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Efecto del trasplante de células orexinérgicas en un modelo murino de narcolepsia

T E S I S

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PRESENTA

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Dedico esta tesis a mi familia:

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Abreviaturas

5-HT	Serotonina
Ach	Acetilcolina
ARAS	inglés para sistema reticular activador ascendente (ascending reticular activating system)
BF	Prosencéfalo basal
CAG-EGFP	Ratón transgénico que expresa la proteina verde fluorescente mejorada bajo control del promotor CAG
CSV	Ciclo sueño-vigilia
DR	Núcleo dorsal del rafe
EEG	Electroencefalograma
EMG	Electromiograma
EOG	Electrooculograma
ESD	Excesiva somnolencia diurna
HL	Hipotálamo lateral
ICSD-3	Clasificación Internacional de Trastornos del Sueño, 3era edición
LC	Núcleo locus coeruleus
LDT	Núcleo laterodorsal tegmental
LPT	Núcleo lateral pontino tegmental
MCH	Hormona concentradora de melanina
MnPO	Núcleo medial preóptico
N1	Fase uno de sueño nREM
N2	Fase dos de sueño nREM
N3	Fase tres de sueño nREM
NA	Noradrenalina
nREM	no REM
NT1	Narcolepsia tipo 1
NT2	Narcolepsia tipo 2
orexina/EGFP	Ratón transgénico que expresa la proteína verde flourescente mejorada bajo control del promotor de orexina
OX1R	Receptor de orexina 1
OX2R	Receptor de orexina 2
OXA	Orexina A
OXB	Orexina B
PB	Núcleo parabraquial
PC	Área pre-coeruleus
POA	Área preóptica
PPT	Núcleo pedunculopontino tegmental
REM	inglés para movimientos oculares rápidos (rapid eye movements)

SCN	Núcleo Supraquiasmático
SLD	Núcleo sublaterodorsal
SOL	Sueño de ondas lentas
TMN	Núcleo tuberomamilar
vlPAG	Núcleo ventrolateral de la sustancia gris periaqueductal
VLPO	Núcleo ventrolateral preóptico
vPAG	Núcleo ventral de la sustancia gris periaqueductal
VTA	Área tegmental ventral

Resumen

La narcolepsia es un trastorno del sueño cuya manifestación se asocia a la degeneración de somas orexinérgicos que se encuentran en el hipotálamo lateral. La falta de estimulación orexinérgica ocasiona síntomas incapacitantes como la excesiva somnolencia diurna y la cataplejía. El manejo de la enfermedad se enfoca en atender los síntomas y esto puede lograrse con un régimen que combina fármacos y cambios en el estilo de vida del paciente. Aun cuando de este modo se puede mejorar la calidad de vida, es un enfoque sintomático que debe observarse crónicamente. Los trasplantes celulares son una alternativa que podrían proveer de alivio duradero y hay evidencia de su éxito en el contexto de otras enfermedades neurodegenerativas, principalmente la enfermedad de Parkinson. En este sentido, proponemos que trasplantes de células orexinérgicas pueden revertir el fenotipo narcoléptico en animales orexina/ataxina-3. Para ello, utilizando un citómetro de flujo, aislamos células de animales que expresan la proteína verde fluorescente mejorada (EGFP) para llevar a cabo trasplantes en núcleos de la formación reticular (pedunculopontino tegmental y laterodorsal tegmental) de animales orexina/ataxina-3. Tras realizar la evaluación 8 días después de trasplantar, nuestros resultados indican que los pequeños trasplantes orexinérgicos realizados son capaces de reducir la severidad de los eventos catapléjicos en términos de reducciones medianas de 30.31 % en duración de episodio, 51.35% para el número de eventos y 69.73 % en el tiempo transcurrido en el estado de evento catapléjico; también se observó que hubo una menor fragmentación del sueño, al evaluarse el número de episodios de vigilia, sueño no REM y sueño REM. Sorpresivamente, el trasplante control compuesto de tejido cerebelar, también disminuyó la severidad de los eventos catapléjicos, aunque modestamente. El estudio de trasplantes en el contexto de la narcolepsia está aún en estadios preliminares, sin embargo nuestra evidencia indica que son capaces de mejorar el cuadro narcoléptico.

Abstract

Narcolepsy is a sleep disorder caused by the loss of orexinergic cells from the lateral hypothalamic area. Lack of orexinergic stimulation leads to dysregulated sleep where excessive daytime sleepiness and cataplexy attacks emerge as two of the most incapacitating symptoms. Patients can achieve disease management with the aid of pharmacotherapy and behavioral changes, nonetheless this approach remains symptomatic and must be observed chronically. Orexin cell replacement therapy, on the other hand, could offer long-term relief as research suggests that stimulation of the orexinergic system can improve narcoleptic symptoms, and the study of similar approaches for other neurodegenerative diseases, such as in the case of Parkinson's disease, has already produced positive results. Therefore, we propose that orexin rich grafts can revert the narcoleptic phenotype of the orexin/ataxin-3 mouse model of narcolepsy. For this purpose, we performed cell grafts into nuclei of the reticular formation (pedunculopontine and laterodorsal tegmental nuclei) of orexin/ataxin-3 mice. Our results indicate that even small grafts can reduce the severity of some aspects of the narcoleptic phenotype. In this regard, we observed median reductions of 30.31% in episode duration, 51.35% for number of events and 69.73% in time spent in the behavioral arrest state 8 days after grafting; additionally, sleep fragmentation, measured as number of bouts per behavioral state (wake, non-REM sleep and REM sleep), was also reduced. Surprisingly, we observed that control grafts (composed of cerebellar tissue) also improved behavioral arrests, modestly reducing their severity. Although still at very early stages, our results indicate that orexinergic grafts have the potential of improving aspects of the narcoleptic phenotype.

El ciclo sueño-vigilia

El sueño

El vocablo sueño viene del latín *somnus,* en referencia al dios romano del sueño, y se entiende como el acto y deseo de dormir. De manera formal esta conducta es difícil de definir porque existe una gran variabilidad en su manifestación, tanto entre las diferentes especies como a lo largo de su ciclo vital. Sin embargo, para los mamíferos terrestres, existe cierto consenso de que dormir es un estado de reducida responsividad a la estimulación ambiental, que tiende a ocurrir de manera regular, es rápidamente reversible y se da con independencia de la disponibilidad de alimento, agua y condiciones ambientales; a diferencia, por ejemplo, de la hibernación (Allada & Siegel, 2008; Krystal et al., 2013).

El sueño es un estado en el cual los mamíferos pasan aproximadamente 1/3 de su vida, y aunque aún no hay respuesta satisfactoria a la pregunta ¿por qué dormimos?, tras muchos años y estudios, se ha logrado responder parcialmente a la pregunta ¿cómo dormimos?

Borbély y el modelo de dos procesos de la regulación del sueño

Hace más de treinta años, Borbély y colaboradores propusieron el modelo de dos procesos de la regulación del sueño que predice la conducta de dormir (Borbély, 1982; Borbély et al., 2016). De acuerdo con este modelo el sueño (duración, profundidad y horario) depende de la interacción de dos procesos independientes: uno homeostático (proceso S) y otro circadiano (proceso C).

El proceso S hace alusión al impulso por dormir, un fenómeno proporcional al tiempo que el individuo pasa despierto. En términos cotidianos esto quiere decir que pasar tiempo despierto incrementa la necesidad de dormir, y de manera inversa, dormir la disminuye. Se estima que esta

relación está mediada por la acumulación de sustancias hipnogénicas como, por ejemplo, la adenosina, el óxido de nitrógeno, la prostaglandina D2 y algunas citosinas (Brown et al., 2012).

El proceso C determina la hora de dormir, está regulado por la cantidad de luz ambiental y bajo control del núcleo supraquiasmático (SCN). Esto implica, que conforme la luz del día se desvanece, se activan procesos fisiológicos que resultan permisivos para la ocurrencia del sueño, algunos de estos son: la producción de melatonina y una reducción en la temperatura corporal (Murphy & Campbell, 1997; Reiter, 1986).

El modelo sostiene que mientras el proceso S aumenta con la vigilia, el proceso C disminuye con la luz diurna, y cuando ambos procesos se encuentran lo más alejados uno del otro, se abre una ventana de oportunidad ideal para la ocurrencia del sueño (Figura 1).

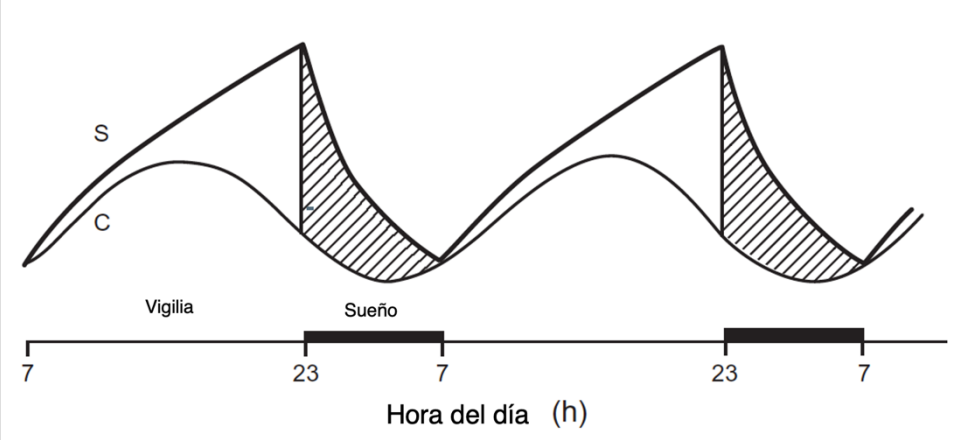


Figura 1 Modelo de dos procesos de la regulación del sueño. Este modelo fue propuesto por Borbély y propone que la ocurrencia del sueño está determinada por la interacción entre el proceso S y el proceso C. El proceso S es homeostático, y describe la acumulación de sustancias hipnogénicas que sucede durante la vigilia y que incrementa la necesidad de dormir. El C es un proceso circadiano dependiente de la luz del sol y regulado por el núcleo supraquiasmático (SCN). Conforme la luz se agota, se desencadenan procesos fisiológicos que preparan al organismo para dormir. Cuando los procesos C y S se encuentran en el punto opuesto, hay una ventana donde la ocurrencia de sueño tiene su máxima probabilidad (zona sombreada por líneas diagonales). Tomado de Cosgrave et al., 2020.

Arquitectura del sueño

En 1924 Hans Berger catalizó la ciencia del sueño con el desarrollo del electroencefalograma (EEG), herramienta que permitió por primera vez analizar la actividad eléctrica de células nerviosas y documentar las diferencias entre los estados de alerta y reposo (Kaplan, 2011). De este modo el sueño comenzó a ser reconocido como un fenómeno activo y altamente organizado.

El ciclo sueño-vigilia (CSV) es un ritmo circadiano donde ocurren tres estados conductuales: vigilia, sueño REM (por las siglas en inglés para movimientos oculares rápidos) y sueño no REM (nREM). Cada uno de estos estados se puede describir a partir de la información recabada mediante registros electrofisiológicos; técnica que consiste en evaluar y contrastar datos obtenidos simultáneamente por el EEG, el electromiograma (EMG) y, en ocasiones, el electrooculograma (EOG).

La vigilia se distingue por presentar un tono muscular alto y trazos desincronizados en el EEG (baja amplitud y alta frecuencia). Los ritmos que se pueden observar son conocidos como ritmos alfa (8-14 Hz), beta (15-30 Hz) y gamma (30-120 Hz). El ritmo alfa es conocido también como la onda Berger y es asociada a un estado de vigilia tranquila.

El sueño nREM se caracteriza por una disminución en el tono muscular y los trazos que se observan en el EEG son amplios y de baja frecuencia. Existen 3 fases de sueño nREM llamadas N1, N2 y N3, cada uno correspondiente a un estado más profundo de sueño. N1 es considerada una fase de somnolencia donde ocurre la transición entre vigilia y sueño. N2 es un sueño ligero, donde es común observar husos de sueño y complejos K. La fase N3, asociada al sueño profundo, es conocida como sueño de ondas lentas (SOL) por los ritmos delta (1-4 Hz) que la acompañan.

El último estado, el sueño REM, presenta actividad desincronizada similar a la de la vigilia y ausencia de tono muscular (atonía muscular de sueño REM), características por las cuales también se le conoce como sueño paradójico. Otras características de la fase son: la presencia de movimientos

oculares rápidos, irregularidad en la respiración y frecuencia cardiaca, y la ocurrencia de ensoñaciones. (Arrigoni & Fuller, 2019; Brown et al., 2012; Equihua-Benítez et al., 2017). En la Figura 2 se muestran los trazos de EEG que caracterizan a las diferentes fases del CSV.

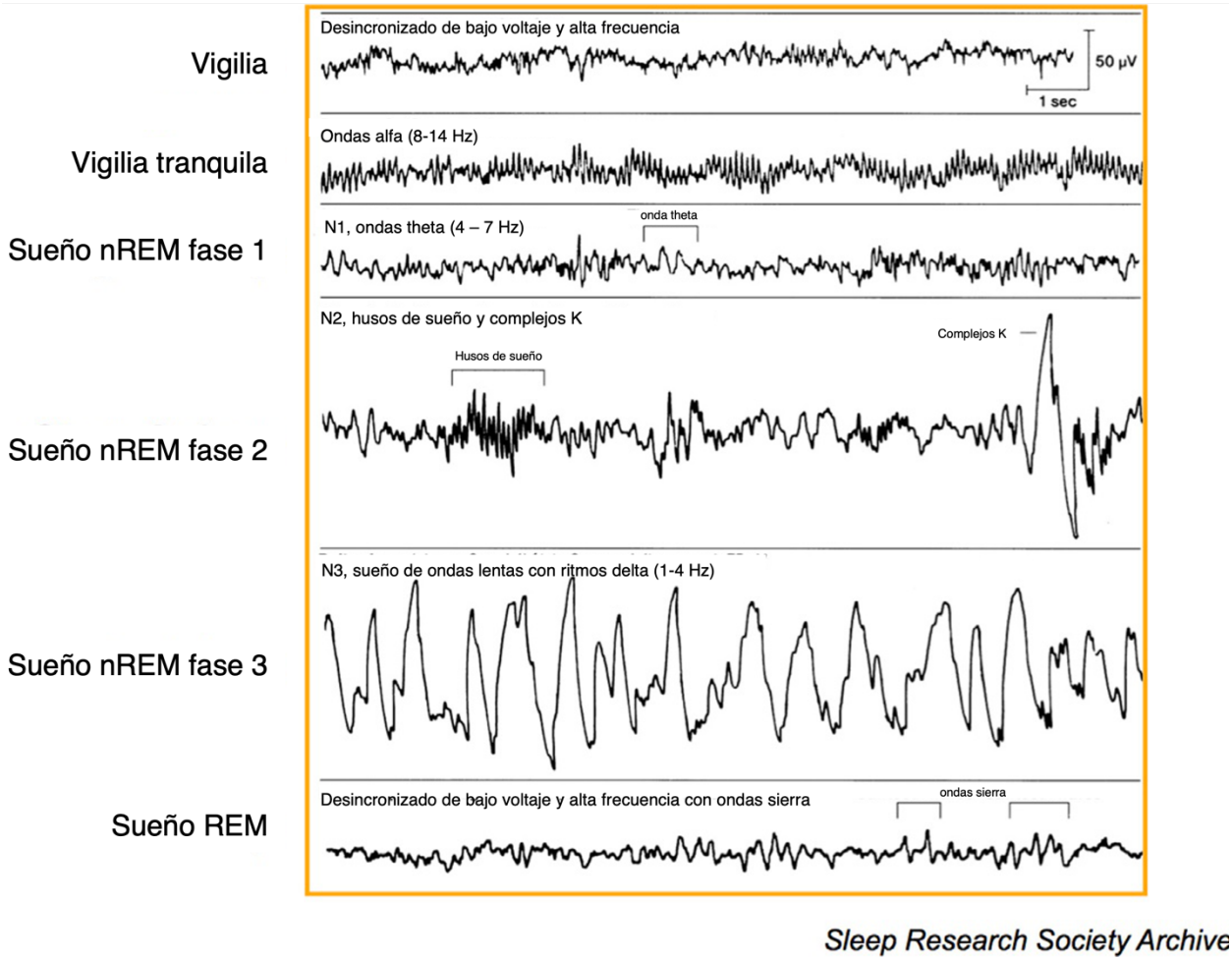


Figura 2 Trazos representativos de actividad eléctrica cortical para cada etapa del ciclo sueño-vigilia. La vigilia se caracteriza por actividad desincronizada de alta frecuencia y baja amplitud. La vigilia tranquila tiene además ritmos alfa del rango 8-14 Hz. El sueño nREM está dividido en fases N1, N2 y N3. Durante la fase N1 el EEG se enlentece y aparecen las ondas theta (4-7 Hz). La fase N2 se caracteriza por la aparición de husos de sueño (ráfagas de 11-16 Hz) y complejos k (una onda bien definida que posee un componente negativo seguido de uno positivo). Durante la fase N3 surgen ondas lentas de gran amplitud conocidas como ondas delta (1-4 Hz). El sueño REM es la última fase en aparecer, y en esta etapa el EEG muestra una actividad de alta frecuencia y baja amplitud que se asemeja a la de la etapa N1, con episodios de movimientos oculares rápidos. Tomado de Arrigoni & Fuller, 2019.

Como puede observarse en la Figura 3, un hipnograma hipotético, en condiciones normales, las fases de sueño nREM (N1, N2, N3) y REM se alternan unas a otras de manera ordenada y secuencial, es decir que todas las fases se suceden predeciblemente formando ciclos que duran alrededor de 100 min. Sabemos, también, que a lo largo de una noche ocurren entre 4 y 6 ciclos de estos ciclos, en los cuales es posible observar que la duración de los episodios de sueño SOL y REM no es constante; mientras que en los primeros ciclos los episodios de SOL son más largos, durante los últimos hay actividad REM de manera predominante.

El sueño REM es normalmente la última fase en aparecer en cada ciclo, y el primer episodio sucede en promedio 90 min después de que la persona comienza a dormir, a esto se le conoce como latencia de sueño REM.

La integridad de la arquitectura del sueño es un indicador tan valioso que su evaluación se utiliza en la clínica para diagnosticar trastornos del sueño tales como la apnea del sueño y la narcolepsia.

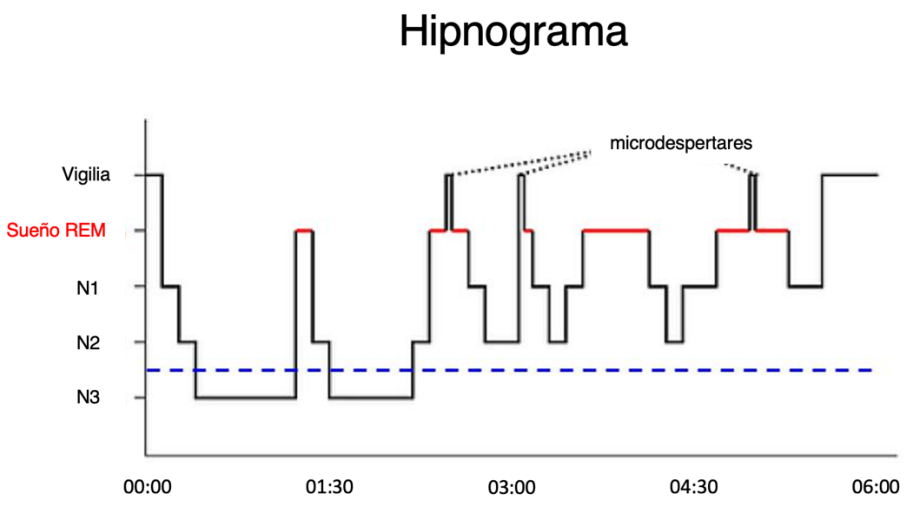


Figura 3 Hipnograma hipotético que ilustra la arquitectura normal del sueño. En rojo se marca el sueño REM, también conocido como sueño paradójico. La línea punteada en azul indica la región donde ocurre el sueño profundo, o sueño de ondas lentas. Los ciclos de sueño se conforman por una serie ordenada de transiciones entre las fases de sueño nREM (N1, N2 y N3) y REM. Esto sucede en promedio entre 4 y 6 veces en una noche. Los primeros ciclos poseen mayor tiempo de sueño nREM y los últimos ciclos presentan mayor duración de sueño REM. Imagen tomada y adaptada de https://www.sleepresolutions.com/blog/what-is-sleep-architecture-stages-and-cycles.

Bases neurofisiológicas del control del sueño y la vigilia

De acuerdo con lo descrito, queda claro que el sueño es un evento complejo y altamente organizado. La sucesión de fases descrita no ocurre de manera azarosa, lo que nos deja entrever que es una conducta promovida de manera activa. Determinar los núcleos involucrados y sus roles en el mantenimiento del estado de sueño y de la alternancia de fases no ha sido tarea sencilla, aunque cada vez tenemos un panorama más completo.

Contribuciones del Barón Von Economo

El neurólogo rumano Constantin von Economo es famoso, entre otras cosas, por los trabajos que realizó durante la epidemia de encefalitis letárgica (1917 a 1928), una enfermedad cuya manifestación clínica incluye fiebre alta, delirio, problemas de visión, y alteraciones del sueño. El Dr. von Economo fue el primero en clasificar las tres variantes de la enfermedad: somnolienta, hyperkinética, y amiostática-akinética (Dickman, 2001). La variante somnolienta fue la más común, aquejando a los pacientes con excesiva somnolencia, lo que ocasionaba que pudieran dormir desde varias horas hasta meses; el sueño los alcanzaba sentados, caminando o realizando otras actividades, y en muchas ocasiones no estaban conscientes de lo ocurrido. En otro grupo de pacientes sucedía lo contrario, les era muy difícil dormir, se sentían inquietos, presentaban movimientos coreicos, convulsiones e insomnio (Lavie, 1993).

Los estudios clínicos y *post-mortem* realizados por von Economo, le permitieron relacionar estos cuadros con lesiones restringidas a la zona del diencéfalo (Figura 4). Los pacientes que presentaban excesiva somnolencia poseían lesiones en la parte posterior del hipotálamo y rostral del mesencéfalo; mientras que pacientes que reportaban gran dificultad para dormir, poseían lesiones localizadas en el área preóptica (POA, por sus siglas en inglés) y prosencéfalo basal (BF, por sus siglas en inglés) (Saper et al., 2001). Estas observaciones permitieron a von Economo especular sobre la

existencia de un centro del sueño con neuronas promotoras del sueño en el POA y BF, y neuronas promotoras de la vigilia en el hipotálamo posterior (Triarhou, 2006).

Eventualmente estudios realizados en modelos animales replicaron las lesiones estudiadas por von Economo, confirmando los hallazgos descritos y dejando entrever el mecanismo regulatorio subyacente.

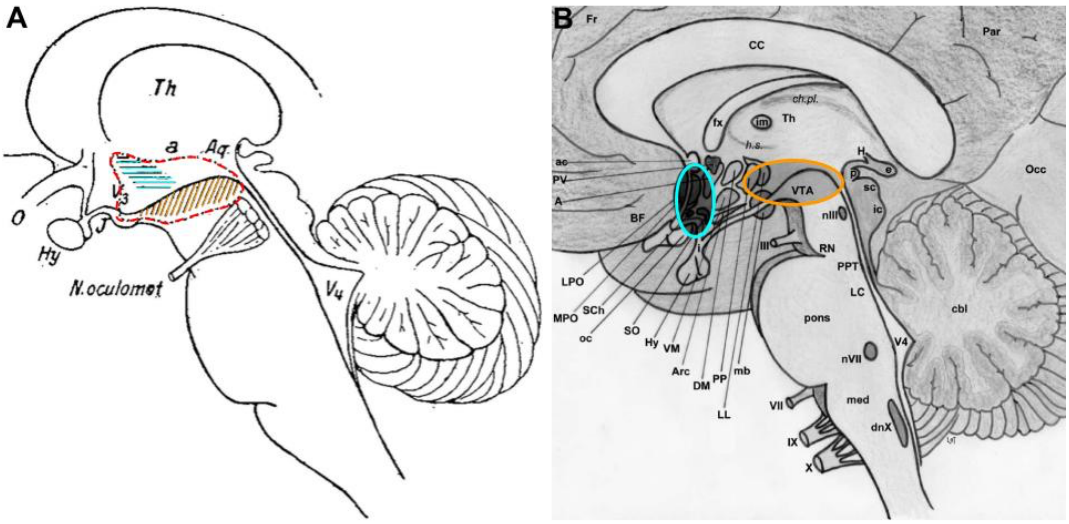


Figura 4 Centros del control del sueño de acuerdo con von Economo. A) Esquema realizado por von Economo ilustrado la región diencefálica y mesencefálica (línea punteada roja) donde sugería se encontraban los núcleos reguladores del sueño. B) Regiones de von Economo indicadas en un esquema con anatomía más reciente. En ambos cuadros, la zona promotora del sueño está indicada en azul y la promotora de la vigilia en naranja. A) Abreviaturas: Aq, acueducto; Hy, hypofisis; J, infundíbulo; O, quiasma óptico; T tálamo óptico; V3, tercer ventrículo; V4, cuarto ventrículo. Abreviaturas B) CC, cuerpo calloso; Fr, Par, Occ, lóbulos frontal, parietal y occipital; cbl, cerebelo; fx, fornix; Th, tálamo; im, masa intermedia del tálamo; ch. pl., plexos coroideos del tercer ventrículo; h.s., surco hipotalámico; H, habénula; e, epífisis (pineal); ac, p, comisura anterior y posterior; oc, quiasma óptico; Hy, hipófisis (pituitaria); i, infundíbulo; mb, cuerpos mamilares; sc, ic, cunículo superior e inferior; III, VII, IX and X, pares craniales (antecedido por n cuando representa su núcleo); dnX, núcleo dorsal of X; BF, prosencéfalo basal; VTA, área tegmental ventral; RN, núcleos del rafe; PPT, núcleo pedunculopontino tegmental; LC, *locus coeruleus*; med, *medulla oblongata*; V4, cuarto ventrículo. Núcleos hipotalámicos: PV, paraventricular; A, anterior; LPO, lateral preóptico; MPO, medial preóptico; SCh, supraquiasmático; SO, supraóptico; VM, ventromedial; Arc, arcuato; DM, dorsomedial; PP, área hipotalámica /nivel posterior; LL, área hipotalámica lateral/nivel lateral. Modificado de Triarhou, 2006.

Modelo 'flip -flop´

Fueron Saper y colaboradores quienes propusieron el modelo 'flip-flop' del control del sueño. Este modelo propone la existencia de un circuito entre los núcleos promotores de la vigilia y promotores del sueño que se inhiben de manera recíproca (Figura 5). De acuerdo a esto, la fase del CSV en la que se encuentra el sujeto corresponde a la de los núcleos cuya actividad predomina sobre los demás (Lu et al., 2006; Saper, 2013; Saper et al., 2001). Hoy en día se reconoce que existen dos interruptores complementarios funcionando de esta manera: uno que regula las transiciones entre vigilia y sueño nREM, y otro que coordina la alternancia de fases entre sueño nREM y sueño REM.

Generación del sueño: transición de vigilia a sueño nREM

La vigilia se caracteriza por ser un estado de alerta y conciencia sobre el entorno, que se cree es mantenida por la actividad de un grupo de núcleos promotores de la vigilia que juntos integran el denominado sistema reticular activador ascendente (SRAA, o ARAS por sus siglas en inglés). El ARAS tiene su origen en la formación reticular y activa la corteza cerebral por dos vías: la dorsal y la ventral (Figura 5 A), (Moruzzi & Magoun, 1949). La vía dorsal del ARAS tiene su origen en los núcleos colinérgicos pedunculopontino tegmental (PPT) y laterodorsal tegmental (LDT por sus siglas en inglés), referidos en conjunto como región peribraquial. La vía dorsal asciende por el tálamo, en particular por los núcleos intralaminar y medial (Yeo et al., 2013), y alcanza la corteza ejerciendo su actividad estimulante (Fairén, 2007; Hur & Zaborszky, 2005).

La vía ventral, comienza a nivel del tallo cerebral y consiste en los núcleos parabraquial (PB), locus coeruleus (LC), dorsales de rafe (DR) y ventral de la sustancia gris periaqueductal (vPAG por sus siglas en inglés); proyecta hacia los núcleos tuberomamilar (TMN por sus siglas en inglés), hipotálamo lateral (HL) y el BF para llegar a la corteza (Jones, 2003).

Además de poseer la capacidad de activar la corteza, los núcleos que conforman el ARAS inhiben a los núcleos promotores del sueño por medio de la liberación de noradrenalina (NA), y acetilcolina (ACh), y en menor grado, serotonina (5-HT) (Chou et al., 2002; Gallopin et al., 2000).

Se considera que importantes núcleos promotores del sueño se encuentran en el POA, donde destacan el núcleo ventrolateral (VLPO por sus siglas en inglés) y medial (MnPO por sus siglas en inglés) (Figura 5 B). La máxima actividad de estos núcleos sucede durante el sueño, periodo durante el cual inhiben la vigilia mediante la liberación de GABA y galanina (Saper et al., 2001; Sherin et al., 1998; Steininger et al., 2001).

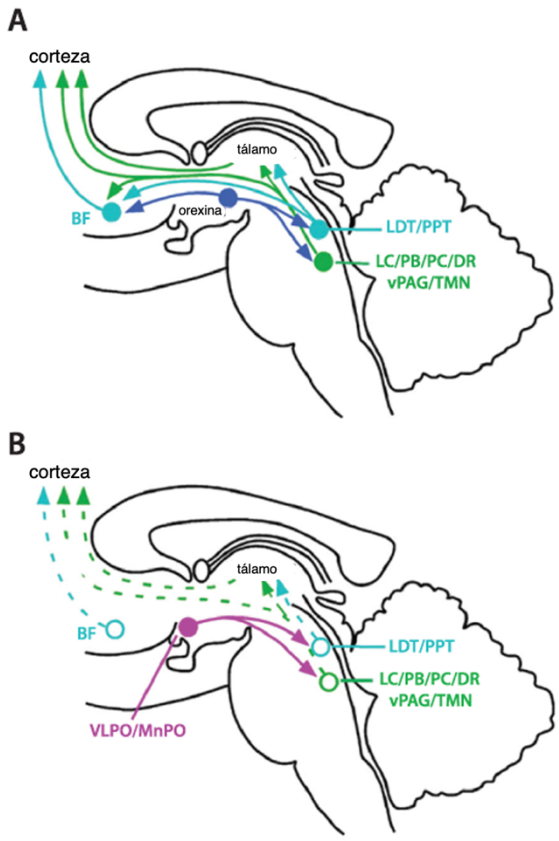


Figura 5 Esquemas ilustrativos para el sistema promotor de la vigilia (A), y el sistema promotor del sueño (B).

A) El sistema reticular activador ascendente (SRAA, ARAS por sus siglas en inglés) posee dos vías, la dorsal (verde) y la ventral (turquesa). La vía dorsal comienza en los núcleos colinérgicos pedúnculopontino tegmental (PPT) y laterodorsal tegmental (LDT), asciende por el tálamo y activa la corteza. La vía ventral incluye núcleos monoaminérgicos como el *locus coeruleus* (LC), dorsales del rafe (DR), ventral de la sustancia gris periaqueductal (vPAG), y tuberomamilar (TMN), que producen noradrenalina, serotonina, dopamina e histamina respectivamente, y los núcleos parabraquial (PB) y precoeruleus (PC) productores de glutamato. Alcanza el prosencéfalo basal (BF) y activa la corteza. Además, neuronas orexinérgicas del hipotálamo lateral inervan todos estos núcleos promoviendo su actividad y ayudando a mantener el estado de vigilia.

B) En el área preóptica se encuentran los núcleos promotores del sueño (magenta), los principales son el ventrolateral (VLPO) y medial (MnPO) cuya actividad inhibe a los núcleos del ARAS.

Estas condiciones dan lugar a un interruptor 'flip-flop' que permite transiciones rápidas y completas entre estados. Tomado y adaptado de Saper, 2013.

Adicional a los núcleos promotores de la vigilia y del sueño, existe estimulación cuyo propósito es el de dotar de mayor estabilidad al sistema 'flip-flop', tal es el caso de las células orexinérgicas y las productoras de la hormona concentradora de melanina (MCH por sus siglas en inglés) del HL. Estos grupos de células inervan de manera extensa a los núcleos involucrados en la regulación del ciclo y poseen periodos de actividad opuestos. Mientras las células orexinérgicas están activas durante la vigilia (Lee et al., 2005), las MCHérgicas se activan durante el sueño REM (Verret et al., 2003). Estas células funcionan como estabilizadoras del interruptor, y previenen la ocurrencia de cambios de fase en momentos inadecuados.

Transiciones del sueño: nREM a REM

Una vez que sucede el cambio de estado, el individuo está dormido. Y como se ha ilustrado, el sueño no es homogéneo, sino que está compuesto por sueño nREM y REM que se alternan de manera regular (Figura 6).

Desde 1962, Jouvet realizó una serie de estudios de transección con los cuales logró determinar que el tallo cerebral es necesario y suficiente para la generación del sueño REM (Jouvet, 1962). Dentro de esta zona, se lograron discernir al núcleo sublaterodorsal (SLD) y el area pre-coeruleus (PC) como promotores del sueño REM (REM-on), y a los núcleos ventrolateral de la sustancia gris periaqueductal (vlPAG) y lateral pontino tegmental (LPT) como supresores (REM-off) (Lu et al., 2006). Estos núcleos se inhiben mutuamente por transmisión GABAérgica, formando un segundo interruptor 'flip-flop' que se mantiene estable debido a la estimulación excitatoria e inhibitoria que reciben los núcleos REM-off de núcleos adicionales.

La ocurrencia de episodios REM está facilitada por la inhibición de estos núcleos REM-off (vlPAG/LPT) por parte de células del LDT/PPT y VLPO e impedida por la actividad excitatoria de las

células orexinérgicas del HL y monoaminérgica de los núcleos LC y DR (Clement et al., 2012; Saper, 2013).

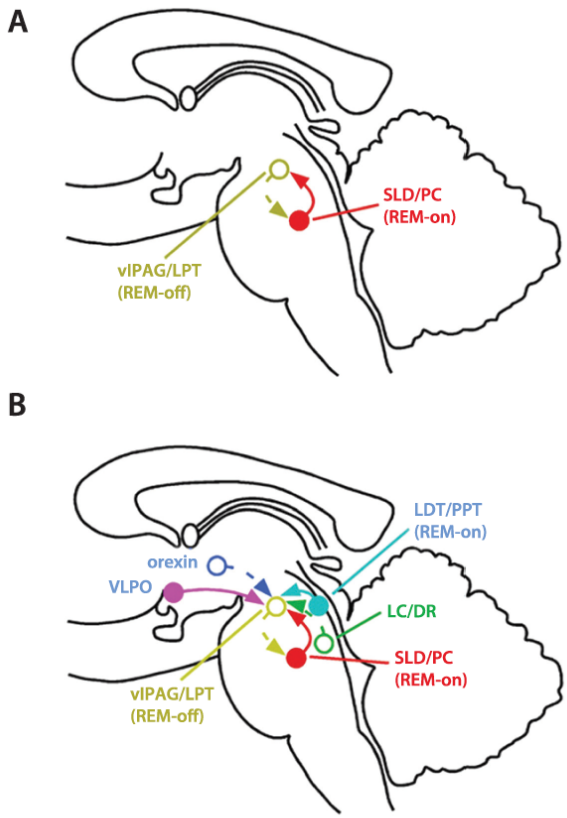


Figura 6 Regulación de las transiciones entre sueño nREM y REM.

A) Núcleos del tallo cerebral son el centro de un interruptor 'flip-flop' que regula las transiciones entre sueños nREM y REM. Tanto el núcleo sublaterodoral y pre-coeruleus (SLD/PC) son conocidos como núcleos REM-on debido a que su actividad promueve el sueño REM. Mientras que los núcleos ventrolateral de la sustancia gris periaqueductal (vlPAG) y lateral pontino tegmental (LPT) inhiben la ocurrencia del sueño REM y son por ello llamados núcleos REM-off.

B) Los núcleos REM-off son a su vez regulados por núcleos externos que, dependiendo de su actividad, promueven o inhiben la ocurrencia de episodios REM. En este sentido, la actividad excitatoria proveniente del *locus coeruleus* (LC), dorsales del rafe (DR) y neuronas orexinérgicas dificulta la aparición de REM; mientras que actividad inhibitoria del núcleo ventrolateral preóptico (VLPO) y colinérgica de los núcleos laterodorsal pontino (LDT) y pedunculopontino tegmental (PPT) la facilitan.

Figura modificada de Saper, 2013.

Trastornos del sueño

En condiciones normales, el sueño es un estado altamente regulado y organizado. Sin embargo, debido a diferentes circunstancias, que van desde hábitos hasta lesión y enfermedad, es posible que se pierda la integridad del sistema, dando lugar a un sueño deficiente o alterado.

Cuando la queja principal del paciente es fatiga durante el día, y esta no puede ser mejor explicada por un factor ambiental u otra enfermedad, se puede sospechar de un trastorno de hipersomnolencia. El trastorno de hipersomnolencia más común es la narcolepsia, una enfermedad asociada a la pérdida de neuronas orexinérgicas (Thannickal et al., 2000).

La narcolepsia

La narcolepsia es una enfermedad del sueño cuyos primeros reportes datan del siglo XIX con la descripción realizada por el médico Jean Baptiste Édouard Gélineau, quien acuñó el término utilizando las raíces griegas para somnolencia y ataque: νάρκης λῆψις (narkes lepsis) (De la Herrán-Arita, Guerra-Crespo, et al., 2011). Desde los primeros reportes que existen sobre la narcolepsia, se ha podido entrever lo incapacitante de la enfermedad. Por ejemplo, Gélineau describió la situación de un paciente de 38 años incapaz de sostener una conversación por más de 30 min sin quedarse dormido, lo que podía ocurrirle hasta 200 veces al día. Debido a esto, le era indispensable la constante compañía de su hijo, encargado de despertarlo cada vez que fuera necesario para que pudiera fungir en su vida cotidiana (Schenck et al., 2007).

Estudios epidemiológicos estiman que la narcolepsia es una enfermedad poco frecuente afectando aproximadamente a 1 de cada 2,000 individuos en EUA (Sakurai, 2013). No obstante, se ha determinado que tiene un impacto socioeconómico significativo cuyos estragos comienzan hasta una década antes del diagnóstico. Las pérdidas económicas son cuantificables en el ámbito personal, ya que los pacientes narcolépticos y sus cónyuges suelen percibir ingresos menores y tienen menor rendimiento laboral, y a nivel social debido a que demandan mayores recursos de los servicios de salud públicos (Jennum et al., 2009, 2012).

Patogénesis y etiología

En 1999 fue puesto de manifiesto una relación entre la degeneración del sistema orexinérgico y la narcolepsia al describirse la mutación del receptor 2 de orexina que ocasiona narcolepsia en perros (Lin et al., 1999). Además, en humanos se observaron niveles casi indetectables de orexina en

líquido cefalorraquídeo y una menor cantidad de células orexinérgicas en el hipotálamo posterior de pacientes narcolépticos (Thannickal et al., 2000).

La causa de la degeneración específica de neuronas orexinérgicas en el humano no ha sido determinada aún. Una de las teorías más estudiadas involucra mecanismos autoinmunes, hipótesis sustentada por varias observaciones.

De estas, destaca que la narcolepsia se manifieste casi exclusivamente cuando se presenta la proteína DQ0602, codificada por el antígeno humano leucocitario haplotipo DQ (DQB1*06:02 y DQA1*01:02) (Mignot et al., 1997) y que una variante específica en el receptor alfa de las células T incrementa el riesgo de desarrollar narcolepsia (Hallmayer et al., 2009). Además, infecciones estacionarias, como la influenza o por estreptococos, coinciden con un incremento temporal en la incidencia de la enfermedad (Kornum et al., 2011).

Estas observaciones han permitido sugerir que cuando ciertas células T son activadas por infecciones virales sucede degeneración de células orexinérgicas; lo que a su vez deriva en un fenotipo narcoléptico.

Cuadro clínico

La narcolepsia está catalogada en la tercera edición de la clasificación internacional de trastornos del sueño (ICSD-3) como un desorden central de hipersomnolencia. Generalmente la queja principal de los pacientes es la excesiva somnolencia diurna (ESD) con la que cursa la enfermedad, sin embargo, el cuadro es más complejo.

En general, los síntomas de la enfermedad pueden dividirse en dos grupos de fenómenos patológicos relacionados con manifestaciones inapropiadas de sueño durante la vigilia (Hasegawa

et al., 2014). El primer grupo considera la intrusión de fenómenos de sueño REM y comprende parálisis de sueño, alucinaciones hipnagógicas y cataplejía. La parálisis del sueño es la inhabilidad para producir movimientos voluntarios durante las transiciones entre sueño y vigilia. Las alucinaciones hipnagógicas son ensoñaciones vívidas que ocurren durante los momentos de transición entre vigilia y sueño (Nishino, 2007). La cataplejía, es la pérdida temporal y súbita del tono muscular con preservación de la conciencia; su factor desencadenante son emociones fuertes, particularmente las emociones positivas y en menor medida las negativas como el enojo (Krahn et al., 2005).

El segundo grupo de fenómenos se relaciona con la activación inapropiada de sueño nREM y se presenta como ESD con irrupciones abruptas de sueño nREM. La ESD se manifiesta como fatiga durante el día y la severidad puede ser tal que se vuelve difícil para el paciente permanecer largos periodos de tiempo despierto, debido a esto es frecuente que los pacientes experimentan ataques de sueño, donde caen dormidos de manera involuntaria, y micro-sueños, episodios breves de sueño (≤30 s) de los que no son conscientes (Morrison & Riha, 2012).

Existen además otros síntomas menos evidentes que están relacionados con alteraciones en la arquitectura del sueño de los pacientes narcolépticos (Figura 7). Entre estos síntomas se puede observar menor latencia para sueño REM, incremento en el número de microdespertares a lo largo de la noche y fragmentación de la arquitectura del sueño, donde las fases de sueño aparecen de manera desordenada (Sakurai, 2013).

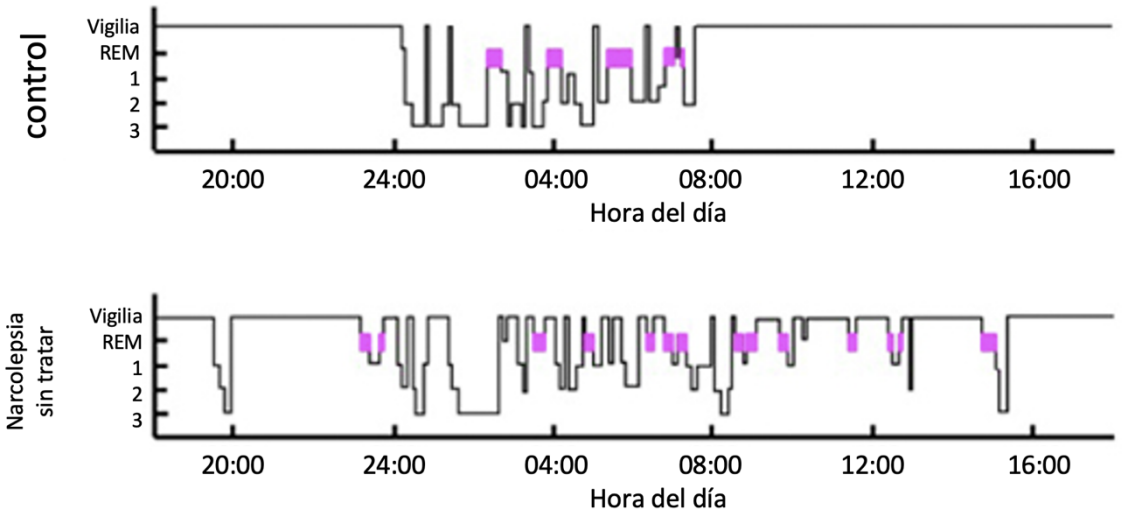


Figura 7 Hipnogramas comparativos de pacientes con y sin narcolepsia. El hipnograma control, pertenece a un individuo saludable, donde se observa el patrón normal del ciclo sueño-vigilia (CSV). Existe un patrón regular de aparición de sueño de movimientos oculares rápidos (REM) y sueño no-REM (fases N1, N2 y N3) que suceden durante un bloque continuo del CSV. Por el contrario, en el hipnograma de narcolepsia sin tratamiento se puede apreciar la fragmentación del sueño con ciclos irregulares y desorganización del sueño REM que ocurre incluso durante el periodo de vigilia. Adaptado de Rogers el al. Sleep 1994; 17:590 y tomado de https://healthysleep.med.harvard.edu/narcolepsy/what-is-narcolepsy/understanding

De acuerdo con el ICSD-3, existen dos tipos de narcolepsia: tipo 1 (NT1) y tipo 2 (NT2). Para clasificar el cuadro como NT1, es indispensable que el paciente presente cataplejía y/o deficiencia de orexina en líquido cefalorraquídeo (<110pg/mL); en ausencia de esto el diagnóstico es NT2 (American Academy of Sleep Medicine, 2014; Sateia, 2014).

El sistema orexinérgico y la narcolepsia

Las orexinas

Desde su primera descripción el sistema orexinérgico ha sido ampliamente estudiado por su papel en el mantenimiento de la vigilia. Por ejemplo, se ha demostrado que la estimulación orexinérgica incrementa el tiempo que diferentes organismos permanecen alertas y activos (Bisetti et al., 2006; De la Herrán-Arita, Zomosa-Signoret, et al., 2011). De este modo, y como vimos previamente, las orexinas juegan un papel de estabilizadoras de la vigilia al facilitar la actividad de los núcleos que integran el ARAS.

El sistema orexinérgico está conformado por una poca cantidad de somas, alrededor de 50,000 en el humano y 3,000 en la rata, que producen dos péptidos excitatorios: la orexina A (OXA) y B (OXB) a partir de un precursor común, la preproorexina. A pesar de la restringida ubicación de los somas orexinérgicos, estos poseen aferencias que inervan de manera amplia al sistema nervioso central, incluidos los núcleos importantes para el control del CSV (Figura 8) (Peyron et al., 1998).

La actividad orexinérgica está mediada por dos receptores acoplados a proteína G, conocidos como OX1R y OX2R que se encuentran diferencialmente distribuidos. El LC posee principalmente OX1R, el TMN OX2R y los núcleos DR, LDT y PPT y área tegmental ventral (VTA por sus siglas en inglés) poseen ambos receptores (Figura 8). Además, mientras el receptor mientras OX2R es selectivo para OXB, OX1R presenta afinidad por OXA y OXB (Sakurai et al., 1998).

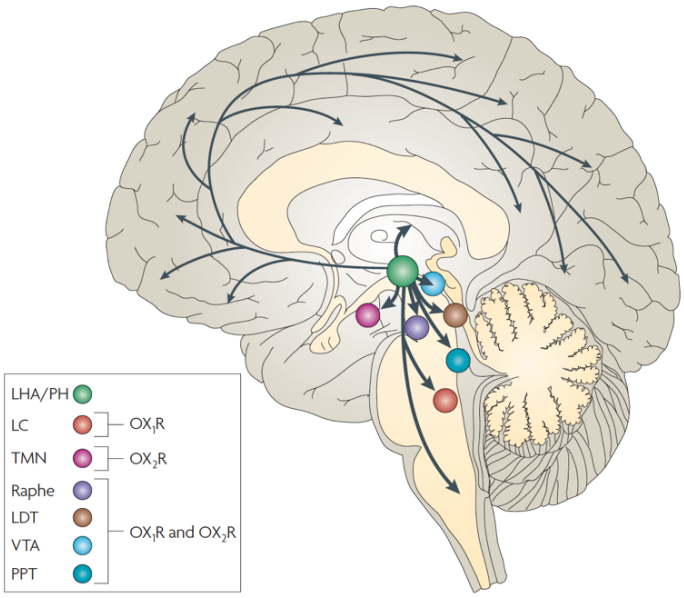


Figura 8 Sistema orexinérgico: inervación y distribución de receptores en núcleos involucrados en el control del ciclo sueño-vigilia. Los somas de neuronas orexinérgicas están restringidos al área hipotalámica (LHA por sus siglas en inglés) desde donde inervan extensamente el sistema nervioso central incluyendo a los centros involucrados en el control del sueño y la vigilia. En estos sitios las orexinas A y B son capaces de ejercer su actividad excitatoria por medio de dos receptores acoplados a proteína G y conocidos como OX1R y OX2R. La expresión de estos receptores difiere entre núcleos, mientras el *locus coeruleus* (LC) expresa OX1R; el núcleo tuberomamilar (TMN) tiene OX2R; y por su parte los núcleos dorsal del rafe (raphe), laterodorsal tegmental (LDT), pedunculopontino tegmental (PPT) y área tegmental ventral (VTA por sus siglas en inglés) poseen ambos receptores. Figura tomada de Sakurai, 2007

El sistema orexinérgico promueve la vigilia

La evidencia de manera consistente ha demostrado que el sistema orexinérgico juega un papel determinante en la consolidación y mantenimiento de la vigilia. Diversos estudios animales han permitido esclarecer los mecanismos subyacentes al manipular partes del sistema de manera selectiva. Por ejemplo, en animales knockout para OX2R se ha observado un incremento en el tiempo que los animales pasan durmiendo (Willie et al., 2003), mientras que animales doble knockout (OX1R y OX2R) expresan un fenotipo que recuerdan a la narcolepsia observada en humanos con fragmentación del sueño y eventos conductuales que asemejan a la cataplejía (Kalogiannis et al., 2011). Un cuadro similar se presenta en animales deficientes para OXA y OXB en el modelo knockout para preproorexina (Chemelli et al., 1999) y en los animales orexina/ataxina-3, un modelo donde hay neurodegeneración orexinérgica de manera posnatal y paulatina (Hara et al., 2001).

El sistema orexinérgico y la narcolepsia

De acuerdo con lo descrito en apartados anteriores, sabemos que alteraciones en el sistema orexinérgico están íntimamente ligadas al desarrollo de la narcolepsia. En este sentido, la aparición de los síntomas narcolépticos posiblemente tienen su origen en la inestabilidad de los interruptores 'flip-flop' ocasionada por una deficiente transmisión orexinérgica (Kantor et al., 2009).

En la Figura 9 se presentan simultáneamente los dos interruptores 'flip-flop' que se han propuesto como reguladores de las transiciones entre sueño y vigilia, y entre sueño nREM y REM, y sus interacciones. De esta manera se puede apreciar como la ausencia de estimulación orexinérgica permite cambios inadecuados de estado que dan lugar a los diferentes síntomas que conforman el cuadro clínico de la narcolepsia.

De entre los diferentes síntomas que son comunes en la narcolepsia, se considera a la cataplejía como patognomónico. La cataplejía es ocasionada por la inhibición de motoneuronas en momentos inapropiados. En condiciones normales, la atonía muscular durante el sueño es deseable debido a que de lo contrario los organismos literalmente recrean los movimientos con los que están soñando, como sucede en el caso de un trastorno del sueño conocido como trastorno del sueño en fase REM (Luppi et al., 2011).

La atonía muscular de sueño REM sucede cuando el SLD libera glutamato en los núcleos ventromediales medulares e interneuronas espinales, quienes a su vez liberan glicina o GABA e inhiben a las motoneuronas (Clément et al., 2011; Hajnik et al., 2000; Krenzer et al., 2011). Generalmente, este circuito no se activa durante la vigilia debido a que la estimulación de los núcleos vlPAG/LPT y neuronas orexinérgicas, inhiben al núcleo SLD (Lu et al., 2006). Sin embargo, en pacientes narcolépticos, la deficiencia orexinérgica permite que esta inhibición motriz se desencadene abruptamente (De la Herrán-Arita et al., 2013) y es común que ocurra en contextos

donde el paciente experimenta fuertes emociones positivas como son la sorpresa y alegría (Krahn et al., 2005; Lammers et al., 2000).

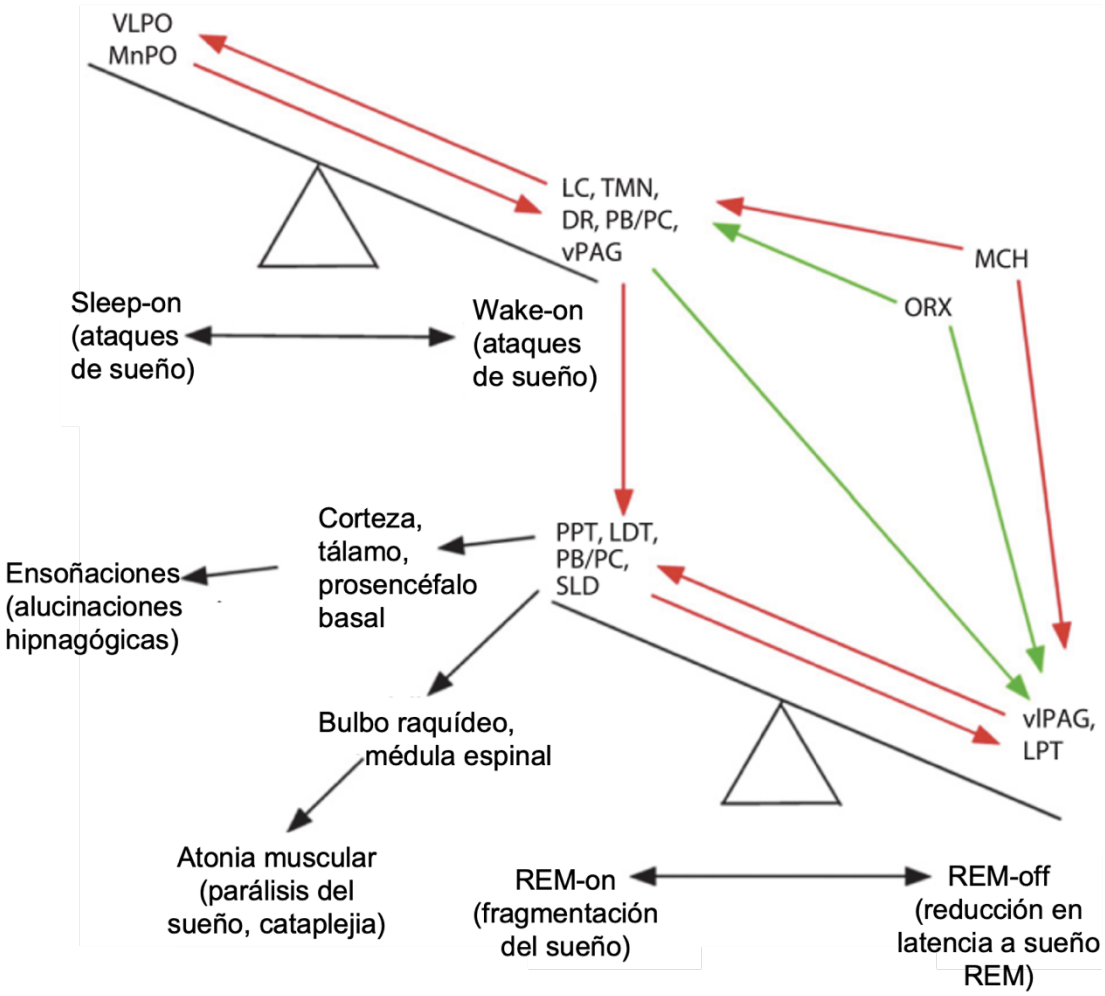


Figura 9 Relación entre la deficiencia orexinérgica y la manifestación de los síntomas de narcolepsia. De acuerdo con la teoría, existen dos interruptores 'flip-flop', uno que regula las transiciones entre la vigilia.y el sueño, y otro para las transiciones entre sueño nREM y REM. En cada interruptor, hay núcleos en oposición formando un circuito de inhibición recíproca, y el estado conductual del individuo está determinado por el grupo de núcleos cuya actividad predomina. En el caso del primer interruptor, existen núcleos promotores del sueño (sleep-on) y núcleos promotores de la vigilia (wake-on). El estado de vigilia se mantiene estable, entre otras cosas, gracias a la estimulación excitatoria de neuronas orexinérgicas (ORX). En el segundo interruptor, hay núcleos promotores del sueño REM (REM-on) e inhibidores del sueño REM (REM-off). En este caso, el sueño REM es inhibido por la actividad ORX. La ausencia de estimulación orexinérgica reduce la estabilidad de los interruptores en sus fases de wake-on y REM-off, permitiendo cambios abruptos de fase a lo largo del ciclo sueño vigilia, y el surgimiento de los síntomas narcolépticos como son los ataques de sueño, fragmentación del sueño, alucinaciones hipnagógicas, parálisis del sueño, cataplejía, y reducción en la latencia de sueño REM. Abreviaturas: *locus coeruleus* LC; tuberomamilar TMN; dorsal del rafe DR; parabraquial/pre-*locus coeruleus* PB/PC; ventral de la sustancia gris periaqueductal vPAG ; ventrolateral preóptico VLPO; medial preóptico MnPO; pedunculopontino tegmental PPT; laterodorsal tegmental LDT; sublaterodorsal SLD; ventrolateral de la sustancia gris periaqueductal vlPAG; y lateral pontino tegmental LPT. Imagen tomada y adaptada de Saper, 2013.

Tratamientos para la narcolepsia

Terapias actuales

Todavía no existe una cura para la narcolepsia y los tratamientos que hay tienen un enfoque sintomático que implican terapia farmacológica y no farmacológica.

Las intervenciones no farmacológicas son intervenciones que ayudan al paciente a ajustar su estilo de vida para disminuir las molestias inherentes al trastorno. Incluyen educación sobre la enfermedad y grupos de apoyo para aprender estrategias de afrontamiento, higiene del sueño, siestas programadas, y el evitar substancias que puedan alterar el CSV o desencadenar o empeorar alguno de los síntomas. La terapia farmacológica es variada y generalmente el fármaco administrado depende del tipo de síntoma que se va a controlar: ESD o cataplejía. (Equihua-Benítez & Drucker-Colín, 2019; Scammell, 2017).

La ESD se puede tratar con modafinilo y estimulantes tipo anfetamina, mientras que la cataplejía responde a antidepresivos tricíclicos e inhibidores selectivos de la recaptura de serotonina y noradrenalina. Hay, además, algunos fármacos capaces de tratar ambos tipos de síntomas: el oxibato sódico y el pitolisant. La terapia farmacológica tiene la desventaja de poseer una amplia gama de efectos secundarios según el fármaco y la respuesta del organismo. En la Tabla 1 se ha hecho un esfuerzo por presentar la información sobre tratamientos aprobados y en el caso de las opciones farmacológicas se enlistan algunos de los efectos secundarios reportados.

No obstante las dificultades, con seguimiento puntual y llevando a cabo los ajustes necesarios para cada caso individual, es posible encontrar un régimen terapéutico que mejore la calidad de vida del paciente de manea sustancial.

Tabla 1 Opciones terapéuticas actuales para el manejo de la narcolepsia

Terapias no-farmacológicas			
Aspecto procurado	Intervención		Descripción del abordaje
Excesiva somnolencia diurna	Siestas programadas		Una o dos siestas cortas y controladas
	Higiene del sueño		Rutinas adecuadas y regulares de sueño
	Evasión de sustancias		Benzodiacepinas, opioides, antipsicóticos, alcohol, teofilina, cafeína
Cataplejía	Evasión de sustancias		Antagonistas de Alpha-1
Día a día	Apoyo psicosocial		Red de soporte, educación sobre la enfermedad y desarrollo de estrategias de afrontamiento
Intervenciones farmacológicas			
Aspecto procurado	Categoría farmacológica	Nombre genérico (mg/day)	Efectos secundarios reportados
Excesiva somnolencia diurna	Estimulantes no anfetamínicos	Modafinilo[a,b] (100-400) Armodafinilo[a] (10-250) Metilfenidato[a,b] (20-40)	Erupción o alergia Reacción eosinofílica y síntomas sistémicos Síntomas psiquiátricos Eventos cardiovasculares
	Estimulantes anfetamínicos	dextroanfetamina[a] (5-60)	Dependencia, abuso y tolerancia Hipertensión y otras condiciones cardiovasculares Síntomas psiquiátricos Vasculopatía periférica Síndrome serotoninérgico
Cataplejía	Antidepresivos	Clomipramina (10-150) Venlafaxina (37.5-150) Atomoxetina (10-60) Fluoxetina (10-40)	Entre otros: Empeoramiento de la depresión e incremento en el riesgo de suicidio Síndrome serotoninérgico Convulsiones Efectos cardiovasculares
Excesiva somnolencia diurna y cataplejía	Agonistas inversos de la histamina	Pitolisant[b] (9-36)	Entre otros: Pensamientos erráticos y cambios conductuales Síndrome de piernas inquietas Aporto espontáneo Cambios en el apetito
	Depresor	Oxibato sódico[a,b] (4.5-9)	Potencial de abuso y mal uso Depresión respiratoria y trastornos respiratorios del sueño Depresión e ideación suicida Síntomas psiquiátricos Parasomnias
	Inhibidores de la monoaminooxidasa	Selegilina (10-40)	Síntomas extrapiramidales Dolor y sensación alterada Síntomas cardiovasculares Desórdenes gastrointestinales
	Estimulantes	Mazindol[c] (1-6)	Anorexia Palpitaciones Apnea Boca seca

a. Aprobado por la Food and Drug Administration (FDA) para el tratamiento de la narcolepsia y síntomas asociados.
b. Aprobado por la European Medicines Agency (EMA) para el tratamiento de narcolepsia y sus síntomas.
c. Mazindol recibió en el 2015 designación de medicamento huérfano para el tratamiento del a narcolepsia por la EMA y en el 2016 por la FDA

Fuentes: Abad & Guilleminault, 2017; Barateau et al., 2016; Scammell, 2017
tabla adaptada de Equihua-Benítez & Drucker-Colín, 2019

Terapias experimentales

El objetivo final de un tratamiento es ser cura de la enfermedad, pero lamentablemente la oferta actual para la narcolepsia no tiene esta propiedad. Además de la terapia empleada de manera tradicional, se están explorando terapias alternativas que incluyen agonistas orexinérgicos, inmunoterapia, terapia genética y trasplantes celulares.

Agonistas orexinérgicos

De acuerdo con la evidencia disponible, la estimulación del sistema orexinérgico tendría un efecto positivo en el cuadro clínico que aqueja a los pacientes narcolépticos. Algunas posibles vías de administración para moléculas estimulantes del sistema orexinérgico son la oral, intravenosa, intratecal, intranasal, etc. Hoy en día también se trabaja con terapias génicas y de trasplante celular cuyo objetivo es que sea el mismo sistema el que produzca la proteína.

En principio, la administración oral de agonistas orexinérgicos sería la más conveniente. Sin embargo, hasta hace muy poco, ninguna de las moléculas que se habían sintetizado era permeable a la barrera hematoencefálica. Sin embargo, actualmente ya se han logrado sintetizar los primeros agonistas orexinérgicos y están siendo estudiados para determinar su seguridad y capacidad de promover y consolidar la vigilia (Tabla 2). Es probable que alguno sea aprobado para el tratamiento de la narcolepsia en algún momento de la siguiente década, sin embargo, sus efectos terapéuticos continuarán siendo sintomáticos.

Tabla 2 Agonistas orexinérgicos como potencial tratamiento para la narcolepsia.

Farmacéutica	Nombre interno de la molécula	Fase clínica, número identificador	Mecanismo de acción	Notas
Orexia Therapeutics (Hertfordshire, UK)	Orexin OX_2	preclínico	Agonista para OX2R	Se trabaja con una fórmula oral y otra intranasal
Takeda (Tokyo, Japan)	TAK-925	1, NCT03332784		Completado desde 2018, sin nuevos estudios clínicos reportados
	TAK-994	2, NCT04096560 y NCT04820842		Bajo estudio para el tratamiento de NT1y NT2
	TAK-861	preclínico		-
Zhang et al., 2021	RTOXA-43	preclínico	Agonista dual para OX1R y OX2R	Descrita por primera vez en 2021

Fuentes: Clinicaltrials.gov, páginas web de las compañías farmacéuticas, y Zhang et al., 2021.
Tabla tomada y adaptada de: Equihua-Benítez & Drucker-Colín, 2019

Inmunoterapia

La hipótesis autoinmune de la narcolepsia sugiere que hay área de oportunidad para la inmunoterapia cuyo objetivo sería detener o desacelerar la degeneración orexinérgica. Actualmente, hay algunos estudios donde se ha explorado el uso de esteroides, inmunoglobulinas y plasmaféresis (Chen et al., 2005; Coelho et al., 2007; Lecendreux et al., 2003). Entre los resultados observados, los prometedores se obtuvieron con la administración intravenosa de inmunoglobulinas, donde se observó una mejoría en los parámetros de sueño y una disminución en el número de eventos catapléjicos (Knudsen et al., 2010). La efectividad de la inmunoterapia está ligada a la detección temprana, por lo que a la par de mejores tratamientos es fundamental mejorar el diagnóstico.

Terapia génica

La terapia génica ofrece la oportunidad de permitir al sistema producir orexina de manera endógena. Entre las ventajas para esta terapia destaca el hecho de que los somas orexinérgicos se

encuentran restringidos a una misma zona, el HL, y es posible que la inserción de genes de orexina en neuronas hospederas de esta zona restablezca la producción de orexina (Sun et al., 2019).

Entre la evidencia disponible, se puede observar que hay resultados preliminares favorables. Por ejemplo en el modelo knockout para orexina, donde hay ausencia de péptido pero el andamiaje del sistema orexinérgico permanece intacto, se realizó la transferencia del virus en la zona diana y se logró detectar orexina en líquido cefalorraquídeo y una disminución en el número de eventos catapléjicos durante el periodo de vida del vector (Liu et al., 2008). Sin embargo, transferencias hechas en este mismo sitio pero dirigidas hacia las neuronas MCHérgicas del modelo orexina/ataxina-3, no arrojaron resultados tan favorables, probablemente debido a que al usar el andamiaje celular de las células MCHérgicas, el péptido producido no pudo llegar en tiempo y forma a las zonas necesarias para estabilizar el interruptor del control del sueño (Liu et al., 2011).

Las mejorías observadas con esta aproximación terapéutica no están limitadas al HL, ya que también se han observado al realizar transferencias en núcleos pontinos y la amígdala (Blanco-Centurion et al., 2013; Liu et al., 2016).

Conforme la hipótesis autoinmune de la enfermedad siga vigente, la terapia génica seguirá siendo una buena apuesta, sin embargo, es importante incrementar la vida media de los vectores y realizar más estudios en modelos más cercanos a lo que ocurre en la narcolepsia humana, como lo es, por ejemplo, el modelo orexina/ataxina-3.

Trasplante de células orexinérgicas

El principio que sostiene la noción de los trasplantes celulares se basa en la idea de que al introducir células en el sistema estas podrían producir y liberar la, o las, proteínas faltantes de manera similar a como ocurriría en el contexto saludable. Entre las dificultades a las que se enfrentan los

trasplantes, están el origen, supervivencia e integración de las células trasplantadas y lo invasivo de la técnica. A pesar de estas dificultades, esta vertiente ha producido resultados positivos para otras enfermedades neurodegenerativas como la enfermedad de Parkinson (Boronat-García et al., 2016; Kefalopoulou et al., 2014).

En el marco de la narcolepsia, el sistema afectado opera con pocas células que se encuentran restringidas a una misma zona, lo que supone una gran ventaja para esta aproximación ya que, aunque la sobrevivencia sea baja, pocas células podrían ser suficientes para desencadenar un efecto terapéutico. En línea con esto, ya se ha reportado el efecto de un trasplante hecho a partir de tejido hipotalámico en un modelo de narcolepsia. De acuerdo con los resultados, en ratas lesionadas con saporina acoplada al receptor OX2R, se observó una mejoría en los parámetros de sueño hasta 21 días post-trasplante (Arias-Carrión & Murillo-Rodríguez, 2014).

Justificación y planteamiento del problema

De acuerdo con la evidencia disponible, la degeneración orexinérgica subyace al desarrollo de la narcolepsia. Hoy en día su manejo se logra por medio de intervenciones farmacológicas y conductuales que buscan consolidar el sueño y disminuir la ESD. Con el régimen adecuado, los pacientes pueden lograr recuperar calidad de vida, sin embargo, estas terapias son sintomáticas y limitadas por la adherencia al tratamiento.

Una aproximación terapéutica diferente propone estimular la producción endógena de orexina, para lo cual se estudia la terapia genética y se han propuesto entre otras aproximaciones, trasplantes con células orexinérgicas.

Hipótesis

El trasplante de células orexinérgicas es capaz de revertir el fenotipo narcoléptico en el modelo animal de narcolepsia orexina/ataxina-3.

Objetivos

- Establecer una colonia de animales orexina/ataxina-3.
- Estandarizar la obtención de poblaciones celulares ricas en células orexinérgicas
- Trasplantar células orexinérgicas a animales orexina/ataxina-3
- Analizar el sueño de animales trasplantados con respecto al de animales silvestres.
- Analizar la conducta de animales trasplantados con respecto a la de animales no trasplantados.

Materiales y Métodos

Animales

Para los experimentos descritos en este trabajo, se utilizaron tres líneas de animales transgénicos: orexina/ataxina-3, CAG-EGFP y orexina/EGFP. Los animales orexina/ataxina-3 y orexina/EGFP fueron obtenidos mediante donación por parte de los laboratorios de los Dres. Kilduff y Van den Pol, respectivamente, previa autorización del Dr. Sakurai, iniciador de ambas líneas. Los animales CAG-EGFP y C57BL/6 se obtuvieron del bioterio del Instituto de Fisiología Celular (IFC), UNAM.

Animales de la línea orexina/ataxina-3 portadores del transgen, sufren de una degeneración gradual y selectiva de células orexinérgicas, por lo que son ampliamente utilizados como modelo de narcolepsia (Hara et al., 2001). Los animales CAG-EGFP y orexina/EGFP expresan la proteína verde fluorescente mejorada (EGFP por sus siglas en inglés) de manera general bajo control del promotor CAG (CAG-EGFP), o exclusivamente en células que sintetizan péptidos orexinérgicos (orexina/EGFP) (Yamanaka et al., 2003), de estas cepas se obtuvieron las células que conformaron los trasplantes. Los animales C57BL/6 se utilizaron para establecer las colonias de animales transgénicos.

Los animales fueron alojados en condiciones estándar con ciclos de luz/oscuridad de 12h y acceso libre a agua y alimento. Todos los protocolos fueron autorizados por el CICUAL del IFC cuyo permiso tiene el número de identificación RDC111(13)-17.

Genotipificación

Para identificar a los animales portadores del transgén en las líneas orexina/ataxina-3 y orexina/EGFP, realizamos la PCR con ADN tomado de muestras de cola en todas las crías.

Cada muestra de cola fue digerida en 200 µL de STE con 3 µL de proteinasa K (Invitrogen; 17916) a 37 °C por una noche. Posteriormente el ADN fue purificado adicionando 100 µL de una solución de

NaCl 6 M. La muestra fue agitada ligeramente por 30 s en un vortex, y centrifugada a 13 000 rpm por 12 min. Se recuperó el sobrenadante y se pasó a tubos de microcentrífuga eppendorfs nuevos de 2.5 ml rotulados apropiadamente; se agregaron 300 µL de etanol frío al 100 %, se ladearon las muestras y se centrifugaron nuevamente a 13 000 rpm por 12 min adicionales. Al terminar, el etanol fue decantado y las muestras se dejaron secar a temperatura ambiente. Posteriormente se adicionaron 50 µL de tris y el ADN fue resuspendido a 65 °C por 1 h, agitando las muestras a intervalos de 30 minutos.

La PCR se llevó a cabo con muestras de ADN diluidas a 25 ng/µL, mastermix DreamTaq (Thermo Scientific; K9021) y los oligonucleótidos: HOXPRO N 5'- GCAG CGGC CATT CCTT GG y HOXPRO A 5'- AAGT CGAC GGTG TCTG GCGC TCAG GGTG. Los resultados se visualizaron con una electroforesis en un gel de agarosa (USB; 32803) al 2 % utilizando una corriente de 90 V por 10 min, seguida de una de 60 V por 50 min. El gel se visualizó y fotografió utilizando un transiluminador para discriminar entre animales positivos y negativos.

Para la identificación de animales CAG-EGFP, los neonatos se observaron bajo microscopio de epiflourescencia para detectar señal EGFP.

Grupos animales y secuencia experimental

De acuerdo con los resultados de la genotipificación, un total de 49 animales adultos de la cepa orexina/ataxina-3 fueron divididos en 5 grupos. Animales orexina/ataxina^{-} fueron considerados silvestres y asignados al grupo WT; animales orexina/ataxina^{+} fueron organizados en los grupos restantes: narcolepsia basal (NB), sham, trasplante control (TXCB) y trasplante orexina (TXOX). Todos los animales fueron implantados con electrodos para poder realizar registros electroencefalográficos, mientras que los grupos sham, TXCB y TXOX fueron además sometidos a cirugía esterotáxica de trasplante celular (TXCB, TXOX) o infusión de medio enriquecido (sham). El

número total de individuos por grupo se presenta en la Tabla 3, mientras que el flujo experimental puede apreciarse mejor en la Figura 10.

Tabla 3 Individuos analizados para cada grupo experimental

Nombre del grupo	Total de animales por grupo	Animales evaluados	
		Registros electrofisiológicos	Análisis conductual
Silvestre (WT)	10	6	5
Narcolepsia Basal (NB)	9	6	6
sham	10	6	6
Trasplante control (TXCB)	8	7	5
Trasplante orexinérgico (TXOX)	12	6	6

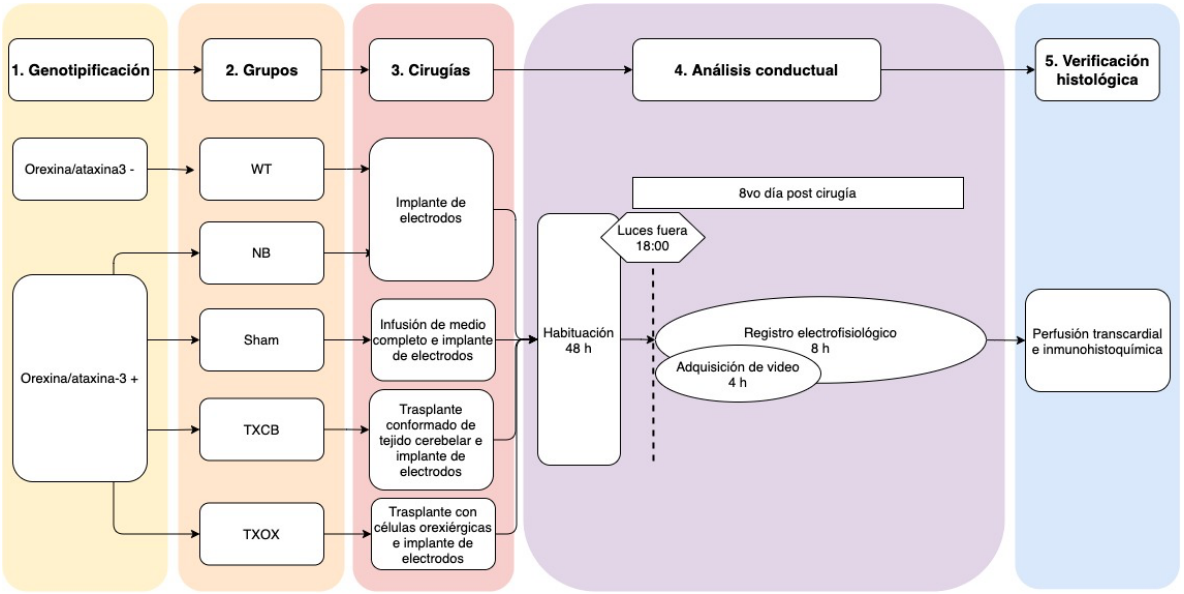


Figura 10 Secuencia experimental. Para los experimentos llevados a cabo en este trabajo, los animales fueron genotipificados (1) y de acuerdo con los resultados + vs -, los animales se distribuyeron en uno de 5 grupos (2). Animales adultos fueron sometidos a cirugía estereotáxica correspondiente a su grupo experimental (3), y posteriormente evaluados de manera conductual (4). Previa eutanasia, se realizó evaluación histológica para verificar el estatus genético (5). Adaptado de (Equihua-Benítez et al., 2020).

Trasplantes celulares y cirugía sham

Preparación de suspensiones celulares para trasplantes

Neonatos de entre 8 y 10 días de nacidos, de ambas líneas de transgénicos, fungieron como donadores de células. Células cerebelares de animales CAG-EGFP se usaron para los trasplantes del grupo TXCB, mientras que de los animales orexina/EGFP se obtuvieron células orexinérgicas del hipotálamo lateral para completar el grupo TXOX.

Para obtener tejido hipotalámico, los cerebros extraídos se colocaron en matrices de corte con el lado ventral expuesto y se obtuvieron cortes coronales de 2 mm de grosor de la zona alrededor del quiasma óptico. Las rebanadas se colocaron en cajas Petri frías y bajo un microscopio Leica EZ4 D se recuperó el tejido en la zona ventral utilizando el 3er ventrículo como referencia anatómica.

Posteriormente, y teniendo cuidado de no contaminar las muestras, el tejido cerebral fue recuperado en 1x PBS (Sigma; D8537) con antibiótico (100 U/ml Penicillina-estreptomicina; Sigma P3539) y resuspendido en 1x PBS con EDTA 0.5 mL (USB; 15694) con antibiótico y centrifugado entre 8 y 10 min a 1 000 rpm.

En la campana de flujo laminar se recuperó sobrenadante para ser disociado con una mezcla de tripsina 2.5 % (Gibco; 15090046) con DNAsa 1.5 mg/mL (Sigma; DN-25) a 37 °C por 20 min. Posteriormente se detuvo la reacción con DMEM (Gibco; 31600034) con 10 % suero fetal bovino (Sigma; A-7030) y antibiótico (DMEM completo). Se centrifugó nuevamente para recuperar el sobrenadante y resuspender en DMEM sin suero fetal bovino, tras lo cual se filtró (poro de 40 µm) la suspensión. Se volvió a centrifugar y se retiró el sobrenadante y las células fueron resuspendidas en 1x PBS y conservadas en hielo para su posterior análisis en el citómetro de flujo.

Las muestras fueron transportadas en frio al Laboratorio Nacional de Citometría de Flujo donde se hizo uso del citómetro MoFlo XDP (Beckman-Coulter, IN, USA) para seleccionar las células $DAPI^-$

/GFP$^+$. DAPI (dilución 1 : 10 000; Thermo Fisher Scientific Cat# D1306, RRID:AB_2629482) se añadió 5 min previos al procesamiento con el objetivo de descartar las células inviables y de cada vial se eligieron las células EGFP más brillantes. Las muestras fueron recuperadas en DMEM completo y concentradas a 8 000 células/µL.

Cirugía esterotáxica

Tanto trasplantes celulares como cirugías sham, se realizaron en animales machos adultos (> 10 semanas) positivos de la línea orexina/ataxina-3. Para este fin los animales fueron anestesiados con un coctel de 100-10 mg/kg xylazina/ketamina intraperitoneal. Después de verificar insensibilidad, los animales fueron montados en un aparato esterotáxico y su cráneo expuesto. Guiándonos por el atlas del cerebro de ratón de Paxinos & Franklin (2ed), se utilizaron las coordenadas AP -4.5, ML -1.4 V -3.9 para dirigir los trasplantes e infusiones sham a los núcleos PPT/LTD de la formación reticular. Se realizó un trépano y con la guía de un inyector y una aguja Hamilton se dispersó un volumen total de 0.5 µL compuesto de 0.25 µL de suspensión celular no orexinérgico (TXCX), orexinérgico (TXOX) o DMEM completo (sham) y 0.25 µL de solución salina como buffer. El volumen fue liberado en un lapso de 5 s, tras lo cual se mantuvo la cánula en su lugar durante 5 min y luego fue retirada lentamente a lo largo de otros 5min para evitar arrastrar la suspensión. Finalmente, el trépano fue sellado con cera para hueso.

Análisis electrofisiológico

Implante de electrodos EEG/EMG

Todos los grupos fueron implantados con electrodos para poder realizar registros electrofisiológicos. Los electrodos se fabricaron manualmente en este laboratorio con dos pines para recolectar información cortical (EEG y referencia) y conectados a dos alambres de acero

inoxidable (A-M Systems; 792500) y un pin conectado a un alambre de plata (A-M Systems; 786500) para obtener la información de EMG. Se abrieron dos trépanos en las coordenadas AP -1.6, ML -1.4 y AP +0.6, ML -1 para colocar tornillos de acero inoxidable (PlasticsONE, Roanoke, VA, USA) y recolectar información EEG y de referencia, respectivamente. La información EMG se registró en los músculos extensores del cuello colocando el cable de plata subcutáneamente. Los electrodos se fijaron con una mezcla de cianoacrilato y cemento dental. Se permitió a los animales convalecer 5 días antes de comenzar los procedimientos experimentales.

Registro electrofisiológico

Para llevar a cabo los registros electrofisiológicos, los animales fueron anestesiados ligeramente con isoflurano y conectados a un conmutador giratorio (Plastics One, Roanoke, Va, USA) en cabinas individuales. En estas condiciones fueron habituados durante 48 h y posteriormente registrados por 8 h consecutivas comenzando al apagar luces, obteniendo así registros que correspondieron al octavo día post cirugía. La señal EEG/EMG se amplificó con un polígrafo (Grass Instruments Model 78, West Warwick, RI, USA) y filtrado (EEG: 0.3-100 Hz. EMG: 30-300 Hz) y la señal se digitalizó con un equipo de adquisición (National Instruments, Austin, TX, USA) con una frecuencia de 128 Hz.

Análisis de registros

Los registros se calificaron manualmente en épocas de 12 s con el software ICELUS (University of Michigan) registrando vigilia, sueño nREM y REM en concordancia con el método descrito (Murillo-Rodríguez et al., 2017; Radulovacki et al., 1984). En total, para los grupos WT, NB, sham, y TXOX se evaluaron n = 6 registros, mientras que para el grupo TXCB se evaluaron n = 7 registros.

Posteriormente, se llevaron a cabo pruebas de Kruskal-Wallis con análisis post-hoc de Dunnett para encontrar diferencias con respecto al grupo WT en los siguientes parámetros: tiempo total y por

hora pasados en vigilia, nREM y REM; latencia de sueño nREM y REM; duración y número de episodios de vigilia, sueño nREM y REM; y número total de transiciones en el periodo evaluado.

Análisis de eventos catapléjicos

Evaluación de la conducta

Para evaluar los eventos catapléjicos, se instaló un sistema de monitoreo infrarrojo con cámaras comerciales y los animales fueron registrados al octavo día post cirugía comenzando al apagar luces. Los videos fueron calificados manualmente en concordancia con los criterios establecidos en el consenso alcanzado por el equipo de trabajo de Scammell et al (2009). De acuerdo con el consenso citado, cuando los eventos catapléjicos son determinados exclusivamente con evidencia videográfica el término apropiado es “behavioral arrest” para lo cual usaremos la abreviatura BA. Un evento es considerado BA cuando se cumplen las siguientes condiciones: un cese abrupto de la conducta que dura por lo menos 10 s, tras lo cual es frecuente que se reanude la conducta como si la interrupción no hubiera sucedido; a todo BA le anteceden por lo menos 40 s de conducta que corresponde a vigilia. Para cada uno de los grupos sham, NB y TXOX n = 6, mientras que para los grupos WT y TXCB n = 5. Los videos obtenidos tuvieron una duración de entre 3 y 4 h, diferencias que fueron ponderadas al realizar el análisis Bayesiano.

Estadística Bayesiana para análisis de conducta

Las diferencias entre grupos fueron evaluadas utilizando modelos jerárquicos bayesianos. En particular se utilizó el lenguaje de programación R (Team, 2018) y el paquete BMRS (Bürkner, 2017), el cual es una interfaz de alto nivel para el lenguaje de programación probabilístico Stan (Carpenter et al., 2017). A diferencia de la aproximación frecuentista, la estadística Bayesiana no utiliza valores-p para determinar significancia estadística, en su lugar se utilizan intervalos creíbles (BCI). Es decir, por ejemplo, al trabajar con un modelo lineal, sus coeficientes tendrán asociados intervalos creíbles,

que en las ocasiones en que no incluyan al cero se considerarán significativos y de acuerdo con la terminología bayesiana se dirá que hay diferencias notables.

Para el análisis del efecto del tratamiento sobre BA, se desarrollaron tres modelos cuya convergencia y ajuste fueron aceptables:

1. Un modelo para predecir la duración de BA. Esta variable (la respuesta) se consideró log-normal, la variable grupo el parámetro poblacional de interés y se usó un término de intercepción para cada sujeto.
2. Un modelo para predecir el número total de BAs en las horas muestreadas (el tiempo total de muestreo varió ligeramente para cada sujeto). La respuesta se consideró Poisson y la variable grupo el parámetro poblacional de interés. Como lo que se modeló fueron tasas se introdujo un multiplicador log(horas).
3. Un modelo para predecir el porcentaje total de tiempo en el estado de BA. Como esta respuesta está acotada entre 0 y 1 se consideró una distribución Beta. De nuevo, la variable grupo es el parámetro poblacional de interés.

Las bases de datos, así como las especificaciones de cada modelo y el código utilizado para realizar el análisis, se pueden consultar en el material suplementario de Equihua-Benítez et al., 2020.

Evaluación histológica postmortem

Al concluir los procedimientos experimentales, a todos los animales se les practicó eutanasia con una dosis letal de pentobarbital y perfundidos transcardialmente. El sistema vascular fue lavado con PBS 0.1 M seguido de la posfijación con paraformaldehido (PFA) al 4 % y posteriormente incubados en concentraciones ascendentes de sacarosa (10, 20 y 30 %) para poder obtener cortes coronales de 40 µm con criostato Leica CM1900 (Leica Microsystems, Weztlar, Germany). De cada individuo, se recolectaron consecutivamente en 4 pozos, secciones coronales de toda la zona del 3er

ventrículo. Las muestras fueron preservadas en anticongelante (50 % PBS, 25 % glicerol y 25 % etilenglicol) hasta el momento de su uso.

Para verificación del estatus transgénico, se realizaron inmunohistoquímicas en todos los animales. Se tomó un pozo de cada individuo y las muestras fueron lavadas tres veces con PBS antes de bloquear la actividad de la peroxidasa endógena durante un minuto con solución de 0.28 % ácido periódico. Inmediatamente después, se realizaron tres lavados de 5 min con PBS para proceder con el bloqueo de sitios inespecíficos con solución de bloqueo: 2.5 % albúmina (Sigma; 9048-46-8) + 0.1 % Tritón-X-100 (Sigma; 9008-93-1) en PBS durante 2 h para después incubar en solución de bloqueo con anticuerpo primario rabbit-α-orexinA (1: 2000; Phoenix Pharmaceuticals Cat# H-003-30, RRID:AB_2315019) toda la noche a 4 °C. Se realizaron tres lavados de 5 min y se incubaron las muestras en anticuerpo secundario biotinilado goat-α-rabbit (1:250; Vector Laboratories Cat# BA-1000, RRID:AB_2313606) por 2 h. Se hicieron nuevamente tres lavados de 5 min para realizar una incubación de 30 min en Vectastain (Vector Labs; PK-6100), antes de realizar nuevos lavados y llevar a cabo el revelado con diaminobenzidina (Sigma; D5637) por 5 min. Se realizó una última serie de lavados en PBS y los cortes fueron montados en porta laminillas SuperFrost (ThermoFisher; 5951 PLUS), dejado secar y posteriormente deshidratados en un tren de alcohol-xileno, para después proteger con Cytoseal XYL (Richard-Allan Scientific; 8312-4) y cubreobjetos. Los resultados fueron documentados con un microscopio estereoscópico Leica EZ4D para determinar la presencia o ausencia de somas orexinérgicos en cada animal. Imágenes representativas fueron obtenidas con un microscpio Zeiss Axio Zoom V16.

Resultados

Animales orexina/ataxina-3^{+} presentan degeneración de células orexinérgicas en el hipotálamo lateral

Al finalizar los procedimientos experimentales, todos los animales fueron perfundidos previa anestesia y su estatus génico verificado mediante inmunohistoquímica para orexina A (Figura 11). En el tejido obtenido de la región hipotalámica de los ratones orexina/ataxina-3 se confirmó que la presencia del transgén coincide con ausencia de somas orexinérgicos en la zona del HL. Los animales control orexina/ataxina^{-}, por el contrario, muestran la distribución esperada de somas en la región.

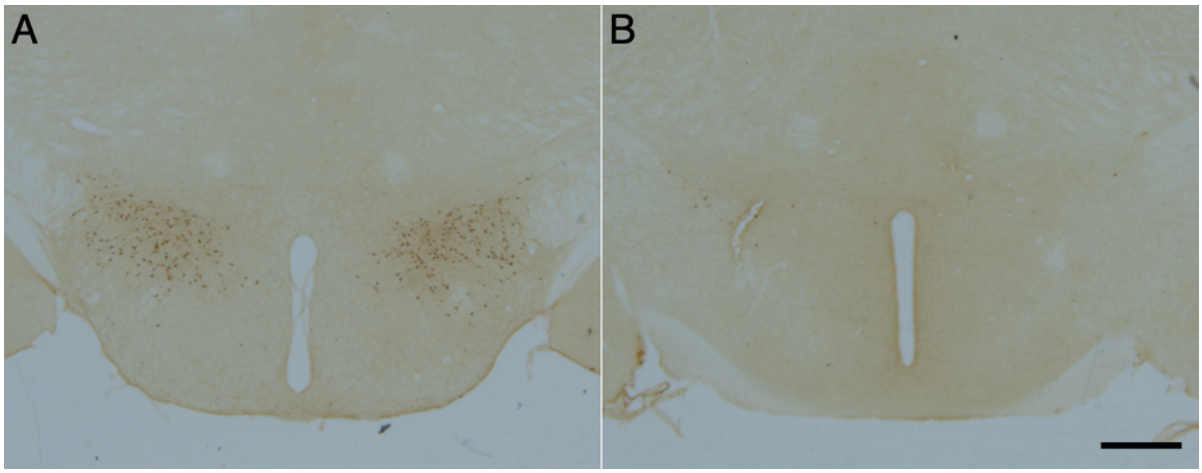


Figura 11 Inmunohistoquímica de la región hipotalámica para verificación de la denervación orexinérgica. Se muestran imágenes representativas de la zona hipotalámica de un animal orexina/ataxina-3^{-} (A) y uno orexina/ataxina-3^{+} (B). Se observa que animales negativos para el transgén poseen señal orexinérgica que incluye somas en el área hipotalámica, mientras que animales positivos, por el contrario, no muestran esta pigmentación, lo que indica la ausencia de somas orexinérgicos en la misma región. La barra de escala representa 500 µm

Obtención de poblaciones celulares para trasplante

Las suspensiones celulares utilizadas en los trasplantes se conformaron por células $DAPI^-/EGFP^+$, las cuales fueron seleccionadas utilizando citometría de flujo. La adición de DAPI previo a la clasificación celular permitió descartar células dañadas e incrementar la viabilidad de la muestra.

Utilizando este método, se obtuvo 994,000 ± 243,269 y 17,160 ± 6,129 células de tejido cerebelar; e hipotálamo respectivamente.

El trasplante de células redujo la fragmentación del sueño en animales narcolépticos

El CSV en los ratones se caracteriza por tener un periodo de "mayor actividad" que coincide con el periodo de oscuridad, y un periodo de "menor actividad" que corresponde al periodo de luz. Para evaluar el efecto del trasplante en la arquitectura del sueño durante el periodo de actividad, obtuvimos 8 h continuas de registros electrofisiológicos (EEG/EMG) que comenzaron al apagar luces.

Tras calificar los datos se analizaron diferentes parámetros del sueño utilizando la prueba no-paramétrica de Kruskal-Wallis seguida de la prueba post-hoc de Dunnett. Por cada estado conductual, vigilia (wake), sueño nREM y sueño REM se evaluó el tiempo total y por hora, así como la duración y número de episodios; también se analizó la latencia para sueño nREM y REM y el número total de transiciones ocurridas a lo largo del periodo de 8 h. En la **¡Error! No se encuentra el origen de la referencia.** se presenta la evaluación por hora y en la Tabla 4 se resumen los resultados del resto de los análisis.

En general, los grupos de animales narcolépticos (narcolepsia basal NB; sham; trasplante control TXCB y trasplante orexina TXOX) tuvieron valores por encima de los observados en el grupo control

(WT) lo que indica desorganización del sueño. Sin embargo, el número de episodios de wake, nREM y REM del grupo TXOX estuvieron dentro del rango de los del grupo WT, lo que indica menor fragmentación. En el resto de los parámetros, las diferencias significativas encontradas hablan de un fenotipo narcoléptico que no fue revertido.

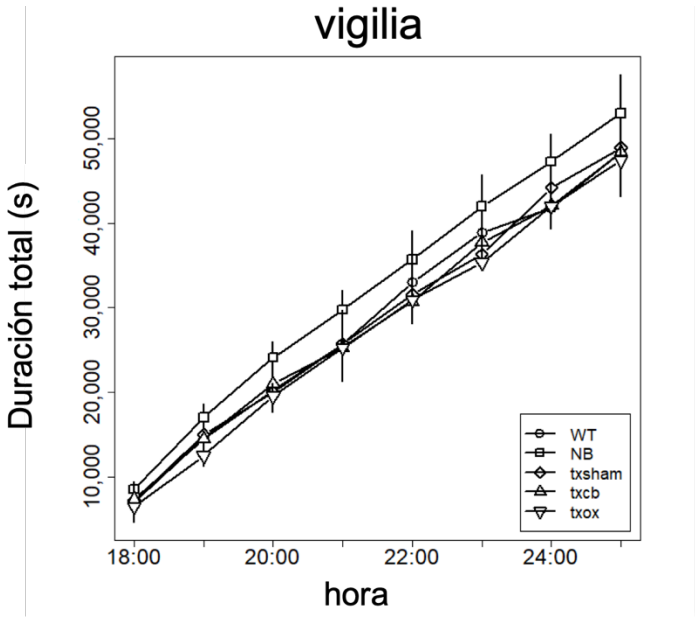
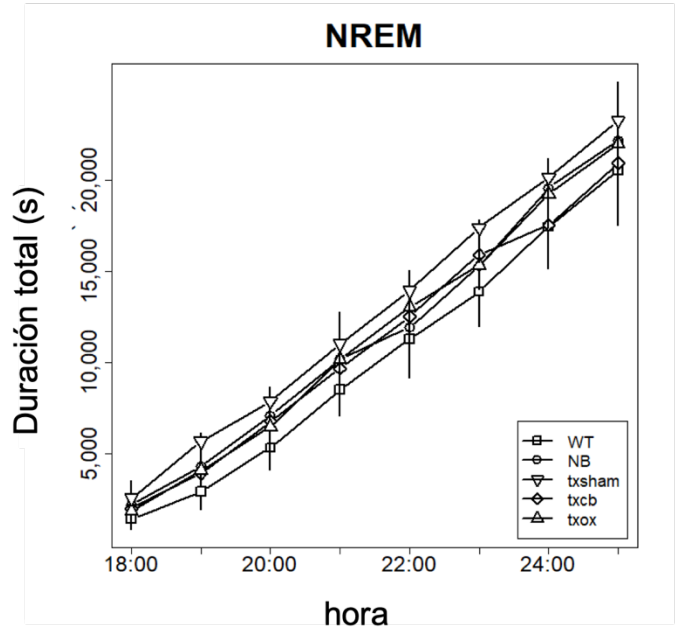


Figura 12 Análisis por hora de tiempo pasado en vigilia, nREM y REM. Los registros electrofisiológicos (EEG/EMG) comenzaron al apagar luces y duraron 8 h continuas. La información se presenta como acumulados por hora. Las figuras sólidas negras indican diferencias significativas ($p < 0.05$; Kruskal-Wallis con prueba post-hoc de Dunnett) al compararse con el grupo silvestre (WT). Los grupos WT, narcolepsia basal (NB), sham y trasplante orexina (TXOX) tuvieron n = 6, mientras que el grupo trasplante control (TXCB) tuvo n = 7.



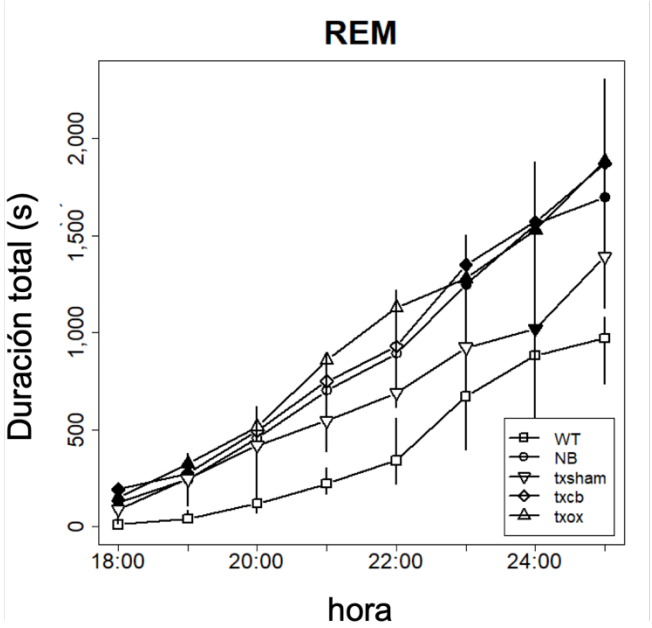


Tabla 4 Análisis de 8 h de registros electrofisiológicos comenzando al apagar las luces

	WT		NB		SHAM		TXCB		TXOX	
Tiempo total (min)										
Wake	294.1	(275.1, 319.55)	268.4	(245.2, 297)	263.3	(243.45, 276.85)	275.8	(265.2, 292)	268.7	(239.9, 285.8)
NREM	171.1	(146.1, 187.7)	185	(158.25, 201.55)	194	(181.55, 203.3)	172.8	(169.1, 181.1)	183.5	(169, 211.65)
REM	16.2	(12.3, 18)	*28.3	(26.25, 31.55)	23.2	(18.8, 33)	**31.2	(27.85, 38.45)	*31.4	(26.1, 37.6)
Duración de episodio (s)										
Wake	84	(36, 540)	96	(36, 312)	84	(48, 294)	72	(36, 372)	108	(36, 372)
NREM	192	(96, 360)	***132	(72, 264)	***156	(78, 246)	***132	(72, 216)	***156	(84, 288)
REM	60	(36, 96)	60	(36, 96)	60	(36, 96)	60	(36, 96)	72	(48, 96)
Latencia de sueño (min)										
NREM	4.2	(2.9, 14.65)	1.2	(0.45, 9.75)	3.8	(3.05, 14.9)	6.4	(2.65, 15.25)	8	(4.45, 10.95)
REM	87.7	(44.85, 149.3)	28.2	(7.45, 35.75)	35.8	(15.75, 79.4)	14.4	(12.7, 25.9)	43.9	(23.4, 45.05)
Número de episodios										
Wake	38	(34.74, 41.25)	*47	(42.25, 68.75)	*55.5	(48.25, 68.75)	*60.5	(51.5, 71)	51.5	(49.5, 58.75)
NREM	39	(36.25, 41)	*51.5	(50.25, 73)	*57	(51.75, 74.5)	**64.5	(50.75, 74.5)	53	(50, 59)
REM	14.5	(10, 17.5)	22	(20.25, 27.5)	16.5	(15.25, 25.25)	*25	(23.25, 30.5)	21.5	(18.75, 28)
Número total de transiciones										
8 h	85.5	(84.25, 94.25)	*117.5	(115, 170.25)	*127	(122.5, 154)	**157.5	(128.75, 169.75)	*127.5	(114.25, 140)

Los valores estadísticos presentados están expresados en medianas con su correspondiente rango intercuartílico 25-75 entre paréntesis. Las comparaciones se realizaron contrastando contra el grupo silvestre (WT) y las diferencias significativas encontradas están indicadas con asteriscos ($p < 0.05$*, 0.01**, 0.001***; Kruskal-Wallis seguida de la prueba post-hoc de Dunnett). NB: narcolepsia basal; TXCB: trasplante control; TXOX trasplante orexina.

El trasplante de células orexinérgicas disminuyó la severidad de los eventos catapléjicos

El análisis conductual se realizó a partir de registros de video obtenidos al inicio del periodo de oscuridad. Para determinar mejorías conductuales en los grupos trasplantados, se ajustaron tres modelos jerárquicos Bayesianos para analizar la duración de episodios (Figura 12), el número de eventos BA por hora (Figura 13) y el porcentaje de tiempo en BA (Figura 14). La revisión exhaustiva de los videos obtenidos del grupo control (WT), demostró que no presentan ninguna conducta que pudiera ser clasificada como eventos de BA, debido a esto las comparaciones se realizaron con respecto al grupo NB. En la Tabla 5 están resumidos los resultados del análisis Bayesiano.

Tabla 5 Resultados del análisis conductual para eventos catapléjicos (BA)

Grupo	Duración de episodio (s)		Número de eventos		% de tiempo en BA	
NB	35	(17, 59)	14.5	(13.25, 15.75)	4.42 %	(3.85 %, 5.71 %)
SHAM	26	(17, 47)	14.5	(11.75, 15.75)	3.40 %	(2.9 %, 4 %)
TXCB	27	(20.5, 45.5)	*9	(4, 11)	*2.42 %	(1.75 %, 2.6 %)
TXOX	*20.5	(15, 34)	*8	(5.5, 9.75)	*1.36 %	(1.07 %, 1.79 %)

Los valores estadísticos presentados están expresados en medianas con su correspondiente rango intercuartílico 25-75 entre paréntesis. Las comparaciones se realizaron usando al grupo narcolepsia basal (NB) como control, las diferencias notables (95 % intervalo creíble) se indican con asteriscos. Trasplante control TXCB; trasplante orexina TXOX.

De acuerdo con los resultados obtenidos, se observa que la conducta BA tuvo su mayor cambio en el grupo TXOX. Con reducciones notables en duración de episodios (95 % BCI [-0.64,-0.07), número de eventos 95 % BCI [-1.12,-0.35]) y porcentaje de tiempo en BA (95 % BCI [-1.82,-0.71]). Estas reducciones se midieron en términos de medianas, y respectivamente corresponden a 13.34 s (30.31 %), 7.49 (51.35 %) eventos y 3.42 % (69.73 %) de tiempo en BA. Inesperadamente, el grupo TXCB también demostró mejorías en BA en dos evaluaciones: número de eventos (95 % BCI [-0.99,-

0.2]) y porcentaje de tiempo en BA (95 % BCI [-1.30,-0.27]), que significan disminuciones medianas de 6.37 (43.68 %) eventos y 2.56 % (52.23 %) de tiempo en BA respectivamente.

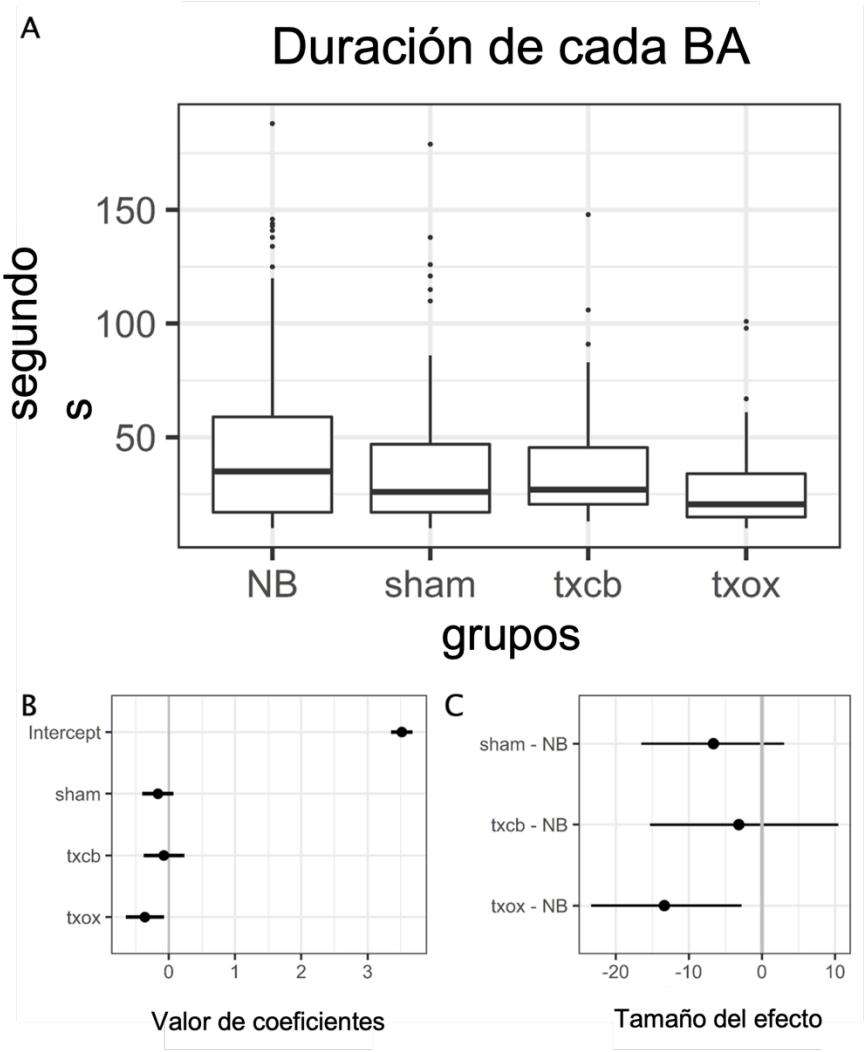


Figura 12 Duración de eventos catapléjicos (BA). Para el estudio de la duración de los eventos catapléjicos (BA), se ajustó un modelo bayesiano que permitió analizar la información obtenida a partir de la conducta de los animales observada en los registros videográficos. Los resultados indican que el mayor cambio ocurrió en el grupo trasplante orexina (TXOX) donde la diferencia notable indica una reducción en la duración de los episodios de 30.31% en comparación con el grupo narcolepsia basal (NB). A) Distribución de la duración de BA para cada grupo; las líneas horizontales indican la mediana y los bigotes el rango intercuartílico. B) Valor de los coeficientes; los coeficientes nos indican diferencias notables cuando la distribución no abarca el cero (línea sólida vertical). C) Tamaño del efecto; nos muestra la magnitud del cambio ocasionada por el tratamiento sobre la duración del episodio. Los grupos NB, sham y TXOX fueron de n.= 6, mientras que para el grupo trasplante control (TXCB) n = 5.

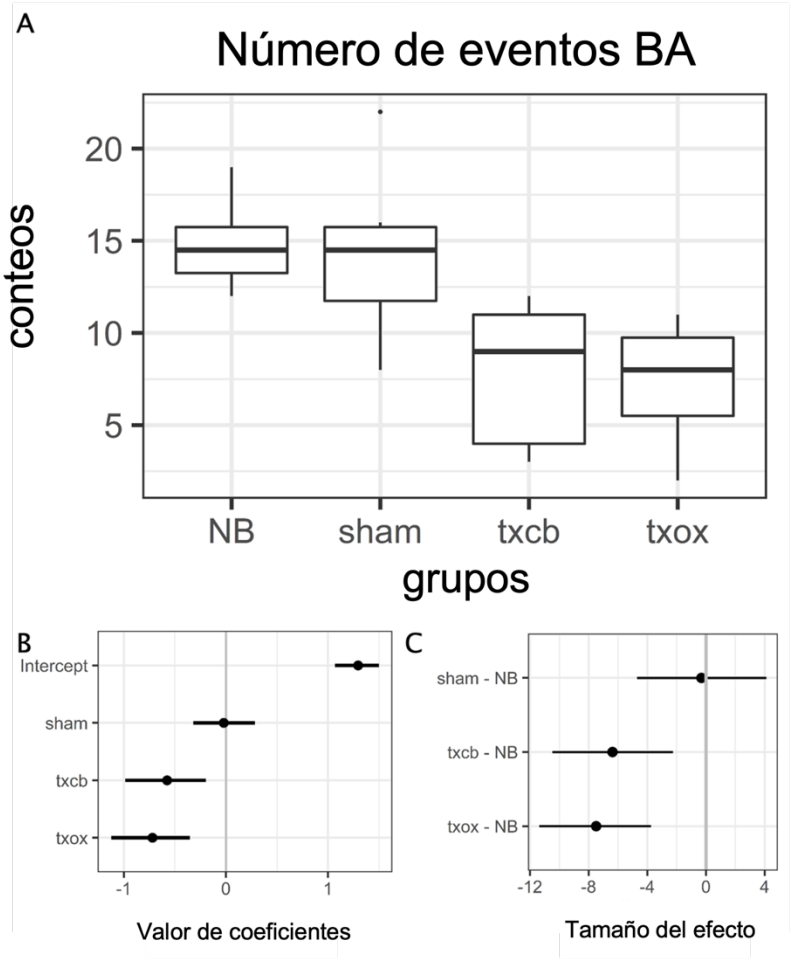


Figura 13 Número de eventos catapléjicos (BA). Para el estudio del número de eventos catapléjicos (BA), se ajustó un modelo bayesiano que permitió analizar la información obtenida a partir de la conducta de los animales observada en los registros videográficos. Los resultados indican que tanto en el grupo trasplante control (TXCB) como en el grupo orexina trasplante (TXOX), el tratamiento redujo notablemente el número de eventos catapléjicos en 46.68 % y 51.35 % respectivamente, al realizar comparaciones con respecto al grupo narcolepsia basal (NB). A) Distribución del número de eventos BA en cada grupo; las líneas horizontales indican la mediana mientras que los bigotes denotan el rango intercuartílico. B) Valor de los coeficientes; los coeficientes nos indican diferencias notables cuando la distribución no abarca el cero (línea sólida vertical). C) Tamaño del efecto; nos muestra la magnitud del cambio ocasionada por el tratamiento sobre el número de eventos. Los grupos NB, sham y TXOX fueron de n = 6, mientras que el grupo TXCB fue de n=5.

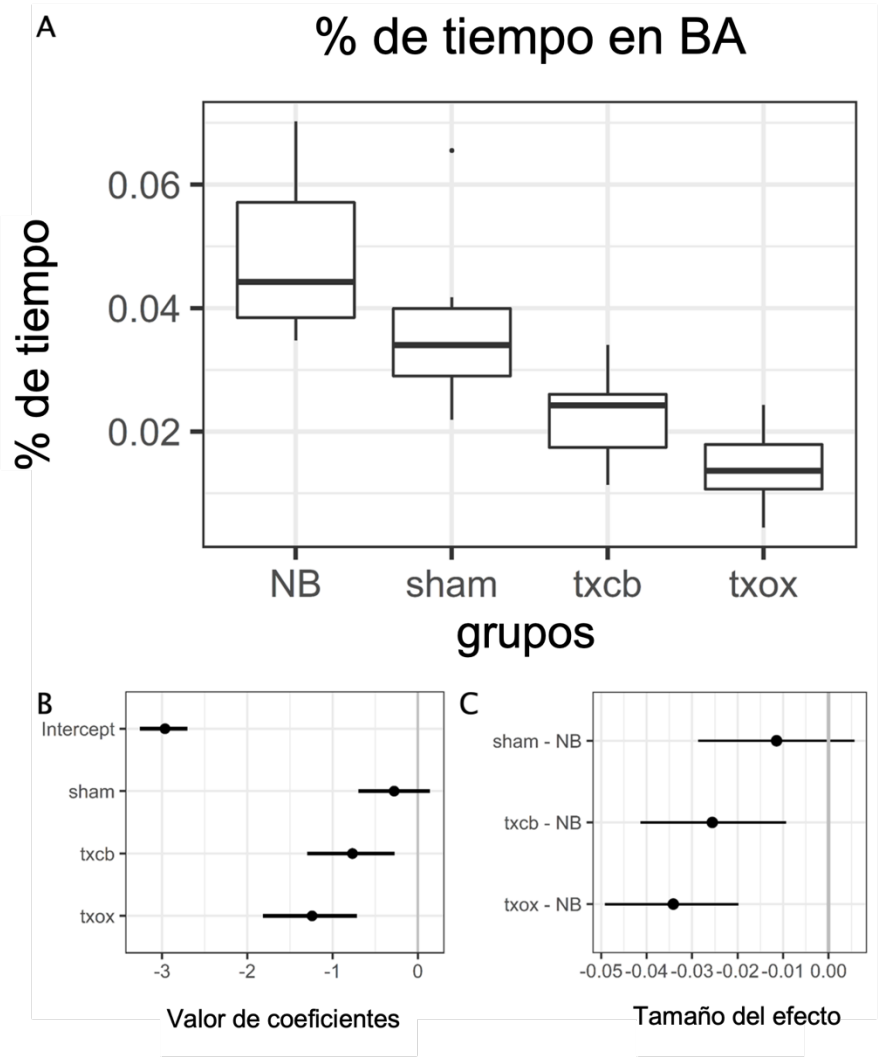


Figura 14 Porcentaje de tiempo en BA. Se estudió el tiempo total que los grupos pasaron en el estado de evento catapléjico (BA) usando un modelo bayesiano. Los resultados indican que tanto el grupo trasplante control (TXCB) como el grupo trasplante orexina (TXOX) redujeron notablemente el tiempo pasado en BA cuando se compararon con el grupo narcolepsia basal (NB). Estas reducciones fueron de 69.73 % para el grupo TXOC y 52.23 % en el grupo TXCB. A) La distribución del tiempo pasado en BA para todos los grupos; las líneas horizontales indican el valor de la mediana y los bigotes denotan el rango intercuartílico. B) Valor de los coeficientes; los coeficientes nos indican diferencias notables cuando la distribución no abarca el cero (línea sólida vertical). C) Tamaño del efecto; nos indica la magnitud del cambio en el porcentaje del tiempo que los animales mostraron la conducta BA. Los grupos NB, sham, y TXOX tuvieron n = 6 individuos, mientras que el grupo TXCB fue de n = 5.

Discusión

La mayoría de la información disponible sobre trasplantes neuronales y enfermedades neurodegenerativas surge a raíz del estudio de la enfermedad de Parkinson. Esta enfermedad se caracteriza por la pérdida de somas dopaminérgicos en la sustancia *nigra pars compacta*, lo que disminuye el suministro de dopamina en el estriado y da lugar a la sintomatología motora clásica. En la década de los 80s, estudios en roedores y primates no humanos, demostraron que trasplantes de células dopaminérgicas, obtenidas de células fetales del mesencéfalo ventral, tienen la capacidad de sobrevivir, integrarse y disminuir parkinsonismos en los modelos estudiados (Borlongan et al., 1999).

Una historia similar se observa en el curso de la narcolepsia, donde sabemos que el cuadro clínico está relacionado con la desorganización del sueño, resultado de la degeneración de somas orexinérgicos. En este caso, los trasplantes orexinérgicos representan una gran área de oportunidad terapéutica, debido a que incluso en condiciones normales la población orexinérgica es pequeña y se encuentra, además, restringida a una misma zona en el HL.

La evidencia disponible indica que la sustitución orexinérgica tendrá efectos positivos en la sintomatología de la narcolepsia y por ende actualmente se trabaja arduamente en encontrar una opción farmacológica basada en orexina. En el campo de los trasplantes no hay muchos estudios que se hayan realizado trabajando con esta aproximación. El antecedente más relevante fue realizado por Arias-Carrión & Murillo-Rodríguez (2014), quienes evaluaron el efecto de un trasplante conformado por células de tejido hipotalámico y dirigido hacia el HL en un modelo de ratas lesionadas con la neurotoxina saporina acoplada a OXB. Con este arreglo experimental, Arias-Carrión et al., describieron una disminución en la ESD que perduró hasta el día 21 post trasplante.

A pesar de los resultados positivos consideramos que, debido a su composición heterogénea, el empleo de tejido hipotalámico para llevar a cabo los trasplantes hace difícil ligar el efecto observado con actividad orexinérgica. Aunado a esto, las alteraciones en el CSV obtenidas a partir del modelo de lesión con saporina, surgen tras la degeneración de células que expresan el OX2R, independientemente de su linaje; por este motivo la lesión es inespecífica y no recrea correctamente la patofisiología de la enfermedad.

Trabajando sobre las observaciones de Arias-Carrión et al, se decidió utilizar el citómetro de flujo para trabajar con suspensiones celulares purificadas y utilizar de hospederos animales de la línea orexina/ataxina-3, un modelo ampliamente estudiado en el contexto de la narcolepsia. Entre las ventajas del modelo se cuenta la degeneración específica, postnatal y paulatina de células orexinérgicas, lo que se asemeja a lo que se ha descrito entre pacientes humanos.

Con estas condiciones, realizamos trasplantes dirigidos a los núcleos PPT/LDT del ARAS donde especulamos que la actividad orexinérgica puede ayudar a revertir el fenotipo narcoléptico al facilitar la actividad de este sistema activador. En este sentido, obtuvimos 8 h continuas de registros electrofisiológicos, comenzando al apagar luces en el 8vo día post trasplante. Los resultados nos indican que nuestros trasplantes tuvieron un modesto efecto de mejoría. Entre los parámetros estudiados, solo el número de episodios para vigilia, nREM y REM del grupo TXOX estuvieron dentro de los rangos del grupo control (WT), lo que sugiere un sueño menos fragmentado.

Creemos que estos resultados, aunque modestos, son prometedores ya que indican que el trasplante tiene la capacidad de incidir de manera positiva sobre el CSV. Creemos, además, que el mínimo efecto observado se debe a limitaciones del arreglo experimental empleado, entre los que destaca la dificultad para purificar la suspensión celular orexina/EGFP que orilló a elegir trasplantes pequeños de solamente 2,000 células $DAPI^{-}/EGFP^{+}$ en 0.25 µL.

La baja tasa celular obtenida se explica, al menos en parte, por la reducida población de células orexinérgicas, la cual se estima entre las 1,100 y 4,000 células por cada cerebro de roedor (Kilduff & Peyron, 2000; Peyron et al., 1998). Para contrarrestar esto, incrementar el número de donadores sería la alternativa más evidente y directa, sin embargo, creemos que se requeriría emplear una gran cantidad de crías donadoras para lograr una concentración de células significativamente mayor, una solución que no consideramos aceptable. En su lugar, creemos que sería mejor buscar fuentes celulares alternas que permitan trabajar con un mayor número de células orexinérgicas. En este sentido, ya hay algunos reportes de éxito en la inducción de células orexinérgicas a partir de células troncales embrionarias de ratón (Hayakawa et al., 2013) y humano (Merkle et al., 2015).

En cuanto al análisis conductual, nos parece interesante que, a pesar de usar trasplantes pequeños, la severidad de los eventos catapléjicos (evaluados como BA y con valores expresados en medianas), se redujo notablemente en los grupos TXCB y TXOX en comparación con el grupo control (NB). Y debido a que no se observó un efecto similar en el grupo sham, estamos confiados en que estos resultados se relacionan con los trasplantes realizados.

En particular, el efecto más marcado se observó en el grupo TXOX, donde el trasplante fue capaz de reducir la duración (30.31 %) y frecuencia (51.35 %) de BA, lo que además implicó una reducción de 69.73 % en el tiempo pasado en BA con respecto a los animales no tratados (NB). En línea con lo comentado con respecto a los registros electrofisiológicos, será interesante evaluar el efecto de un trasplante de mayor densidad celular en BA.

Un resultado inesperado, pero interesante, se observó en el grupo TXCB, donde los trasplantes también redujeron la severidad de los eventos BA, aunque menos marcadamente que en el grupo TXOX. Tejido cerebelar trasplantado fue capaz de reducir la frecuencia de eventos BA en 43.68 % sin

observarse cambios en la duración de los episodios. Asimismo, los animales del grupo TXCB tuvieron una reducción de 52.23 % en el tiempo pasado en BA.

Dentro de nuestro arreglo experimental, el grupo TXCB se empleó como un control del trasplante, en donde se utilizó tejido cerebelar que no contendría células orexinérgicas. De este modo, el trasplante realizado en el grupo TXCB nos permite, por un lado saber que el efecto observado no se debe totalmente a actividad orexinérgica, pero por otro, al ser de composición heterogénea, nos impide saber específicamente qué células son las que están contribuyendo. Sin embargo, al tomar en cuenta que el cerebelo es rico en GABA y glutamato, dos neurotransmisores que hemos visto juegan un papel importante en la regulación del CSV, es plausible que el trasplante contuviera uno u ambos, y que el efecto observado esté relacionado a estos.

Vale la pena, además, recalcar que para todos los trasplantes (TXOX y TXCB) se utilizó la misma concentración de trabajo con el propósito de mantener las condiciones experimentales similares, por lo que, si en el futuro se llegara a trabajar con trasplantes más grandes en el grupo TXOX, necesariamente se debe hacer el ajuste para el TXCB.

En resumen, con los resultados obtenidos, sostenemos que se pueden considerar como una prueba de concepto para los trasplantes en la narcolepsia. Esto debido a que los resultados descritos respaldan la idea de que los trasplantes celulares tienen la capacidad de mejorar el fenotipo narcoléptico. Más aún, no necesariamente requieren ser trasplantes orexinérgicos para promover mejorías en uno de los síntomas que se considera más incapacitante: la cataplejía.

Aun cuando estos resultados preliminares son alentadores, hay aun un largo camino por recorrer antes de poder imaginar a los trasplantes como opción terapéutica. Entre lo que queda pendiente, está la evaluación de un injerto de mayor tamaño conformado por células troncales inducidas, y la persistencia de los efectos en el tiempo.

Además, deben explorarse otros sitios de trasplante para poder determinar cuál sería el de máximo efecto, y en este sentido el siguiente sitio a estudiar debiera ser el HL donde tienen su asiento natural las células orexinérgicas. En este sitio el mayor reto es que las células sean capaces de extender proyecciones a los múltiples y distantes núcleos que naturalmente inervan las orexinas. Pero de ser posible, teoréticamente resultarían en un alivio integral del fenotipo.

Por otro lado, los resultados observados en el grupo TXCB, sugieren que trasplantes no-orexinérgicos tienen la capacidad también de mejorar algunos aspectos del fenotipo, esto podría ser interesante de estudiar ya que podría evaluarse la posibilidad de utilizarlos para mejorar aspectos del fenotipo narcoléptico a sabiendas de que el beneficio es sobre síntomas particulares, como fue en este caso el número de eventos de BA.

Ahora bien, a pesar de que somos optimistas con respecto a las implicaciones clínicas que estos estudios puedan tener a futuro, todavía quedan muchos obstáculos que sortear como el hecho de que la evidencia sugiere que la narcolepsia tiene un origen autoinmune, lo que significa que debe evaluarse la respuesta inmune al trasplante si es que se pretende que sea exitoso. Hay además un obstáculo más tangible, donde debe tomarse en cuenta que los trasplantes requieren una neurocirugía, un procedimiento invasivo que acarrea riesgos que deben ser sopesados con cuidado.

Conclusiones

La evidencia disponible indica que la terapia de reemplazo orexinérgico es una opción terapéutica viable para el tratamiento de la narcolepsia. En este sentido, se ha trabajado arduamente en el desarrollo de agonistas orexinérgicos capaces de permear la barrera hematoencefálica, y en los últimos años algunas de estas moléculas han avanzado a pruebas clínicas. A pesar de que probablemente los agonistas orexinérgicos demuestren ser un tratamiento eficaz para la narcolepsia, su actuación continuará siendo sintomática. Una alternativa que también aborda al

sistema orexinérgico, pero que ofrece la oportunidad de ser un remedio duradero e integral, es el trasplante de células orexinérgicas. Las dificultades a las que se enfrenta esta perspectiva incluyen desde sobrevivencia de trasplante, hasta lo invasivo de la técnica. Sin embargo, en este primer acercamiento hemos demostrado que un pequeño trasplante es capaz de revertir parcialmente el fenotipo narcoléptico de animales orexina/ataxina-3, destacando la notable mejoría en la severidad de los eventos catapléjicos.

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Anexos

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Research report

Orexin cell transplant reduces behavioral arrest severity in narcoleptic mice

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HIGHLIGHTS

- Orexin cell grafts show promise for reducing the narcoleptic phenotype.
- The use of flow cytometry allows for successful grafts.
- Grafts from cerebellar tissue also reduced behavioral arrest severity.

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ABSTRACT

Narcolepsy is a sleep disorder that has been associated with the loss of orexinergic neurons from the lateral hypothalamic area. This loss leads to dysregulated sleep and cataplexy attacks. Therapeutic options are currently limited to symptom management with pharmacotherapy and nonpharmacological approaches. Nonetheless, cell replacement therapy could offer relief, and research in the field has yielded positive results for other neurodegenerative disorders, such as Parkinson's disease. Thus, we propose that orexin cell rich grafts could help improve narcoleptic symptoms in the orexin/ataxin-3 mouse model of narcolepsy. For this purpose, we isolated EGFP + cells from either orexin/EGFP or CAG-EGFP mice with the use of a flow cytometer and grafted them into the pedunculopontine and laterodorsal tegmentum nuclei (PPT/LDDT) of orexin/ataxin-3 mice. Our results show that even small orexinergic grafts can reduce the severity of behavioral arrests, with a median reduction of 30.31% in episode duration, 51.35% for number of events and 69.73% in time spent in the behavioral arrest state and help with sleep fragmentation measured in number of bouts per behavioral state. Surprisingly, control grafts made from cerebellar tissue also reduced behavioral arrest severity, but to a lesser degree. Although still at a very early stage, these results show that there is potential in cell grafts for improving aspects of the narcoleptic phenotype and further research could help elucidate realistic expectations of an orexin cell replacement therapy for narcolepsy.

1. Introduction

The orexinergic system (alternatively known as the hypocretinergic system) is comprised by relatively few somas located in the lateral and dorsal hypothalamic areas that extensively innervate the central nervous system (Peyron et al., 1998). These hypothalamic orexin cells produce two excitatory peptides, orexin A and B, that exert their action by binding to two G coupled receptors, the OXR1 and OXR2. Orexinergic activity has been observed to help sustain wakefulness by stimulation of wake-promoting neurons, including those that conform the

Abbreviations: ARAS, ascending reticular activating system; BCI, Bayesian Credible Intervals; BA, behavioral arrest; IQR, interquartile range; PPT/LDT, pedunculopontine and laterodorsal tegmental nuclei; NB, narcolepsy-basal; NREM, non-rapid eye movement; WT, orexin/ataxin-3^{-}; TXOX, orexin transplant; TXCB, transplant control

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ascending reticular activating system (ARAS), such as the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) of the mesopontine tegmentum (Mahoney et al., 2019; Weber and Dan, 2016). Conversely, lack of orexinergic input has been associated with the development of narcolepsy, a chronic sleep disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis (American Academy of Sleep Medicine, 2014).

Traditionally, management of narcoleptic symptoms has been achieved with a combination of pharmacological and non-pharmacological approaches. Pharmacotherapy includes stimulants (modafinil, methylphenidate, and some types of amphetamines), antidepressants (such as tricyclic antidepressants and serotonin and norepinephrine reuptake inhibitors) and sodium oxybate (Bassetti et al., 2019; Scammell, 2017). Although evidence supports the notion that stimulation of the orexinergic system improves narcoleptic phenotypes (Mieda and Sakurai, 2013), an orexin-based therapy is still absent from the clinical setting. One of the mayor obstacles to bypass has been the blood brain barrier, nonetheless intracerebroventricular, intrathecal, intranasal and intraperitoneal, administration routes have been tested and indicate that orexin replacement tends to improve narcoleptic symptoms by reducing the number of cataplexic events and consolidating wakefulness in animal models (Baier et al., 2011; De la Herrán-Arita et al., 2011; Hasegawa et al., 2014; Schatzberg et al., 2004). More recently, progress in the field has finally allowed for the synthesis of orexinergic agonists capable of crossing the blood brain barrier, and at least one of them is currently being studied in animal models (Irukayama-Tomobe et al., 2017).

Although pharmacotherapy aimed at the orexinergic system will most likely help narcoleptic patients, therapeutic effect is bound to patience adherence. Alternative approaches for orexin replacement therapies include gene therapy and stem cell and orexin cell transplants (Dauvilliers et al., 2014; Takenoshita et al., 2018). The rationale behind cell transplants raises from the idea that if grafted cells survive and integrate into existing circuits, they could potentially produce and release the missing proteins in a manner that resembles the naturally occurring liberation in healthy individuals. Evidence from research carried out in the field for other neurodegenerative disorders, such as Parkinson's disease (Boronat-García et al., 2016; Kefalopoulou et al., 2014), suggests that this is possible. Overall, there are few reports that focus on orexinergic cell transplants (Arias-Carrión et al., 2006, 2004; Arias-Carrión and Murillo-Rodríguez, 2014), and although they report graft survival and improved narcoleptic symptoms, there are concerns regarding the model used (lesion with HCRT2/sap), and the cell population (non-specific hypothalamic tissue) utilized. Additionally, it is posible that grafts aimed at the PPT/LDT nuclei in the mesopontine tegmentum could yield better results, as evidence indicates that stimulation to this region can promote wakefulness (Kroeger et al., 2017) and that direct orexin infusion to the site can prevent the unfolding of cataplexic events (Takakusaki et al., 2005). In the present study we tested whether orexin cell transplants aimed at the PPT/LDT nuclei can improve the narcoleptic phenotype of the orexin/ataxin-3 mouse model of narcolepsy.

2. Results

2.1. The presence of the orexin/ataxin-3 transgene is linked to orexinergic denervation

For each animal from the orexin/ataxin-3 line, we carried out PCR analysis for sorting into experimental groups and immunocytochemical verification of the orexinergic denervation at the end of experimental protocols. Results confirm that adult mice carrying the orexin/ataxin-3 transgene display an evident absence of orexin somas in the lateral hypothalamic area versus the normal distribution observed in their orexin/ataxin-3$^-$ littermates (WT) (Fig. 1).

2.2. Orexin/EGFP cells are notoriously difficult to isolate

To maximize the viability of the cell transplants, we added DAPI to the sample prior to flow cytometry analysis; this allowed us to discard compromised cells before isolating EGFP$^+$ cells. With this process, the mean number of DAPI$^-$/EGFP$^+$ cells obtained from the cerebellum of the CAG-EGFP mouse line was $\bar{x}$ = 994, 000(SD = 243, 269.4), and from the lateral hypothalamus of orexin/EGFP mice was $\bar{x}$ = 17, 160(SD = 6, 129.03).

2.3. Orexin cell transplants reduced sleep fragmentation

Since mice are most active after lights-out, and most behavioral alterations are evident during this period, we sampled 8 h of electrophysiological data starting at lights-out and performed Kruskal-Wallis and Dunnett's post-hoc tests on several common sleep parameters.

The parameters evaluated consisted in total and hourly time spent in WAKE, NREM and REM sleep, episode duration of each behavioral state, the total number of bouts during the 8 h period, the latency to NREM and REM sleep, and the overall number of transitions per group. The hourly analysis is presented in Fig. 2 while the rest of the results are summarized in Table 1. Overall, narcoleptic groups (narcolepsy basal NB, sham, transplant control TXCB, and orexin transplant TXOX) displayed altered sleep, with several parameters scoring higher than those of the WT group. Noteworthy is the number of bouts of WAKE, NREM and REM sleep of the TXOX group, as scores are in the same range than those of the WT group, indicating a less fragmented sleep. For other parameters, significant differences indicate that the narcoleptic phenotype was not restored, such as in the case of the amount of REM sleep both across the 8 h (Table 1) and for some hours of the night (solid shapes in Fig. 2).

2.4. Behavioral arrest severity is reduced in the group treated with orexinergic cells

For the analysis of behavioral arrests (BAs), we fitted three Bayesian multilevel models and analyzed episode duration (Fig. 3), BA events per hour (Fig. 4) and percentage of time spent in BA (Fig. 5). Analysis of video footage obtained from the WT group demonstrated that intact animals do not display behaviors that meet the criteria for BA, thus comparisons were carried out against the NB group. Table 2 summarizes the findings of the Bayesian analysis.

Results of our Bayesian analysis, show that BAs in the TXOX varied the most, with notable reductions in episode duration (95% Bayesian Credible Intervals BCI [−0.64, −0.07]), number of events (95% BCI [−1.12, −0.35]) and percentage of time spent in the BA state (95% BCI [−1.82, −0.71]), furthermore the median reductions consisted of 13.34 s (30.31%), 7.49 (51.35%) events and 3.42% (69.73%) of time spent in BA, respectively. Unexpectedly, the TXCB group also displayed reductions in two parameters: number of events (95% BCI [−0.99, −0.2]) and percentage of time spent in BA (95% BCI [−1.30, −0.27]), in this case, median drops consisted of 6.37 (43.68%) events and 2.56% (52.23%) of time spent in BA, respectively.

3. Discussion

Cell transplants for the treatment of neurodegenerative diseases is not a new field of research. Factors such as cell source, survival and integration to existing circuits, mean they are a complicated therapeutic approach. Nonetheless, technological advances have improved the odds for cell replacement therapy to succeed, and positive results have already been reported for other disorders such as Parkinson's disease. Regarding narcolepsy, there is only one report, albeit with positive results (Arias-Carrión and Murillo-Rodríguez, 2014). The results of this report face two main concerns: a graft composed of a heterogenic cell suspension from hypothalamic tissue and a narcolepsy model achieved

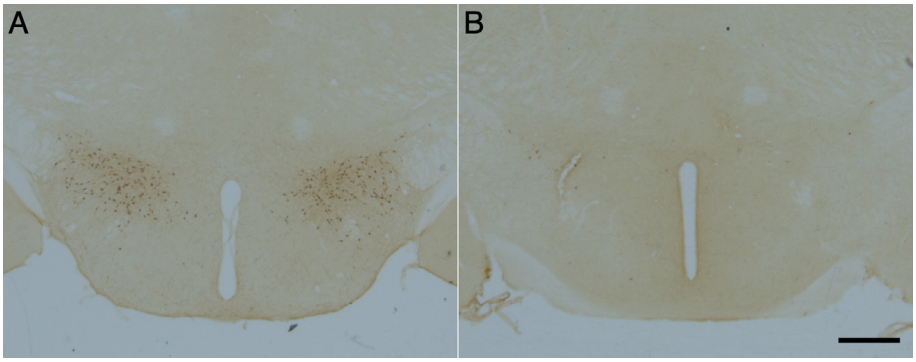

Fig. 1. Immunohistochemical verification of orexinergic denervation. A) Orexin/ataxin-3 - animals have intact orexinergic signal, including cell bodies in the hypothalamic area. B) Orexin/ataxin-3+ animals lack orexinergic signal in this same area. Scale bar = 500 μm.

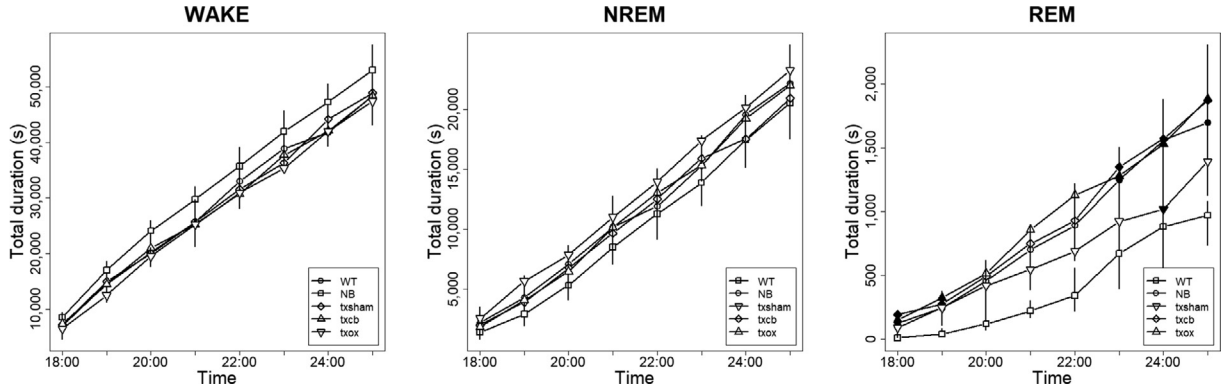

Fig. 2. Hourly analysis of total time spent in Wake, NREM and REM. Electrophysiological recordings (EEG/EMG) started at lights-out and lasted for 8 continuous hours, data is presented in cumulative times. Solid black figures represent significant differences ($p < 0.05$; Kruskal-Wallis with Dunnett's post-hoc tests) when compared to the wildtype (WT) group. For the orexin/ataxin-3$^-$ (WT), narcolepsy-basal (NB), sham, and orexin transplant (TXOX) n = 6, and for transplant control (TXCB) n = 7.

by lesion of the lateral hypothalamus of rats using the toxin saporin coupled to the orexin B peptide (hcrt2/sap). While heterogenic grafts make it difficult to link the observed effects to orexinergic activity, the lesion model employed is not specific of orexinergic cells, thus does not accurately recreate the pathogenesis of narcolepsy. In this report, we attempted to bypass both drawbacks by using a cell suspension rich in orexinergic cells for grafts, and mice of the well-established orexin/ataxin-3 mouse model of narcolepsy as hosts.

Regarding our results, analysis of the electrophysiological data we collected showed that transplants into the region of the PPT/LDT nuclei only modestly modified sleep parameters. Of all the parameters we analyzed, the number of bouts for wake, NREM and REM of the TXOX group appeared to be in the range of the WT group, indicating a less fragmented sleep. We believe that the modest effect of the transplants

Table 1
Analysis of sleep parameters for 8 h of sleep recordings starting at lights-out.

Total time (min)										
	WT		NB		SHAM		TXCB		TXOX	
Wake	294.1	(275.1, 319.55)	268.4	(245.2, 297)	263.3	(243.45, 276.85)	275.8	(265.2, 292)	268.7	(239.9, 285.8)
NREM	171.1	(146.1, 187.7)	185	(158.25, 201.55)	194	(181.55, 203.3)	172.8	(169.1, 181.1)	183.5	(169, 211.65)
REM	16.2	(12.3, 18)	*28.3	(26.25, 31.55)	23.2	(18.8, 33)	**31.2	(27.85, 38.45)	*31.4	(26.1, 37.6)
Episode duration (s)										
Wake	84	(36, 540)	96	(36, 312)	84	(48, 294)	72	(36, 372)	108	(36, 372)
NREM	192	(96, 360)	***132	(72, 264)	***156	(78, 246)	***132	(72, 216)	***156	(84, 288)
REM	60	(36, 96)	60	(36, 96)	60	(36, 96)	60	(36, 96)	72	(48, 96)
Latency (min)										
NREM	4.2	(2.9, 14.65)	1.2	(0.45, 9.75)	3.8	(3.05, 14.9)	6.4	(2.65, 15.25)	8	(4.45, 10.95)
REM	87.7	(44.85, 149.3)	28.2	(7.45, 35.75)	35.8	(15.75, 79.4)	14.4	(12.7, 25.9)	43.9	(23.4, 45.05)
Number of bouts										
Wake	38	(34.74, 41.25)	*47	(42.25, 68.75)	*55.5	(48.25, 68.75)	*60.5	(51.5, 71)	51.5	(49.5, 58.75)
NREM	39	(36.25, 41)	*51.5	(50.25, 73)	*57	(51.75, 74.5)	**64.5	(50.75, 74.5)	53	(50, 59)
REM	14.5	(10, 17.5)	22	(20.25, 27.5)	16.5	(15.25, 25.25)	*25	(23.25, 30.5)	21.5	(18.75, 28)
Total number of transitions										
8 h	85.5	(84.25, 94.25)	*117.5	(115, 170.25)	*127	(122.5, 154)	**157.5	(128.75, 169.75)	*127.5	(114.25, 140)

All statistical values are expressed as medians with their corresponding 25–75 interquartile (IQR) range. Comparisons were carried out using the wildtype (WT) group as control and significant differences are indicated with asterisks ($p < 0.05$*, 0.01**, 0.001***; Kruskal-Wallis with Dunnett's post-hoc tests). NB: narcolepsy basal; TXCB: transplant control; TXOX orexin transplant.

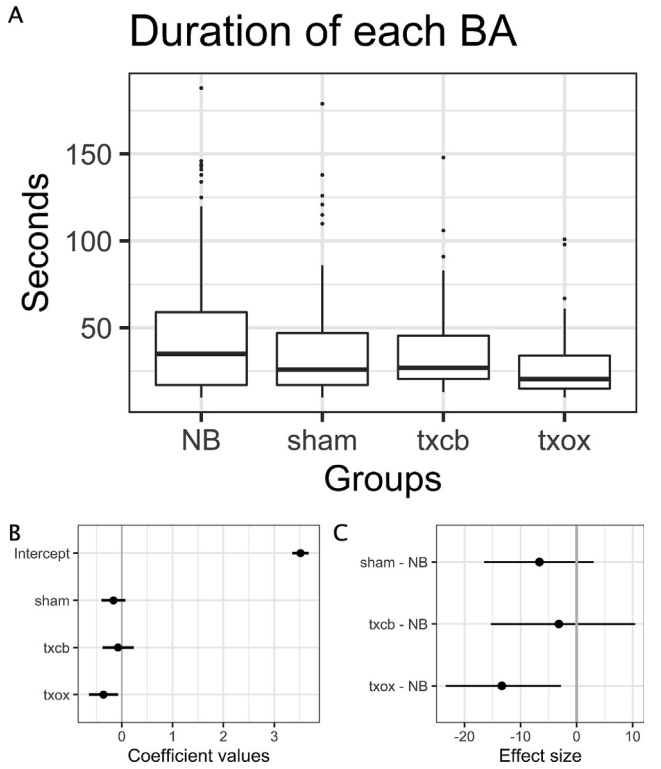

Fig. 3. Median duration of Behavioral Arrest. A Bayesian model was fit for the analysis of behavioral arrest (BA) episode duration, all video recordings started at lights out. Results indicate that the orexin transplant (TXOX) notably reduced the length of BA episode duration by 30.31% when compared to the narcolepsy-basal (NB) group. A) Distribution of BA episode duration among groups, vertical lines indicate medians, whiskers indicate the first and third quartile; B) Coefficient values for episode duration indicate notable variations when the distribution doesn't cross the cero mark; and C) Effect size of treatment on episode duration indicates the magnitude of change. Group size n = 6 for NB, sham and TXOX, and n = 5 for transplant control (TXCB).

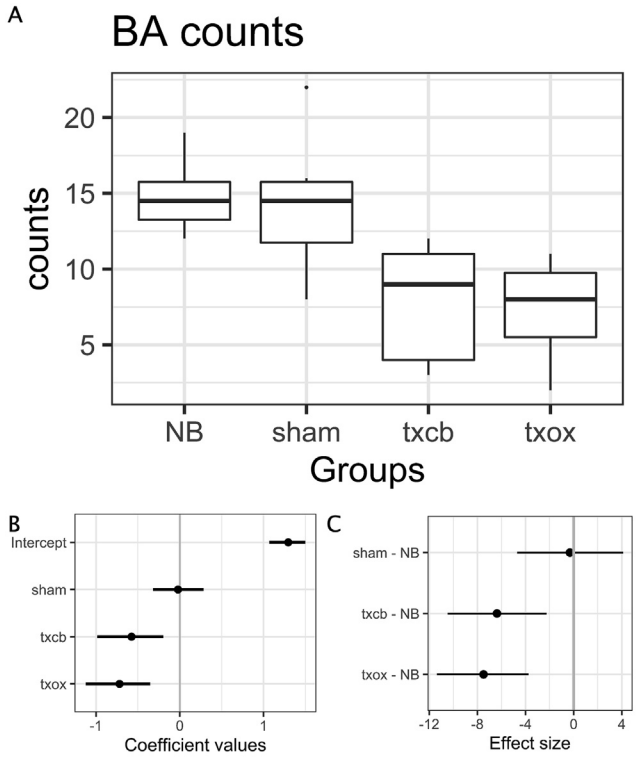

Fig. 4. Median number of Behavioral Arrests per hour. A Bayesian model was fit for the analysis of number of behavioral arrest (BA) per group, all video recordings started at lights out. Results indicate that both the transplant control (TXCB) and orexin transplant (TXOX) notably reduced the BA episode duration by 43.68% and 51.35% respectively, when compared to the narcolepsy-basal (NB) group. A) Distribution of BA events per group, horizontal lines indicate median values, while whiskers indicate the first and third quartile; B) Coefficient values for number of BA events indicate notable variations when the distribution doesn't cross the cero mark; and C) Effect size of treatment on BA events indicates the magnitude of change. Group size n = 6 for NB, sham and TXOX, and n = 5 for TXCB.

of sleep parameters, could be, in part, related to the small size of the grafts. We decided in favor of performing small grafts (2,000 cells in 0.25 μL) due to the low yield of orexinergic cells ($DAPI^{-}/EGFP^{+}$) obtained after harvesting the hypothalamic area of donor pups and sorting through flow cytometry. This result is most surely influenced by the small size of the orexinergic system, where the population has been estimated to be between 1,100 and 4,000 cells per rodent brain (Kilduff and Peyron, 2000; Peyron et al., 1998). While increasing the amount of pup donors could increase the yield, we believe that the increase would be moderate and not enough to outweigh the bioethical issues of increasing the number of donors, therefore a different source of cells is required to be able to work with a larger orexinergic graft. Fortunately, stem cell research could offer a better source, as some groups have already reported success developing induced orexinergic cells while working with embryonic stem cells from mice (Hayakawa et al., 2013) and humans (Merkle et al., 2015).

On the other hand, despite the use of small grafts, analysis of video footage demonstrated that in the TXOX and TXCB groups, BA severity was notably reduced. Because we did not observe any considerable change in BA manifestation in the sham group when compared to the NB group, we believe that these observed effects are most likely related to the grafts performed.

In the TXOX group the results we obtained, show that grafts performed with a small population of orexinergic cells reduce the duration (by 30.31%) and frequency (by 51.35%) of BA events, in a way that outperformed grafts made of cerebellar tissue (TXCB). Overall, animals that received orexin rich grafts spent 69.73% less time in the BA state than those of the untreated NB group. In line with the discussion of results from the analysis of electrophysiological data, we believe that the results observed in the TXOX group could be improved with the use of a larger graft.

Unexpectedly, cerebellar grafts (TXCB) also reduced BA severity, although to a lesser degree than when grafts were performed with orexinergic cells (TXOX). We found that cerebellar tissue can reduce the frequency (by 43.68%) of BAs but does not appear to alter their duration, in line with this result, animals of the TXCB group showed a median reduction of 52.23% of time spent in the BA state when compared to the NB group. As we used the TXCB group as a no-orexin control, heterogenic cerebellar cell suspensions were used for grafting, therefore we do not have specific information of the cell types that conformed these grafts. Nonetheless, the cerebellum is rich in GABA and glutamate, two neurotransmitters that also play a role in the regulation of the sleep-wake cycle, and it is therefore plausible that our transplants contained both. In addition, TXCB grafts were the same size as those performed in the TXOX group, thus we would also suggest that larger grafts should be tested.

Overall, we are confident our findings offer proof-of-concept for the notion that cell grafting has the potential of improving the narcoleptic phenotype. There is still much more that remains to be determined, such as the effect of larger grafts of both cell types, and the evaluation of different time periods and graft sites. For the testing of different graft sizes, we believe larger orexinergic grafts will need to be obtained from a different source than the one described here, for that instance

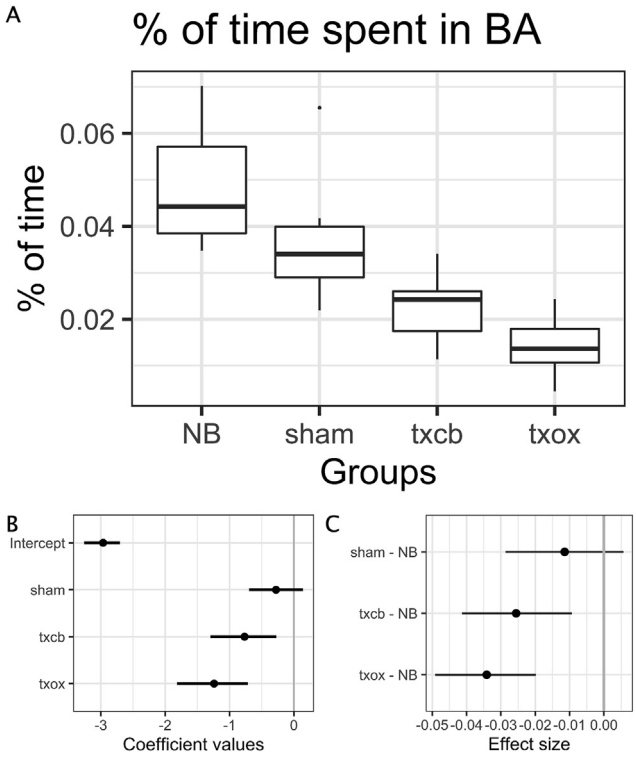

Fig. 5. Percentage of time spent in Behavioral Arrests. A Bayesian model was fit for the analysis of the overall time spent in the behavioral arrest (BA) state per group, all video recordings started at lights out. Results indicate that the transplant control (TXCB) and orexin transplant (TXOX) notably reduced the amount of time animals spent in BA when compared to the narcolepsy-basal (NB) group, furthermore the reduction was accentuated in the TXOX (69.73%) over the TXCB (52.23%) group. A) Distribution of time spent in BA per group, horizontal lines indicate median values while box whiskers indicate the first and third quartiles; B) Coefficient values of time spent in BA per group indicate notable variations when the distribution doesn't cross the cero mark; and C) Effect size of treatment on percentage of time spent in BA per group indicates the magnitude of change. Group size n = 6 for NB, sham and TXOX, and n = 5 for TXCB.

Table 2
Behavioral Arrest analysis results.

Group	Episode duration (s)		Number of events		% time spent in BA state	
NB	35	(17, 59)	14.5	(13.25, 15.75)	4.42%	(3.85%, 5.71%)
SHAM	26	(17, 47)	14.5	(11.75, 15.75)	3.40%	(2.9%, 4%)
TXCB	27	(20.5, 45.5)	*9	(4, 11)	*2.42%	(1.75%, 2.6%)
TXOX	*20.5	(15, 34)	*8	(5.5, 9.75)	*1.36%	1.07%, 0.72%)

All statistical values are expressed as medians with their corresponding 25–75 interquartile (IQR) range. Comparisons were carried out using the narcolepsy basal (NB) group as control and notable differences (95% Bayesian Credible Interval) are indicated with asterisks.

embryonic stem cells seem plausible at this point. For cerebellar grafts, the same protocol described in this report could allow for larger grafts, although it would be worthwhile to further purify the cell suspension as to find the cell population that has the most contribution to the observable effect.

Another set of interesting studies would involve grafts into the lateral hypothalamic area, the naturally occurring site for orexinergic cells. Here, the main challenge would be for grafted cells to extend their projections to the many and distant nuclei where orexins are released; nonetheless, if this was to be achieved, it would theoretically offer the most complete recovery.

As it can be inferred, regenerative therapy for narcolepsy is still very hypothetical and nowhere near the clinical setting. Moreover, even if all results were to provide strong evidence in favor of cell replacement therapy for narcolepsy, concerns related to the autoimmune hypothesis of narcolepsy and the invasiveness of the approach remain and would require to be addressed.

4. Conclusions

Research suggests that orexin replacement therapy is a promising area for the treatment of narcolepsy. While the development of orally active orexinergic agonists would remain a symptomatic approach, cell transplants could offer a long-lasting therapeutic effect. Graft survival is one of the main concerns for cell transplants, nonetheless the orexinergic system is small, suggesting it does not require many cells to perform its function. In line with this, we have found that a small graft can reduce the severity of BA in orexin/ataxin-3 narcoleptic mice. Further research should be carried out to determine the graft that can best improve the observed effects and in addition restore other sleep parameters as well.

5. Methods

5.1. Animals

Experimental protocols were revised and approved by the local ethics committee (CICUAL) under the project number RDC111(13)-17, adhering to national and international standards of animal care. All animals were kept at our *vivarium* under standard housing conditions of 12 h light/dark cycles, 22 °C temperature and humidity 40–70% with *ad libitum* access to food and water. Animals were initially housed collectively (2–4 animals per cage), and then individually after surgeries were performed.

For the experiments described in this report we worked with the orexin/ataxin-3, orexin/EGFP and CAG-EGFP lines of transgenic mice. Animals of the orexin/ataxin-3 are born with intact orexinergic systems and progressively suffer orexinergic degeneration (Hara et al., 2001), for this reason they are used as a model of narcolepsy. Orexin cell suspensions used for grafts were obtained from orexin/EGFP mice, a strain that expresses EGFP solely in orexin-producing cells (Yamanaka et al., 2003). Control grafts were performed with cell suspensions composed of cerebellar tissue of CAG-EGFP mice, a strain that has widespread EGFP fluorescence. Founder orexin/ataxin-3 and orexin/EGFP mice were donated to our institution by Dr. Kilduff and Dr. Van den Pol, respectively, while CAG-EGFP mice were obtained from the local animal facility. Donated mice were initially bred with C57BL/6 mice obtained from our animal facility and then endogamically reproduced.

5.2. Experimental groups

PCR analysis was carried out using tail DNA to identify the presence of the orexin/EGFP and orexin/ataxin-3 transgenes using the HOXPRO N 5′- GCAG CGGC CATT CCTT GG and HOXPRO A 5′- AAGT CGAC GGTG TCTG GCGC TCAG GGTG primer sequences, while CAG-EGFP pups were observed under an epifluorescence microscope to determine transgenic status.

According to results of PCR analysis, a total of 49 orexin/ataxin-3 mice were sorted into the following groups: orexin/ataxin-3^{+} mice were used for the narcolepsy-basal (NB), sham transplant (sham), transplant control (TXCB) and orexin transplant (TXOX) groups, while orexin/ataxin-3^{-} animals were used as wildtype controls (WT). All groups were implanted with electrodes for sleep studies while only the sham, TXCB and TXOX groups were infused with either supplemented medium (sham) or cell suspensions (TXCB and TXOX). To illustrate the

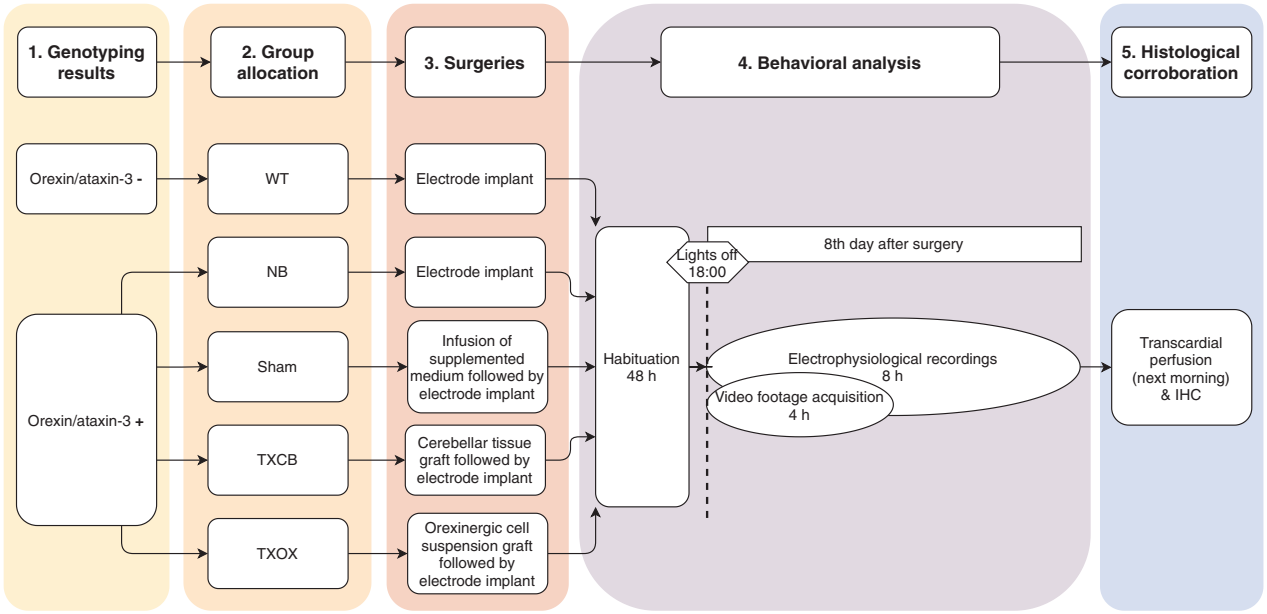

Fig. 6. Experimental flow. For the experiments carried out, we first genotyped all animals of the orexin/ataxin-3 line (1) and according to the transgenic status, positive vs negative, animals were sorted into five experimental groups (2). Surgeries for electrode implantation and grafts were performed exclusively in adult male animals (3) and after a convalescence period, on the eight day after surgeries, behavioral studies were registered (4). The next morning following recordings, all animals were transcardially perfused for immunohistochemical (IHC) verification of transgenic status (5). WT wildtype; NB narcolepsy basal; TXCB control transplant; TXOX orexin control.

Table 3
Individuals analyzed per study per group.

Group Name	Animals per group	Animals per evaluation	
		Electrophysiological recordings	Behavioral arrest analysis
Wildtype (WT)	10	6	5
Narcolepsy basal (NB)	9	6	6
sham	10	6	6
Transplant control (TXCB)	8	7	5
Orexin transplant (TXOX)	12	6	6

experimental flow, we refer to Fig. 6, and for details regarding the number of individuals used for each analysis to Table 3.

5.3. Preparation of cell suspensions

Transplants were carried out using EGFP$^+$ cell suspensions obtained from either the cerebellum of CAG-EGFP animals or the lateral hypothalamic area of orexin/EGFP pups (8–10 days). For collection of the lateral hypothalamic area, brains were placed ventral side up in an acrylic matrix and 2 mm coronal sections were taken around the optic chiasm; the slices were then placed on the surface of an ice cold petri dish and with the help of an Leica EZ4 D stereo microscope and a scalpel, the tissue surrounding the ventral 3rd ventricle was obtained. Collected brain tissue was suspended in an ice-cold mixture of PBS (Sigma; D8537) and 100 U/mL Penicillin-Streptomycin (Sigma; P3539). Brain tissue was enzymatically dissociated using trypsin 2.5% (Gibco; 15090046) and DNAse 1.5 mg/mL (Sigma; DN-25), filtered through a 40 μm mesh, and retrieved in PBS for posterior analysis in the flow cytometer. DAPI$^-$/GFP$^+$ cells were selected using a MoFlo XDP (Beckman-Coulter, IN, USA) cell sorter and retrieved in DMEM (Gibco; 31600034) culture medium supplemented with 10% fetal bovine serum (Sigma; A-7030) and Penicillin-Streptomycin. Cells were then centrifuged, and the suspension volume reduced to achieve a concentration of 8,000 cells/μL. Cell suspension was kept ice cold until stereotaxic surgery.

5.4. Cell transplants and sham injections

Cell grafts were performed on adult (> 10 weeks) orexin/ataxin-3$^+$ males. For this purpose, animals were anesthetized with a 100–10 mg/kg ketamine/xylazine cocktail administered intraperitoneally. Following the atlas of the mouse brain by Paxinos & Franklin (2ed), cell transplants and sham assays were aimed at the PPT/LTD nuclei in the mesopontine tegmentum region at AP −4.5, ML −1.4 and V −3.9 stereotaxic coordinates. With the aid of a Hamilton syringe (Hamilton; 8929C51), a 0.25 μL volume of the cell suspension (TXOX and TXCB groups), or supplemented medium only (sham), behind an equal amount of saline solution was injected at a rate of 1 μL/min (Lee et al., 2008). The syringe was held in place for 5 min and then slowly retrieved for another 5 min.

5.5. Electrophysiological analysis

5.5.1. EEG/EMG surgeries.

All groups were chronically implanted with a custom-made electrode for electrophysiological recording; for sham, TXCB and TXOX groups, electrode implant was carried out after grafting. To monitor electrical brain activity, electroencephalographic data was collected through a screw (PlasticsONE, Roanoke, VA, USA) placed at AP −1.6, ML −1.4 connected to the EEG electrode, and a second screw was placed at AP + 0.6, ML −1, connected to the reference electrode. Electrical muscular activity was monitored through a silver wire (A-M Systems; 786500) inserted into the nuchal muscles and connected to the EMG electrode. Electrodes were fixed in place by mixing a cyanoacrylate-based glue and dental acrylic. After surgery animals received an intramuscular dose of buprenorphine (0.05 – 0.2 mg/kg) were housed individually and allowed to convalesce for at least 5 days.

5.5.2. Sleep-wake recordings setup

On the eight day after surgeries electrophysiological recordings were carried out. For habituation, animals were lightly anesthetized with isoflurane and a lightweight cable was attached to the head-mounted connector and then coupled to a slip ring commutator (Plastics One, Roanoke, Va, USA). Habituation to the setting lasted 48 h and then each mouse was recorded for 8 consecutive hours starting at lights-off. EEG/EMG signals were amplified using a Grass Model 78 polygraph (Grass Instruments, West Warwick, RI, USA) and filtered (EEG: 0.3–100 Hz. EMG: 30–300 Hz). Signals were digitized with a data acquisition device (National Instruments, Austin, TX, USA) at a sampling rate of 128 Hz

5.5.3. Analysis of sleep recordings

Data was scored off-line using the software ICELUS (University of Michigan) into 12 s epochs of Wake, NREM and REM stages in agreement with the parameters described elsewhere (Murillo-Rodríguez et al., 2017; Radulovacki et al., 1984). In total, there were n = 6 individuals for the WT, NB, sham and TXOX groups, and n = 7 for the TXCB group.

After sleep scoring, we used the Kruskal-Wallis and the Dunnett post-hoc tests to find differences relative to the WT group for total and hourly time spent in WAKE, NREM and REM, latency of NREM and REM, bout duration and number of bouts for WAKE, NREM and REM, and total number of transitions from one state to another.

5.6. Behavioral arrest evaluation

5.6.1. CCTV setup

For the evaluation of BAs, a CCTV infrared recording system was set up to monitor animals starting at lights-off on the eight day after grafting and/or EEG/EMG surgery. Videos were manually scored by identifying BA in accordance with the consensus definition reached by Scammell et al (Scammell et al., 2009). In short, BA was considered when there was an abrupt cessation of locomotor activity that lasted at least 10 s, after a period of at least 40 s of sustained wakefulness. For the duration of the video, behaviors were logged into one of the following categories: eating, climbing, drinking, grooming, otherwake and sleep. We analyzed 6 individuals for the sham, NB and TXOX groups, and 5 for the WT and TXCB groups; for all animals we obtained at least 3 h and up to 4 h of video footage; these differences were considered in the Bayesian analysis.

5.6.2. Bayesian analysis of behavioral arrests

Group differences were analyzed using Bayesian multilevel models. These analyses were carried out using the R language (Team, 2018) by means of the brms package (Bürkner, 2017) which is a high level interface to the Stan probabilistic programming language (Carpenter et al., 2017). Bayesian models do not have p-values as is common in a frequentist approach, instead, to test whether regression coefficients are different from zero, BCI are used. Aside from the interpretation of uncertainty, interpretation of regression coefficients doesn't differ much from frequentist models.

For the analysis of treatment effect on BAs three models were fitted. All models showed a good convergence and fit.

1. A model fit to predict BA duration, with response distribution set to log-normal, with the variable group as the population-level parameter of interest and a varying-subject intercept, following the terminology of (Bürkner and Vuorre, 2019).
2. A model to predict the total number of BAs per sampled hour as the total sampled time varied slightly for each subject. Response distribution is set to Poisson, also with the variable group as the population-level parameter of interest. But as what is modeled are rates, we introduced a log(hours) offset.
3. A model to predict the percentage of total time spent in a BA state. As this response is limited to 0 and 1, it was set to be a Beta distribution. Again, the variable group is the population-level parameter of interest.

For model specifications and code that can be used to replicate the results, please refer to the Supplementary materials.

5.6.3. Immunohistochemical postmortem verification

Following sleep-wake recordings, all animals were sacrificed with a lethal dose of pentobarbital and transcardially perfused with 0.1 M PBS and 4% PFA (Sigma; P6148). Brains were dissected from the cranium and post-fixed overnight consecutively in each of the following: 4% PFA, 10%, 20% and 30% sucrose dissolved in 0.1 M PBS. All samples were sectioned into 40 μm slices with a Leica CM1900 cryostat (Leica Microsystems, Weztlar, Germany). Consecutive coronal sections comprehending the extent of the ventral 3rd ventricle were collected successively in four wells with PBS; sections were rinsed again in PBS and then stored at −20 °C in antifreeze (made of 50% PBS, 25% ethylene glycol and 25% glycerol) until immunohistochemical procedures were carried out.

For postmortem verification of the orexin/ataxin-3 mouse status, one well from each mouse was selected and rinsed (three 5 min rinses with PBS). Endogenous peroxidase activity was blocked with 0.28% periodic acid for one minute and rinsed. Blocking of off-target sites was done with a 2.5% albumin (Sigma; 9048-46-8) and 0.1% Triton X-100 (Sigma; 9008-93-1) in PBS blocking solution overnight at 4 °C and followed with an overnight incubation at 4 °C in primary antibody rabbit-α-orexin A (1:2000 dilution; Phoenix Pharmaceuticals Cat# H-003-30, RRID:AB_2315019). Sections were then rinsed and incubated in the secondary antibody, biotinylated goat-α-rabbit (1:250 dilution; Vector Laboratories Cat# BA-1000, RRID:AB_2313606), for 2 h at room temperature. Sections were rinsed before a 30 min incubation in Vectastain (Vector Labs; PK-6100), rinsed again and finally revealed using diaminobenzidine (Sigma; D5637) for 5 min. After rinsing, sections were mounted on slides, dehydrated and protected with Cytoseal XYL (Richard-Allan Scientific; 8312-4) mounting media. All treated sections were examined under a Leica EZ4D stereoscopic microscope and presence or absence of orexin somas was registered for each animal. Representative images were acquired with an Axio Zoom V16 Zeiss microscope, images were assembled with the ImageJ (Rueden et al., 2017) software using the FigureJ (Mutterer and Zinck, 2013) plug-in.

CRediT authorship contribution statement

Ana Clementina Equihua-Benítez: Investigation, Methodology, Data curation, Writing - original draft. **Julián A. Equihua-Benítez:** Formal analysis, Visualization, Validation. **Khalil Guzmán-Vásquez:** Data curation. **Oscar Prospero-García:** Writing - review & editing, Funding acquisition. **René Drucker-Colín:** Conceptualization, Supervision, Funding acquisition, Resources.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2020.146951.

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CHAPTER

41

Drug Discovery and Emerging Treatments for Sleep Disorders☆

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I INTRODUCTION

Sleep is an often overlooked and underestimated physiological state. However, when the duration or quality of sleep is compromised, health and productivity are negatively impacted. Short-term sleep loss can cause impaired judgment and reduced alertness, while chronic loss has been associated with a higher risk of developing pathological conditions such as obesity, diabetes, cancer, depression, and cardio- and cerebrovascular disease (Watson et al., 2015).

Problems associated with sleep are widespread; available statistical data from countries such as Australia, Canada, the United States, and the United Kingdom indicate that roughly 1/3 of the population have problems in this area (Adams, Appleton, Taylor, McEvoy, & Antic, 2016; Chaput, Wong, & Michaud, 2017; Knutson et al., 2017; The Sleep Council, 2016). Furthermore, the prevalence of clinically significant sleep disorders has been estimated at around 10% (Ram, Seirawan, Kumar, & Clark, 2010).

The main aim of sleep disorder treatment is to consolidate sleep and improve daytime functioning. However, despite advances achieved in the field, physicians are still faced with the difficult challenge of identifying and prescribing treatments that best balance therapeutic effects and drawbacks, including significant side effects, abuse potential, and the development of drug tolerance. In an effort to meet the demand for better therapies, many new pharmacological and nonpharmacological options have recently become available, even though they are also associated with their own limitations. Therefore, a significant research effort continues, with many promising developments currently in preclinical and early clinical stages. In this chapter, we will discuss investigations regarding recently approved and currently developing treatments for the management of the four most common sleep disorders: insomnia, obstructive sleep apnea (OSA), narcolepsy, and restless legs syndrome (RLS).

II SLEEP DISORDERS

A Insomnia: Chronic Insomnia

The high number of people that complain about restless nights makes insomnia an extremely common condition; an estimated 30% of the population has experienced insomnia symptoms at some point, while the prevalence for insomnia disorder has been narrowed down to between 5% and 10% (Ohayon, 2002; Roth, 2007).

In general, difficulty with sleep initiation, duration, or consolidation or obtaining sleep of unsatisfying quality qualifies for insomnia when it leads to some degree of daytime impairment. The International Classification of Sleep Disorder—Third Edition (ICSD-3) recognizes three types of insomnia: short-term, chronic, and other. Short-term insomnia refers to sleep complaints for a period of less than 3 months, while chronic insomnia arises when complaints are present at least three times a week for at least 3 months. The category of "other insomnia" refers to conditions where symptoms of insomnia are present, but the criteria for the other two insomnia types are not fully met (American Academy of Sleep Medicine, 2014).

Although the pathogenesis of insomnia is not well understood, several studies point to insomnia as a

☆ This chapter is dedicated to the memory of Dr. René Drucker-Colín, who passed away on September 17th, 2017. He is still sorely missed.

[a] In memoriam Dr. René Drucker-Colín who passed away on September the 17th, 2017.

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disorder of sustained hyperarousal across the sleep-wake cycle. Evidence obtained using the multiple sleep latency test (MSLT) has shown that insomnia sufferers appear less sleepy than controls during daytime testing (Edinger et al., 2003). Further, several studies have documented elevated measures of physiological arousal in insomnia patients, including heart rate; body temperature; oxygen consumption; and the secretion of cortisol, adrenaline, and adrenocorticotropin (Bonnet & Arand, 2010; Monroe, 1967).

Traditional management options for insomnia include pharmacological and nonpharmacological strategies; their aim is to improve sleep quality and quantity and to reduce or reverse daytime impairment. Table 41.1 summarizes the currently available management options for insomnia.

The American Academy of Sleep Medicine and the American College of Physicians recommend cognitive behavioral therapy for insomnia (CBTi) as the first line of treatment for chronic insomnia (Qaseem et al., 2016; Schutte-Rodin, Broch, Buysse, Dorsey, & Sateia, 2008). CBTi reliably improves sleep by allowing the patient to break away from unhealthy sleeping habits and, when compared with pharmacotherapy, has better efficacy and a longer duration of therapeutic effects (Mitchell, Gehrman, Perlis, & Umscheid, 2012). However, CBTi can be expensive, and sleep health professionals are not always readily available. In an effort to increase the accessibility of CBTi, nonsleep specialists have been trained in CBTi (Manber et al., 2012), and delivery methods have become more diversified. Traditionally, CBTi is carried out in person and individually, but group therapy, computerized systems (either mobile apps, video conference, or web-based modules), telephone, and self-help books have also shown to be effective (Arnedt et al., 2013; de Bruin, Bögels, Oort, & Meijer, 2015).

When symptom relief is suboptimal with an initial intervention of CBTi, the short-term use of pharmacotherapy as a complement can help achieve the desired therapeutic effect. There are a number of pharmacological options for the treatment of insomnia, falling into one of three categories: Food and Drug Administration (FDA)-approved drugs, over-the-counter (OTC) medications, and off-label drugs. Although pharmacological approaches have demonstrated some success, they are not without limitations; the main concerns with pharmacotherapy are tolerance, abuse, allergic reactions, and undesired side effects. Thus, although many options for the management of insomnia exist, research for novel drugs that combine amicable safety profiles and improved therapeutic effects continues. Recently, the FDA has approved three new drugs: ramelteon (2005), doxepin (2010), and suvorexant (2014).

The orexinergic antagonist suvorexant is a particularly noteworthy example of drug discovery in the field of sleep medicine. Suvorexant is the first drug that targets the orexinergic system to be approved by the FDA for the management of a sleep disorder. Cataloged as a dual orexin receptor antagonist (DORA), suvorexant exerts its therapeutic effect by inhibiting the activity of both orexin receptors (OX1R and OX2R) that comprise the system. Despite being received with high expectations, concerns have been raised that it might not be much more effective than other currently available options (Kripke, 2015). Others have argued that the approved dosages of suvorexant fall below the therapeutic effect threshold and that its side-effect profile is harsh (Moore, Cohen, Furberg, & Mattison, 2016). Common side effects associated with the use of suvorexant include those usually observed with other sleep aids (e.g., next-day impairment and abnormal thoughts and behavior) but with the addition of an increased risk of developing sleep paralysis, hypnagogic hallucinations, and cataplexy, all of which are symptoms of another sleep disorder: narcolepsy.

It is reasonable to assume that some of the issues with the use of suvorexant are due to its ability to block both types of orexin receptors. In this regard, the single orexin receptor antagonist (SORA) seltorexant (MIN-202) is currently being studied as an alternative. Seltorexant is a selective OX2R antagonist, currently in phase 2 clinical trials for the treatment of chronic insomnia. Studies carried out in transgenic mouse models demonstrate that the selective blockade of OX2R might be sufficient to promote sleep (Bonaventure et al., 2015). Thus, it is possible that seltorexant could help consolidate sleep in insomnia patients while exhibiting a less aggressive side-effect profile relative to DORAs.

In a novel and innovative insomnia management approach, the FDA has approved a device that aims to relieve insomnia symptoms by maintaining the temperature of the forehead within a specific range. The basis for the Ebb® insomnia therapy stems from functional brain imaging studies that have shown that insomniacs display elevated brain metabolism (Nofzinger et al., 2004). The authors speculated that this higher metabolic rate could cause brain temperature to rise and, in turn, interfere with the optimal physiological conditions for sleep. The proposed thermal system appears to offer proof of concept, since data obtained through clinical trials have shown that the induction of cerebral hypothermia with this device is effective in reducing the latency to nonrapid eye movement sleep stages 1 and 2 in insomnia patients (Nofzinger, Miewald, Price, & Buysse, 2009; Roth et al., 2017). Furthermore, its use appears to be well tolerated, with patients only reporting mild headache as a device-associated adverse effect. The novelty of this product means that information is still scarce, but the increasing availability of temperature-based devices and therapies will allow for a more detailed assessment of their effectiveness and of potential adverse events associated with their use.

In summary, with the approval of the melatonin receptor agonist ramelteon, the antidepressant doxepin,

TABLE 41.1 Therapeutic Options for the Treatment of Insomnia

Nonpharmacological treatments			
Category	**Therapy**	**Description**	**Components**
Behavioral therapy	CBTi[a]	The patient is educated in the techniques necessary for strengthening the association between bed and sleep	Stimulus control, sleep restriction, sleep hygiene, relaxation training, cognitive therapy, sleep diaries, relapse prevention
Devices	Ebb®	The patient wears a head device that cools the temperature of the forehead with the aim of facilitating sleep	Bedside temperature controller, headband, and fluid cartridge

Pharmacological treatments[b]			
Category	**Drug class**	**Generic name (mg/day)**	**Warnings and precautions**
Food and Drug Administration approved	Barbiturates[c]	Butabarbital (50–100) Secobarbital (100)	Severe anaphylactic and anaphylactoid reactions Habit forming Sleep driving and other complex behaviors CNS depressant effects
	Benzodiazepines	Temazepam (7.5–30) Triazolam (0.125–0.5) Estazolam (0.5–2) Quazepam (7.5–15) Flurazepam (15–30)	Concomitant use with opioids carries a risk of profound sedation, respiratory depression, coma, and death CNS depressant effects and daytime impairment Benzodiazepine withdrawal syndrome Severe anaphylactic and anaphylactoid reactions Abnormal thinking and behavior changes Worsening of depression
	Nonbenzodiazepines	Zaleplon (2.5–10) Zolpidem (5–20) Eszopiclone (1–3)	Serious anaphylactic and anaphylactoid reactions Abnormal thinking, behavior changes, and complex behaviors Withdrawal effects CNS depressant effects and next-day impairment
	Melatonin receptor agonists	Ramelteon (8)	Severe anaphylactic and anaphylactoid reactions Abnormal thinking, behavior changes, and complex behaviors CNS depressant effects
	Tricyclic antidepressants	Doxepin (3–6)	Abnormal thinking and behavioral changes Suicide risk and worsening of depression CNS depressant effects
	Orexin receptor antagonists	Suvorexant (10–20)	Daytime impairment Abnormal thinking and behavioral changes Worsening of depression/suicidal ideation Sleep paralysis, hypnagogic/hypnopompic hallucinations, cataplexy-like symptoms
Off-label	Sedating antidepressants[c]	Trazodone (25–100) Mirtazapine (7.5–30)	Among others: Worsening of depression and suicide risk Serotonin syndrome Agranulocytosis
	Atypical antypsychotics[c]	Quetiapine (≤200)	Among others: Weight gain Diabetes mellitus Extrapyramidal effects Neuroleptic malignant syndrome Withdrawal
Over the counter	Antihistamines[c]	Diphenhydramine (50) Doxylamine (25)	Next-day sedation, drowsiness, dry mouth, tolerance

Abbreviations: *CBTi*, cognitive behavioral therapy for insomnia; *CNS*, central nervous system.

[a] *CBTi is the first line of treatment for chronic insomnia.*

[b] *Medication is appropriate for short-term insomnia and as an adjunct to CBTi.*

[c] *Despite their sedating effects, these medications are not recommended for the treatment of insomnia.*

Data obtained from Bonnet, M. H., & Arand, D. L. (2018). Treatment of insomnia in adults. In R. Benca & A. F. Eichler (Eds.), UpToDate (pp. 1–35). Waltham, MA: UpToDate Inc. Retrieved from https://www.uptodate.com/contents/treatment-of-insomnia-in-adults?search=treatment of insomnia in adults&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1; Buysse, D. J., Rush, A. J., & Reynolds, C. F. (2017). Clinical management of insomnia disorder. Journal of the American Medical Association, 318*(20), 1973–1974. doi: 10.1001/jama.2017.15683; Qaseem, A., Kansagara, D., Forciea, M. A., Cooke, M., Denberg, T. D., Barry, M. J., et al. (2016). Management of chronic insomnia disorder in adults: a clinical practice guideline from the American college of physicians.* Annals of Internal Medicine, ***165*** *(2), 125–133. doi: 10.7326/M15-2175.*

the orexinergic receptor antagonist suvorexant, and the recently approved thermal system, insomniacs now have an increasing range of options at their disposal to help improve their quality of life. In addition, seltorexant is a promising compound that could soon become an alternative to suvorexant and offer sleep improvement with fewer undesired side effects.

B Sleep-Related Breathing Disorders: Obstructive Sleep Apnea

Sleep disorders of this category share the common characteristic of abnormal breathing patterns during sleep. Among them, OSA is the most common sleep-related breathing disorder with an estimated prevalence of 15% for males and 5% for females (Young et al., 2009). There are numerous known risk factors for OSA, the most important ones being obesity, gender, age, and craniofacial and upper airway abnormalities; smoking, diabetes, a family history, and asthma are also risk factors but to a minor degree (Strohl, 2018). Considering that obesity is one of the most significant risk factors for OSA, it is not surprising that there has been an increase in the prevalence of OSA in parallel with the increase in obesity rates, an association thought to be related to increased fat deposits in the tongue (Peppard et al., 2013).

The major event of OSA is the temporary but repetitive interruption of airflow during sleep, due to the collapse of the upper airways. This leads to episodes of apneas and hypopneas that are responsible for sleep fragmentation, oxygen desaturation, and excessive daytime sleepiness (EDS). The standard diagnostic tool for OSA is a full-night polysomnography, although in some cases, home sleep apnea testing can also be recommended. The aim of these tools is to determine the frequency of apneas, hypopneas, and respiratory effort-related arousals during sleep (RERAs); this information yields the apnea-hypopnea index (AHI = apneas + hypopneas/total sleep time in hours) and the respiratory disturbance index (RDI = apneas + hypopneas + RERAs/total sleep time in hours). In a polysomnography, an AHI or RDI > 5 in addition to other symptoms consistent with OSA (sleepiness, waking up gasping, habitual snoring, hypertension, and among others) or an AHI or RDI > 15 in an otherwise asymptomatic patient is indicative of OSA. Furthermore, based on the AHI score, OSA is subdivided into mild (5–15), moderate (15–29), and severe (30 or more) categories, with each category having an increased degree of symptom severity and higher risk for cardiovascular complications, cancer, motor vehicular accidents, and among others.

OSA is a chronic condition requiring long-term management. Although many different expressions and complications of OSA require tailored interventions, the general aim of treatment is to reduce nighttime disturbances of sleep by maintaining an open airway. Some interventions involve positive airway pressure (PAP) machines, oral appliances, or implants. In addition, because of the recognized risk factors for OSA, healthy lifestyle changes are often recommended, including regular physical activity, maintaining a healthy weight, and avoiding alcohol and smoking. The currently used treatments are compiled in Table 41.2.

Continuous PAP (CPAP) is the first line of treatment for OSA. Nonetheless, CPAP efficacy is limited by patient adherence, with studies showing that up to 83% of patients do not practice regular use of their device (Canadian Agency for Drugs and Technologies in Health, 2016). Fortunately, feedback to patients about the use of CPAP could help many of them with improving their constancy. Initial supporting evidence for the effectiveness of education and feedback was provided by the pilot trial for the SleepMapper app, a program designed to provide educational material and facilitate real time access of the patient's AHI and PAP use data. Results of the trial found that patients using this program for 11 weeks logged more days of PAP use and used their device for longer periods (Hostler et al., 2017).

Although CPAP is effective and safe, several patients complain of pressure-related side effects, such as nasal congestion, rhinitis, or runny noses, indicating that further improvements are necessary to increase comfort levels associated with this treatment approach. In this regard, the use of heliox therapy has recently been tested. Heliox is a gas combination of helium and oxygen that possesses significantly lower density than air and, therefore, is easier to inhale. In the first study of its kind, heliox administered to OSA patients significantly increased mean oxygen saturation (MSO_2) and lowest oxygen saturation (LSO_2) while also producing a modest (not significant) decrease of apnea and hypopnea events (Mahgoub, Abou Yassine, Harris, & Chalhoub, 2017). Although these findings are promising, more research needs to be conducted to determine the effect of these improvements on EDS and to find a successful means to further reduce the number of apnea and hypopnea events.

Although CPAP is the mainstay treatment for OSA, a significant portion of patients do not benefit from this approach. For this subset of patients, alternative treatment, such as surgical interventions, can be offered. Nasal and upper airway surgery, maxillomandibular advancement, and, in extreme cases, tracheostomy are surgical options that improve OSA symptoms.

Another treatment alternative is hypoglossal nerve stimulation, which, while requiring surgical intervention, is a much less invasive procedure. This treatment approach consists of an electronic device, similar to a pacemaker, which delivers electric stimulation directly

TABLE 41.2 Therapeutic Options for the Treatment of Obstructive Sleep Apnea

Nonpharmacological[a]			
Type	**Intervention**	**Description**	**Notes**
Behavior modification	Patient education	Education regarding risk and consequences	–
	Lifestyle changes	Weight loss, exercise, sleep position	–
	Substance avoidance	Alcohol, medication with inhibitory effects (benzodiazepines, barbiturates, sedating antidepressants, antihistamines, and opiates) and that cause weight gain (mirtazapine)	–
Positive airway pressure therapy	Continuous (CPAP)	Delivers a steady flow of pressurized air through the airway preventing collapse	First choice of treatment. Efficacy hampered by low adherence
	Bilevel (BPAP)	A different airway pressure for inspiratory and expiratory events	Not necessarily better than traditional CPAP
	Autotitrating (APAP)	Pressure is adjusted automatically in response to changes in airflow, circuit pressure, or a vibratory snore	
Oral appliances	Mandibular advancement devices Tongue-retaining devices	Mouthpieces designed to keep a clear airway, by preventing either the tongue or the throat muscles from collapsing	For mild to moderate cases
Sleep surgery	Upper airway surgery	Uvulopalatopharyngoplasty Radiofrequency volumetric tissue reduction Septoplasty and turbinate reduction Genioglossus advancement Hyoid suspension Midline glossectomy and lingualplasty Maxillomandibular osteotomy and advancement Palatal implants	Best for severe cases caused by a correctable alteration (tonsillar hypertrophy, adenoid hypertrophy, or craniofacial abnormalities)
Implants	Inspire®	Electric stimulation to the hypoglossal nerve restores tongue muscle tone and opens the upper airway	First implant approved by the Food and Drug Administration

[a] *To date, pharmacological interventions are limited to the management of residual daytime sleepiness.*
Data obtained from Canadian Agency for Drugs and Technologies in Health. (2016). Interventions for the treatment of obstructive sleep apnea in adults: A health technology assessment, 6(1b), 556.; Kryger, M. H., & Malhotra, A. (2018). Management of obstructive sleep apnea in adults. In N. Collop & G. Finlay (Eds.), UpToDate. Waltham, MA: UpToDate Inc. Retrieved from https://www.uptodate.com/contents/management-of-obstructive-sleep-apnea-in-adults?search=management of obstructive sleep apnea&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1.

to the hypoglossal nerve. The first studies of the safety and efficacy of this type of device were carried out by Inspire Medical Systems in 2010 with the launch of the Stimulation Therapy for Apnea Reduction (STAR) trial in medical centers of the United States and Europe. With the start of the STAR trial, patients received the first implants of the Inspire® upper airway stimulation system. In 2014, this system obtained the first FDA approval for an implant designed for the treatment of moderate to severe OSA. According to the evaluation by the FDA, the use of this system resulted in clinically significant reductions in the severity of OSA symptoms and an increase in quality of life for most patients. Additionally, although the frequency of nonserious adverse events was high, most of these events could be resolved with stimulation adjustments and other measures (Strollo et al., 2014; US Food and Drug Administration, 2014).

Research related to effectiveness of implants for OSA treatment has continued, with a subsequent clinical trial assessing an implant developed by ImThera Medical, Inc. The Aura6000® system is composed of only two implantable components (the implant and the lead) and a handheld remote control unit. In the spring of 2015, the first two patients were implanted with this invention, and the trials for the evaluation of safety and effectiveness continue to this date. The last device we know to be in clinical trials is being developed by Nyxoah. Their device, The Genio™, consists of a battery-free implant placed on the genioglossus muscle, where it can bilaterally stimulate the hypoglossal nerve. The electrode is wirelessly powered by a rechargeable chip adhered to the chin of the patient each night. These devices offer the opportunity for an effective, minimally invasive, and discrete treatment that could greatly benefit patients.

An important but often underemphasized approach for the treatment of OSA is pharmacotherapy. Medications have mostly been used as adjuncts for improving the outcome of traditional treatment measures. For example, nonresponsive EDS can be improved with the administration of stimulants, such as modafinil or armodafinil. In some cases, because OSA is often worse during REM sleep, REM-suppressing antidepressants can be of assistance. Recently, desipramine, dronabinol, and yohimbine have emerged as promising options for reducing the AHI.

Desipramine is a tricyclic antidepressant for which initial phase 2 clinical trials have been completed in both healthy individuals and OSA patients, with the aim of establishing the effect of this drug on the activity of the genioglossus muscle. Results are promising, as they show that desipramine improved muscle tone during sleep for both groups while also reducing the AHI in some cases (Taranto-Montemurro et al., 2016; Taranto-Montemurro et al., 2016). However, significant limitations of these studies include the protocol duration (single dose) and number of subjects (10 healthy individuals and 14 OSA patients of 16 enrolled in the trial).

Dronabinol is a synthetic form of tetrahydrocannabinol, historically used for the treatment of anorexia in the context of HIV/AIDS and for refractory nausea and vomiting during chemotherapy. The therapeutic potential of dronabinol for OSA was suggested by animal studies that demonstrated reduced spontaneous sleep-disordered breathing following drug administration. In a subsequent proof-of-concept study, it was observed that a regimen of weekly increasing doses of dronabinol (from 2.5 to 5 and finally 10 mg/day) had a significant effect in lowering the AHI of OSA patients (Prasad, Radulovacki, & Carley, 2013). Based on these findings, the US federal government granted funds to initiate the "pharmacotherapy of apnea by cannabimimetic enhancement (PACE) study," a phase 2, multisite, fully blinded, parallel group, randomized placebo-controlled clinical trial for patients with moderate to severe OSA. The first reports of this study have been consistent with the initial findings, in that the AHI was reduced and, furthermore, patients reported reduced sleepiness and increased satisfaction with the treatment. Additionally, dronabinol appears to be well tolerated by patients; the most common adverse events reported so far are sleepiness/drowsiness, headache, nausea/vomiting, and dizziness/lightheadedness (Carley et al., 2018).

Lastly, although yohimbine is used as a nutritional supplement and has no FDA-approved indication, its mechanism of action as a α_2-adrenergic receptor antagonist made it reasonable to believe that it could be of use for OSA. Studies have revealed that norepinephrine is one of the most important excitatory neuromodulators involved in the activity of the hypoglossal nerve that, in turn, facilitates the tone of the genioglossus muscle (Funk, Zwicker, Selvaratnam, & Robinson, 2011). Recent studies in animal models show that systemic administration of yohimbine was able to reduce inhibition of A5 and A7 noradrenergic brain stem neurons, hence facilitating the activity of the hypoglossal nerve, an effect that was also observed in an experimental animal model of OSA (Song & Poon, 2017).

In summary, even though research in the field of pharmacotherapy for OSA has presented some intriguing results in recent years, none of the tested drugs has demonstrated the therapeutic effects necessary for them to be considered an acceptable replacement for standard OSA treatments. At the same time, all currently available treatments, including the CPAP, are associated with at least some limitations and side effects. Thus, much more research is required to optimize and develop effective treatments in an effort to restore the quality of life in OSA patients.

C Central Disorders of Hypersomnolence: Narcolepsy

The defining trait for this category of sleep disorders is the occurrence of severe daytime sleepiness when nocturnal sleep is not otherwise compromised. Although narcolepsy is considered a rare disease, with prevalence estimated at about 1 in 2000 worldwide (Dauvilliers, Arnulf, & Mignot, 2007), it is more common than other disorders covered in this section, such as idiopathic hypersomnia and Kleine-Levin syndrome, for which exact prevalence rates are unknown but estimates place them in the range of 1–5 per 10,000 and 3.19 per million, respectively (Dauvilliers & Vecchierini, 2009; Habra, Heinzer, Haba-Rubio, & Rossetti, 2016).

In recent years, the development of most cases of narcolepsy has been explained as a consequence of the specific loss of orexinergic (also known as hypocretinergic) cells in the lateral hypothalamic area (Mignot et al., 2002; Thannickal et al., 2000); other causes of narcolepsy include brain injury, tumors, and genetic factors. Although the underlying mechanisms of orexin neurodegeneration are not fully understood, evidence such as the high prevalence of the DQB1*0602 HLA haplotype among narcoleptic patients (Mignot, Hayduk, Black, Grumet, & Guilleminault, 1997; Watson, Ton, Koepsell, Gersuk, & Longstreth, 2010), and the overlap in timing of viral infections with disease onset (Nellore & Randall, 2016; Partinen et al., 2014), among others, support an autoimmune hypothesis.

Clinicians agree that the cardinal symptoms of the disease are EDS, cataplexy, sleep paralysis, and hypnagogic hallucinations. EDS is a complaint of irresistible sleepiness throughout the day that can cause patients to nod

off without warning, an event known as "sleep attack." Cataplexy is a sudden and transient muscle weakness, often elicited by strong emotions, among which laughter stands out as the classic trigger (Krahn, Lymp, Moore, Slocumb, & Silber, 2005). Sleep paralysis is the temporal absence of voluntary movements during sleep-wake transitions. Hypnagogic hallucinations are vivid hallucinations (visual, tactile, or auditory) that occur during wake-sleep transitions. EDS is thought to be a consequence of night sleep fragmentation, while cataplexy, sleep paralysis, and hypnagogic hallucinations are thought to be inappropriate manifestations of REM sleep during waking. Since two-thirds of patients do not display the classic tetrad of symptoms for narcolepsy, narcolepsy can be suspected even in cases where EDS is the only apparent symptom (Scammell, 2018).

In the ICSD-3, narcolepsy is classified as either type 1 (NT1) or type 2 (NT2). The difference between these diagnoses is that NT1 presents with cataplexy and/or orexin peptide deficiency (<110 pg/mL in cerebrospinal fluid), while patients with NT2 have no evidence of either.

Even though the management of narcolepsy warrants pharmacological intervention, patients can also increase their quality of life by simultaneously employing nonpharmacological therapies. Beneficial nonpharmacological approaches include avoiding drugs that are known for exacerbating symptoms, well-timed naps, maintaining sleep hygiene techniques, psychosocial support, and general health maintenance (Scammell, 2017).

Pharmacotherapy for narcolepsy usually involves a specific medication for each set of symptoms. Stimulants such as modafinil, methylphenidate, and dextroamphetamine are aimed at reducing EDS, while REM-suppressing antidepressants such as clomipramine, venlafaxine, and fluoxetine are effective in reducing the number of cataplectic events. Additionally, there are a small number of other options that have demonstrated varying degrees of efficacy for both types of symptoms: sodium oxybate, pitolisant, selegiline, and mazindol. As with other drug treatments, there are safety concerns associated with their use, particularly the occurrence of undesirable side effects, allergic reaction, and the potential for abuse (Scammell, 2017; Syed, 2016). An overview of the available therapeutic options for narcolepsy is outlined in Table 41.3.

Despite narcolepsy now being considered a sleep disorder caused by an underlying orexin deficiency, there is currently no treatment that targets the orexinergic system. Although evidence obtained from animal models suggests that orexin replacement therapy could be a viable approach (España, Baldo, Kelley, & Berridge, 2001), blood-brain-barrier-pervious molecules have only recently been successfully synthesized. In 2015, the first molecule of this kind was described; YNT-185 (initially known as "compound 26") is a potent OX2R agonist that has proved to be effective in promoting wakefulness and ameliorating the number of cataplexy attacks in narcoleptic mice (Irukayama-Tomobe et al., 2017; Nagahara et al., 2015). Although this OX2R agonist has not yet been studied in the context of human narcolepsy and, thus, no information regarding its safety or efficacy is available, some pharmaceutical companies have already unveiled several other orexin receptor agonists. For example, the Takeda Pharmaceutical Company initiated phase 1 clinical trials for their OX2R agonist TAK-925 in the autumn of 2017, and Heptares Therapeutics lists an orally active OX2R among their current molecules under study. As different compounds advance to clinical trials, we are excited about obtaining new information regarding the specific advantages and drawbacks of orexin replacement therapy as a new approach for the treatment of narcolepsy.

While orexin replacement therapy is expected to soon become the center of a new generation of medications for narcolepsy, it will nonetheless constitute another example of conventional treatments and remain a symptomatic approach. Based on the generally accepted autoimmune hypothesis of narcolepsy, some research has been carried out with immunotherapy regimes based on steroids, immunoglobulin, or plasmapheresis (Chen, Black, Call, & Mignot, 2005; Coelho, Pradella-Hallinan, Alves, Bittencourt, & Tufik, 2007; Lecendreux, Maret, Bassetti, Mouren, & Tafti, 2003). Although there are only a small number of published reports, the therapeutic effect observed with these treatments has generally been modest and/or short-lived.

Among the therapeutic options that have been evaluated, intravenous immunoglobulin (IVIg) therapy has been studied most extensively. In the limited number of available case reports, the most promising results were obtained when IVIg was administered close to disease onset, even though sleep parameters remained abnormal and cataplexy improved only temporarily (Knudsen, Mikkelsen, Bang, Gammeltoft, & Jennum, 2010). These results suggest that the window of opportunity for immunotherapy to be effective is small, and early intervention is key. However, it is important to acknowledge that the autoimmune hypothesis of narcolepsy has not yet been fully corroborated. Thus, more research is required to both confirm and describe the exact autoimmune mechanism involved to allow the development of a successful immunotherapy intervention for this condition.

D Sleep-Related Movement Disorders: Restless-Leg Syndrome

Among the other sleep disorders discussed in this section, RLS, also known as Willis-Ekbom disease, is one of the most prevalent. Estimates indicate that between 5% and 15% of the adult population live with RLS; among

TABLE 41.3 Therapeutic Options for the Treatment of Narcolepsy

Nonpharmacological measures		
Improves	**Intervention**	**Description**
EDS	Scheduled naps	One or two short well-timed naps as needed
	Sleep hygiene	Regular and adequate sleep schedules
	Substance avoidance	Benzodiazepines, opiates, antipsychotics, alcohol, theophylline, caffeine
Cataplexy	Substance avoidance	Alpha-1 antagonists
Day-to-day living	Psychosocial support	Coping skills and patient education

Pharmacological			
Indication	**Drug class**	**Generic name (mg/day)**	**Warnings and precautions**
EDS	Nonamphetamine stimulants	Modafinil[a,b] (100–400) Armodafinil[a] (10–250) Methylphenidate[a,b] (20–40)	Serious rash or allergic reaction Drug reaction with eosinophilia and system symptoms Psychiatric symptoms Cardiovascular events
	Amphetamine stimulants	Dextroamphetamine[a] (5–60)	Dependence, abuse, and tolerance Hypertension and other cardiovascular conditions Psychiatric symptoms Peripheral vasculopathy Serotonin syndrome
Cataplexy	Antidepressants	Clomipramine (10–150) Venlafaxine (37.5–150) Atomoxetine (10–60) Fluoxetine (10–40)	Among others: Worsening of depression and increased suicidal risk Serotonin syndrome Seizures Cardiovascular effects
EDS and cataplexy	Histamine inverse agonist	Pitolisant[b] (9–36)	Among others: Abnormal thinking and behavioral changes Restless legs syndrome Spontaneous abortion Changes in appetite
	Depressant	Sodium Oxybate[a,b] (4.5–9)	Abuse and misuse potential Respiratory depression and sleep-disordered breathing Depression and suicidality Psychiatric symptoms Parasomnias
	Monoamine oxidase inhibitors	Selegiline (10–40)	Extrapyramidal symptoms Pain/altered sensation Cardiovascular symptoms Gastrointestinal disorders
	Stimulant	Mazindol[c] (1–6)	Anorexia Palpitations Apnea Dry mouth

[a] *Food and Drug Administration (FDA) approved for the treatment of narcolepsy or its symptoms.*
[b] *European Medicines Agency (EMA) approved for the treatment of narcolepsy or its symptoms.*
[c] *Mazindol has long been used off-label for narcolepsy; nonetheless in 2015, it received orphan drug designation for the treatment of narcolepsy by the EMA and in 2016 by the FDA.*
Data obtained from Abad, V., & Guilleminault, C. (2017). New developments in the management of narcolepsy. Nature and Science of Sleep, ***9***, *39–57. doi: 10.2147/NSS.S103467; Barateau, L., Lopez, R., & Dauvilliers, Y. (2016). Management of narcolepsy.* Current Treatment Options in Neurology, ***18***(*10*), *1–13. doi: 10.1007/s11940-016-0429-y; Scammell, T. E. (2017). Treatment of narcolepsy in adults. In R. Benca (Ed.), UpToDate. Waltham, MA: UpToDate. Retrieved from https://www.uptodate.com/contents/treatment-of-narcolepsy-in-adults.*

these, elders and women tend to exhibit particularly high prevalence rates (Colten & Altevogt, 2006).

The hallmark symptom of RLS is an urge to move the legs that is usually, but not necessarily, associated with an unpleasant or uncomfortable sensation. This urge and any accompanying unpleasant sensations are particularly notable and intense during periods of inactivity, such as when the individual is resting or trying to sleep.

Movement is effective in partially or completely relieving this urge and any accompanying sensations; however, this effect might only last as long as the activity itself. The presence of symptoms follows a circadian pattern, in that they commence or become more pronounced in the evening and are almost or totally absent in the morning. A proper diagnosis of RLS must establish that another condition does not better explain the symptoms; some examples of other conditions that should be considered, due to the similarity of symptoms, are myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, and habitual foot tapping (Allen et al., 2014).

In clinically significant cases, the EDS-related daytime impairment is an additional complaint of RLS. EDS is a result of insomnia and frequent night awakenings due to RLS discomforts, but up to 80% of patient unknowingly also experience involuntary and repetitive limb movements during sleep, usually of the lower extremities, known as periodic limb movements (PLM), that further aggravate daytime impairment (Bogan, 2006; Montplaisir et al., 1997).

The etiology of idiopathic RLS has not been fully established, but mounting evidence suggests the involvement of alterations in iron and dopamine pathways, as well as genetic factors. The available evidence has given rise to the so-called iron model of RLS, which proposes that iron deficiency causes dopamine abnormalities that, in turn, give way to RLS (Earley et al., 2014). Further, there is abundant evidence for genetic factors involved in the pathophysiology of RLS. Classic studies have shown that a family history is present in at least 50% of patients and that monozygotic twins are highly concordant for RLS (Ondo, Dat Vuong, & Wang, 2000; Winkelmann et al., 2000).

There are two recognized types of RLS: primary and secondary. Primary or idiopathic RLS is considered "early onset" (before 40 years of age), has a slow progression, and tends to run in families; hence, it is also known as familial or hereditary RLS. In contrast, secondary RLS is of later onset (after 40 years of age) and faster progression and usually seen in the context of another medical condition such as iron deficiency anemia, kidney failure, neuropathy, or pregnancy (Khan, Ahlberg, Chow, Shah, & Koo, 2017). Each type of RLS requires a different course of treatment.

The management of primary RLS is generally chronic and symptomatic, while secondary RLS improves when the underlying condition receives proper intervention. This latter is observed, for example, in the case of iron deficiency anemia treated with iron supplements and RLS during pregnancy, where symptoms recede within 4 weeks of delivery.

Pharmacotherapy is usually the first line of treatment for clinically relevant primary RLS, while nonpharmacological therapy can help reduce symptoms in cases of mild and moderate RLS and improve outcomes as a coadjutant to pharmacotherapy for more severe cases. Nonpharmacological treatments include maintaining a normal sleep pattern; practicing moderate exercise regularly; limiting the consumption of caffeine, tobacco, and alcohol; avoiding medications that can aggravate RLS symptoms; and using of medical devices (Colten & Altevogt, 2006; Tarsy & Silber, 2018).

The primary drugs used for the treatment of RLS are dopaminergic agents; α2δ ligands; opioids; and, to a lesser extent, benzodiazepines and hypnotics. Dopaminergic agents are considered the first line of treatment; pramipexole, ropinirole, and the rotigotine patch are widely used and approved in many countries, while the sporadic use of levodopa can be prescribed for RLS with intermittent symptoms (Tarsy & Silber, 2018). The most worrisome complication of dopaminergic therapy is the augmentation of symptoms over time, as it has been observed that long-term medication can induce an overall increase in symptom severity, including daytime onset, increased intensity and spreading of symptoms to other body parts, and a shorter duration of drug action (Kurlan, Richard, & Deeley, 2006).

In some cases, dopamine therapy cannot be administered, either due to augmentation or because it conflicts with a history of psychiatric disorders; in these cases, α2δ ligands, such as pregabalin and gabapentin, are an effective alternative. Opioids are reserved for RLS cases unresponsive to dopamine therapy and α2δ ligands or when patients cannot tolerate the side effects of the initial treatments (Garcia-Borreguero, Pumarega, & Marulanda, 2018; Trenkwalder, Winkelmann, Inoue, & Paulus, 2015). The therapeutic options currently available for RLS are summarized in Table 41.4.

Effective nonpharmacological options for the treatment of RLS are largely lacking, but recently, the first device to control RLS has been cleared by the FDA. The Relaxis® is a stimulation-based therapy that is programed to deliver vibratory stimulation through a pad placed under the legs when needed, essentially creating a diversion that, in theory, should interrupt annoying episodes of RLS. The device has been available since 2014, with the current evidence regarding its effectiveness revealing a paradoxical finding that its therapeutic effect is not related to a reduction of RLS symptoms, but rather to its ability to improve sleep quality (Mitchell, 2015). Furthermore, although the device is able to shut off on its own after a vibration session, it requires the patient to manually turn it on every time the urge to move arises, thus hindering its practicality.

Another device using counterstimulation that is currently under study for RLS is the scrambler device. Inspired by the results achieved in the treatment of chronic pain, this device is already in clinical trials, with the first study expected to conclude in the summer of

TABLE 41.4 Therapeutic Options for Restless Legs Syndrome

Nonpharmacological options		
Type	**Intervention**	**Description**
Behavioral strategies	Substance avoidance	Caffeine, nicotine, alcohol, and medication that worsens symptoms (antidepressants, neuroleptic agents, dopamine-blocking antiemetics, and sedating antihistamines)
	Sleep hygiene	Regular and adequate sleep schedules
	Lifestyle changes	Regular exercise, mental alerting activities
Devices	Pneumatic compression	Sleeves placed around the foot and calf that apply pressure in a cyclic manner help promote blood flow
	Near-infrared light	Enhanced circulation with the application of light in the 750–1000 nm helps reduce symptoms
	Relaxis® pad	A pad placed under the legs provides 35 min cycles of vibration therapy as needed

Pharmacological options			
		Side effects	
Drug class	**Generic name (mg/day)**	**Common**	**Serious**
Intravenous iron	Ferric carboxymaltose (1000)	Allergic reactions	Hypertension
Oral iron	Ferrous sulfate (325)	Overdose	
Dopamine agonists	Pramipexole[a] (0.125–0.750) Ropinirole[a] (0.5–4) Rotigotine[a] (1–3)	Drowsiness Symptomatic hypotension Hallucinations	Rebound and augmentation Compulsive behavior
	Carbidopa-levodopa (25/100–50/200)	High risk of augmentation	
Benzodiazepines	Clonazepam (0.5–2)	Not for long-term use Interference with cognitive and motor performance	Suicidal behavior and ideation
Alpha-2 delta calcium channel ligands	Gabapentin enacarbil[a] (600)	Driving impairment Somnolence/sedation and dizziness	Suicidal thoughts and behaviors
	Pregabalin (150–450) Gabapentin (300–1800)	Angioedema Hypersensitivity reactions Suicidal behavior and ideation	Peripheral edema Dizziness and somnolence
Opioids[b]	Codeine (30–180) Tramadol (50–200) Oxycodone (5–30)	Addiction, abuse, and misuse potential Respiratory depression	Increased intracranial pressure

[a] *Food and Drug Administration approved medications for the treatment of restless legs syndrome.*
[b] *Helpful for cases of refractory restless legs syndrome. Doses in this case are far lower than those used for pain management.*
Data obtained from Mitchell, U. H. (2015). Medical devices for restless legs syndrome—clinical utility of the relaxis pad. Therapeutics and Clinical Risk Management, *11, 1789–1794. doi: 10.2147/TCRM.S87208; Tarsy, D., & Silber, M. H. (2018). Treatment of restless legs syndrome and periodic limp movement disorder in adults. In H. I. Hurtig & A. Y. Avidan (Eds.), UpToDate. Waltham, MA: UpToDate Inc.; Trenkwalder, C., Winkelmann, J., Inoue, Y., & Paulus, W. (2015). Restless legs syndrome—current therapies and management of augmentation.* Nature Reviews Neurology, 11*(8), 434–445. doi: 10.1038/nrneurol.2015.122.*

2018. The scrambler device consists of noninvasive electrodes placed on the skin of patients to deliver varying, weak electric currents, based on the physiological properties of normal action potentials. Although no results have been reported in the context of RLS, it is expected to offer long-time reduction of the urges and uncomfortable sensations characteristic of RLS, based on the experience with other applications of scrambler therapy (Lipford, 2018).

With regard to the development of pharmaceutics, it is unfortunate that there appears to be a lack of novel compounds to treat or control RLS and the associated symptoms. Instead, there are a number of trials of already approved drugs that assess new presentations and doses, their use in comorbid conditions and different clinical populations, or for complications that arise from other primary treatments. As one of the few examples of a new compound, the Bioprojet dopaminergic D3 receptor partial agonist BP 14979 is currently undergoing clinical testing. The rationale for the development of this drug is based on the effects of other dopaminergic agonists, but it acts upon a different receptor subtype;

in theory, BP 14979 should be effective in mitigating RLS symptoms, but detailed information will not be available until Bioprojet concludes their ongoing phase 2 clinical trial.

The antiepileptic drugs gabapentin and pregabalin are thought to exert their therapeutic effect in RLS through a reduction of glutamatergic transmission. Along the same rationale, the use of another antiepileptic drug, but with a different mechanism of action, has been proposed for the treatment of RLS. Results from a pilot study carried out in 22 patients with idiopathic RLS indicate that the selective, noncompetitive, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, perampanel, administered at 2–4 mg/day doses, is effective in consolidating sleep and reducing RLS symptoms and PLM while displaying an apparently safe side-effect profile (Garcia-Borreguero, Cano, & Granizo, 2017). While these initial results are promising, additional trials, including trials of longer duration and on larger samples, are required to further determine the efficacy and safety of this RLS treatment candidate.

Finally, anecdotal experience suggests that the cannabinoid system is worth studying as a possible new target for the treatment of RLS. A recently published report documents the experience of six patients with severe refractory RLS. These patients had previously been unsuccessfully treated with dopaminergic agonists, α2δ ligands, and opioids. Surprisingly, after the use of cannabis (five cases inhaled and one administered sublingual cannabidiol), these patients reported remission of RLS symptoms and improved sleep quality (Megelin & Ghorayeb, 2017). Even though the subjective evaluation of these cases limits their validity, further rigorous trials could help determine if cannabinoids are a worthwhile target for medication aimed at improving RLS. Thus, AXIM Biotechnologies has recently introduced a chewing gum formulation of dronabinol (MedChew RL) and, as of April 2017, is preparing a phase 1 clinical trial.

To date, RLS continues to be an enigmatic affliction. Classic treatments do offer some degree of relief, but efficacy is hindered by undesired side effects, tolerance, augmentation, rebound RLS, or conflicting treatment of comorbid conditions. Further, while there are a number of nonpharmacological therapies available, controversy exists over whether the observed therapeutic effects could be better attributed to a placebo effect or publication bias, rather than to the intervention itself (Harrison, Keating, & Morgan, 2018).

III CONCLUDING REMARKS

It is fortunate that an intense research effort has yielded important new information regarding the mechanisms of sleep disorders, allowing physicians to better understand, recognize, and treat these conditions. Among the over 60 currently recognized sleep disorders in the ICSD-3, insomnia, OSA, narcolepsy, and RLS are the sources of most of the clinically relevant sleep-related complaints. For these disorders, several new treatment approaches have been introduced, and a number of additional, promising options are currently in preclinical test phases; some of the most noteworthy pharmacological advances are summarized in Table 41.5.

Significant progress has been made for the treatment of insomnia, with new technological developments allowing improved access to CBTi. Further, the study of the mechanisms of narcolepsy has led to the generation of a new pharmacotherapy aimed at the orexinergic system. The treatment of OSA with CPAP is noninvasive, effective, and with relatively few adverse events; nonetheless, compliance to treatment is very low. For some patients, the introduction of new mobile apps that offer PAP therapy feedback and training may be sufficient for treatment to become successful. For others, electric stimulation of the hypoglossal nerve is a helpful intervention, even though current stimulation devices rely on battery-powered implants with a limited duration of use; excitingly, technology now exists for remotely powering such devices, and patients could be offered a smaller implant with increased service life. Regarding pharmacotherapy for OSA, the therapeutic effects of current drug treatments do not exceed those seen with PAP therapy or surgical options, even though yohimbine and dronabinol have the potential to become a more comfortable and discrete therapy for OSA.

Even though narcolepsy is now recognized to be a consequence of orexinergic cell loss, there are currently no pharmacological options targeting this system. However, orexin agonists as treatments are under development, and it is likely that orexin-based therapies will be available in the near future. In the case of RLS, case reports reveal that cannabis can cause symptom remission, suggesting that the cannabinergic system could become a new drug target for the treatment of RLS.

As a final note, it is important to recognize that, despite efforts to increase the ratio between therapeutic effect and treatment drawbacks, every new treatment developed and applied will also come with new challenges and complications. The high prevalence of sleep-related complaints and the complexity of individual needs mean that the demand for new and improved treatments will continue to be high. The field of sleep medicine continues to work in expanding the options available in an effort to offer the best relief possible for each patient.

TABLE 41.5 Drug Development Pipeline for Sleep Disorders

Developer	Drug name (alternative names)	Clinical trial phase	Trial number identifier	Reported mechanism of action	Novelty
Insomnia					
Taisho Pharmaceutical (Tokyo, Japan)	TS-142	2	JapicCTI-173570	Undefined	–
Minerva Neurosciences Inc. (Waltham, MA, the United States)	Seltorexant (MIN-202, JNJ-42847922)	2	NCT03375203	Orexin receptor type 2 antagonist	Single orexin receptor antagonist
Alexza Pharmaceuticals Inc. (Mountain View, CA, the United States)	AZ-007 (Staccato zaleplon)	Preclinical	–	$GABA_A$ receptor agonist	Aerosol delivery
Obstructive sleep apnea					
University of Illinois at Chicago & Collaborators	Dronabinol	2	NCT01755091	Cannabinoid receptor agonist	Promising pharmacological option for this disorder
Narcolepsy					
Heptares Therapeutics (Hertfordshire, the United Kingdom)	Orexin OX_2	Preclinical	–	Orexin receptor type 2 agonist	Targets the orexinergic system
Takeda (Tokyo, Japan)	TAK-925	1	NCT03332784		
NLS Pharma (Stans, Switzerland)	NLS-0 (mazindol)	Orphan drug designation	–	Reuptake inhibitor of norepinephrine, dopamine, and serotonin	A drug already used off-label for the treatment of narcolepsy
Balance Therapeutics (San Bruno, CA, the United States)	BTD-001 (pentylenetetrazole)	2	NCT02512588	$GABA_A$ receptor antagonist	An untapped mechanism of action for EDS in narcolepsy
Jazz Pharmaceuticals Inc. (Dublin, Republic of Ireland)	Oxybate once-nightly dosing	Preclinical	–	Possibly GHB and $GABA_B$ receptor agonist	Single administration
	JZP-258 (oxybate midex salt solution)	3	NCT0330599		90% less sodium than original sodium oxybate
	JZP-110 (solriamfetol, ADX-N05)	New drug application	NCT02348593 NCT02806908 NCT02348632	Norepinephrine-dopamine reuptake inhibitor	Promising drug for excessive sleepiness associated with narcolepsy
Taisho Pharmaceutical (Tokyo, Japan)	TS-091	2	NCT03267303	Undefined	–
Restless legs syndrome					
AXIM Biotechnologies (New York, NY, the United States)	MedChew RL	Preclinical	–	Cannabinoid receptor modulator	Targets the endocannabinoid system
Hisamitsu Pharmaceutical (Tosu, Japan)	HP 3000 (ropinirole)	2	JapicCTI-132281	Dopamine D2 receptor agonist	Transdermal delivery method
Bioprojet (Paris, France)	BP 14979	2	NCT03345953	Dopamine D3 receptor partial agonist	Targets a different dopamine receptor subtype

Abbreviations: GABA gamma, aminobutyric acid; GHB gamma, hydroxybutyric acid.
Data obtained from Clinicaltrials.gov, adisinsight.com, and web pages of the mentioned pharmaceutical companies.

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REVIEW

Understanding sleep-wake mechanisms and drug discovery

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ABSTRACT

Introduction: Although not discernible at first glance, sleep is a highly active and regulated brain state. Although we spend practically one third of our lifetimes in this stage, its importance is often taken for granted. Sleep loss can lead to disease, error and economic loss. Our understanding of how sleep is achieved has greatly advanced in recent years, and with that, the management of sleep disorders has improved. There is still room for improvement and recently many new compounds have reached clinical trials with a few being approved for commercial use.

Areas covered: In this review, the authors make the case of sleep disorders as a matter of public health. The mechanisms of sleep transition are discussed emphasizing the wake and sleep promoting interaction of different brain regions. Finally, advances in pharmacotherapy are examined in the context of chronic insomnia and narcolepsy.

Expert opinion: The orexinergic system is an example of a breakthrough in sleep medicine that has catalyzed drug development. Nevertheless, sleep is a topic still with many unanswered questions. That being said, the melanin-concentrating hormone system is becoming increasingly relevant and we speculate it will be the next target of sleep medication.

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1. Introduction

Sleep is an essential life function often taken for granted. However, the absence of sleep has been associated with many undesired conditions such as: reduced alertness, impaired judgment, depression, and weight gain, as well as putting people at greater risk of stroke, heart attack, and diabetes. In the worst case scenario, sleep deprivation can ultimately lead to death (for more information on the noxiousness of sleep deprivation please refer to [1]).

According to the National Center on Sleep Disorders Research, an estimated 1/3 of the world population suffers from some sort of sleep disorder. A study carried out in Australia in 2004 concluded that the economic burden of chronic sleep disorders was around $7.5 billion [2], and a reevaluation in 2010 found that this had increased to around $36.8 billion [3]. In the US alone sleep-related problems add an estimated $15.9 billion to the US national health-care bill each year, and this does not include the cost of related health problems, lost worker productivity, and accidents [4]. Thus, inadequate sleep is a costly public problem that is on the rise.

Beyond the economic toll of sleep disorders, sleepiness can lead to serious injury for oneself and others. For example, in the case of motor vehicle accidents, around 41% of crashes that occur in low-speed roads (<60 km/h) are related to driver sleepiness [5] and the odds of being injured increases six times for sleepy drivers [6]. In the workplace, sleepiness has been associated with errors in judgment that can have dire consequences, such as industrial accidents (the Exxon *Valdez* oil spill is a widely known example) and medical errors [7], to cite some.

Sleepiness can arise from lack of sufficient opportunity to sleep or as a consequence of a sleep disorder. Among the most prevalent sleep disorders are obstructive sleep apnea, narcolepsy, restless leg syndrome, rapid eye movement (REM) sleep behavior disorder and insomnia [8].

Sleep is finally garnering the relevance it deserves as a public health issue. For the study of sleep, it is important to understand how sleep is generated and how disrupted sleep can lead to sleep disorders. In this review we will try to paint a picture covering these topics with an emphasis on treatment opportunities.

2. Sleep physiology

2.1. Sleep architecture

Sleep is a difficult behavior to define; it can broadly be described as a resting state where animals exhibit a reduced response to environmental stimuli that, unlike coma, is rapidly reversible [9]. Since the introduction of the electroencephalogram (EEG) by Hans Berger in 1924, sleep has also been studied by characterizing brain electrical activity throughout the sleep–wake cycle (SWC) [10].

Analysis of combined recordings of EEG and electromyogram (EMG, muscle activity) demonstrates that the SWC is divided into three states: wakefulness, REM sleep, and non-REM (nREM) sleep [11]. Wakefulness is characterized by high muscle tone and desynchronized EEG (high-frequency and low-amplitude activity). nREM sleep displays high-amplitude, low-frequency activity and decreased muscle tone. In humans, nREM sleep is further

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Article highlights

- Sleep disorders are a widespread problem with great economic and social repercussions
- Sleep is a highly regulated behavior initiated through a complex interaction of different levels of phenomena (genetic, environmental, hormonal and neurotransmission, to cite some).
- Therapeutic approaches for sleep disorders have many shortcomings including harsh side-effect profiles, tolerance and next-day impairment.
- The orexinergic system is the newest target for drug development for sleep disorders
- The melanin-concentrating hormone system in the near future might become the target of a new generation of sleep medication.

This box summarizes key points contained in the article.

divided into three stages: N1, N2, and N3. N1 is considered the period of transition into sleep; stage N2 is light sleep where sleep spindles and K-complexes are visible; and the deepest stage of sleep is stage N3, also known as slow-wave sleep (SWS). REM sleep is characterized by EEG activity similar to that of the wake period (desynchronized), but with the absence of EMG activity (REM muscle atonia) and with eye movements of the saccadic type which occurs in bursts. Additionally, it is during REM sleep that most dreaming occurs [12].

The organization of sleep during a normal night of human rest follows a predictable pattern. nREM and REM alternate in sleep cycles of about 90–110 min that repeat throughout the night. In addition, at the beginning of the night, the bouts of SWS are longer than those of REM sleep, and as the night weans this trend is inverted [13].

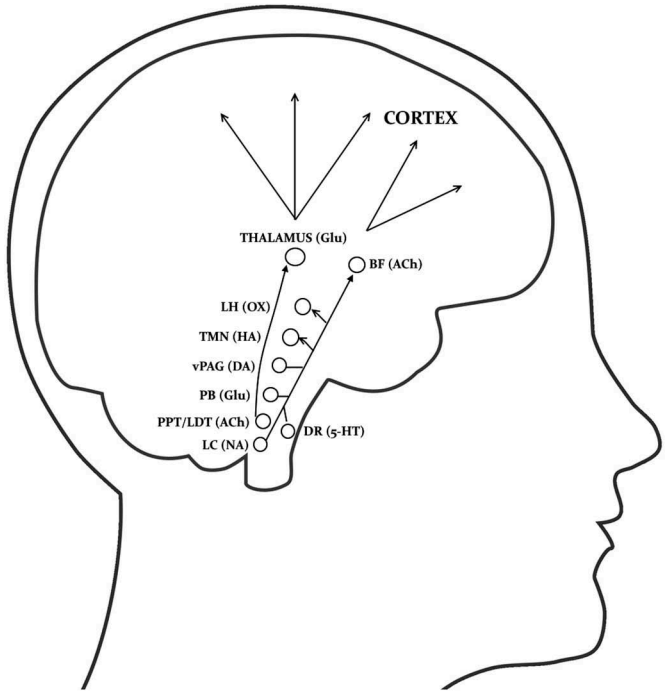

Figure 1. Wake-promoting pathway. The ARAS arises from the brainstem and promotes wakefulness by cortical activation. The ARAS comprises the dorsal and ventral pathway.
Dorsal pathway: Cholinergic PPT and LDT nuclei project through the thalamus to reach the cortex. Ventral pathway: PB, DR, LC and vPAG cell groups project to the histaminergic tuberomammillary nucleus, orexinergic hypothalamus, and to the basal forebrain, ultimately reaching the cerebral cortex causing activation.
ARAS, ascending reticular activating system. BF, Basal Forebrain. DR, Dorsal Raphe. LC, Locus Coeruleus. LDT, Laterodorsal Tegmental nuclei. LH, Lateral Hypothalamus. PB, Parabrachial Nucleus. PPT, Pedunculopontine nuclei. TMN, Tuberomammillary Nucleus. vPAG, ventral Periaqueductal Gray Matter. 5-HT, Serotonin. Ach, Acetylcholine. DA, Dopamine. Glu, Glutamate. HA, Histamine. NA, Noradrenaline. OX, Orexin.

2.2. Sleep generation: wake to nREM

Once opportunity for sleep arises, a transition from wake to sleep can occur. According to the flip-flop switch model, there are sleep-promoting and wake-promoting groups of cells that exert mutually inhibitory activity [14]. Depending on environmental and physiological circumstances, one group of cells can surmount the inhibition of the other and the switch then transitions swiftly among states.

Wakefulness is thought to be sustained by the ascending reticular activating system (ARAS), a neural pathway associated with arousal of the cerebral cortex (Figure 1). The ARAS arises from the reticular formation in the brainstem and causes cortical activation either via the thalamus (dorsal pathway) or through the hypothalamus and basal forebrain (BF) (ventral pathway) [15].

The dorsal pathway of the ARAS emanates from the cholinergic pedunculopontine tegmental (PPT) and laterodorsal tegmental (LDT) nuclei (collectively these are called the peribrachial nuclei), goes through the thalamus (specifically the midline and intralaminar nuclei), and reaches the cortex [16]. The thalamic innervation of the cortex is ample and when activated, glutamate is released causing arousal [17,18].

The ventral pathway, at the level of the brainstem, is primarily comprised of the glutamatergic parabrachial nucleus (PB) and the monoaminergic dorsal raphe (DR), locus coeruleus (LC), and ventral periaqueductal gray matter cell groups (serotoninergic; noradrenergic; and dopaminergic, respectively). From the brainstem, these cells project to the histaminergic tuberomammillary nucleus (TMN), orexinergic hypothalamus, and to the BF (rich in acetylcholine [ACh] and gamma-aminobutyric acid [GABA]), ultimately reaching the cerebral cortex causing activation [19]. In addition to reaching the cortex and eliciting cortical activation, wake-promoting nuclei extensively target the sleep-promoting centers [20] and inhibit their activity mainly via noradrenaline (NA) and ACh stimulation and to a lesser extent serotonin (5-HT) [21].

Orexinergic cells have received a lot of attention for their role in the maintenance of wakefulness [22]. Although they are not thought to be part of the mutually inhibitory loop of sleep- and wake-promoting centers, they act as a stabilizer of the switch by their excitatory input to wake-promoting nuclei [23,24].

Because orexinergic neurons fire predominantly during active wakefulness [25], they can ensure a consolidated wake episode by stimulating the activity of the wake-promoting centers. In fact, loss of orexinergic cells has been linked to a sleep disorder known as narcolepsy [26], where sleeping and waking are not properly differentiated.

The best-regarded sleep-promoting center is contained in the preoptic area (POA), where the ventrolateral preoptic area (VLPO)

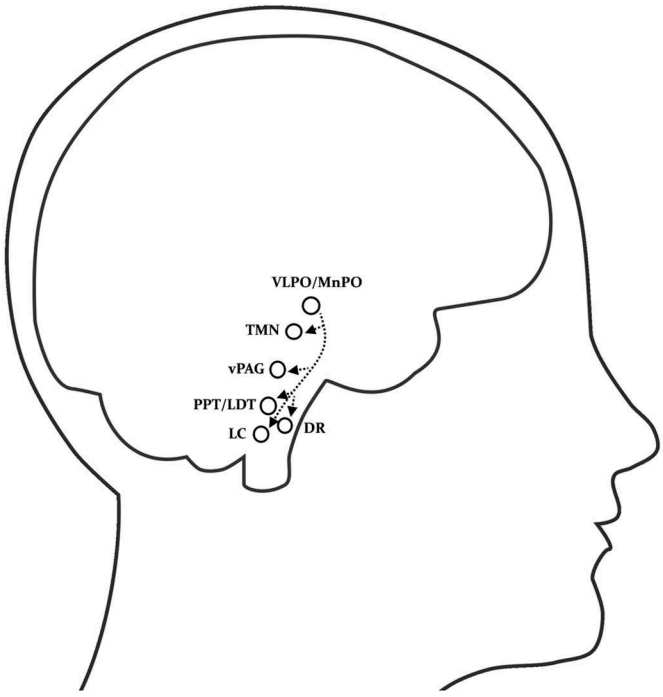

Figure 2. Sleep-promoting pathway. The Ventrolateral preoptic area (VLPO) and the median preoptic nucleus (MnPO) innervate most of the wake-promoting centers, and inhibit arousal with the release of GABA.
DR, Dorsal Raphe. LC, Locus Coeruleus. LDT, Laterodorsal Tegmental nuclei. MnPO, median Preoptic Nucleus. PPT, Pedunculopontine nuclei. TMN, Tuberomammillary Nucleus. VLPO, Ventrolateral Preoptic area. vPAG, ventral Periaqueductal Gray Matter.

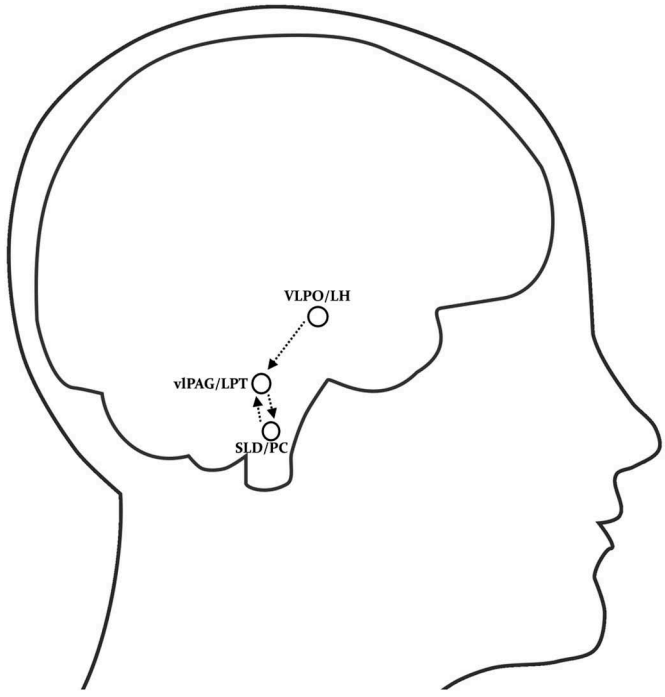

Figure 3. REM sleep-promoting pathway. The SLD/PC nuclei promote REM sleep while the vlPAG/LPT nuclei inhibit it. During REM sleep, SLD inhibits the vlPAG through GABA projections. On the other hand, GABA from the VLPO and MCH from hypothalamic MCH cells inhibit vlPAG/LPT.
REM, rapid eye movement. LH, Lateral Hypothalamus. LPT, Latero Pontine Tegmentum. PC, Precoeruleus area. SLD, Sublaterodorsal nucleus. vlPAG, ventrolateral Periaqueductal Gray matter. VLPO, Ventrolateral Preoptic area.

and the median preoptic nucleus (MnPO) are especially relevant (Figure 2). These nuclei are mainly active during sleep, innervate most of the wake-promoting centers, and inhibit arousal with the release of GABA and galanin [14,27,28]. Studies also show that the MnPO is active before the transition to sleep is achieved, while the VLPO is activated once sleep begins [29]. Thus, it is possible that the MnPO is monitoring the extracellular accumulation of somnogens, such as adenosine, and in turn promoting the activation of VLPO when need to sleep reaches certain threshold.

Besides the POA as sleep-promoting centers, melanin-concentrating hormone (MCH) cells from the hypothalamus have recently been the focus of research per their involvement in sleep promotion. Release of MCH has been found to cause inhibition of orexinergic and aminergic wake-promoting cells, and to increase the amount of nREM and REM sleep [30,31].

2.3. nREM to REM transitions

As mentioned earlier, once sleep takes place, there are also organized transitions from nREM sleep to REM sleep. Since 1962 we know, by the transection studies carried out by Jouvet, that the brainstem is essential and sufficient for REM sleep generation [32]. More recently, the REM control area has been further linked to the sublaterodorsal nucleus (SLD) and precoeruleus area (PC) (Figure 3). While the SLD/PC promotes REM sleep, the ventrolateral periaqueductal gray matter (vlPAG) and the lateral pontine tegmentum (LPT) inhibit it [33]. The aforementioned nuclei extend GABAergic mutually inhibitory projections and form the core of a second flip-flop switch that alternates the transitions from nREM to REM and vice versa.

This flip-flop switch is stabilized by other cell groups that provide excitatory and inhibitory stimulation. Specifically from the hypothalamus, the vlPAG/LPT is inhibited by GABA from the VLPO and MCH from hypothalamic MCH cells [34]. These different cell groups are inactive during wakefulness and have similar firing patterns during sleep that peak during deep and REM sleep [35]. This means that REM sleep is facilitated by the inhibition of the vlPAG/LPT by MCH and VLPO cells.

Of particular interest is the generation of REM muscle atonia. This REM sleep event has been linked to glutamatergic release from the SLD/PC REM-on toward the ventromedial medulla and interneurons of the spinal ventral horn. These structures in turn inhibit motor neurons via glycine and GABA [36].

2.4. Borbély and the two-process model of sleep regulation

Over three decades ago, Borbély et al. proposed the two-process model of sleep regulation which is still used today to predict sleeping behavior [37]. This model states that the timing, depth, and duration of sleep are under the control of the interaction of the independently regulated homeostatic process (Process S) and the circadian pacemaker (Process C).

Process S represents sleep debt and is proportional to the amount of time the individual spends awake; conversely, the pressure to sleep is relieved when an adequate amount of sleep occurs. Sleep debt is thought to arise from the

accumulation of somnogens, and some examples of these sleep factors are: adenosine, nitric oxide (NO), prostaglandin D_2, and some cytokines (the following reference has further information on sleep factors [38]).

Process C, on the other hand, is entrained to the amount of environmental light (day/night cycle) and under control of the suprachiasmatic nucleus (SCN). Process C determines the time of sleep, as nighttime sets in, physiological changes occur that allow for the proper initiation of sleep, and some examples of these changes are a drop in core body temperature [39] and melatonin production [40].

As Process S rises during time spent awake, Process C lowers as the daylight runs out. When both processes are the furthest from each other, the so-called sleep gate opens and sleep is privileged.

3. Sleep disorders

When normal sleep architecture is altered, sleep disorders arise. Although there are more than 100 different types of sleep disorders, we will briefly review only two: insomnia and narcolepsy. The first is relevant because, due to its high prevalence, many pharmaceutical companies are continually developing compounds for its management. The second is not so frequent, but its relationship to the orexinergic system has propelled a whole new field of drug research for the management of sleep disorders.

3.1. Insomnia

Insomnia is one of the most frequent sleep complaints in the world. The prevalence for insomnia is estimated at about 10% in the general population [41]. According to the International Classification of Sleep Disorders 3rd (ICSD-3), insomnia is a persistent difficulty with sleep initiation, duration, consolidation, or quality that occurs despite adequate opportunity and circumstances for sleep, and results in some form of daytime impairment. Insomnia disorders are diagnosed as short-term, chronic, or other (when the diagnosis of insomnia does not appropriately fit the other two criteria) [42].

Short-term insomnia is overwhelmingly common in modern society. Difficulty initiating or maintaining sleep during a short period of time (less than 3 months) happens to practically everyone at least once in a lifetime. Short-term insomnia disorder can be triggered by a variety of reasons that range from, but are not limited to, stress, excitement, anticipation, pain, illness, jet lag, and noise. This type of insomnia typically resolves itself when the stressor does or when the patient develops adequate coping mechanisms or adapts to the stressor. In some cases, short-term insomnia can evolve into chronic insomnia. Furthermore, the diagnostic criteria for chronic insomnia require that dissatisfaction with sleep be present for at least three times a week for at least 3 months [43].

Treatment for chronic insomnia includes: cognitive behavioral therapy (CBTi), pharmacotherapy, or both. There is evidence that all three options of treatment are effective for the management of insomnia; nonetheless, some approaches of CBTi are usually recommended as the first line of treatment [44].

The main goal of CBTi is to break unhealthy sleeping habits that might be reinforcing maladaptive changes associated with chronic insomnia. This includes sleep hygiene education, cognitive restructuring, stimulus control, sleep restriction therapy, and relaxation training [45,46]. Although CBTi is an effective approach for the treatment of chronic insomnia, it relies on patient adherence to the protocol and health-care availability. Recently, an interesting CBTi aid has been put forth in the form of a smartphone app, 'CBT-I Coach Mobile App,' that has the potential of improving the outcome of CBTi by tackling both these issues [47].

The pharmacotherapy approach is broadly categorized as Food and Drug Administration (FDA)-approved prescription, off-label medications, and over-the-counter (OTC) sleep aids. FDA prescription medications are: benzodiazepines, nonbenzodiazepine hypnotics, the melatonin receptor agonist ramelteon, the tricyclic antidepressant (TCA) doxepin, and the dual orexin receptor antagonist (DORA) suvorexant. Commonly, off-label treatments are antidepressants and atypical antipsychotics. Finally, the most consumed OTC sleep aids include antihistamines, alcohol, melatonin, and valerian [46,48,49].

The etiology of chronic insomnia is thought to be associated with physiological hyperarousal across the SWC. Under this theory, stressors induce an exacerbated physiological reaction that interferes with sleep. Some of the exacerbated physiological levels include: heart rate, cortisol, body temperature, and oxygen consumption [50]. Therefore, it is not surprising that sleep-inducing medications tend to depress the central nervous system (CNS). For example, benzodiazepines, nonbenzodiazepines, and alcohol enhance GABA activity [51,52]; activation of melatonin receptors appears to facilitate GABA activity [53]; doxepin and antihistamines inhibit histaminergic receptors [54]; and off-label medications have 5-HT and histamine (HA) antagonistic effects [55].

The side effect profile of available treatments for chronic insomnia includes: abnormal thoughts and behavior, next-day impairment, and physical dependence (details in Table 1). In the abnormal thoughts category, suicidal thoughts deserve special attention due to the high comorbidity of chronic insomnia and depression [56]. Next-day impairment and sedation are particularly paradoxical because the main objective of the treatment for insomnia is to promote a restful night and allow the patient to function properly during the day. Physical dependence is a constant concern with the use of benzodiazepine-type therapies, and melatonin use has been proposed as an alternative to phase out their use [57].

3.2. Narcolepsy

Narcolepsy was first described long ago by Gélineau in the 1880s [58]. The cardinal symptoms of narcolepsy are: excessive daytime sleepiness (EDS), cataplexy, hypnogogic hallucinations, and sleep paralysis. Cataplexy is a transient loss of muscle tone usually triggered by positive emotions [59]. Hypnagogic hallucinations are vivid hallucinations (visual, tactile, or auditory) that occur while the patient is falling asleep and less frequently when they are waking up (hypnopompic

Table 1. Pharmacological options for the management of chronic insomnia.

Drug class	Generic name	Usual dose (mg/day)	Mechanism of action mediating sedation	Common side effects
FDA approved				
Benzodiazepines	Flurazepam	15–30	Positive allosteric modulator of GABA A	Dizziness, drowsiness, next-day sedation,
	Estazolam	0.5–2		Rebound insomnia
	Temazepam	7.5–30		Allergic reactions
	Triazolam	0.125–0.5		Complex sleep-related behaviors: sleep-driving, making phone calls, eating
	Quazepam	7.5–15		Abnormal thoughts and behavior: worsening of depression, suicidal thoughts or actions, increased aggressiveness
				Physical dependence
Nonbenzodiazepines	Eszopiclone	1–3	Positive allosteric modulator of GABA A	Dizziness, headache, drowsiness, nausea, vomiting
				Complex sleep-related behaviors
	Zaleplon	2.5–10		Abnormal thoughts and behavior
	Zolpidem	5–20		Physical dependence
Melatonin receptor agonists	Ramelteon	8	MT1 & MT2 receptor agonist	Somnolence, dizziness, fatigue, nausea, exacerbated insomnia
				Allergic reactions
				Complex sleep-related behaviors
				Abnormal thoughts and behavior
				Hormone effects: decreased interest in sex, problems getting pregnant
Sedating antidepressants	Doxepin	3–6	H1 receptor antagonist	Sedation, nasopharyngitis, gastrointestinal effects, hypertension
				Complex sleep-related behaviors
				Abnormal thoughts and behavior
Orexin receptor antagonists	Suvorexant	10–20	OX1 & OX2 receptor antagonist	Next-day sedation, memory loss, anxiety
				Abnormal thoughts and behavior
				Temporary inability to move or talk (sleep paralysis)
				Temporary weakness in legs
Off-label				
Sedating antidepressants	Amitriptyline	25–150	5-HT2 & H1 receptor antagonist	Rebound insomnia, drowsiness, headache, nausea, loss of concentration, anticholinergic effects
	Mirtazapine	7.5–30		
	Trazodone	50–100		
Atypical antipsychotics	Quetiapine	≤200	H1 receptor antagonist	At the low doses used for insomnia: dry mouth, drowsiness, weight gain
				Case reports of: hepatotoxicity, restless leg syndrome, akathisia
Over-the-counter				
Antihistamines	Diphenhydramine	50	H1 receptor antagonist	Next-day sedation, drowsiness, dry mouth, tolerance
	Doxylamine	25		
Alcohol	Ethanol	–	Positive allosteric modulator of GABA A	Dependency, tolerance, negative impact on sleep quality, disturbed sleep cycle
Melatonin		0.1–0.5	MT1 & MT2 receptor agonist	Drowsiness, headache, dizziness or nausea
Valerian		300–600	Possibly mediated by GABA A activity	Headache, dizziness, gastrointestinal disturbances, nervousness, anxiousness, heart palpitations

GABA: gamma-aminobutyric acid; MT: melatonin; H: histamine; OX: orexin; 5-HT: serotonin.
Information is available from the US FDA website and the following references [46,48,49]

hallucinations). Sleep paralysis is an inability to move usually after waking. Only about one-third of patients present with all four symptoms [60].

The etiology of narcolepsy was unknown until canine narcolepsy was found out to be secondary to a genetic mutation of orexinergic receptors [61]. Not long after, orexin cell loss was confirmed in human narcolepsy [26]. An autoimmune mechanism is thought to be involved in the selective degeneration of orexinergic neurons, but the exact process is still unclear [62].

Once the link between orexinergic cell loss and narcolepsy was made, diagnostic criteria were updated to include the orexinergic system defect. The ICSD-3 classifies narcolepsy as a central disorder of hypersomnolence that can be either type 1 (NT1) or type 2 (NT2). NT1 includes patients that have a clear history of cataplexy and/or are orexin A (OXA) deficient (<110 pg/mL in cerebrospinal fluid), while the NT2 group includes patients that have the symptoms of narcolepsy but no evidence yet of OXA deficiency or cataplexy [42]. Eventually, some patients with NT2 will develop cataplectic episodes, low OXA in cerebrospinal fluid or both, and be recategorized as NT1.

Narcolepsy symptoms are managed with non-pharmacological interventions and pharmacological therapy. The non-pharmacological approach includes avoiding drugs that can worsen EDS (benzodiazepines, opiates, antipsychotics, alcohol, and caffeine) or cataplexy (certain alpha-1 antagonists), taking well-timed naps, following an adequate sleep schedule, and participating in support groups [63].

Although many patients benefit from a non-pharmacological approach, most require a combination therapy with medication. Pharmacotherapy aims to control EDS and cataplexy, and usually each set of symptoms requires a different pharmacological approach. In the US, stimulants such as modafinil, methylphenidate, and amphetamines are approved for the treatment of EDS, while in the EU the HA inverse agonist pitolisant has recently been approved by the European Medicines Agencies [64]. Antidepressants that suppress REM sleep, such as TCAs, and sodium oxybate are effective at reducing cataplectic events [65,66]. Among the available pharmacotherapy for narcolepsy, sodium oxybate is the only FDA-approved drug indicated for the treatment of both EDS and cataplexy; nonetheless, there are off-label options such as selegiline [67], and mazindol [68], a wake-promoting agent that has recently been granted orphan drug designation for the treatment of narcolepsy by the FDA. In Table 2, the current pharmacological options for narcolepsy are revised.

4. Pharmacological opportunities for the treatment of sleep disorders

As we have already stated, sleep is often taken for granted. Nonetheless, its importance should not be underestimated. It is a behavior we dedicate approximately one-third of our lives to and when not met properly can make the rest of our time miserable. It is not surprising then that there is a continued need for new and better drugs.

Novel drugs in the pipeline for sleep disorders act upon already studied neurotransmitter systems: melatonin, histaminergic, and orexinergic. We will briefly describe these systems and their current pharmacological developments.

Finally, we will discuss the MCHergic system as a possible novel target for the treatment of sleep disorders. As a summary, we present in Table 3 the current status of several compounds undergoing clinical trials for the treatment of sleep disorders.

4.1. Targeting the melatonergic system

Melatonin is a natural hormone primarily produced by the pineal gland. The production of melatonin maintains an inverse relationship with the amount of environmental light: during the night production spikes and during the day it is inhibited. This pattern is observed regardless of the sleeping habits (nocturnal vs. diurnal) of the species in question [69,70]. Due to this, melatonin has been referred to as the hormone of the night.

How melatonin influences sleep is still not completely understood. In humans, the production of melatonin and sleep behavior tend to happen at similar times; nonetheless, the current consensus is that melatonin is not by itself a hypnotic. Instead, melatonin is thought to form part of Process C of the two-process model of sleep regulation, and, as predicted by this model, helps promote sleep by modulating the activity of the SCN and opening the so-called sleep gate [71]. In this regard, several studies have shown that administration of melatonin facilitates entrainment to new patterns of light and dark. An example of this occurs with jet lag, where administration of melatonin reduces the time it takes to adjust to new time zones [72].

In humans, melatonin binds to two G-protein-coupled receptors : mel1a (MT1) and mel1b (MT2). These receptors are the target of melatonin agonists that aim to improve sleep quality in people with different sleep disorders including insomnia and circadian rhythm disorders. Specifically for insomnia, ramelteon was approved by the FDA in 2005 and has since demonstrated a very modest efficacy. In this regard, ramelteon has proven to particularly benefit patients that have trouble initiating sleep by reducing sleep latency; although, it has also proved to increase total sleep time and quality of sleep [73,74]. Additionally, ramelteon offers an excellent safety profile and the therapeutic effect has been observed even after long-term use [75].

In addition to ramelteon, there are other drugs in the market that also target the melatonergic system. The most well-known are: tasimelteon (indicated for the treatment of non-24 h sleep–wake disorder), agomelatine (a melatonergic antidepressant), and Circadin® Neurim Pharmaceuticals (a prolonged-release melatonin preparation for the treatment of insomnia in patients aged 55 years or older). Finally, piromelatine is a new melatonin agonist under development that has so far completed phase 2 clinical trials for the treatment of insomnia. At the end of the clinical trial, the pharmaceutical company issued a statement claiming positive results; nonetheless, there have been no further studies.

The sleep-inducing effects of this class of drugs have proven to be modest causing consumers to consider them

Table 2. Pharmacological options for the management of Narcolepsy.

Drug class	Generic name	Usual dose (mg/day)	Common side effects	Serious side effects
For the treatment of excessive daytime sleepiness				
Nonamphetamine stimulants	Modafinil[a]	100–400	Headache, back pain, nausea, nervousness, stuffy nose, diarrhea, anxiousness, insomnia, dizziness, upset stomach	Serious rash Serious allergic reaction Psychiatric problems: depression, hallucinations, mania, suicidal thoughts, aggressiveness Cardiac symptoms
	Armodafinil[a]	10–250	Headache, nausea, dizziness, trouble sleeping	Serious rash Serious allergic reaction Psychiatric problems Cardiac symptoms
	Methylphenidate[a]	20–40	Headache, stomach ache, trouble sleeping, nausea, decreased appetite, nervousness	Heart-related problems Psychiatric problems: new or worse behavior and thought problems, bipolar illness, aggressiveness. Circulation problems in fingers and toes Slowing of growth Seizures Eyesight changes Priapism
Amphetamine stimulants	Dextroamphetamine[a]	5–60	Stomach ache, decreased appetite, nervousness	Heart-related problems Psychiatric problems Circulation problems in fingers and toes Slowing of growth Seizures Eyesight changes
Histamine inverse agonist	Pitolisant[b]	9–36	Insomnia, headache, nausea, anxiety, irritability, dizziness, depression, tremor, sleep disorders, fatigue, vomiting, vertigo, dyspepsia, weight increase, abdominal pain	Abnormal weight decrease Spontaneous abortion
For the treatment of cataplexy				
Tricyclic antidepressants	Clomipramine	10–150	Anticholinergic effects	Cataplexy rebound
Serotonin norepinephrine reuptake inhibitors	Venlafaxine	37.5–375	Anxiety, agitation, panic attacks, insomnia, irritability, hostility, aggressiveness, impulsivity, akathisia, hypomania and mania	Suicidal thinking and behavior Serotonin syndrome
Norepinephrine reuptake inhibitors	Atomoxetine	10–60		Suicidal thinking Liver injury Allergic reactions
Selective serotonin reuptake inhibitors	Fluoxetine	10–40	Unusual dreams, sexual problems, loss of appetite, diarrhea, indigestion, nausea or vomiting, weakness, dry mouth, flu symptoms, tiredness, changes in sleep habits, sinus infection, tremor, sweating, anxiousness, hot flashes, rash	Cataplexy rebound Suicidal thinking and behavior Serotonin syndrome Severe allergic reactions Abnormal bleeding Visual problems Seizures Manic episodes Changes in appetite or weight Low sodium levels in blood Changes in cardiac electric activity

(*Continued*)

Table 2. (Continued).

Drug class	Generic name	Usual dose (mg/day)	Common side effects	Serious side effects
For the treatment of both excessive daytime sleepiness and cataplexy				
Depressant	Sodium oxybate[a]	4.5–9	Nausea, dizziness, vomiting, bedwetting, diarrhea	Breathing problems Mental health problems: confusion, hallucinations, abnormal thinking, anxiousness, depression, suicidal thoughts Sleep walking
Monoamine oxidase inhibitors	Selegiline	10–40	Headache, dizziness, insomnia, nausea	Drug–drug interaction Dietary restrictions: tyramine
Stimulant	Mazindol	1–6	Dry mouth, diarrhea, sweating, irritability, anorexia, nightmares, headache, nausea, anorgasmia, muscle pain, nightmares, dysuria	Allergic reaction Irregular heartbeat Hallucinations Abnormal behavior Confusion

[a]Food & Drug Administration approved drugs for the treatment of narcolepsy and its symptoms.
[b]European Medicines Agencies approved drug for the treatment of narcolepsy.
Information is available from the US Food and Drug Administration website and the following references [64,66–68].

Table 3. Current and former compounds in the pipeline for the treatment of sleep disorders.

Generic name	Developer	Therapeutic indication	Clinical trial phase	Possible mechanism of action
Piromelatine (Neu-P11)	Neurim Pharmaceuticals (Tel-Aviv, Israel)	Chronic insomnia	2 (completed in 2013)	MT and 5-HT receptor agonist
JZP-110	Jazz Pharmaceuticals (Dublin, Republic of Ireland)	Narcolepsy (EDS) Obstructive sleep apnea	2 (recruiting) 3 (active not recruiting)	DA and NE transporter inhibitor
JZP-258		Narcolepsy (EDS & cataplexy)	3 (not yet recruiting)	DA receptor agonist
JZP-507		Narcolepsy (EDS & cataplexy)	Expected to initiate pivotal BE in 2017 and NDA in 1Q18	DA receptor agonist
THN-102	Theranexus (Orsay, France)	Narcolepsy (EDS)	2 (active, recruiting)	Alpha-1 adrenergic receptor agonist; sodium cannel antagonist
BTD-001	Balance Therapeutics (San Bruno, CA, US)	Narcolepsy	2 (active, recruiting)	GABA A receptor antagonist
JNJ-42847922	Janssen Research & Development (Raritan, NJ, US)	Insomnia	2 (completed)	OX2 receptor antagonist
ACT-541468	Actelion Pharmaceuticals (Allschwill, Switzerland)	Insomnia	2 (recruiting)	OX1 receptor antagonist
Lemborexant	Eisai Inc. (Tokyo, Japan)	Insomnia	3 (recruiting)	OX1 and 2 receptor antagonist
APD916	Arena Pharmaceuticals (San Diego, CA, US)	Narcolepsy	1 (completed in 2010)	H3 receptor inverse agonist
Filorexant (MK-6069)	Merck & Co (Kenilworth, Nj, US)	Insomnia	2 (completed in 2009)	OX1 and 2 receptor antagonist
LY2624803	Eli Lilly (Indianapolis, IN, US)	Insomnia	2 (discontinued)	H1 receptor antagonist
Esmirtazapine	Merck & Co	Insomnia	3 (terminated)	H1 receptor inverse agonist

MT: melatonin; 5-HT: serotonin; EDS: excessive daytime sleepiness; DA: dopamine; NE: norepinephrine; BE: bioequivalence; NDA: new drug approval; Q: quarter; GABA: gamma-aminobutyric acid; OX: orexin; H: histamine.

ineffective. Despite this misconception, objective improvements can be measured. If we consider these results in addition to the desirable safety profile and sustained therapeutic effect of long-term administration, melatonin agonists are a viable option for the treatment of sleep disorders.

4.2. Targeting the histaminergic system

HA is a wake-promoting neurotransmitter that binds to four G-coupled receptors: H1, H2, H3, and H4. The histaminergic cells found in the TMN of the posterior hypothalamus are the sole source of HA in the CNS. From this area, the TMN innervates the forebrain and brainstem. Similar to other wake-promoting nuclei, the firing pattern of TMN histaminergic cells is highest during wakefulness and lowers during nREM and REM sleep (the histaminergic system is reviewed in reference [76]).

Evidence in favor of the waking properties of HA comes from several studies. For example, wakefulness follows intracerebroventricular administration of HA and can be observed through EEG desynchronization, increased motor activity in normal rats, and a reduced duration of narcosis in pentobarbital-treated rats. Additionally, H1R antagonists abolish the observed arousal of intracerebroventricular administration of HA [77,78].

Pharmacological studies have exposed the potential of H1 and H3Rs as targets for drug development in sleep disorders. In this regard, H1R antagonists decrease wakefulness, and agonists dose dependently promote it. Moreover, H3 stimulation has an inverse effect: antagonism causes wakefulness, and pretreatment with an agonist inhibits the elicited arousal [79,80].

In the case of H2 [79] and H4Rs, effects on the SWC have not been observed. For H4Rs, even their presence in the brain is still matter of debate [81]. Consequently, there are no compounds that target H2 or H4Rs for the treatment of sleep disorders, thus we will discuss the available treatments that target H1 and H3Rs.

4.2.1. H1Rs

It has been almost a hundred years since the sedating effects of antihistamines were first noted. To this day, sleep aids that target H1Rs are commonly used for the treatment of chronic insomnia.

Existing chronic insomnia treatments that target H1Rs can fall in one of the three categories: OTC, off-label, and FDA approved. Some of the most used OTC antihistamines for their soporific effects are: diphenhydramine and doxylamine. Off-label treatments such as the antidepressants trazodone, amitriptyline, and mirtazapine, and the antipsychotic quetiapine are H1R antagonists. The FDA-approved TCA doxepin, when used at low doses (3–6 mg), is a potent H1R antagonist [44,46,54,82].

There is at least one H1R antagonist that has reached phase 2 clinical trials: LY2624803. However, there are no open clinical trials for this drug since 2013. Another H1R antagonist, esmirtazapine, reached phase 3 clinical with promising results. Nonetheless, the pharmaceutical company dropped the development, citing strategic reasons.

4.2.2. H3Rs

Molecules that target the H3R are a new generation of drugs in the catalog of sleep disorder treatments. In 2016, pitolisant became the first H3 inverse agonist approved for the treatment of narcolepsy with (NT1) or without (NT2) cataplexy in the EU. In the US, pitolisant is undergoing clinical trials (phase 3) for EDS and cataplexy. Where approved, pitolisant is recommended at a dosage of 18–36 mg/day. Common side effects for pitolisant are insomnia, headache, nausea, anxiety, irritability, dizziness, depression, tremor, vertigo, and dyspepsia.

The effect of pitolisant on EDS was comparable to the improvement observed with the administration of the FDA-approved drug modafinil [83]. For the management of cataplexy, clinical trials showed a 75% reduction in the weekly frequency of cataplectic episodes when compared to baseline [84]; the mechanism involved in this effect is not well understood as HA activity is not altered during cataplectic events [85], a phenomenon thought to mediate in the maintenance of wakefulness during these episodes.

Not only is pitolisant the first H3R inverse agonist indicated for the treatment of EDS and cataplexy, it has also just recently hit the markets and therefore user experience information is still scarce. We expect to learn more as US clinical trials progress and EU consumption increases.

Beyond pitolisant, also the H3R inverse agonist, APD-916 completed phase 1 clinical trials in 2010 but further studies were suspended.

4.3. Targeting the orexinergic system

The orexinergic system was first described in 1998 by two independent laboratories, each of whom gave them their own names: de Lecea et al. prefer the term hypocretins, while Sakurai et al. use the orexin nomenclature [24,86]. This system is comprised by a relatively small group of 50,000–80,000 neurons [26] that can be found in the lateral hypothalamus (LH), mainly in the perifornical area. These cell bodies have widespread projections throughout the central CNS, including the nuclei previously mentioned as involved in the control of sleep–wake and nREM–REM transitions: hypothalamus, LC, TMN, DR, POA, PB, PPT/LDT, and periaqueductal gray, to mention some [23].

Orexin neurons produce two excitatory peptides (OXA and OXB) that differentially stimulate two G-coupled receptors (OXR1 and OXR2) [86]. While OXA binds to both receptors (EC50 30 and 34 nM, respectively), OXB binds selectively to OXR2 (EC50 2500 nM for OXR1 vs. 60 nM for OXR1) [86]. In congruence with orexinergic afferents stemming from the LH, there are orexin receptors in these sites, albeit with a differential distribution.

Since the discovery of the orexinergic system, it has been made clear that orexins are involved in the promotion of wakefulness. Furthermore, defects in the system have been associated with the sleep disorder narcolepsy in animals [61,87] and humans [26], and orexin overexpression in mice and fish has been suggested to interrupt sleep and even cause an insomnia like phenotype [88,89].

These results make the orexinergic system a good candidate for a next generation of drugs that could offer relief for conditions such as narcolepsy and chronic insomnia. In fact, attempts have already been successful for the development of orexin antagonists for the treatment of chronic insomnia, and orexin replacement therapy is under study for the treatment of narcolepsy.

4.3.1. Orexin antagonists

Since it came apparent that the orexinergic system was involved in the maintenance of wakefulness, great expectation developed around the notion of a new generation of sleep aids that could target this system and be useful for the management of insomnia. Among several other DORAs developed, the first, and only one thus far, to reach FDA approval for the treatment of chronic insomnia is suvorexant [90].

Suvorexant proved during clinical trials to be effective at increasing total sleep time, reducing sleep onset latency, and nighttime waking [91]. In 2014, the FDA approved the commercialization of suvorexant with a recommended initial dose of 10 mg and a maximum dosage of 20 mg daily. Although the best improvements in nighttime sleep were observed at 40 and 80 mg, driving tests suggested that even at 20 mg suvorexant could cause a level of impairment similar to that observed with ≈0.05 of ethanol in the blood stream. Possibly, next-day impairment is associated with the 12 h half-life of suvorexant causing working concentrations to be present in plasma even after 8 h of sleep; worse so is the finding that the half-life increases with frequent administrations reaching up to 20 h in an older woman after 7 days of administrating 40 mg of suvorexant. This caused the FDA to consider higher than 20 mg doses unsafe due to next-day impairment [92].

The main concern with the approved low doses is the possibility of not observing the therapeutic effect. In such a direction, a 2016 study of the Institute for Safe Medication Practices (ISMP) found that almost half (ã42.2%) of the complaints submitted by users during the first two quarters of 2015 claimed that suvorexant was ineffective, and this was consistent among all dosages available [93].

Since orexin antagonists became the new possible treatment for sleep disorders such as chronic insomnia, concerns arose about the possibility of them eliciting narcolepsy like symptoms as a side effect. This fear was confirmed during clinical trials when reports of sleep paralysis, hypnagogic hallucinations, and excessive daytime somnolence were materialized [91]. Although the number of reports was small, the FDA included a warning in the label for sleep paralysis and temporary weakness of legs as a possible side effect of suvorexant. Furthermore, as expected, when the use of suvorexant was made more widespread, more events were reported. The ISMP in their analysis of submitted complaints found evidence for all symptoms of narcolepsy including the most worrisome one: cataplexy [93]. The circumstances in which these symptoms arise are still not clear and while the use of suvorexant continues, the manufacturer and FDA should pay a lot of attention to the pattern of their occurrence.

Suvorexant was met with a lot of expectation, but it is possible that it will not prove to be superior to other available treatments [94]. Suvorexant is only the first DORA to reach the market, so attention must be paid to the other compounds in the pipeline, such as: JNJ-42847922 (phase 2), lemborexant (phase 3), and ACT-541468 (phase 2).

Among these, JNJ-42847922 is different to the other antagonists in that it is a selective orexin receptor antagonist (SORA) with selectivity for the OXR2 (2-SORA). As mentioned earlier, there are two orexin receptors: OXR1 and OXR2. Among these, OXR2 seems to carry more weight in the wake-promoting effects of orexin peptides [95]. Conversely, antagonism of only OXR2 is more effective in sleep promotion than OXR1 antagonism [96]. Hence, the potent 2-SORA JNJ-42847922 could prove to have therapeutic effects while displaying milder side effects than DORAs such as suvorexant [97].

Presently, suvorexant is in clinical trials for additional uses beyond chronic insomnia. It is being tested for its safety and efficacy in treating insomnia associated with Parkinson's disease (phase 4), Alzheimer's disease (phase 3), post-traumatic stress disorder (phase 4), bipolar disorder (phase 4), and fibromyalgia (phase 4). It should be noted that it is also being proposed as an addition to antidepressant therapy in major depression (phase 4), for improving daytime sleep in shift workers (phase 4) and as an anxiolytic in cocaine users (phase 2).

4.3.2. Orexin agonists

An accumulation of evidence suggests that orexinergic agonists should be effective for the management of narcolepsy. In this regard, a hallmark study demonstrated that replacement of orexin peptides can effectively prevent narcolepsy symptoms such as cataplexy and other REM sleep abnormalities in the orexin/ataxin-3 mouse model of narcolepsy [98]. Despite the association of narcolepsy with a degeneration of the orexinergic system, none of the currently available drugs target it.

Orexin replacement therapy is an exciting therapeutic opportunity for narcolepsy. For a long time only native and enhanced orexinergic peptides were available, but they are only used in the experimental setting. Their short half-lives, low blood–brain barrier penetrance, and limited intestinal absorption deemed them unsuitable for clinical use. Moreover, synthesizing functional molecules has also proven to be difficult as evidenced by a lack of available non-peptidergic orexin receptor agonists. Recently, a selective receptor agonist for OXR2, 'compound 26,' was developed and described by Nagahara et al. [99]; this development came after high-throughput screening ~250,000 molecules. The slow discovery rate of orexin agonists might be partially solved since the development of a screening approach designed to increase the rate of orexin receptor ligands found [100].

There are still no reports of the behavioral effects of compound 26 on narcoleptic phenotypes, but available evidence supports the notion of a direct involvement of OXR2 in the generation of narcolepsy. For instance, knockout (KO) OXR2 mice have disrupted wakefulness and cataplexy attacks [101]

and intracerebroventricular infusion of OXA in OXR1 KO mice caused an increase in wakefulness and suppression of nREM sleep although these effects were significantly smaller than those observed in wild-type mice after the same administration of OXA [95]. These results suggest that stimulation of OXR2 can improve wakefulness, albeit modestly.

4.4. Targeting the MCH system

At the level of the hypothalamus, MCH cells are intermingled with orexinergic neurons [102], virtually project to the same sites [103], are inhibitory [104], and possess an opposite firing pattern: they are silent during wakefulness, start activity during nREM sleep, and reach a peak during REM sleep [105]. Thus, MCH cells appear to be the counterpart of orexinergic cells.

The effect of MCH is mediated by two G-coupled receptors: MCHR1 and MCHR2. MCHR1 is functional in several species from mice to humans, while MCHR2 is only functional in dogs, ferrets, monkeys, and humans [106]. From the hypothalamus, MCH has been studied for their involvement in the control of feeding behavior [107], mood [108], and the SWC.

Although there is still much research needed, in the case of the SWC, MCH stimulation has proven to promote sleep. The mechanisms are still unclear but MCH stimulation appears to preferentially promote REM sleep. In rats, it has been observed that intracerebroventricular injection of MCH causes a dose-dependent increase of nREM and REM sleep, of up to 70% and 200%, respectively, when compared to controls [31]. Additionally, the influence of MCH activity for sleep promotion appears to be strong, as optogenetic activation of about half of MCH cells in rats can elicit a significant increase in the amount of REM and nREM sleep [109]. In mice, ablation of MCH cells by diphtheria toxin A caused increased wakefulness and reduced nREM sleep duration, without changes in REM sleep duration [110].

To date, there is little information of the effect of MCH agonists and antagonists across the SWC. For studying their role in sleep, Ahnaou et al. carried out a systemic administration of two MCHR1 antagonists in rats and observed an increase in the amount of wakefulness with a concurrent reduction of nREM and REM sleep [111]. Studies surrounding the role of MCHR2 in the SWC are not available, possibly due to the lack of MCHR2 expression in mice and rats.

MCH agonism in the experimental setting has been traditionally achieved by intracerebroventricular injection of the peptide; nonetheless, several compounds have been developed that have selective and non-selective agonistic and antagonistic effects on MCHRs [112,113]. Some of these compounds are even being studied for their potential clinical applications. For example, MCHR1 antagonists are a new target for the treatment of obesity [114].

In the case of the SWC, the little evidence that is available suggests that the MCHergic system plays an important role in sleep promotion. As more research is carried out, we are confident that pharmacological studies will prove that the MCHergic system is also a good target system for the treatment of sleep disorders.

5. Conclusion

Our understanding of sleep–wake mechanisms relies on models such as the two-process and flip-flop switches. Notwithstanding, they do a fair job in predicting the occurrence of wake and sleep states. The neurotransmitter systems involved have allowed for the development of compounds capable of altering the SWC architecture and offering aid in the treatment of sleep disorders. Traditional targets for sleep medications include the GABA, melatonin, HA, dopamine (DA), 5-HT, and NA systems. More recently, the discovery of the orexinergic system has allowed for the development of suvorexant, a DORA indicated for the treatment of chronic insomnia. Despite the many available treatments for chronic insomnia and narcolepsy, their efficacy/side effect profile is poor at best, highlighting the need for better therapeutic options.

6. Expert opinion

Sleep is a very complicated and highly regulated behavior that relies on orchestrated events that range from the genetic machinery to environmental factors. Just at the level of brain neurotransmission, it is clear that several systems are working together without any single one being indispensable. Thus, it is very hard to pinpoint an exact target for drug development, and even if that is achieved, the evoked effects are not limited to the sleep or wake realm. Despite its complexity, the study of sleep is indispensable, due to its importance in everyday life.

Understanding of sleep–wake mechanisms has come very far, and new target systems are being proposed as candidates for drug development. For instance, the relatively recent discovery of the orexinergic system and its association with the sleep disorder narcolepsy has revitalized the pipeline for sleep aides. Paradoxically, this discovery has benefitted chronic insomnia more than narcolepsy itself.

Suvorexant is the first DORA to receive FDA approval for the treatment of insomnia. Although it was a land-breaking drug, it has, in our opinion, fallen short of expectations. It has displayed limited efficacy with a comparable side effect profile to other already available treatments, with the added risk for developing narcoleptic symptoms. Regardless, there are other DORAs and SORAs in the pipeline that could offer relief in better circumstances.

For narcolepsy, H3R inverse agonists are the newest molecules. Pitolisant has recently been approved in EU where it has demonstrated therapeutic effects on EDS and cataplexy with a very mild side effect profile. The mechanisms through which pitolisant is capable of exerting its therapeutic effect remains unclear, but further study will offer interesting insight into the etiology of the most exemplary symptom of narcolepsy: cataplexy.

Additionally, we strongly believe that the role of the MCHergic system in the SWC will become much more relevant in the near future. It has already been suggested as the complement of the orexinergic system, and the potential for drug development in that regard is ample. MCHR1 stimulation appears to enhance REM sleep preferentially, thus antagonism of MCHRs should be capable of offering aid for sleep disorders, i.e. parasomnias and narcolepsy symptoms such as cataplexy.

The role of MCHR2 has been more difficult to elucidate because these receptors are not functional in mice and rats.

One of the main objectives of sleep pharmacotherapy is to restore a natural sleep architecture that would in turn allow the patient to feel refreshed the next day. Nonetheless, next-day impairment is a common side effect of sleep medication even when the patient reports an improvement in sleep quality. We believe this is one of the biggest pitfalls of current sleep medicine.

We speculate that current sleep pharmacotherapy does not emulate the naturally occurring flip-flop switch between wake- and sleep-promoting systems, thus there is a lag in the transition that could be responsible for the 'groggy' feeling that often accompanies sleep remedies. It could be possible that, in the future, a combination therapy of sleep-inducing and wake-promoting agents be used in hopes of facilitating the transition among states.

Successful management of sleep disorders increases quality of life for individuals and reduces the economic burden for society; therefore, the search for better and improved therapies is an ongoing challenge.

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Treatment of cataplexy

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Introduction: The term cataplexy originates from the Greek $\kappa\alpha\tau\acute{\alpha}$ (kata, 'down'), and $\pi\lambda\eta\xi\iota\Omega$ (plexis, 'stroke'). Cataplexy, a sudden loss of muscle tone in response to strong emotions is the most specific symptom of narcolepsy. It is thought to be due to disturbed rapid eye movement (REM) sleep regulation and portrayed as REM sleep atonia occurring at the wrong time. However, there are several arguments against including cataplexy in the 'state boundary control' hypothesis. Cataplexy is most likely secondary to the disruption of different neurological pathways and eliciting the mechanisms by which cataplexy unfolds still remains an undergoing challenge.

Areas covered: In this review, the authors appraise the neurobiology of cataplexy and emphasize the prospective approaches for the treatment of this ailment. The authors describe current pharmacological approaches that address this symptom, such as serotonergic and noradrenergic agents and γ-hydroxybutyrate. In addition, drugs that are still in the preclinical phase, such as triple reuptake inhibitors, thyrotropin-releasing hormone, histamine agonists and hypocretin are also reviewed. Finally, the authors conduct a comprehensive analysis of the various promising future treatments for cataplexy.

Expert opinion: Cataplexy remains a fascinating symptom, showing complex interactions between systems involving emotions and those involved in motor control. Incorporating and integrating this knowledge will be imperative to further understand and treat this phenomenon.

Keywords: cataplexy, hypocretin, narcolepsy, rapid eye movement sleep

Expert Opinion on Orphan Drugs [Early Online]

1. Background

1.1 Narcolepsy and cataplexy

The history of cataplexy is closely related to that of narcolepsy. Gélineau first introduced the term narcolepsy in 1880 to encompass two kinds of occurrences, one of overpowering drowsiness and sleep during the day and another of sudden muscle weakness. These symptoms were assumed to share a common origin, even if they were not always observed together. It was 1916 when Henneberg coined the term 'cataplexy', referring to a loss of muscle tone elicited by emotions, which also yielded the term narcolepsy with cataplexy (NC) [1].

The current definition of cataplexy has not changed much from Henneberg's first description. According to the second edition of the International Classification of Sleep Disorders, cataplexy is defined as 'a sudden and transient loss of muscle tone triggered by emotions that happens without loss of consciousness'. With exception of the respiratory and eye muscles, cataplexy involves all muscle groups and can be either generalized, affecting the entire body, or partial with buckling of the knees, dropping of the head, flickering of facial muscles or sagging of the jaw. The frequency of cataplexy attacks varies across patients, with some having an episode only once a year to others experiencing many throughout the day [2,3].

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Article highlights.

- Cataplexy is the pathognomonic and most intriguing symptom of narcolepsy.
- Hypocretin agonists may represent the best candidate for treating cataplexy.
- ASI might help prevent NC onset.

This box summarizes key points contained in the article.

1.2 Dissecting narcolepsy and its symptoms

The study of cataplexy has benefitted from its close association with narcolepsy. The four cardinal symptoms of narcolepsy are excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations and sleep paralysis [4]. EDS is manifested by the tendency of patients to fall asleep at any moment during the day as a consequence of an improper sleep–wake regulation; while cataplexy, hypnagogic hallucinations and sleep paralysis are considered as partial manifestations of rapid eye movement (REM) sleep occurring at the wrong time. Cataplexy refers to attacks of flaccid muscle weakness triggered by a variety of emotions such as humor or anger. Hypnagogic hallucinations are vivid dream-like experiences occurring during sleep–wake transitions. Sleep paralysis is the inability to perform voluntary movements during the transition from waking to sleep or *vice versa* [5,6].

Although the mechanisms underlying the symptomatology of NC remained unknown for a long time, tremendous progress has been made in our understanding of the neurobiology of NC in the last few years [7]. One of the most important breakthroughs came with the discovery of two excitatory neuropeptides named hypocretins (also known as orexins) that are exclusively synthesized in the lateral hypothalamus (LH) [8,9]. Hypocretin neurons virtually project throughout the entire neuraxis; this arrangement makes the hypocretin system particularly suitable to orchestrate various functions, particularly sleep–wake boundary control and REM sleep [10]. Hypocretins play essential roles in maintaining wakefulness and regulating transitions between sleep and wake, and dysfunctions of these neurotransmitters or their receptors result in narcolepsy in humans, dogs and mice [11-14].

NC is caused by the specific loss of hypocretin neurons, and both mRNA and protein are dramatically reduced in the brain of narcoleptic patients. In addition, there are also undetectable levels of hypocretin peptide in the cerebrospinal fluid of these patients [15]. An autoimmune basis for the hypocretin cell loss in narcolepsy has long been suspected based on its strong genetic association with selected human leukocyte antigen (HLA) alleles. Narcolepsy nearly exclusively occurs with DQ0602, a heterodimeric a/b class II protein encoded by *HLA DQB1*06:02* and *DQA1*01:02*, two gene variants found together on the same haplotype [15].

Even though there has been a remarkable improvement in dichotomizing the physiopathology of narcolepsy, we still have not been able to pinpoint the exact mechanism by which cataplexy is generated. Loss of state boundary control is a convincing explanation for EDS, but it does not address the appearance of REM sleep at inappropriate times, especially cataplexy which is triggered by emotional stimuli. Hence, cataplexy remains the most elusive symptom of narcolepsy, making it an entity on its own.

2. Neurobiology of REM sleep atonia and cataplexy

Decreased muscle tone as seen in cataplexy resembles the normal muscle atonia present during REM sleep. When an individual is in REM sleep, all skeletal muscles, with exception of ocular and respiratory muscles are paralyzed. Similar mechanisms may cause the muscle paralysis in REM sleep and cataplexy.

The neurochemical basis of REM sleep is complex, with pharmacological studies indicating the involvement of many neurotransmitter systems and neuronal populations in the brainstem. In 1962, Jouvet determined that REM sleep persists after dissecting the structures rostral to the pons, proving that the brainstem is essential and sufficient for REM sleep generation [16].

Based on extensive neuropharmacological experiments, it has been determined that REM sleep atonia is a result of the sublaterodorsal nucleus (SLD) glutamatergic excitatory input to the ventromedial medullary nuclei and spinal cord interneurons that in turn release glycine or GABA and inhibit spinal motoneurons [17-19]. Lesions of these pathways in animals produce REM sleep without atonia [20,21] and similar injuries could be responsible for REM sleep behavior disorder in humans, a parasomnia in which REM sleep paralysis fails, causing patients to act out their dreams [22].

During normal wakefulness, atonia-producing pathways are inhibited by noradrenergic, serotonergic and GABAergic neurons of the ventrolateral periaqueductal gray and the lateral pontine tegmentum (vlPAG/LPT) [23], while hypocretin peptides released from the LH help prevent REM sleep atonia by, respectively, increasing or reducing the required threshold for inhibitory and excitatory muscle control nuclei to discharge [24-27]. During cataplexy, noradrenergic and serotonergic neuron activity is suppressed, while wake-promoting histaminergic neurons of the tuberomammillary nucleus remain active, preventing loss of consciousness [28,29].

In summary, an imbalance between pontine monoaminergic and cholinergic neuronal populations, secondary to a disruption in the hypocretin system, is thought to underlie the pathophysiology of cataplexy, although it does not yet explain the characteristic emotional trigger.

2.1 The emotional trigger

Among the events that most frequently trigger an episode of cataplexy is laughter, which reminds us of the transient feeling of losing strength when heartedly laughing [30].

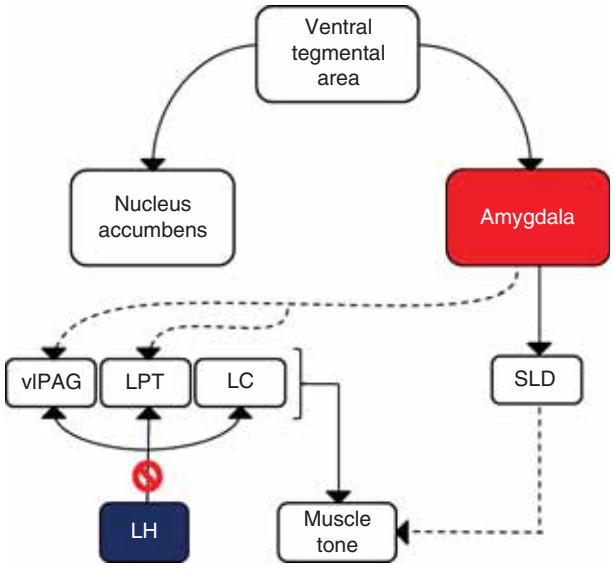

Figure 1. Proposed cataplexy pathway; overactivation of the NAcc and amygdala, accompanied by a decreased activation of the LH when presented with humorous material. The amygdala sends excitatory projections to the SLD and inhibitory projections to the vlPAG/LPT. Positive emotions activate these limbic pathways and promote the onset of muscle atonia. In healthy individuals, this would be offset by the atonia suppressing effects of the hypocretin peptides; however, in people with narcolepsy, these emotional signals would be unopposed, resulting in sustained activation of the SLD and downstream pathways that lead to paralysis.
Dashed line: Inhibition; Solid line: Excitation.

Laughter is an expression of mirth and amusement, and the rewarding feeling that follows is associated with the activation of the mesolimbic dopaminergic pathway that projects from the ventral tegmental area (VTA) to the *nucleus accumbens* (NAcc), amygdala and hippocampus [31,32]. The amygdala plays an important role in the detection and consolidation of emotionally significant events and has projections to the LH and structures of the pons relevant for the control of muscle tone, such as the SLD [33-36]. The activity of the amygdala has been studied both during cataplexy episodes and REM sleep and appears to be consistent among these stages. One landmark study, carried out with cataplectic dogs, showed that there is a specific cell population in the amygdala whose activity increases during cataplexy attacks, REM sleep and non-REM sleep with simultaneous inactivation of the *locus coeruleus* (LC) [29,37].

In humans, increased limbic activity, specifically within the amygdala and hippocampus, has been reported during sleep and is thought to account for the emotional content of dreams [38,39]. The use of neuroimaging techniques, such as functional magnetic resonance imaging, to study patients diagnosed with NC revealed an altered activation of the structures involved in the reward process, this altered activity includes over activation of the NAcc and amygdala, accompanied by a decreased activation of the hypothalamus when presented with humorous material [40-42].

If we take into consideration that the central nucleus of the amygdala sends excitatory projections to the SLD and inhibitory projections to the vlPAG/LPT, it is very likely that strong, positive emotions activate these limbic pathways and promote the onset of muscle atonia. In healthy individuals, this would be offset by the atonia suppressing effects of the hypocretin peptides, resulting in no more than a fleeting sense of mild weakness. However, in people with narcolepsy, these emotional signals would be unopposed, resulting in sustained activation of the SLD and downstream pathways that lead to paralysis (**Figure 1**) [33,43].

Taking all of the above into account, one proposed explanation for cataplexy suggests that sudden motoneuron inhibition is caused by an exacerbated physiological reaction to humor mediated by the amygdala and facilitated by the lack of hypocretinergic input. The relationship between the pathways that trigger atonia in cataplexy and REM sleep is yet to be fully understood.

3. Current treatment for cataplexy

The treatment for cataplexy is habitually an integral part of the treatment for narcolepsy; however, different approaches are generally used for the different sets of symptoms (i.e., EDS vs cataplexy). While EDS is addressed with stimulants, such as modafinil or amphetamine-like stimulants, cataplexy has traditionally been treated with antidepressants.

In the next part of this review, we will conduct an appraisal of current pharmacological approaches for treating cataplexy.

3.1 Tricyclic antidepressants

Tricyclic antidepressants (TCAs) are the most commonly used anticataplectic agents (**Table 1**). Akimoto was the first to describe the benefit of TCAs for the treatment of cataplexy after reporting the efficacy of imipramine [44]. In addition to relieving cataplexy, TCAs are also helpful for treating other REM sleep abnormal phenomena, such as sleep paralysis and hypnagogic hallucinations, but do little or nothing for EDS. The beneficial effects of TCAs are related to their monoaminergic reuptake inhibition and anticholinergic effects.

The majority of the TCAs act as serotonin (5-HT) and noradrenaline (NA) reuptake inhibitors by blocking the 5-HT transporter (SERT) and the NA transporter (NAT). The degree of uptake inhibition of NA and 5-HT is quite variable depending on the compound and the existence of active metabolites (mostly active on adrenergic uptake). In addition to their reuptake inhibition, many TCAs also act as antagonists of 5-HT (5-HT_2, 5-HT_6, 5-HT_7), α_1-adrenergic and NMDA receptors and as agonists of sigma receptors (σ_1 and σ_2), some of which may contribute to their therapeutic efficacy, as well as their side effects. TCAs also have a varying high affinity for antagonizing the histamine

Table 1. Current anticataplectic agents.

Drug	Mechanism of action	Usual daily dose	Side effects
TCAs			
Imipramine	Block reuptake of NA ≈ 5-HT	10 – 100 mg	Dry mouth, anorexia, sweating, constipation, drowsiness (anticholinergic effects)
Clomipramine	Block reuptake of 5-HT > NA	10 – 150 mg	Anticholinergic effects
Desipramine	Block reuptake of NA > 5-HT	25 – 200 mg	Anticholinergic effects
Protryptiline		5 – 60 mg	Anticholinergic effects
SSRIs			
Fluoxetine	Block reuptake of 5-HT ≫ NA = DA	20 – 60 mg	Anhedonia, gastrointestinal effects
Fluvoxamine		50 – 300 mg	Gastrointestinal effects
SNRIs			
Venlafaxine	Block reuptake of 5-HT ≥ NA	150 – 375 mg	Anorexia, drowsiness, nausea
Milnaciplan		30 – 50 mg	Anorexia, drowsiness, nausea
MAOIs			
Brofaromine	Reversible MAO-A inhibitor	75 – 150 mg	Dry mouth, sleep disturbance, headache, nausea, vertigo/dizziness
Selegiline	Irreversible inhibitor of MAO-B	15 – 30 mg	Dry mouth, headache, insomnia, sweating, muscle twitching, dizziness, irritability, restlessness and tremor
Others			
Sodium Oxybate	Unknown May act via GABA-B or specific GHB receptors	4 – 6 g	Dizziness, nausea, vomiting, confusion, agitation, epileptic seizures, and hallucinations and coma with bradycardia

5-HT: Serotonin; DA: Dopamine; GABA: γ-Aminobutyric acid; GHB: Sodium oxybate; MAOI: Monoamine oxidase inhibitor; NA: Noradrenaline; SNRIs: Selective noradrenaline reuptake inhibitors; SSRIs: Selective serotonin reuptake inhibitors; TCAs: Tricyclic antidepressant.

1 and histamine 2 (H_1 and H_2) receptors, as well as the muscarinic acetylcholine receptors [45].

Despite their effectiveness, the use of TCAs in the treatment of cataplexy has been hampered by a number of problems. The first one is the relatively poor side effect profile of most tricyclic compounds. These are mostly due to their anticholinergic properties, leading to dry mouth (and associated dental problems), tachycardia, urinary retention, constipation and blurred vision (**Table 1**). Additional side effects are weight gain, sexual dysfunction (impotence and/or delayed orgasm), tremors, antihistaminergic effects leading to sedation and occasionally orthostatic hypotension due to a1 adrenergic blockade effects of some compounds. Even though antidepressants achieve anticataplectic effects quickly and at very low doses (doses under antidepressant effects) the number and severity of side effects can cause the patient to abruptly discontinue TCAs use, which in turn can provoke cataplexy rebound [46].

3.2 Selective reuptake inhibitors

The introduction of newer antidepressants with selective serotonin (SSRIs) and adrenergic reuptake inhibitions (SNRIs) with no anticholinergic effects achieves the control of cataplexy with fewer side effects when compared to TCAs.

SSRIs, such as fluoxetine, fluvoxamine, paroxetine, sertraline, femoxamine, zimelidine, trazodone, achieve the control of cataplexy with fewer side effects when compared to TCAs. However, the potency of the serotonergic compounds on cataplexy is not as good as expected. This experience parallels experiments in canine narcolepsy suggesting that adrenergic, and not serotonergic, uptake inhibition mediates the anticataplectic effects of most antidepressant medications. Fluoxetine, one of the most commonly used therapeutic agents is effective for addressing cataplexy, but higher doses closer to the antidepressive effective doses are needed (e.g., 20 – 60 mg/day) [47].

SNRIs, such as milnacipran and venlafaxine, are a preferred class of medications for treatment of cataplexy over TCAs because they lack the more significant anticholinergic and cardiac risk factors of TCAs, yet still have the benefit of affecting release of NA. They have no significant affinity for muscarinic, cholinergic, histaminergic or alpha-adrenergic receptors. The most common side effects include headache, nausea and dry mouth. Even though the side effects are minimal and similar to those of SSRIs, there is an important dose-dependent risk of hypertension [48]. Worthy of notice, SSRIs compounds are generally not as effective as SNRIs. For this reason, SNRIs, such as venlafaxine, are commonly used and are probably the best option. Venlafaxine is typically started at 37.5 mg (extended release) in the morning, but is most often effective at doses of 75 – 150 mg extended release/day. With these compounds, doses inferior to those typically used for depression or anxiety treatment are generally sufficient [49,50].

This newer generation drugs have lower affinities for the abovementioned receptors, and therefore are less prone to cause adverse effects as those usually seen with the older generation of antidepressants. These drugs are frequently rapidly active on cataplexy when compared with anxiety or depression, but an important problem is the cataplexy rebound after their discontinuation.

3.3 Monoamine oxidase inhibitors

Monoamine oxidase inhibitors (MAOIs) are known as potent REM sleep inhibitors and are therefore considered anticataplectic agents. The extracellular effect of naturally released catecholamine is terminated by reuptake or enzymatic degradation, either by MAO or catechol-*O*-methyl transferase. MAO is a flavin-containing dominating enzyme located in the outer membranes of neural and glial mitochondria. There are two forms – MAO-A and MAO-B. MAO-A is blocked by clorgyline and has high affinity for NA and 5-HT, while MAO-B is insensitive to clorgyline and denotes high affinity for phenylethylamine and dopamine (DA) [51].

The first generation of MAOIs has been rarely used in clinical practice due to their poor safety profile. It is also dangerous to use other drugs with sympathomimetic effects (TCA and amphetamine-like compounds) in patients treated with MAOIs due to drug interactions that are impossible to predict. In addition, the first generation of MAOIs (irreversible MAOIs) has the unique property of covalently binding to the active site of the enzyme leading to long-term enzymatic inhibition even after a single dose [51].

A safer generation of MAOIs is now available. These include compounds with selective MAO-A (brofaromine) or MAO-B (selegiline) inhibition and/or a reversible enzymatic inhibition profile. In contrast to irreversible MAOIs, reversible MAOIs are substrates for the MAOs and compete with the endogenous monoamines, and therefore are less prone to cause hypertensive crisis when taken with tyramine-containing foods [51]. Selegiline is a potent irreversible MAO-B selective inhibitor used in the treatment of Parkinson's disease. This compound is essentially a methamphetamine derivative and is metabolized into amphetamine and methamphetamine. Usually, 20 – 30 mg reduces cataplexy, an effect comparable to amphetamine at the same dose range, suggesting that its effects are also due to its active metabolites [52].

Some of these new reversible MAOIs have shown to be more effective and safe for the treatment of cataplexy with fewer side effects; however, the benefit this group of drugs provides has yet to be proven.

3.4 Sodium oxybate

Sodium oxybate, also known as gamma-hydroxybutyrate (GHB), is a metabolite of GABA first studied as an anesthetic and later found to be effective for the treatment of all the symptoms of NC [53,54]. Nightly doses (500 mg) of GHB have proved to reduce the occurrence of cataplexy events during the day and the frequency of nocturnal awakenings and to promote sleep [55]. Owing to its short half-life (1 – 2 h), GHB is administrated twice during the night, the first dose at bedtime and a second one between 2.5 and 4 h later. Unlike the treatment with antidepressants, there is no cataplexy rebound after withdrawal and in 2002 the Food and Drug Administration approved GHB for the treatment of cataplexy associated with narcolepsy and has become a first-line treatment for NC. It has been reported that GHB is safe and efficacious when prescribed within the active dose range and when administration is limited to nighttime hours. This compound should be considered as first line for any patient with disturbed nocturnal sleep, cataplexy and obesity [56]. Nonetheless, GHB is given to patients under close medical supervision and after being extensively educated about the dangers of an overdose. Intoxication with GHB can lead to profound sedation, euphoria, decreased inhibitions, enhanced sex drive, anterograde amnesia and even coma.

The mechanism of action of GHB is still unclear. GHB binds to specific GHB receptors and is thought to regulate GABA release. Moreover, GHB is a GABA metabolite; when excessive release of GABA occurs, the concentration of GHB increases, which in turn acts on presynaptic GHB receptors, increasing the membrane potential and therefore reducing the amount of GABA released [57]. The physiological effects of GHB appear to be related to the activity of $GABA_B$ receptors – a finding that requires further study [58]. GHB has also been shown to enhance DA and NA release in a biphasic way, first by inducing its accumulation in synaptic vesicles and later enhancing its release [59] – an effect that could account for the improvement on EDS. The most common side effects are nausea and weight loss.

3.5 Clinical considerations for children and pregnant women

The treatment of children with NC is similar to that of adults with a few cautions. First, in children, antidepressants with adrenergic effects can slightly reduce growth. Regarding pregnancy and anticataplectic drug administration, there are not a lot of studies addressing this matter.

Venlafaxine is category C (studies on animals show adverse effect and toxicity on fetus).

GHB is category B (studies on animal reproduction have not demonstrated a fetal risk, but there are no controlled studies in pregnant women) [56]. These ratings suggest that there is no obvious teratogenicity, although it is impossible to exclude small developmental effects that could manifest later in life. As an example, animal studies have shown that rodents treated in pregnancy with antidepressants gave birth to animals with a behavioral profile consistent with depression. It is also clear that most of the drugs access the fetal brain, and in many cases, neonates may experience mild symptoms consistent with withdrawal. In addition, the potential impact of any given drug varies depending on the pregnancy timing (for a complete overview, see Ref. [56]).

4. Drugs in developmental phase with potential anticataplectic properties

4.1 Triple reuptake inhibitors

It has been hypothesized that triple reuptake inhibitors (TRIs), or so-called broad-spectrum antidepressants, may be more efficacious for treating a wide variety of disorders by enhancing three different neurotransmitter systems. In the

context of cataplexy, TRIs could represent a better alternative than TCAs and MAOIs by incorporating inhibition of 5-HT, NA and DA uptake transporters in one compound.

However, incorporation of equivalent levels of DA compared to 5-HT and NA can increase the potential for DA-mediated adverse events, such as drug abuse liability, hypomania, insomnia and emesis [60], so a reduced relative effect of DA may make a more tolerable compound. Such a compound is amitifadine (EB-1010), which has been shown to be a 5-HT-preferring TRI [61,62].

It is important for the anticataplectic drug that incorporates multiple neuronal activities into its pharmacology to produce additive/synergistic activity and reduce adverse events, and not just add redundant effects that could entail additional side effect burdens. As compounds with selective action at 5-HT, NA or DA transporters have anticataplectic activity [47,48,51], one can hypothesize that drugs simultaneously interacting with all three tracts could have additive effects for controlling cataplexy. However, it is very likely that these compounds may never be used in clinical trials due to side effects and abuse potential, among other considerations.

4.2 Histamine 3 receptor antagonists/ inverse agonists

Histaminergic neurons have a key role in maintaining the brain awake under normal conditions and in the presence of behavioral challenges. They promote wakefulness through their direct widespread projections to the cerebral cortex and indirectly via their subcortical targets in the thalamus, basal forebrain and brainstem [63]. H_3 receptors are autoreceptors in presynaptic histaminergic neurons and also control histamine turnover by feedback inhibition of histamine synthesis and release. Moreover, H_3 receptors control the release of a variety of other neurotransmitters involved in sleep–waking regulation, including biogenic amines [64], acetylcholine [65], glutamate [66], GABA and peptides [67].

Currently, several pharmaceutical companies are evaluating the use of H_3 antagonists/inverse agonists to promote wakefulness and cognition for various indications. Animal studies have demonstrated that thioperamide, a potent H_3-receptor antagonist, significantly enhances wakefulness and decreases direct REM onset events in hypocretin-deficient mice [68]. In addition, a study on narcoleptic canines showed that H_3-receptor antagonists and $_{H3}$-receptor inverse agonists are effective for sleepiness and cataplexy [69]. Whether the promising results in animal models will also extend to humans, and whether these compounds will also be able to control cataplexy, remains to be established [68,70].

4.3 Hypocretin agonists

In the past decade it has been made clear that NC is the result of hypocretin deficiency [15]. Therefore, the ideal treatment for addressing every symptom in narcolepsy, including cataplexy, would be based on the replacement of hypocretin peptides. Unfortunately, hypocretin-1 and hypocretin-2 are medium-sized peptides, 33 and 28 amino acids, respectively, and the blood–brain barrier is impermeable to these peptides.

In an attempt to circumvent this limitation, several alternatives have been analyzed. Intracerebroventricular (ICV) administration of hypocretin A peptide has proven to increase arousal and to reduce the number of cataplexy attacks in model mice of NC [14,71]. Intrathecal administration also bypasses the blood–brain barrier and is somewhat less invasive but proved unsuccessful for preventing cataplexy attacks in a single sporadic case of canine NC [72]; however, further studies should be carried out in order to discard insensitivity to the peptide, given that narcoleptic dogs have a null mutation of the hypocretin-2 receptor [73]. Intranasal is a noninvasive route of administration, and although there is no information available on the effect of intranasal hypocretin on cataplexy in NC patients, preliminary studies showed an improvement in night sleep by stabilizing REM bouts [74].

An obvious solution, considering the lack of central nervous system (CNS) penetration of exogenous hypocretin peptides, is to develop centrally acting hypocretin agonists. Substitution scans, truncated peptide analysis and cross-species comparisons indicate that the C-terminal amide portion of both hypocretin peptides, particularly the last eight amino acids (a region of high homology between both hypocretin peptides), is of most importance for eliciting a response [75].

The manufacture of precursor molecules (prodrugs) or modification of the native hypocretin peptide may be able to overcome administration route barriers; however, to the best of our knowledge, no one has developed such molecules.

5. Orphan drugs for cataplexy

A medication designated as an orphan drug is one that has been developed specifically to treat a rare medical condition, the condition itself being referred to as 'orphan disease'. Hypocretin deficient NC is still considered a rare sleep disorder even though prevalence is estimated to be 1:2,000. In all probability, narcolepsy is an underdiagnosed disease, given that the average time between the disease onset and the diagnosis is 10 years. Fortunately, the treatment of human narcolepsy is rapidly evolving and novel therapies are being developed.

In the next part of this review, we will describe some molecules that are plausible anticataplectic agents and that are a promising alternative for conventional medications.

5.1 Melanin-concentrating hormone receptor antagonists

In mammals, melanin-concentrating hormone (MCH) is a cyclic neuropeptide with 19 amino acids that has been predominantly found in neurons localized in the LH that are intermingled with hypocretin neurons [76,77]. MCH is generated by the cleavage of a precursor of 165 amino acids, the prepro-MCH. Prepro-MCH contains other peptides in

Table 2. Drugs with potential anticataplectic effects.

Drug	Mechanism of action	Status
TRIs		
Amitifadine	Block reuptake 5-HT > NA ≫ DA	Currently in clinical trials for the treatment of major depressive disorder
GSK372475	Block reuptake 5-HT ≈ NA ≈ DA	
Histamine antagonists		
Bevisant (JNJ-31001074)	H_3 antagonist	Have shown potential for promoting wakefulness in animal models
ABT-239	Inverse agonist H_3	
GSK189254		
Thioperamide	Inverse agonist H_3/H_4	
MCH antagonists		
AMG-076	MCH1R antagonist	Currently under clinical trials for the treatment of obesity
NGD-4715		
GW-856464		
ALB-127158		
TRH agonists		
NS-3 (CG3703)	Enhances release of DA and NA	Effects include increased wakefulness and reduced cataplexy in narcoleptic dogs
CG3509		
TA0910		

5-HT: Serotonin; NA: Noradrenaline; DA: Dopamine; H: Histamine; MCH: Melanin-concentrating hormone.

addition to MCH, designated as neuropeptide EI (NEI) and neuropeptide GE [78,79].

MCHergic neurons project widely throughout the CNS, particularly to areas involved in the control of the sleep–wake cycle, such as the thalamus, the tuberomammillar nucleus of the hypothalamus, the preoptic area of the hypothalamus, VTA, vlPAG and LC. Furthermore, projections to other structures such as the cerebral cortex, amygdala and motor nuclei have also been described [76,77]. MCH receptors (MHCR) are distributed in diverse areas of the CNS. There is a widespread distribution of the MCH1R mRNA, and like the MCHergic fibers, this receptor is present in limbic structures and in areas related to the control of sleep and wakefulness. The distribution of MCH2R nearly overlaps with that of MCH1R, but the latter shows higher relative levels of density and a wider distribution pattern [80].

It is strikingly interesting that MCH and hypocretin neurons discharge in a reciprocal manner across the sleep–wake cycle and in relation to muscle tone. Whereas the MCH neurons are silent during wakefulness and are most active during REM sleep, hypocretin neurons are most active during wakefulness and virtually silent during REM sleep [81]. Additionally, ICV injection of MCH significantly increases time spent in REM sleep [82], and mice lacking the MCH peptide [83] or receptor [84] display altered sleep patterns. These data strongly demonstrates that the MCHergic system promotes sleep, but especially REM sleep. In concordance with the latter, several studies have found that microinjection of MCH into brain nuclei with known involvement in sleep induces a significant increase of REM sleep [82,85]. Somewhat surprisingly, there are no studies focused on how MCH antagonism affects sleep.

Despite a limited understanding of the role of endogenous MCH signaling in the regulation of sleep and the absence of detailed mechanistic studies into the nature of MCH1R antagonism, the MCH1R appears to be a promising new target for the treatment of cataplexy. A particular compound named ATC0175 showed a high affinity for MCH1R and 5-HT receptors (5-HT2B and 5-HT1A) [86,87]. This molecule is a potential candidate for treating both sleepiness and cataplexy; however, further antagonist studies are needed to elucidate how endogenous MCH signaling is involved in sleep.

5.2 Thyrotropin-releasing hormone

The use of thyrotropin-releasing hormone (TRH) direct or indirect agonists may also be potentially interesting for treating cataplexy. TRH is a hypothalamic tripeptide hypophysiotropic hormone of three amino acids that was originally isolated as a releasing hormone that stimulated pituitary thyrotropin release and, ultimately, thyroxin [88]. Subsequently, TRH was also found in axons terminating on hypothalamic neurons and in other regions of the brain [89]. Small peptide derivatives with agonistic properties and increased blood–brain barrier penetration (**Table 2**) have been developed – a success facilitated by the small nature of the parent TRH peptide. TRH and TRH agonists increase alertness and have been shown to be promoting wake and anticataplectic in the narcoleptic canine model [90,91]. One mode of action of TRH is that it increases neurotransmission of DA and NA and has excitatory effects on motoneurons [92].

Other studies suggest that TRH may increase alertness by directly interacting with thalamocortical networks. TRH itself and TRH receptor type 2 are abundant in the reticular thalamic nucleus, and local thalamic application of TRH abolishes spindle wave activity. In slices, TRH depolarizes

thalamocortical and reticular/perigeniculate neurons by inhibiting a leak K^+ conductance [93]. Another study found that the activity of hypocretin cells is modulated by TRH, and hypocretin cell excitation may contribute to the arousal-enhancing actions of TRH [94]. In addition to the hypocretin cell excitation, TRH also produces an inhibitory action on MCH neurons [95]; hence, TRH could have a significant role in preventing cataplexy by a dual mechanism of hypocretin cell activation and MCH cell inhibition in the LH. Unfortunately, however, human clinical studies of NC still need to be conducted.

5.3 Autoimmunity, narcolepsy and antigen-specific immunopharmacology

NC is a life-long sleep disorder caused by the loss of ~ 70,000 hypocretin-producing neurons in the hypothalamus. Onset is typically around puberty and displays a seasonal pattern of incidence, with highest rates in spring and summer. Likely triggering factors are influenza A, notably the pandemic H1N1 2009 variant, and *Streptococcus pyogenes* infections [94-96]. The condition is almost completely associated with human HLA DQ0602, a heterodimeric protein encoded by the DQA1*01:02-DQB1*06:02 haplotype. The overwhelming effect of this haplotype on risks suggests the importance of antigen presentation by DQ0602 and that narcolepsy could be secondary to an autoimmune attack against the hypocretin neurons. In humans, more than 20 polymorphic HLA genes encode multiple subtypes of MHC, which present foreign peptides to T cells during infections, triggering immune responses. In the case of autoimmunity, self-peptides are mistakenly seen as foreign, instigating cell destruction. Resting autoreactive T cells may have reactivity toward hypocretin cells, having escaped negative selection in the thymus. The concept of molecular mimicry has been hypothesized as a possible mechanism for the induction of autoimmunity. Most likely in NC, an autoimmune reaction is produced against the hypocretin population because the antigen on a pathologic organism closely resembles the structure of an antigen on the hypocretin cells [97]. This probable mistaken recognition would result in a functional loss of self-tolerance and the destruction of hypocretin neurons by the host's immune system.

Current treatment options for autoimmunity, such as immunosuppressive drugs (e.g., cyclosporine) and anti-T-cell antibodies (e.g., anti-CD3 antibodies), have shown varying degrees of success in the suppression of B-cell autoimmunity in diabetic mice [98]. However, these strategies require repeated drug administration and may cause nonspecific harmful effects, such as interference with normal immune system functions. By contrast, antigen-specific immunopharmacology (ASI) makes use of inverse vaccination for a specific autoantigen. Application of ASI has a major advantage of permitting selective inactivation of autoreactive T cells without interfering with normal immune function [99]. This therapy could prevent the degeneration of the hypocretin population and, thus, the appearance of narcolepsy symptoms and cataplexy. The development of vaccine for hypocretin-specific autoimmune disease will be an ongoing challenge; however, we still have to determine if the hypocretin cell loss is secondary to an autoimmune reaction and the mechanisms involved in such process.

6. Conclusion

While substantial challenges remain to be solved before these new ligands can be used as therapeutic agents for cataplexy, the latent value for exploiting their modulatory and integrative role is tremendous; however, the therapeutic potential of these rather diverse drugs still remains to be determined.

The discovery of novel drugs for treating NC remains the primary focus of study in sleep medicine research. New therapies will arise through the discovery of new molecular targets, which will offer new insights into the understanding of its physiopathology. Patients with NC may look at the future with hope and growing optimism that sometime soon we might unravel the mystery behind cataplexy.

7. Expert opinion

Cataplexy remains the most fascinating and complex symptom of narcolepsy, and thus far, we have not been able to completely elucidate the mechanism behind its genesis. Treatment of cataplexy remains a challenge and in the majority of patients, current therapy is either inadequate or lavish with side effects.

The pharmacological treatments used for cataplexy are diverse; however, the quality of the published pieces of clinical evidence supporting them varies widely, and studies comparing the efficacy of different substances are lacking. Several treatments are used on an empirical basis, especially antidepressants for cataplexy, as these medications are already used widely in depressed patients, leaving little motivation from the manufacturers to investigate their efficacy in relatively rare indications.

Taking the latter into consideration, we are certain that GHB is without any doubt one of the most effective medications available for NC, having effects on all the symptoms (cataplexy, sleepiness, disturbed nocturnal sleep, sleep paralysis, hypnagogic hallucinations). The use of GHB is greatly limited by its side effects and the fact that it is difficult to prescribe.

Based on several class I evidence (level A rating) studies, the first-line pharmacological treatment of cataplexy should be GHB at a starting dose of 4.5 g/night divided into two equal doses of 2.25 g/night. The dose may be increased to a maximum of 9 g/night, divided into two equal doses of 4.5 g/night, by increments of 1.5 g at 2-week intervals. Adverse effects may need the dose to be reduced and titrated more slowly. As indicated above, the drug should not be used in association with other sedatives, respiratory

depressants and muscle relaxants; vigilance should be held for the possible development of sleep-disordered breathing, and depressed patients should not be treated with the drug.

Second-line pharmacological treatments are antidepressants. TCAs, particularly clomipramine (10 – 75 mg), are potent anticataplectic drugs. However, they have the drawback of anticholinergic adverse effects. The starting dosage should always be as low as possible. SSRIs are slightly less active but have fewer adverse effects. The NA/5-HT reuptake inhibitor, venlafaxine, is now widely used, but it lacks any published clinical evidence of efficacy. The NA reuptake inhibitors also lack published clinical evidence. Given the well-evidenced efficacy of GHB and antidepressants, the place for other compounds is fairly limited.

Even though there are no studies evaluating the combination of medications for treating cataplexy, this should be avoided considering that most of the current drugs for treating this symptom have similar mechanisms of action and, thus, may have a synergistic effect.

The basic research that is leading to a better understanding of narcolepsy and its symptoms is quite extensive, and it is through this emerging knowledge that drug development for cataplexy will progress.

It is difficult to predict which drug targets will be most beneficial to patients. In the area of symptomatic treatment of cataplexy, a diverse number of new drug classes, such as TRIs, H_3 antagonists, hypocretin agonists, MCHR antagonists and TRH, could come forward and replace traditional medication (TCAs, SSRIs, SNRIs and MAOIs). These new drugs might have an improved efficacy, tolerability and sensitivity.

There is an established pharmacotherapy for symptomatic treatment of NC; nonetheless, cures for this disease have not yet been found. Consequently, a large research effort is sustained in this field.

We believe that the substantial evidence suggests that NC is an autoimmune disease that targets the hypocretin neurons. Almost certainly, in NC, genetic predispositions as well as environmental triggers play a role in the development of the disease; the identity of these factors has been largely elusive, but we are currently in the path of elucidating them. The identification of the most common genetic and environmental factors that set off autoimmunity may lead to a better understanding of the ensuing pathogenesis and will offer improved therapies and, ultimately, cures or vaccines.

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Declaration of interest

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