activity) and 15–20 Hz (α band), suppression of cortical fast rhythm (β + γ, 20–60 Hz), and increased amount of slow wave sleep (SWS) induced by injection of triprolidine (5 mg/kg, i.p, at 8 p.m., indicated by the arrow) only in an H1+/+ mouse, but not in an H1−/− mouse as compared to a saline injection. EEG, electroencephalogram; EMG, electromyogram.

increase in SWS more statistically significant in KO than in WT mice during a 4 h period. PS decreased with a delayed latency, which was longer in KO than in WT mice: 141 ± 12 vs 108 ± 8 min (Fig. 6A; Table 3). Thus, sleep-wake quantitative data also show, to some extent, greater effects of scopolamine in the KO than in WT mice.

In contrast to scopolamine, application of physostigmine (0.3 and 0.5 mg/kg, i.p.) an indirect cholinergic agonist, enhanced cortical fast rhythms (30–60 Hz) and suppressed slow oscillations (0.8–5 Hz) (Fig. 7 A,B) in both genotypes. As a result, W (Table 3) and behavioral activities were promoted. Like scopolamine, the effects of physostigmine on the EEG were more prominent in H1−/− than in WT mice (Fig. 7B) even though the effects on the sleep-wake stages during a 4 h analysis did not show any difference between genotypes. As shown in Fig. 7, physostigmine displayed significantly greater slow activity-suppressing effects in H1−/− than in WT mice both during W and SWS. Moreover, there was also a greater enhancement of cortical fast rhythms during W.

Together, both data sets with scopolamine and physostigmine indicate that H1−/− mice responded to cholinergic ligands in a more pronounced manner than their WT littermates.

Table 4
Levels of cholinergic muscarinic receptor (M1-M5) mRNA genes in the frontal cortex of H1+/+ and H1−/− mice, as measured by quantitative real time PCR. Total RNA from mouse frontal cortex was extracted. Reverse transcription was then performed following measurement of total RNA yield using OD260. Quantitative real-time PCR was performed and hybridization and elongation at 60 °C for 20 s on a 7900HT Fast Real-Time PCR System. The levels of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) transcript were used to normalize the potential amount variation of sample cDNAs. For all genes, the relative expression ratio was then determined using the 2ΔΔCt calculation method. Results are expressed as mean gene expression levels ± SEM from five mice of each genotype. Note no significant difference in terms of gene expression of all muscarinic receptors between genotypes (Scheffe post hoc test after significance in ANOVA).

	M1	M2	M3	M4	M5
H1 +/+	1.01 ± 0.08	1.13 ± 0.30	1.08 ± 0.21	1.04 ± 0.14	1.06 ± 0.17
H1 −/−	0.89 ± 0.10	1.36 ± 0.19	1,17 ± 0.10	0.96 ± 0.11	0.91 ± 0.13
P-values	0.34	0.52	0.71	0.66	0.53

3.8. Gene expression of muscarinic M1-M5 receptors evaluated by quantitative real time PCR

Because of the greater effects seen with the cholinergic ligands in H1−/− mice and in order to assess whether this genotype-related difference was linked to gene expression, we quantified

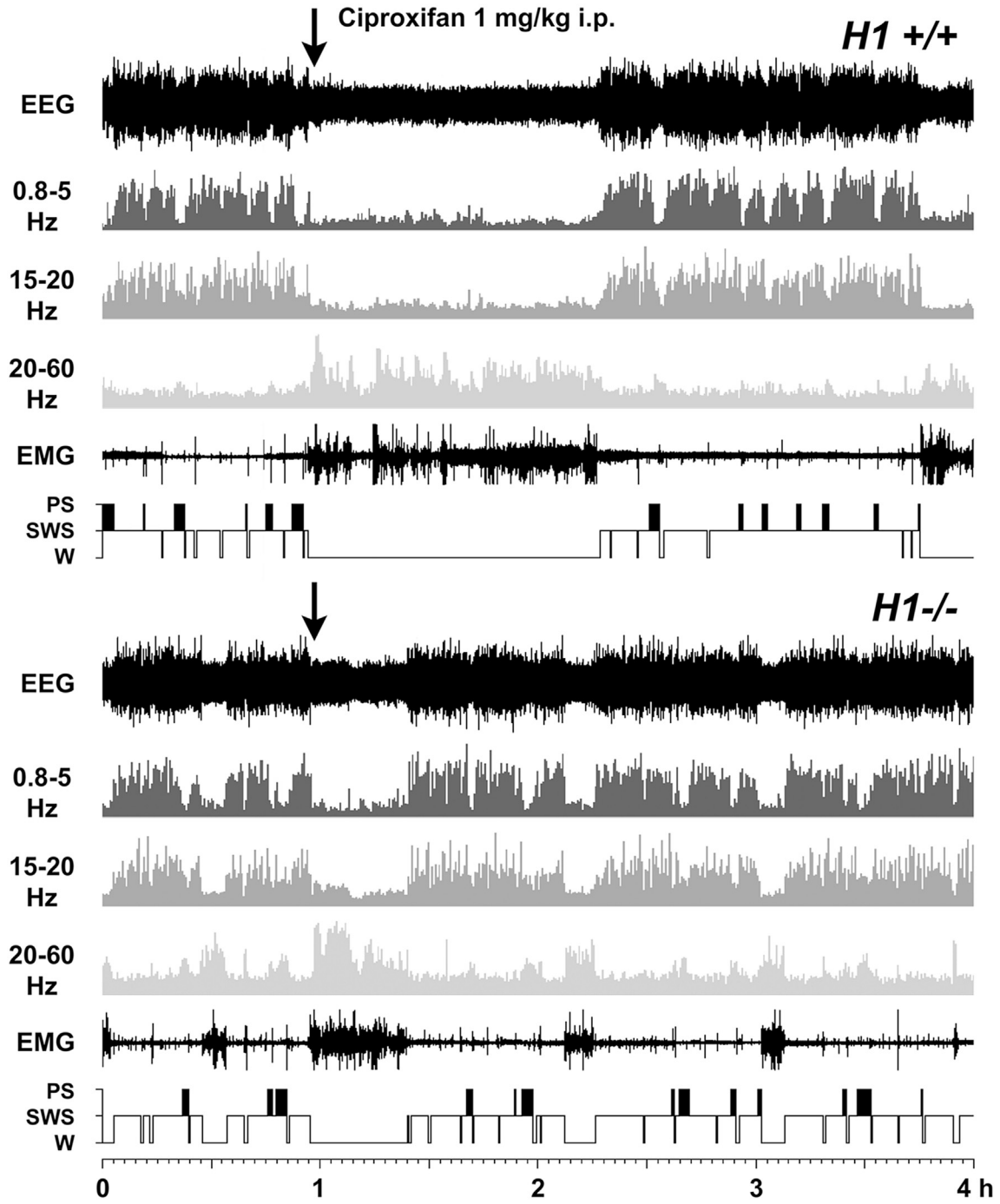

Fig. 5. Effects of ciproxifan on the cortical EEG and sleep-wake states in H1+/+ and H1−/− mice. Upper traces, H1+/+ mouse; lower traces, H1−/− mouse. Examples of polygraphic recordings, cortical EEG power density (μV^2) in different frequency bands, and the corresponding hypnograms illustrating suppression of cortical EEG power at 0.8–5 Hz (slow activity) and 15–20 Hz (α band), marked enhancement of cortical fast rhythms (β + γ, 20–60 Hz), and an increased amount of wakefulness (W) induced by injection of ciproxifan (1 mg/kg, i.p, at 10 a.m., indicated by the arrow) in a H1+/+ mouse, but not in a H1−/− mouse. EMG, electromyogram.

muscarinic receptor (M1–M5) genes because of the importance of this receptor category in cortical activation and sleep-wake control (Imeri et al., 1991, 1996; Vanni-Mercier et al., 1991; Velazquez-Moctezuma et al., 1991; Sakai and Onoe, 1997; Brown et al., 2012). The frontal cortex was chosen for this assay because the cerebral cortex is the ultimate target for cortical activation and because the frontal cortex is known for its predominant role in cognitive functions during W. As shown in Table 4, quantitative PCR analysis did not reveal any significant difference in any muscarinic receptor gene expression in the frontal cortex between H1+/+ and H1−/−mice. Because of the relative importance of M2 and M3 receptors in cortical activation, we also carried out *in situ* hybridization of their mRNA in order to compare their cerebral distribution using the same protocol for H1-receptor used in the present study. Although well contrasted and heterogeneous cerebral autoradiograms were obtained in both H1+/+ and H1−/− mouse brains, we were unable to detect any differences between genotypes in terms of M2 and M3 receptor mRNA density and distribution (data not-

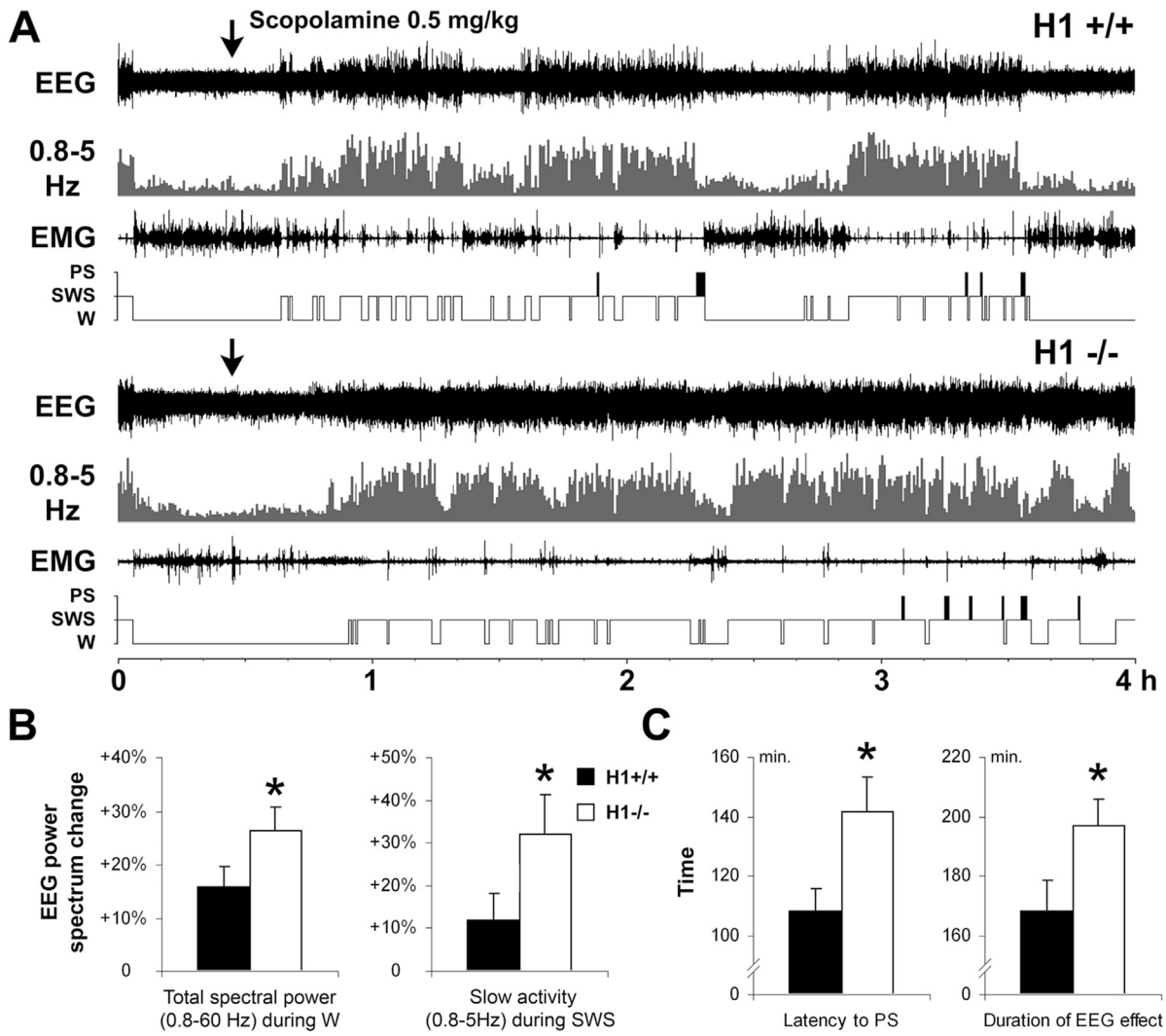

Fig. 6. Effects of scopolamine on cortical EEG and sleep-wake states in H1+/+ and H1−/− mice. A, Upper, H1+/+ mice; lower, H1−/− mice. Examples of polygraphic recordings, cortical EEG power density (μV^2) in slow activity (0.8–5 Hz), and the corresponding hypnograms illustrating the increase in cortical slow activity induced by injection of scopolamine (0.5 mg/kg, i.p, at 8 p.m., indicated by the arrow) in a H1+/+ mouse or a H1−/− mouse. Note a more important quantitative increase in cortical slow activity and slow wave sleep (SWS) in the H1−/− mouse compared to that in the H1+/+ mouse. EMG, electromyogram. B, EEG power spectra density at 0.8–60 and 0.8–5 Hz range expressed as a mean percent change (±SEM) relative to value of saline injection in both H1+/+ and H1−/− mice. Note a significant greater increase in EEG total power (0.8–60 Hz) during wakefulness (W) and also in cortical slow activities (0.8–5 Hz) during slow wave sleep (SWS) in H1−/− than in H1+/+mice. C, Comparison of mean durations (in min ± SEM) of the cortical EEG-synchronizing and paradoxical sleep (PS) suppressing effects of scopolamine in H1+/+ and H1−/− mice. The duration on the EEG effect is defined as the time between injection and the end of the significant increase in the power spectral density at 0.8–5 Hz while that on PS suppression is represented by latency to the appearance of the first PS episode lasting for >30 s after the injection. Note significant longer lasting effects in H1 −/− than in H1 +/+ mice (n = 18 in 9 pairs of animals; *, $p < 0.05$, two-tailed *t*-test between genotypes after significance in a two-way ANOVA for repeated measures).

shown). Given this absence of any difference in terms of muscarinic receptor gene expression in H1−/−mice, further studies are required in order to assess whether such a genotype-related difference in the response to cholinergic ligands results from a differential regulation at the level of receptor protein expression/functionality or Ach synthesis and/or release.

4. Discussion

4.1. Major sleep-wake phenotypes of H1-receptor−/− mice

We showed here that the sleep-wake cycle in H1−/− mice was affected both quantitatively and qualitatively. On the one hand, these mice exhibited a deficit of W just after lights-off in spite of an unaffected daily amount. On the other hand, their cortical EEG showed a reduced SWS/W power ratio and a significant deficit of θ rhythms (3–9 Hz) during W. These changes likely impacted the animal's behavior, as they presented signs of sleepiness, uncovered through a significant decrease in sleep latencies when facing several behavioral stimuli. All these phenotypes are reminiscent of those identified in HDC KO mice (Parmentier et al., 2002). Finally, pharmacological characterization revealed different responses, between H1+/+ and H1−/− littermates, exclusively to ligands acting on histaminergic or cholinergic transmission, results that further our understanding of sleep-wake control in KO or brain-deficient models. To the best of our knowledge, the present study is the first to report such sleep-wake, behavioral and pharmacological phenotypes in H1−/− mice.

The present study used exclusively littermate animals to avoid any mis-interpretation due to the genetic background (Valatx and

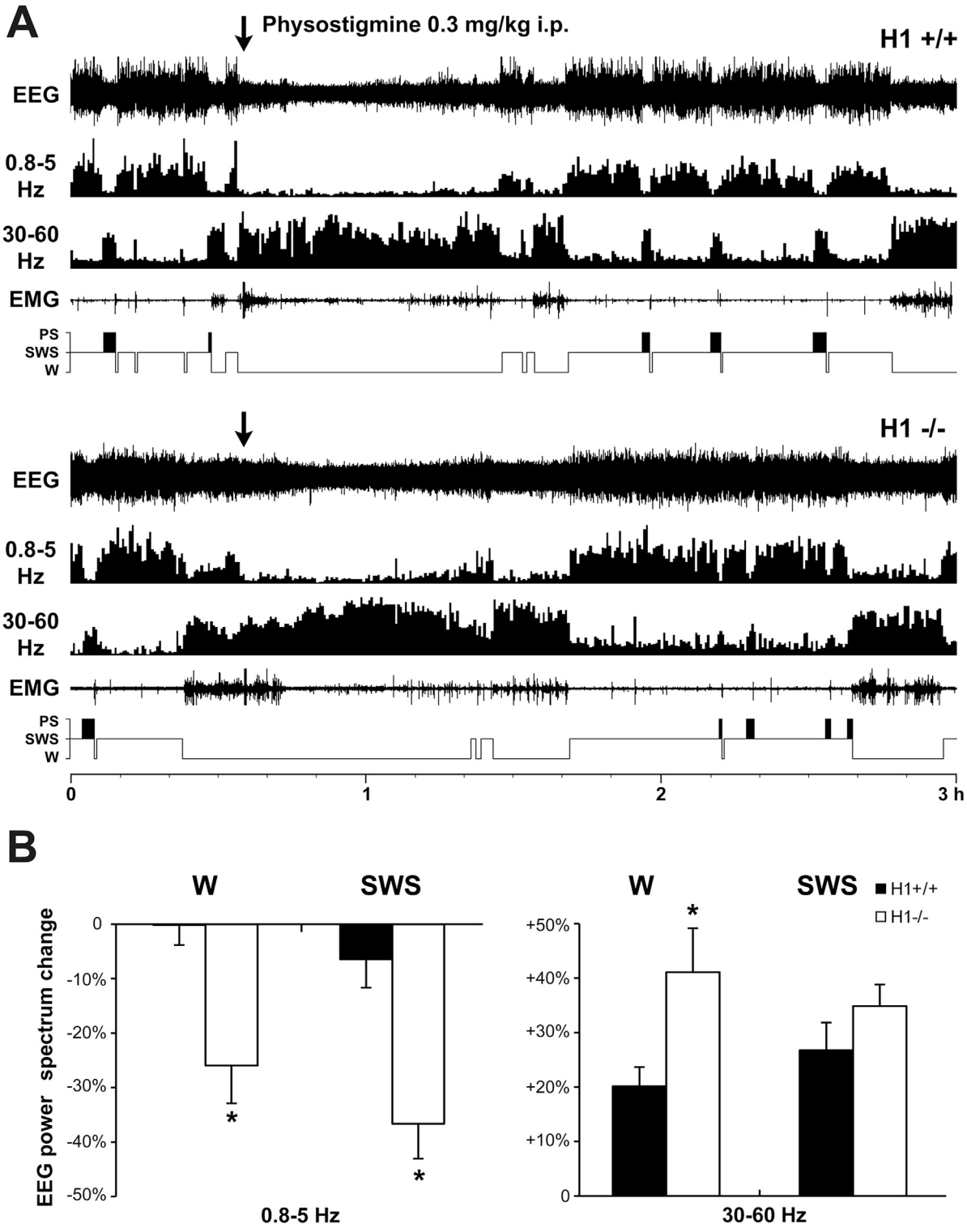

Fig. 7. Effects of physostigmine on cortical EEG and sleep-wake states in H1+/+ and H1−/− mice. A, Examples of polygraphic recordings, cortical EEG power density (μV^2) of slow activity (0.8–5 Hz) and fast rhythms (30–60 Hz) and the corresponding hypnograms illustrating the decrease in cortical slow activity and increase in fast rhythms induced by injection of physostigmine (0.3 mg/kg, i.p, at 10 a.m., indicated by the arrow) in a H1+/+ or H1−/− mouse. Upper, samples form a H1+/+ mouse; lower, form a H1−/− mouse. B, EEG power spectra density at 0.8–5 Hz (left side bars) or 30–60 Hz (right side bars) range expressed as a mean percentage change (±SEM) relative to value after saline injection in H1+/+ and H1−/− mice. Note, in the H1−/− mice, markedly greater effects of physostigmine in suppressing slow activity both during wakefulness (W) and slow wave sleep (SWS) and in enhancing fast rhythms during W. There was also a greater but not statistically significant enhancement of cortical fast rhythms during SWS in the H1−/− mice (n = 9 for both genotypes; *p < 0.05; two-tailed *t*-test between genotypes after ANOVA).

Bugat, 1974; Franken et al., 1999). Application of triprolidine, a selective H1-receptor antagonist (Nicholson et al., 1991), had no effect in H1−/− mice, whereas, in H1+/+ mice, it produced sedation and increased SWS. Similarly, ciproxifan, a HA H3-receptor inverse agonist/antagonist, elicited cortical activation and enhanced W in H1+/+ mice, as in cats (Ligneau et al., 1998), but had no effect in

H1−/− mice. Whereas these data validate pharmacologically the absence of H1−receptor and so the KO model used, they also show that the effects of triprolidine or ciproxifan depend on the availability of the H1-receptor. This confirms their pharmacological selectivity toward HA transmission. Finally, application of terfenadine, a H1-receptor antagonist that does not pass through the blood brain barrier (Simons et al., 1996), had no effect on the sleep-wake cycle in either genotype, indicating that cortical arousal does not depend on peripheral H1-receptors. These results, together with the role of H1-receptors in arousal already demonstrated by a great deal of data, designate the loss of the brain H1-receptor as responsible for the EEG, sleep-wake and behavioral effects observed in the present study using H1−/− mice.

4.2. H1-receptor and spontaneous sleep-wake states

Like other KO mice, such as those lacking orexin (Chemelli et al., 1999; Anaclet et al., 2009), 5HT receptors (Boutrel et al., 2002), or HDC (Parmentier et al., 2002), the H1−/− mice exhibited no major change in the daily amount of spontaneous W (≤15%/24 h) in spite of the excitatory mode of action of the H1-receptor on many cerebral cell targets. Nevertheless, H1−/− mice present, in the early dark period (rodents' active phase), a deficit of W similar to that identified in HDC−/− mice lacking HA (Parmentier et al., 2002), although in HDC KO mice this deficit occurs earlier and is detected before lights-off. These data together with the well-known sedative effects of H1-receptor antagonists, confirm the role of HA in maintaining the brain awake in the crucial situation where an increased alertness is required and also indicate that this function is largely mediated by H1-receptors. The slight temporal discrepancy observed in this deficit between HDC and H1−/− mice could reflect either an involvement of other HA receptors, or their different genetic background: 129Sv for the HDC KO and C57BL/6J for the H1−/−mice.

With regards to PS, because histaminergic neurons cease firing during this sleep stage (Sakai et al., 1990; Vanni-Mercier et al., 2003; Takahashi et al., 2006), the PS rate increase in HDC−/− mice (Parmentier et al., 2002) would point out a contribution of HA neurons to the permissive mechanisms of PS. In this study, H1−/− mice did not display such an increase. In addition to a different genetic background, we must consider the possibility that the abolition of postsynaptic H1-receptors only is not sufficient to release the histaminergic permissive mechanisms. Therefore other receptors must be involved. Since the number of HA neurons appear greater in 129Sv (Parmentier et al., 2002) than that in C57BL/6J (Takahashi et al., 2006) mice, it remains to be determined whether such an anatomic variation reflects a difference in terms of functional outputs and whether in the 129Sv mouse strain the HA neurons constitute a more powerful permissive system to PS.

4.3. Cortical EEG and role of the H1-receptor in the qualitative aspect of wakefulness

While only subtle quantitative changes of W have been observed in various KO mice (e.g., Chemelli et al., 1999; Boutrel et al., 2002; Parmentier et al., 2002; Anaclet et al., 2009), remarkable qualitative changes were uncovered in H1−/− mice, which displayed EEG characteristics similar to HDC−/− mice (Parmentier et al., 2002), and notably 1) a deficit of power in θ rhythms during W, 2) a reduced EEG amplitude during SWS and 3) a significant reduction in the SWS/W EEG power ratio, an effect that is also observed following acute pharmacological inactivation of either HDC by α-fluoromethylhistidine (Parmentier et al., 2002) or H1-receptor by triprolidine in this study. Taken together, these results show a crucial involvement of either presynaptic (HDC) or postsynaptic (H1-receptor) mechanisms in the shaping of several aspects of the EEG, which are apparently not compensated by upregulation of other brain activating systems.

Among the EEG phenotypes seen with HDC and H1R KO mice, emphasis should be made on the decreased EEG SWS/W ratio. Interestingly, the EEG SWS/PS ratio remained unchanged in H1 KO mice, indicating that the control exerted by the H1R is specific to the balance between SWS/W and that this reduced EEG SWS/W ratio is largely attributable to SWS. In addition to an impaired waking EEG characterized by reduced θ rhythms, indeed, both HDC and H1-receptor KO mice have no fully synchronized/inactivated SWS, as confirmed by decreased amplitudes and reduced slow components within the θ rhythms. Thus, these KO strains unlike WT mice have a less clear state-dependent change across the sleep−wake cycle and reduced contrast between SWS and W. Because the EEG power and cortical inactivation of SWS depend on the duration and EEG activation of previous W episodes (Tobler, 2000; Borbély and Achermann, 2000; Vyazovskiy et al., 2011), it appears that, in HDC−/− or H1−/− mice, an incomplete and deficient activation during W episodes leads to poor quality of SWS, which, in turn, compromises brain activity and excitability during subsequent W episodes producing behavioral signs of sleepiness. At the cellular level, loss of the H1-receptor would reduce synaptic potentiation during W, leading to reduced slow activity during SWS most likely through synaptic homeostatic mechanisms (Tononi and Cirelli, 2014). We qualify this decreased EEG SWS/W ratio as the cortical expression of somnolence, and also as the EEG correlates of behavioral deficits identified in HA-transmission deficient models.

4.4. H1-receptor and wakefulness in the presence of behavioral challenges

In keeping with the well-known drowsiness and impaired performance caused by H1R antagonists (Douglas, 1985; Yanai et al., 1999; Nicholson and Stone, 1986; Schwartz et al., 1991), H1−/− mice presented signs of sleepiness, as evidenced by decreased sleep latencies following, e.g., a routine change of litter or simulation of injection. This decreased arousal reaction is also consistent with their deficit of W after lights-off under baseline conditions. Moreover, H1−/− mice placed in a new environment were unable to remain awake in the light (sleepy) phase demonstrated by their decreased latencies to sleep. Nevertheless, during the dark phase, when mice are mostly awake, no significant difference in the latencies to sleep was observed. H1-receptor is supposed to promote wake induction given its major mode of action, i.e., to cause a switch of neuronal discharge from rhythmic bursts to tonic activity and to activate other neural systems involved in cortical activation such as the mesopontine and basal forebrain cholinergic systems (reviewed in McCormick, 1992; Lin, 2000; Brown et al., 2001; Jones, 2004). Here, when mice are already awake, induction of a more awake state presumably requires mobilization of not only H1-receptors but likely also other ones, such as the H2-receptor, despite its minor arousing role suggested by cellular and in vivo studies (reviewed in Lin, 2000; Brown et al., 2001, 2012). Finally, whereas HDC−/− mice are unable to remain awake during the 4 h exposure to the new environment, H1−/− mice display the same W amount compared to that of their WT littermates. Here, again, the involvement of other receptor(s) appears to be a prerequisite to ensure W-associated exploratory behaviors in the new environment.

4.5. To remain awake facing a deficient brain activating system

Since the concept of reticular ascending cortical activation proposed by Moruzzi and Magoun (reviewed in Moruzzi, 1972),

several neuronal groups such as aminergic, cholinergic and more recently orexinergic cells have been proposed as arousal systems that maintain the brain awake during diverse behavioral situations. Quite surprisingly, classical lesioning or recent KO models invalidating chronically each of the above systems usually results in transitory loss or subtle deficiency of the baseline waking state in spite of various more or less pronounced phenotypes. It is generally believed that, to ensure a vital function like W, brain plasticity is organized in such a way that the selective loss of one system could be compensated by upregulated adaptive mechanisms of the others (see for ex., reviews by Lin, 2000; Brown et al., 2012). Yet, how such compensation occurs and operates remains undetermined. The same question also arises regarding sleep-wake regulation in chronic brain pathological conditions such as neurodegenerative or autoimmune diseases. Using H1−/−mice, the present study addresses this question through a neuropharmacological approach using ligands acting on the above-mentioned systems. In this model, only a subtype of HA receptors is invalidated and so all other arousal systems remain presumably intact allowing our question to be assessed. Moreover, low dose ranges of the ligands were used in order to detect any subtle difference between genotypes.

First, we wondered whether such adaptive mechanisms would occur within the HA system, such as by a postsynaptic compensation with H2-receptor, which exhibits pronounced facilitating action on neuronal firing (Haas et al., 2008) and so could be involved in facilitation of W. Yet, we found that both WT and H1−/− littermates were insensitive to the brain-penetrating H2-receptor zolantidine. Whereas the brain availability and efficiency of zolantidine require further characterization, these data at least suggest that no remarkable hypersensibility of H2-receptors occurs in H1−/−mice.

Then, we extended the tests to the catecholaminergic systems, which are known for their control and modulation of diverse behavioral activities during W and which have multiple interactions with the HA system (reviewed in Haas et al., 2008; Panula et al., 2015. See also Crochet and Sakai, 1999; Yanovsky et al., 2011). Both the α1-adrenoreceptor antagonist prazosin and dopamine D1 or D2 receptor antagonist SCH23390 or haloperidol induced clear sleep-wake, notably SWS enhancing effects. Yet, we were unable to detect any significant difference between genotypes in favor of any upregulation.

Finally, the cholinergic system plays a major role in cortical EEG activation (reviewed in Jouvet, 1972; McCormick, 1992; Vanderwolf, 1992; Sakai et al., 1990; Steriade, 1996; Jones, 2005). Can it compensate for the deficit of the HA system by an increased activity? We found that the muscarinic antagonist scopolamine induced greater effects in H1−/− mice, i.e., a longer EEG effect, a prolonged PS suppression and notably an amplified increase in cortical slow rhythms. These data suggest that the muscarinic antagonist had blocked an increased muscarinic signaling. Similarly, physostigmine, an indirect cholinergic agonist, elicited more pronounced enhancement of EEG fast rhythms and greater suppression of cortical slow activities in H1−/− mice, indicating a higher sensitivity of cholinergic receptors to the agonist. Other results that would support an enhanced cholinergic transmission in H1−/−mice are the identification of higher levels of Ach concentrations in their frontal cortex and neostriatum (Dere et al., 2004; Zlomuzica et al., 2008).

It seems, therefore, that cholinergic transmission is upregulated through a compensatory mechanism that allows H1−/−mice to maintain a quasi-normal baseline waking state indispensable for survival. Such compensation appears relevant in view of the tight interactions between histaminergic and cholinergic neurons in terms of cortical activation (reviewed in Lin, 2000; Jones, 2004. See also Vu et al., 2015). In the cortical and thalamic relay neurons, notably, the H1-receptor and muscarinic receptor share common membrane channels and mechanisms to produce similar postsynaptic activation and to promote arousal (Reviewed in McCormick, 1992, 1993; Haas et al., 2008). Nevertheless, if such compensation appears operational under the baseline conditions, it can be vulnerable during crucial situations and as such, deficient phenotypes may appear when facing behavioral challenges as shown by our results. Moreover, we found that qualitative impairment on the cortical EEG occurs in spite of any possible compensation.

In the future, much remains to be investigated in order to assess how the brain maintains itself awake in spite of deficiencies. Within the cholinergic system, it remains to be determined whether the upregulation takes place at the levels of genes, receptors and/or Ach release and synthesis. A differential gene expression of muscarinic receptors seems unlikely since quantitative real time PCR of M1 to M5 genes did not reveal any detectable difference in H1−/−mice. It would also be important to combine the pharmacological approach with other ones in order to confirm and extend the present findings, such as to find out whether further systems (orexins, 5-HT …) contribute to the compensatory mechanisms. The understanding of such fundamental neurophysiological mechanisms is also of clinical relevance in order to provide strategy for improving vigilance/alertness and sleep-wake control in chronic cerebral diseases, e.g., neurodegenerative and autoimmune ones.

In conclusion, long-term abolition of histamine impairs cortical EEG, affects sleep-wake qualities and causes sleepiness facing behavioral challenges. Such roles are largely mediated by the H1-receptor. An upregulated cholinergic system may account for a quasi-normal daily amount of wakefulness in HDC−/− and H1−/− mice and likely constitutes a major compensatory mechanism when the brain is faced with the deficiency of an activating system. Understanding how the brain remains awake in deficient situations is not only of physiological but also clinical significance.

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