Bogotá, 4 de diciembre de 2025

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E. S. D.

REF.:	**SOLICITANTES**	**:**	SANIA TX LTD
	TITULO	**:**	MÉTODO
	No. de Solicitud Internacional	**:**	PCT/IB2024/054426

ENTRADA A FASE NACIONAL EN COLOMBIA CON LAS MODIFICACIONES QUE SE LLEVARON A CABO EN FASE INTERNACIONAL BAJO ARTÍCULO 34 – RESPUESTA AL IPEA (CAPÍTULO II)

HELENA CAMARGO WILLIAMSON, mayor de edad y vecina de Bogotá D.C., identificada como aparece al pie de mi firma, en mi condición de apoderada de la sociedad **SANIA TX LTD,** adjunto este memorial con el fin de brindar mayor claridad al Examinador a la hora de revisar los documentos radicados. Así, me permito señalar que la presente solicitud entrara a fase nacional en Colombia con las modificaciones que se llevaron a cabo en fase internacional presentadas en Capítulo II bajo le artículo 34, en particular con la respuesta al IPEA.

Así las cosas, me permito adjuntar la traducción de la solicitud con modificaciones bajo artículo 34 (Capítulo II), el cual comprende la descripción y las reivindicaciones modificadas, de manera similar, se adjunta la respuesta al IPEA junto con la descripción y las reivindicaciones modificadas, con las cuales se entrarán a fase nacional en Colombia. Además, de la traducción de la descripción, reivindicaciones y resumen originalmente presentados en fase internacional. Y, por último, adjuntamos algunos de los documentos que se han presentado y emitido durante la fase internacional, tales como la publicación internacional, ISR y WO, el reporte preliminar internacional de patentabilidad (IPRP), los anexos del IPRP, reivindicaciones modificadas en respuesta a la opinión escrita, certificado de cambio de nombre del solicitante, entre otros.

De acuerdo con todo lo anterior, amablemente solicitamos que proceda a llevar a cabo el trámite de la solicitud y el estudio de patentabilidad a las reivindicaciones radicadas y basadas en las modificaciones bajo el artículo 34 (capítulo II) de la solicitud internacional, en particular las modificaciones radicadas en la respuesta al IPEA. Así las cosas, considero que la presente invención cumple con todos los requisitos formales de acuerdo con el numeral 5.2.5. del Título X de la Circular Única de la Superintendencia y la Decisión 486.

De la Señora directora,

POSSE HERRERA RUIZ

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MÉTODO

CAMPO DE LA INVENCIÓN

La presente invención se refiere a métodos para reducir los efectos secundarios de los fármacos neuromoduladores, incrementar la eficacia de fármacos neuromoduladores y a métodos para reducir la excitabilidad de neuronas. La invención también se refiere a vectores de terapia génica útiles para llevar a cabo los métodos de la invención.

ANTECEDENTES DE LA INVENCIÓN

Los trastornos neurológicos se caracterizan por una actividad anormal en las neuronas y circuitos dentro del sistema nervioso central (SNC). Con frecuencia, esta actividad anormal se transmite a las neuronas de salida del SNC, las neuronas motoras, lo que provoca contracciones musculares inapropiadas y disfunción sensitiva y motora.

Se han desarrollado terapias farmacológicas dirigidas a mejorar la actividad anormal típicamente mediante la modulación de los canales iónicos que transportan corrientes excitatorias e inhibitorias. Sin embargo, los fármacos suministrados al sistema nervioso es difícil y las terapias existentes suelen tener efectos secundarios indeseables debido a su baja especificidad.

Así, aunque los fármacos neuromoduladores desarrollados para trastornos neurológicos han mostrado algún beneficio terapéutico, éste se ha sido limitado por efectos no deseados en neuronas no disfuncionales y otros sistemas que expresan el objetivo (cardiovascular, respiratorio, gastrointestinal, etc.).

Canales de potasio (K^+) son proteínas de membrana que permiten el flujo rápido y selectivo de iones K+ a través de la membrana celular, y así generando señales eléctricas en las células. Los canales de K^+ dependientes de voltaje (canales Kv), presentes en todas las células animales excitables, se abren y se cierran en

cambios en el potencial transmembrana. Los canales Kv son uno de los componentes clave en la generación y propagación de impulsos eléctricos en el sistema nervioso. Ante cambios en el potencial transmembrana, estos canales se abren y permiten el flujo pasivo de iones desde la célula para restaurar el potencial de membrana en reposo. La activación de canales Kv es por lo tanto considerada inhibitoria para la acción de generación de potencial.

Existen 40 genes de canales de potasio dependientes de voltaje en humanos, pertenecientes a 12 subfamilias (Kv1–Kv12). Estas subfamilias suelen estar restringidas a ubicaciones específicas o tipos celulares/tisulares y se observan tanto en tejidos excitables como no excitables. La notable diversidad de los canales Kv se debe a la combinación de sus subunidades. Dentro de cada una de las familias Kv1, Kv2, Kv3, Kv4 y Kv7, pueden formarse canales homoméricos y heteroméricos con un rango de propiedades funcionales. Los miembros de la familia Kv2 también pueden ensamblarse con miembros de las familias Kv5, Kv6, Kv8 o Kv9, con más patrones de expresión restringidos en el sistema nervioso y el músculo liso.

En los canales de potasio dependientes de voltaje en neuronas, se dirigen a varios compartimentos subcelulares, y pueden estar presentes canales con diferentes composiciones de subunidades en diferentes subpoblaciones de neuronas.

La estructura tetramérica de los canales Kv se conforma de dos dominios funcional y estructuralmente independientes: un poro de conducción iónica y dominios sensores de voltaje. El poro de conducción se conforma por cuatro subunidades las cuales están dispuestas simétricamente alrededor de la trayectoria de conducción. Los dominios sensores de voltaje se posicionan en la periferia del canal y consiste de cuatro segmentos transmembrana (S1-S4). La reorganización estructural de los dominios sensores de voltaje en respuesta a cambios en el potencial de membrana, y en particular de S4, que incluye aminoácidos cargados positivamente en cada tercera posición, produce cambios conformacionales en el poro de conducción, que pueden abrir u obstruir la trayectoria de conducción iónica. El mecanismo de activación por

voltaje aún no se comprende completamente.

La familia Kv7 de canales de potasio (K^+) dependientes de voltaje consisten de cinco miembros (Kv7.1-7,5) codificados por los genes KCNQ1-5, respectivamente. Los canales Kv7 se ensamblan como tetrámeros de subunidades α idénticas o compatibles, cada subunidad α que consiste de seis segmentos transmembrana (S1-S6) y extremos N- y C-terminales citoplasmáticos. El Kv7 se expresa ampliamente tanto en el SNC como en otros tejidos; con Kv7,2 y 7,3 en particular, se conocen por controlar la excitabilidad neuronal.

Los canales Kv7,2 y 7,3 normalmente forman heterómeros entre sí para incrementar la conductancia y son raramente expresados como homómeros de baja conductancia. Los canales Kv7,2 y 7,3 se expresan en la mayoría de los subtipos neuronales y tienden a localizarse en el segmento inicial del axón, donde se inician los potenciales de acción (espigas). Por lo tanto, las conductancias de Kv7,3 y Kv7,2 se consideran inhibidores particularmente potentes de la generación de potenciales de acción y de la actividad en la misma neurona, así como en sus objetivos (por ejemplo, la inhibición de las neuronas motoras reduce la actividad muscular).

Los fármacos neuromoduladores dirigidos a los canales Kv7 para tratar trastornos asociados a la hiperexcitabilidad neuronal. La retigabina es un anticonvulsivo que actúa principalmente como abridor de canales de potasio Kv7,2-Kv7,5 y, como modulador alostérico positivo del GABA a concentraciones altas. El término “abridor de canal” se refiere a un desplazamiento hacia la izquierda en la dependencia del voltaje para la apertura del canal, hacia potenciales más negativos. En presencia de retigabina, los canales Kv7 se abren a potenciales más negativos, contribuyendo así a la hiperpolarización de la membrana y a la estabilización del voltaje.

También se ha demostrado que la retigabina estabiliza el canal Kv7,2/7,3 (heteromérico) abierto, lo que ralentiza su desactivación con poco cambio en la dependencia del voltaje. El efecto general es una mayor conductancia de potasio, una

hiperpolarización del potencial de membrana y, por consiguiente, la inhibición de la generación del potencial de acción. Este efecto de la retigabina se observa a concentraciones inferiores a 10 μM, *in vitro* (Corbin-Leftwich et al., 2016; Villalba-Galea, 2020). La administración oral de retigabina a dosis de 600-1200 mg al día, que corresponde a una concentración plasmática media de 0,83 μM (Gunthorpe et al., 2012), ha demostrado una eficacia clínica de hasta el 45 % en la reducción de convulsiones en humanos con epilepsia (Brodie et al., 2010; French et al., 2011). Cabe destacar que, mientras el CE50 de retigabina es de 0.6 μM para los canales Kv7,3, la mayoría de los canales del sistema nervioso son heterómeros Kv7,2/7,3, cuya CE50 es de 1,6 μM. Para algunos pacientes, estas dosis resultan en decoloración del ojo y piel lo que conduce a restringir su indicación. Posteriormente, retigabina fue retirada voluntariamente por motivos comerciales (Brickel et al., 2020).

El interés farmacéutico en Kv7 como un objetivo terapéutica persiste. En al menos 3 activadores con potencia incrementada, selectividad incrementada por Kv7,2/7,3 y una carencia de efectos secundarios cosméticos se encuentran en ensayos clínicos y han mostrado resultados favorables. Sin embargo, debido a la ubicuidad de la expresión de Kv7,2/Kv7,3 en el sistema nervioso, los efectos secundarios neuronales (somnolencia, fatiga, etc.) son actualmente inevitables. Si bien los nuevos activadores de Kv7,2/7,3 presentan mejores perfiles de seguridad, los efectos secundarios neuronales solo pueden reducirse aumentando la selectividad exclusivamente por las neuronas disfuncionales.

La quimiogenética es un método mediante el cual se diseñan por ingeniería proteínas para interactuar con activadores químicos de moléculas pequeñas previamente desconocidos. Desde la década de 1990, se han desarrollado numerosas plataformas quimiogenéticas que han sido desarrolladas que han sido particularmente útiles para los neurocientíficos que desean modular la actividad neuronal en poblaciones específicas. Varias clases de proteínas se han diseñado por ingeniería de esta manera que incluye enzimas cinasas, no cinasa, canales iónicos activados por

ligandos y receptores acoplados a proteínas G (GPCR). Las más utilizadas ampliamente se conocen como receptores diseñados activados por fármacos diseñados (DREADD).

Los DREADD son GPCR diseñados por ingeniería para responder a ligandos sintéticos que cruzan la barrera hematoencefálica (tal como el N-óxido de clozapina y la olanzapina), pero no a su ligando natural, la acetilcolina.

En uso, un inserto del vector viral el gen que codifica la proteína DREADD en la célula para ser estudiada. Se pueden utilizar diversos serotipos virales, promotores y rutas de administración para seleccionar las células objetivo. Una vez transducida, la célula infectada toma dos o tres semanas en expresar la proteína receptora diseñada por ingeniería la cual entonces será activada usando un ligando DREADD. De esta manera, la actividad puede ser modulada específicamente en las neuronas objetivo, con una mínima influencia en las células fuera de objetivo.

Aquí se describe un método similar al empleado por los DREADD, en el cual se expresa un canal iónico sensible a fármacos específicos en las neuronas para modular su actividad. Cabe destacar que este método invierte el concepto actual de forma importante, el método cambia el concepto existente de diseñar específicamente emparejados de receptores novo, y fármaco y reutiliza canales iónicos de tipo silvestre y mutantes para reducir o eliminar los efectos secundarios de los fármacos que actúan sobre los canales. El método expresa positivamente canales de tipo silvestre o mutados.

Además, el método descrito beneficiosamente usa canales que pueden ser ya sea abiertas (agonistas) o cerradas (antagonistas) dependiendo del fármaco utilizado. En contraste directo, la activación de DREADD induce cambios unidireccionales en la excitabilidad (ya sean excitatorios o inhibitorios).

BREVE DESCRIPCIÓN DE LA INVENCIÓN

La presente descripción se refiere a un método quimiogenético que permite

modular la actividad en neuronas objetivo, así aumentando la eficacia de los fármacos mientras se reducen sus efectos secundarios, al menos en parte, debido a la capacidad para utilizar una dosis menor. El método involucra usar una metodología de terapia génica mediante un vector de suministro (por ejemplo, AAV) para expresa positivamente (regular positivamente) un canal iónico terapéutico en neuronas objetivo, de modo que la conductancia del canal (y, por lo tanto, la actividad neuronal) pueda modularse con dosis bajas de fármacos activadores e inhibidores específicos. Es decir, el método aumenta específicamente la sensibilidad de las neuronas disfuncionales a los fármacos moduladores.

Se ha mostrado que la expresión positiva, mediada por AAV, del canal de potasio dependiente de voltaje Kv7,3 (codificado por KCNQ3) permite que un activador de Kv7, tal como la retigabina, para reducir significativamente la excitabilidad de las neuronas humanas, tanto a nivel de neurona individual y poblacional. A bajas concentraciones (0,3-3 μM), la retigabina redujo el número de potenciales de acción en neuronas sensoriales y motoras derivadas de hIPSC individuales. A las mismas dosis, la retigabina tiene un efecto mínimo en las neuronas control que expresan únicamente proteína fluorescente verde (GFP). Usando matrices de multielectrodos, hemos mostrado que la retigabina fue también efectiva en la actividad explosiva espontánea de reducir selectivamente de una red de miles de neuronas motoras (MN) que expresa positivamente Kv7,3, pero no en neuronas que expresan GFP. Los resultados in vitro se tradujeron a un modelo de ratón de espasticidad, en el cual las MN hiperactivas provocan contracciones musculares excesivas (espasmos musculares). En este modelo, la expresión positiva de Kv7,3 en neuronas motoras mediante inyección intramuscular de un constructo AAV-Kv7 permitió que una dosis baja de retigabina (2 mg/kg) para reducir la intensidad y la duración de los espasmos musculares. Sin embargo, la misma dosis no redujo significativamente la espasticidad en ratones inyectados con un constructo AAV-GFP. Se confirmó que la reducción de la excitabilidad de MNs se confirmó como el mecanismo para la reducción en

espasticidad, como 3 μM de retigabina reducida en disparo de AP en Kv7,3 que expresa en neuronas motoras de ratón, pero no en aquellas que expresan GFP. Adicionalmente, se ha mostrado que la introducción de una mutación puntual en el residuo de aminoácido 315 (Zaika et al., 2008; Gómez-Posada et al., 2010) de la proteína Kv7,3 reduce la excitabilidad de neurona motora en el valor de referencia in vitro e in vivo, e incrementa aún más los tamaños de efecto de la retigabina a las concentraciones probadas in vitro.

La terapia utilizará AAV para expresa positivamente subunidades del canal iónico Kv7,3 y sus variaciones de las mismas, ya sea como homómeros o heterómeros Kv7,2 /7,3, en neuronas hiperexcitables de pacientes con trastornos neurológicos, y utilizará dosis bajas de moduladores del canal para normalizar la actividad.

De conformidad con un primer aspecto, se proporciona un método para incrementar la eficacia de un fármaco neuromodulador que comprende los siguientes pasos:

a. introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. administrar una dosis menor del fármaco neuromodulador.

Ventajosamente, al incrementar la eficacia del fármaco, se puede administrar una dosis menor para obtener el mismo efecto terapéutico. Esto, beneficiosamente, significa que la ocurrencia de efectos secundarios se pueden reducir.

Así, en un segundo aspecto se proporciona un método para reducir los efectos secundarios de un fármaco neuromodulador que comprende los siguientes pasos:

a. introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótido que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. administrar una dosis menor del fármaco neuromodulador.

Los efectos secundarios de los fármacos neuromoduladores pueden variar de menor a serios y pueden afectar la calidad de vida del paciente. Por ejemplo, para retigabina, los NIH enumeran los efectos adversos más comunes, que se presentan en más del 10 % de los sujetos en los ensayos clínicos, incluyen paso anormal, confusión, mareo, fatiga, dolor de cabeza, náuseas, somnolencia, trastornos del habla, temblor, infección del tracto urinario y visión borrosa (Harris y Murphy, 2011).

En algunos casos, los efectos secundarios (efectos adversos) pueden llevar a la retirada de fármacos del mercado, ya que la dosis necesaria produce efectos adversos inaceptables. Si se reduce la dosis, se pueden disminuir los efectos adversos y, potencialmente, reducir el uso del fármaco puede potencialmente ser restaurado.

En un tercer aspecto se proporciona un método para incrementar el índice terapéutico de un fármaco neuromodulador que comprende los pasos:

a. introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. administrar el fármaco neuromodulador.

Un cuarto aspecto proporciona un vector de terapia génica que comprende una secuencia de nucleótido que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótido que codifica la secuencia de aminoácido de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16, en donde, cuando el vector transduce una neurona objetivo, la expresión de la subunidad del canal iónico Kv7,3 se regula a la alza y se forman canales iónicos funcionales.

Ventajosamente, el suministro de la secuencia de nucleótido a una población de neuronas objetivo específicos resulta en que la carga útil de nucleótidos sólo se transduce en las neuronas que exhiben una excitabilidad incrementada. Estas neuronas expresa positivamenten los canales iónicos, lo que proporciona más objetivos para los fármacos neuromoduladores los cuales, a su vez, permite que una dosis menor del fármaco para ser efectivo, resultando en menos efectos secundarios. Dirigir las neuronas de esta manera también significa que la actividad fuera de objetivo es menos probable.

En un quinto aspecto se proporciona un vector de terapia génica de partículas virales AAV que comprende:

a. una cápside de AAV que transduce selectivamente las neuronas objetivo, y

b. una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16, en donde, cuando el vector transduce las neuronas objetivo, se regula positivamente la expresión de la subunidad del canal iónico Kv7,3 y se forman canales iónicos funcionales.

En otro aspecto, se proporciona un vector de terapia génica que comprende una cápside de AAV que transduce selectivamente las neuronas objetivo y una

secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16, para su uso en la regulación positiva de la expresión de la subunidad del canal iónico Kv7,3 por la neurona objetivo de manera que forme canales iónicos funcionales en un sujeto que tiene un trastorno neurológico asociado con una excitabilidad neuronal incrementada.

En un aspecto adicional, se proporciona un vector de terapia génica que comprende una cápside de AAV que transduce selectivamente las neuronas objetivo y una secuencia de nucleótidos que codifica una secuencia de aminoácido de subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la secuencia de aminoácido de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16, para su uso en el tratamiento de un sujeto que tiene un trastorno neurológico, en donde, una vez que el vector ha transducido la neurona objetivo, la expresión del canal iónico Kv7,3 por la neurona objetivo se regula positivamente, de modo que se puede administrar efectivamente una dosis inferior de un fármaco neuromodulador al sujeto.

En otro aspecto, se proporciona el uso de un vector de terapia génica que comprende una secuencia de nucleótido que codifica una subunidad del canal iónico Kv7,3 que comprende la secuencia de cualquiera de las SEQ ID.1 a SEQ ID.8 o una secuencia de nucleótido que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7,3 de cualquiera de las SEQ ID.9 a SEQ ID.16 en la preparación de un medicamento para uso en el tratamiento de un trastorno neurológico asociado con una excitabilidad incrementada de las neuronas, tal como en donde el trastorno neurológico se selecciona del grupo que consiste en: epilepsia, espasticidad, BNFS, enfermedad de Parkinson, dolor nociceptivo y dolor no nociceptivo, ELA.

BREVE DESCRIPCIÓN DE LAS FIGURAS

Para una mejor comprensión de la invención y para mostrar cómo puede llevarse a la práctica, a continuación se describirán, a modo de ejemplo únicamente, modalidades, métodos y procesos de conformidad con la presente invención con referencia a los dibujos adjuntos en los cuales:

La figura 1 muestra los constructos de AAV utilizados para la transducción de neuronas con Kv7,3 (constructo superior) o Kv7,3 A315T (constructo inferior). Nótese que se incluyó una secuencia de GFP corriente abajo de Kv7,3 y Kv7,3 A315T mediante enlazadores T2A, lo que significa que la fluorescencia de GFP indica la expresión de Kv7,3.

La figura 2 muestra que la transducción AAV de neuronas hIPSC mediante induce a la expresión de proteínas codificadas por transgenes empaquetados en los constructos AAV (A). Específicamente, en el ejemplo presentado, se transdujeron neuronas hIPSC con AAV para inducir la expresión de ya sea la proteína fluorescente verde (AAV-GFP) o de los canales Kv7,3 humanos y GFP (AAV-Kv7,3).

La figura 2 muestra también los puntos finales evaluados usando electrofisiología de fijación en parche en el valor de referencia (expresión de Kv7,3 o GFP sin fármaco activador (B) y siguiendo la exposición al activador (retigabina, C). Los puntos finales evaluados son: reobase (entrada de corriente mínima para evocar un potencial de acción), número de potenciales de acción a 3x la reobase y potencial de membrana en reposo. En la presencia de retigabina, la excitabilidad neuronal se reduce como se muestra por un incremento en la reobase, una disminución en el número de potenciales de acción a 3x de reobase y la hiperpolarización del potencial de membrana en reposo.

La figura 3 muestra que la expresión positiva del valor de referencia de Kv7,3 no altera significativamente la excitabilidad de las neuronas sensoriales hIPSC en comparación con las neuronas control transducidas con AAV-GFP. El panel (A) muestra el número medio de potenciales de acción generados para cada valor de

entrada para AAV-GFP (línea discontinua) y AAV-Kv7,3 (línea continua). Las áreas sombreadas muestran los intervalos de confianza del 95 %. Se determinó que los niveles de excitabilidad eran similares debido a los tamaños del efecto extremadamente pequeños medidos por Aps/500 ms (B), el RMP (C) y reobase (D). En estos gráficos de estimación de Cummings, cada punto representa el número de potenciales de acción generados a 3x la reobase en las neuronas transducidas con AAV-GFP (gris ligero) o AAV-Kv7,3 (gris oscuro). En el lado derecho del gráfico, se representan los tamaños del efecto G de Hedges y los intervalos de confianza del 95 % para datos remuestreados mediante carga inicial de programas (1000 repeticiones).

La figura 4 muestra que la retigabina suministrada a una concentraciones de 0,3 µM, 1 µM y 3 µM tiene un efecto limitado en la excitabilidad de las neuronas sensoriales hIPSC transducidas con AAV-GFP, como se determinó midiendo el número de potenciales de acción a 3x la reobase. Sin embargo, se midieron tamaños del efecto de gran magnitud a las mismas concentraciones en las neuronas transducidas con AAV-Kv7,3. En cada una de las gráficas de estimación (A, B y C), las gráficas superiores son líneas que representan el cambio en el número AP entre los registros realizados antes y después de la exposición a la retigabina. Las gráficas inferiores muestran los tamaños del efecto G de Hedges e intervalos de confianza.

La figura 5 muestra que la retigabina suministrada a concentraciones de 0,3 µM, 1 µM y 3 µM, tiene efectos limitados sobre la excitabilidad de las neuronas sensoriales hIPSC transducidas con AAV-GFP, como se determinó mediante la medición de la reobase (corriente mínima para generar un potencial de acción). Sin embargo, se midieron los tamaños de efecto de gran magnitud a las mismas concentraciones en las neuronas transducidas con AAV-Kv7,3.

La figura 6 muestra que la retigabina suministrada a concentraciones de 0,3 µM, 1 µM y 3 µM, tiene efectos pequeños y moderados sobre la excitabilidad de las neuronas sensoriales hIPSC transducidas con AAV-GFP, como se determinó mediante la medición del potencial de membrana en reposo (PMR). Sin embargo, se midieron

tamaños de efectos grandes y muy grandes a las mismas concentraciones en las neuronas transducidas con AAV-Kv7,3.

La figura 7 muestra que la expresión del valor de referencia de Kv7,3$_{A315T}$ (línea discontinua) reduce significativamente la excitabilidad de las neuronas sensoriales hIPSC. Se mostró una disminución en los niveles de excitabilidad debido a la incapacidad de las neuronas transducidas con AAV-Kv7,3$_{A315T}$ para generar más de 3 potenciales de acción (A, B). El RMP también se encontraba hiperpolarizado en el valor de referencia (C), pero la reobase no presentó cambios sustanciales (D).

La figura 8 muestra que la retigabina suministrada a concentraciones de 0,3 µM, 1 µM y 3 µM causó aumentos muy grandes en la reobase de las neuronas transducidas con AAV-Kv7,3$_{A315T.}$ La magnitud del efecto fue al menos 2-3 veces mayor para AAV-Kv7,3$_{A315T}$ en comparación con AAV-GFP. Obsérvese la diferencia de escala tanto para la reobase (eje primario) como para el tamaño del efecto g de Hedges apareado (eje secundario).

La figura 9 muestra que la retigabina suministrada a concentraciones de 0,3 µM, 1 µM y 3 µM causó efectos muy grandes en la hiperpolarización del RMP de las neuronas sensoriales hIPSC transducidas con AAV-Kv7,3$_{A315T.}$ Los tamaños del efecto, también son al menos 2-3 veces más grandes que en las neuronas hIPSC transducidas con AAV-GFP.

La figura 10 muestra la expresión positiva de Kv7,3 en neuronas motoras derivadas de hIPSC mediante manchado Western (A) y ELISA (B). Obsérvese que las bandas fuertes alrededor de 80 kDa.

La figura 11 muestra que la expresión positiva de Kv7,3 en neuronas motoras derivadas de hIPSC induce una pequeña reducción del valor de referencia en la relación frecuencia-corriente (A-B); sin embargo, la generación de potenciales de acción a 3x de reobase (C) y reobase (D) no se vio afectada. (E-H) La expresión positiva de Kv7,3 aumentó la sensibilidad de las MNs de hIPSC a retigabina. Las concentraciones bajo 3 µM no afectaron la excitabilidad de las neuronas transducidas

con AAV-GFP, pero redujeron la generación de AP hasta en un 80 % en las neuronas transducidas con AAV-Kv7,3. La expresión positiva de Kv7,3 también incrementó la eficacia de la retigabina, como lo demuestra el aumento en los efectos máximos sobre la frecuencia de PA (G) y traslape de FI (H).

La figura 12 muestra que la expresión positiva de Kv7,3 aumenta la sensibilidad de las MN a RTG a nivel poblacional, como se midió usando una placa de arreglo de microelectrodos (MEA). Las MN de hIPSC se cultivan en una placa de cavidades con electrodos grabados de modo que la actividad espontánea se puede registrar. El 50 % de los cavidades se tratan con AAV-GFP y el 50 % con AAV-Kv7,3. Tras un tiempo de transducción suficiente, se registra la actividad espontánea en el valor de referencia (0 μM) y posteriormente con concentraciones incrementadas de RTG. La RTG no afecta la viabilidad de las neuronas motoras, evaluada mediante la impedancia del electrodo (A). Sin embargo, las MN tratadas con RTG muestran una mayor sensibilidad a RTG como es evidenciada por el desplazamiento hacia la izquierda en la curva de respuesta a la dosis para retigabina y la frecuencia de ráfagas de potenciales de acción espontáneos (B), la duración de las ráfagas (C) o el número total de espigas. Los ejemplos representativos mostrados E-F de la actividad registrada en cavidades tratados con AAV-GFP (E) o AAV-Kv7,3 (F) después de la adición de 1.8 μM de RTG. Las líneas verticales en los paneles superiores indican la frecuencia de ráfagas de la red. Abajo se muestran diagramas de puntos de la actividad de los potenciales de acción registrados por cada uno de los 16 electrodos (filas). Los ejemplos representativos G-H muestran explosiones individuales de MNs en cavidades tratadas con AAV-GFP (G) o AAV-Kv7,3 (H). Cada fila es la actividad de acción registrada por un único electrodo. A la derecha de cada gráfico se muestra una leyenda de calor que indica el cambio en la frecuencia de acción.

La figura 13 muestra que, en un modelo neonatal de ratón con espasticidad inducida por lesión medular, se puede lograr la expresión positiva de Kv7,3 en neuronas motoras tras la inyección intramuscular de AAV-Kv7,3. (A) Muestra un

manchado Western de Kv7,3 en médulas espinales obtenidas 2 semanas después de la inyección y la lesión medular. Obsérvese que las bandas fuertes con el peso molecular esperado para Kv7,3 en las muestras de médula espinal de ratones tratados con AAV-Kv7,3, pero no en las de ratones tratados con AAV-GFP. (B) Muestra la expresión de Kv7,3 específicamente en MNs usando inmunohistoquímica. (C-D) Muestra los cambios en la duración de espasmos musculares después de la administración de 2 mg/kg de RTG en ratones tratados con AAV-GFP y AAV-Kv7,3. E-F muestra el cambio en la intensidad del espasmo (número de potenciales de acción por espasmo) después de la administración de 2 mg/kg de RTG en ratones tratados con AAV-GFP y AAV-Kv7,3. Las G-H muestran registros electromiográficos (EMG) representativos del efecto de 2 mg/kg de RTG en ratones tratados con AAV-GFP y AAV-Kv7,3. Los rastros superiores son pre RTG y los rastros inferiores a 20 minutos después de RTG.

La figura 14 muestra que la expresión positiva de Kv7,3 en neuronas motoras mediante inyección intramuscular de AAV-Kv7,3 no afecta significativamente las propiedades electrofisiológicas del valor de referencia de las neuronas motoras en un modelo neonatal de ratón con espasticidad. Se realizaron registros en cortes de médula espinal de ratones con espasticidad. (A) muestra que la excitabilidad de MNs no se redujo, ya que el número de potenciales de acción evocados a 2x la reobase no presentó diferencias entre los controles AAV-GFP y las MNs transducidas con AAV-SRx-C490. (B) muestra que el umbral para evocar un único potencial de acción no presentó diferencias en las MNs transducidas con AAV-SRx-C490. (C) muestra que el potencial de membrana en reposo (PMR) no presentó diferencias entre los grupos. (D) muestra que el tamaño de MN no presentó diferencias entre los grupos. (E-H) muestra que 3 μ M de retigabina redujeron significativamente las MN de ratones tratados con AAV-SRxC490 pero no en ratones tratados con AAV-GFP, como se evaluó por el cambio en el número de potenciales de acción al 2x del umbral y la reobase.

La figura 15 muestra que las neuronas motoras de ratones con espasticidad

tratados con AAV-GFP generan trenes repetitivos de potenciales de acción (A); sin embargo, las neuronas motoras de ratones inyectados con un constructo viral para expresa positivamente una forma mutada de Kv7 (A315T) son incapaces de generar respectivamente. Esto sugiere una reducción significativa y permanente de la excitabilidad de estas neuronas motoras.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

Como se emplea en el presente documento, “incrementar la eficacia” significa un incremento en la capacidad de producir el resultado deseado. Esto puede lograrse mejorando la respuesta al fármaco, en lugar de mejorar su efectividad del fármaco. La eficacia mejorada significa que el mismo nivel de respuesta se puede lograr utilizando menos del fármaco.

Por lo tanto, incrementado la eficacia significa que se puede utilizar una dosis menor.

Como se emplea en el presente documento, el fármaco neuromodulador se refiere a un canal de potasio dependiente de voltaje. Los fármacos conocidos se incluyen, pero no se limitan a, retigabina y derivados de los mismos (véase Musella et al., 2022 por ejemplo), BHV-7000 y Xen496, Xen 1101, flupirtina, diclofenaco, BMS-204352, ácido meclofenámico, ETX -123, linopirdina.

En una modalidad, el fármaco neuromodulador es un abridor de canal.

En una modalidad, el fármaco neuromodulador es un bloqueador de canales.

En una modalidad, el fármaco neuromodulador se selecciona entre: Retigabina o un derivado de la misma, BHV-7000 y Xen496, Xen 1101, flupirtina, diclofenaco, BMS-204352, ácido meclofenámico, ETX -123, linopirdina.

Tal como se emplea en este documento, la introducción de una secuencia de nucleótidos se refiere a la transducción o transfección de una célula, como una neurona, con una o más secuencias de nucleótidos, ya sea para complementar el material genético existente con más de lo que ya está presente, o para proporcionar

nuevas secuencias de nucleótidos.

La subunidad Kv7,3 del canal iónico es un monómero de un canal de potasio dependiente de voltaje que se ensambla para formar un canal iónico tetramérico funcional. El Kv7,3 puede ensamblarse como un homómero o como heterómero con otras subunidades Kv7, que incluye Kv7,2 y Kv7,5, u otras subunidades auxiliares (por ejemplo, KCNE).

Los canales iónicos Kv7,3, tal como se emplean en este documento, se refieren tanto al homómero Kv7,3 como a los heterómeros que contienen Kv7,3.

Como se emplea en el presente documento, una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3 se refiere a una secuencia de nucleótidos (ARN o ADN) que codifica la subunidad del canal iónico Kv7,3.

En una modalidad, la secuencia de nucleótido comprende la secuencia de SEQ ID.1 o una variante de SEQ ID.1 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.1.

En una modalidad, la secuencia de nucleótido comprende la secuencia de SEQ ID.2 o una variante de SEQ ID.2 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.2.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.3 o una variante de SEQ ID.3 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.3.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.4 o una variante de SEQ ID.4 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.4.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.5 o una variante de SEQ ID.5 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.5.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.6 o una variante de SEQ ID.6 que codifica la misma secuencia de

aminoácidos codificada por SEQ ID.6.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.7 o una variante de SEQ ID.7 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.7.

En una modalidad, la secuencia de nucleótidos comprende la secuencia de SEQ ID.8 o una variante de SEQ ID.8 que codifica la misma secuencia de aminoácidos codificada por SEQ ID.8.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con la SEQ ID.9.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con la SEQ ID. 10.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.11.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.12.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.13.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.14.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.15.

En una modalidad, la subunidad del canal iónico Kv7,3 comprende o consiste de la secuencia de aminoácidos de conformidad con SEQ ID.16.

En una modalidad, la secuencia de nucleótidos codifica la subunidad del canal iónico Kv7,3 de conformidad con cualquiera de los ID de SEQ 9 a 16.

En ciertas modalidades, la subunidad del canal iónico Kv7,3 puede comprender una mutación en el residuo de aminoácido 315 de SEQ ID.9. Por ejemplo, A315T, A315S, A315V, A315C, A315N, A315Q o A315Y.

En una modalidad, la subunidad del canal iónico es Kv7,3 de tipo silvestre.

En una modalidad, la subunidad del canal iónico no es Kv7,3 de tipo silvestre.

Un canal iónico funcional como el empleado aquí significa que la subunidad del canal iónico se ha ensamblado en un tetrámero, ya sea como homómero o como heterómero, y está ubicada en la membrana neuronal en una posición adecuada para actuar como un canal de potasio dependiente de voltaje.

Como se emplea en el presente documento, el término neurona incluye una neurona y una porción o porciones de la misma (por ejemplo, el cuerpo celular neuronal, el axón o un dendrito). El término neurona designa las células del sistema nervioso que son eléctricamente activas e incluye un cuerpo celular (o soma) y hasta dos tipos de extensiones o proyecciones: dendritas, mediante las cuales se transmite la mayoría de las señales neuronales al cuerpo celular, y axones, mediante los cuales se transmiten la mayoría de las señales neuronales desde el cuerpo celular a las células efectoras, como las neuronas objetivo o músculo. Las neuronas pueden transmitir información desde los tejidos y órganos al sistema nervioso central (neuronas aferentes o sensoriales) y transmitir señales desde el sistema nervioso central a las células efectoras (neuronas eferentes o motoras). Otras neuronas, denominadas interneuronas, conectan neuronas dentro del sistema nervioso central (el cerebro y la médula espinal). Otras neuronas, denominadas neuronas de proyección, extienden de sus axones de una región del sistema nervioso a otra.

En algunas modalidades, la neurona se involucra en la actividad sensoriomotora.

En algunas modalidades, la neurona es una neurona motora (motoneurona).

En algunas modalidades, la neurona es una neurona sensorial.

En algunas modalidades, la neurona es una neurona eferente autónoma.

En algunas modalidades, la neurona es una neurona sensorial autónoma.

En algunas modalidades, la neurona es una neurona del núcleo subtalámico.

Como se emplea en el presente documento, un vector se refiere a cualquier

vector de transferencia génica adecuado conocido en la técnica. Esto incluye, pero no se limitan a, vectores virales (que incluye el virus adeno asociado, el adenovirus y el lentivirus) y métodos no virales (como el ADN desnudo o los métodos de transferencia química). Típicamente, el vector es un vector de terapia génica.

La expresión positiva, tal como se emplea aquí, es sinónimo de regulación positiva de un gen y se refiere a un aumento en la expresión de un gen relativo a una célula (neurona) que no ha sido transfectada ni transducida. Para que sea efectiva, la neurona debe expresar niveles más altos de canales iónicos Kv7,3 ensamblados, ya sea como homómeros o heterómeros de Kv7,3, por ejemplo, heterómeros Kv7,2 /7,3.

En el presente documento, la expresión “administrar una dosis menor” se refiere a la exposición posterior de una neurona que expresa positivamente el gen introducido a una dosis inferior de un fármaco que puede normalmente ser administrada a un paciente para ser terapéuticamente efectivo. Por ejemplo, para tratar la epilepsia, la retigabina se administraba típicamente a una dosis de 600-1200 mg al día, lo que corresponde a una concentración plasmática media de 0,83 μM. Mediante el método actual, se ha mostrado que las concentraciones inferiores a 0,5 μM son eficaces en neuronas humanas *in vitro.* Sin limitarse a la teoría, los inventores consideran que esto permitiría administrar *in vivo una dosis efectiva inferior a 0,5* μM.

En una modalidad, la dosis terapéuticamente efectiva del fármaco neuromodulador se reduce hasta en un 100%. Por ejemplo, aproximadamente 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 90%, 95%, 90% o 95%.

Si bien no se desea limitarse a la teoría, los inventores creen que la expresión positiva de Kv7,3 puede tener un efecto del valor de referencia que reduce la excitabilidad de las neuronas incluso en ausencia de un fármaco neuromodulador. Por lo tanto, en algunas modalidades, no se requiere la administración de un fármaco neuromodulador.

Se prevé que la reducción de la dosis terapéuticamente efectiva sea

suficiente para disminuir los efectos secundarios del fármaco neuromodulador a niveles clínicamente aceptables. Los expertos en la materia comprenderán qué se entiende por niveles clínicamente aceptables.

Tal como se emplea en este documento, "incrementar la eficacia del fármaco" se refiere al efecto terapéutico incrementado de una dosis determinada del fármaco en las neuronas transducidas que expresa positivamenten el transgén en relación con las neuronas no transducidas.

Tal como se emplea en el presente documento, "reducir los efectos secundarios" se refiere a la disminución o eliminación de los efectos secundarios indeseables relacionados con la administración de un fármaco a un sujeto a una dosis determinada.

Por ejemplo, se descubrió que el fármaco neuromodulador retigabina (ezogabina), el cual se comercializa como anticonvulsivo, causaba somnolencia, mareo, tinnitus y vértigo, confusión y dificultad para hablar a dosis terapéuticas (600-1200 mg). Entre los efectos secundarios menos comunes se incluían temblor, pérdida de memoria, trastornos de la marcha y visión doble. En 2013, la FDA advirtió al público que la ezogabina puede causar decoloración azulada de la piel y anomalías oculares caracterizadas por cambios en la pigmentación de la retina. Si bien no desean limitarse a la teoría, los inventores consideran que el aumento de la expresión de Kv7,3 en las neuronas hiperexcitables asociadas con las crisis epilépticas permitiría ampliar el rango terapéutico de la retigabina a dosis más bajas, reduciendo o eliminando así los efectos secundarios anteriores. Es decir, se incrementaría el índice terapéutico de la retigabina. De hecho, los ensayos que incluyeron dosis más bajas de retigabina (300-1200 mg) informaron menos efectos adversos dermatológicos, oftalmológicos y neurológicos (Lerche et al., 2015; Brickel et al., 2020).

Como se emplea en el presente documento, el vector de terapia génica es un vector adecuado para métodos de terapia génica. Si bien aquí se describen cápsides de AAV, cabe señalar que otros vectores pueden ser adecuados y utilizarse sin que

ello suponga una desviación del alcance de la presente invención.

Típicamente, la terapia génica se utiliza para tratar trastornos o condiciones neurológicas. Un ejemplo de trastorno o condición neurológica que puede tratarse con terapia génica es la espasticidad. La espasticidad es un síntoma neurológico que sufren personas con diversos trastornos neurológicos, que incluyen pero no se limitan a esclerosis múltiple, apoplejía, lesión cerebral traumática, lesión medular, enfermedad de la motoneurona y parálisis cerebral. La espasticidad resulta por la excitación excesiva de los músculos por las neuronas motoras, que, debido a la enfermedad, se vuelven hiperexcitables.

La excitabilidad de las neuronas está determinada por la frecuencia de los potenciales de acción generados en respuesta a un estímulo dado. El incremento de la excitabilidad es indicado mediante una o más de las medidas que se describen a continuación.

El incremento de la excitabilidad, tal como se emplea en este documento, se define como una neurona que presenta al menos una de las siguientes:

a. Un incremento (despolarización) del voltaje de reposo de una neurona (potencial de membrana en reposo - PMR) de tal manera que se acerca al umbral para la generación de un potencial de acción.

b. Una disminución en la magnitud del estímulo requerido para causar que las neuronas generen potenciales de acción (Reobase).

c. Un incremento en el número de potenciales de acción generados por un estímulo dado (Frecuencia) en comparación con una neurona con niveles de excitabilidad “normales”.

d. Generación persistente de potenciales de acción más allá del cese del estímulo responsable de iniciar el fuego.

e. Un aumento en la frecuencia de los potenciales de acción en una población de neuronas (2-100 000) registrados utilizando una matriz de microelectrodos (matriz de microelectrodos-MEA).

f. Un aumento en el número o duración de las ráfagas de potenciales de acción registradas por un MEA, definido como al menos 5 potenciales de acción consecutivos con intervalos de 100 ms o menos entre cada potencial de acción.

Por ejemplo, una neurona hiperexcitable puede tener un RMP de -55 mV, lo cual es con dureza 15 mV más positivo de lo esperado en las neuronas y más cercano al umbral para la generación de un potencial de acción (~-40 mV). Si una magnitud de estímulo de 50 picoamperios (pA) provoca que una neurona normal para generar un potencial de acción (reobase = 50 pA), 25 pA podrían ser suficientes en una neurona hiperexcitable (reobase = 25 pA). De manera similar, si una magnitud de estímulo de 100 pA provoca que una neurona normal genere 10 potenciales de acción en 1 segundo (frecuencia = 10 Hz), una neurona hiperexcitable podría generar 20 con la misma magnitud de estímulo (frecuencia = 20 Hz).

Además, en las neuronas hiperexcitables, la generación de potenciales de acción suele persistir más allá de la desaparición del estímulo, a diferencia de las neuronas normales. Este es el caso en la espasticidad, con ello las neuronas generan más potenciales de acción durante periodos más prolongados, y a menudo en respuesta a un estímulo menor.

Típicamente, el vector de terapia génica es un vector AAV que comprende una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7,3. Esta subunidad de canal iónico puede ser de tipo silvestre, codificada por la EC ID No. 1, o mutante, codificada por la SEQ ID.2, o una secuencia equivalente que codifique las secuencias de aminoácidos de la SEQ ID.3 o ID.4, respectivamente.

Las partículas virales de AAV, tal como se emplean en este documento, son partículas virales de virus adenoasociado que comprenden un genoma de ADN monocatenario empaquetado dentro de una envoltura viral capaz de transducir una célula, por ejemplo, una neurona objetivo. En particular, las partículas virales de AAV empleadas en este documento incluyen la secuencia de nucleótidos de conformidad con SEQ ID.1 o SEQ ID.2. En algunas modalidades las partículas virales pueden

carecer de nucleótidos que codifiquen la replicación; es decir, son deficientes en replicación.

Los AAV son virus pequeños pertenecientes al género dependoparvovirus que contienen una sola hebra de ADN de hasta ~4,9 kb. El genoma del AAV contiene tres proteínas de la cápside: VP1, VP2 y VP3, todas ellas traducidas a partir de un único ARNm mediante empalme alternativo. En la naturaleza, se han identificado múltiples serotipos de AAV, cada uno con secuencias únicas del gen de la cápside y, por lo tanto, con tropismos distintos, si bien los serotipos silvestres suelen ser capaces de infectar múltiples tipos de tejidos y células. Estos serotipos se designan con números: AAV1, AAV2, etc. Se ha demostrado que la modificación de las secuencias de la cápside mediante métodos de recombinación de ADN puede generar secuencias no nativas con propiedades y tropismo específicos, dirigidas hacia (o contra) células o tejidos particulares, y que evaden el sistema inmunitario (Vandenberghe et al., 2009). Como se emplean en el presente, las partículas virales de AAV transducen selectivamente neuronas objetivo, como las neuronas motoras.

Como se emplea en el presente la cápside del AAV, se refiere a la cápside de los virus adenoasociados. Se sabe que la cápside del AAV posee una selectividad notable debido a su composición (está formada por una mezcla de VP1, VP2 y VP3, con un total de 60 monómeros dispuestos en simetría icosaédrica en una proporción 1:1:10) y a modificaciones postraduccionales. Las proteínas de la cápside del AAV contienen 12 regiones de superficie hipervariables, pero el genoma, en general, presenta genes de replicación y estructurales altamente conservados entre los serotipos.

La cápside del AAV empleada aquí puede transducir selectivamente un tipo celular particular, tal como las neuronas, tal como en lugar del tejido muscular, y puede transducir selectivamente neuronas objetivo sobre otras neuronas, incluso partes específicas de neuronas, como el axón.

En uso, la cápside del AAV que contiene la secuencia de nucleótidos de

conformidad con cualquiera de alguna de SEQ ID Nos. 1 a 8 (la partícula viral) se administra a un sujeto, la partícula viral infecta neuronas objetivo, que se convierten en neuronas transducidas. Las neuronas transducidas expresa positivamenten las secuencias de aminoácidos codificadas por las secuencias de nucleótidos, las cuales se ensamblan para formar canales iónicos funcionales que contienen Kv7,3 (ya sea homómeros o heterómeros). En general, el tiempo transcurrido desde el suministro de las partículas virales al sujeto hasta la expresión positiva de los canales iónicos funcionales es de aproximadamente 3 semanas. Una vez transcurrido el tiempo adecuado para que las neuronas transducidas sobreexpresen los canales iónicos, el sujeto puede ser tratado con un fármaco neuromodulador, como retigabina, a una dosis menor.

La transducción selectiva, tal como se emplea aquí, se refiere a la capacidad de la cápside del AAV para transducir selectivamente un tipo celular con preferencia sobre otro. La especificidad puede ser neuronas en preferencia por las células musculares. Alternativamente, puede ser la preferencia por las neuronas motoras en preferencia de las neuronas sensoriales, por ejemplo.

Un sujeto como se emplea en el presente documento, se refiere a un ser humano o a un mamífero no humano. Típicamente, el sujeto presenta un trastorno neurológico asociado a una mayor excitabilidad neuronal.

En algunas modalidades, el sujeto tiene un trastorno neurológico asociado con una disfunción de los canales iónicos Kv7,3.

En algunas modalidades, el sujeto presenta síntomas caracterizados por hiperexcitabilidad neuronal que puede o no estar asociada con disfunción de los canales iónicos Kv7,3.

En algunas modalidades, el sujeto presenta un trastorno neurológico y/o síntomas caracterizados por hiperexcitabilidad neuronal que pueden o no estar asociados con disfunción de los canales Kv.

El término “trastorno neurológico asociado a una mayor excitabilidad

neuronal", tal como se emplea en este documento, se refiere a cualquier afección en la que el exceso de actividad neuronal produce síntomas relacionados con tal trastorno neurológico. Por ejemplo: epilepsia, espasticidad, convulsiones neonatales familiares benignas (CNFB), dolor nociceptivo, dolor no nociceptivo, enfermedad de Parkinson, esclerosis múltiple.

En una modalidad, el trastorno neurológico se asocia con la hiperexcitabilidad de las neuronas, tal como las neuronas motoras.

En una modalidad, el trastorno neurológico se asocia con una mayor excitabilidad de las neuronas.

En una modalidad, el trastorno neurológico se selecciona entre epilepsia, espasticidad, BNFS, dolor nociceptivo, dolor no nociceptivo, enfermedad de Parkinson, esclerosis múltiple.

El término "tratar o tratamiento", tal como se emplea en el presente documento, se refiere a la reversión de una afección, la mejora o el alivio de los síntomas asociados con una afección o la prevención del desarrollo/empeoramiento de una condición.

El término "tratamiento" incluye tratamientos y terapias combinadas, en las que se combinan dos o más tratamientos o terapias, por ejemplo, de forma secuencial o simultánea. Por ejemplo, el vector se puede administrar uno o más días antes de que se administre el fármaco neuromodulador al sujeto.

Una dosis menor, tal como la empleada en este documento, se refiere a una dosis inferior a la considerada efectiva en un sujeto (o en la parte inferior de un rango efectiva) y, por lo tanto, no causa efectos secundarios o reduce su gravedad, manteniendo al mismo tiempo la eficacia terapéutica.

La cantidad terapéuticamente efectiva, tal como se emplea en el presente documento, significa una cantidad del compuesto que es efectiva para tratar el trastorno o la afección mencionados.

En algunas modalidades, los canales Kv7,3 expresados positivamente

pueden ensamblarse como heterómeros, tal como los heterómeros Kv7,2/7,3 o Kv7,3/7,5. Las proporciones de subunidades de estos heterómeros pueden variar y permitir perfeccionar el método descrito en este documento.

De forma similar, en algunas modalidades, la proporción de subunidades Kv7,3 de tipo silvestre y mutantes puede diferir para permitir el refinamiento del método. Esto podría aplicarse tanto a heterómeros como a homómeros.

En algunas modalidades, los canales Kv7,3 expresados positivamente pueden ensamblarse como homómeros.

Los canales homoméricos Kv7,3 pueden tener una conducción mínima, de modo que su efecto sobre la excitabilidad del valor de referencia de la neurona transducida, pero aún permite al fármaco neuromodulador actuar en la neurona transducida a una dosis menor.

La reobase, tal como se emplea aquí, se refiere al valor mínimo de inyección de corriente requerido para despolarizar el potencial de membrana neuronal lo suficiente como para generar un único potencial de acción.

El potencial de membrana en reposo, tal como se emplea aquí, se refiere a la diferencia de voltaje potencial registrada entre los compartimentos neuronales intracelular y extracelular en ausencia de cualquier inyección de corriente. Este valor de voltaje se registra usando técnicas electrofisiológicas como la técnica tal como fijación de parche de la célula completa.

Como se emplea en el presente, “desplazar el potencial de membrana en reposo (PMR) de la neurona objetivo lejos del umbral” significa que el valor de voltaje en estado de reposo de la neurona es más negativo (hiperpolarizado) y, por lo tanto, se requiere una mayor magnitud de estímulo para despolarizarla el voltaje hasta un nivel en el que se generen potenciales de acción. Por ejemplo, un PMR típico de una neurona puede ser de -65 mV, lo que significa que se necesitan 500 pA de corriente (estímulo) para despolarizar la neurona lo suficiente como para alcanzar el potencial de membrana umbral necesario para generar potenciales de acción (-45 mV). Si el

PMR se vuelve más negativo (-75 mV), entonces se aleja aún más del umbral y, por consiguiente, requiere una mayor inyección de corriente para alcanzar el umbral y generar potenciales de acción.

La reducción de la excitabilidad de la neurona objetivo, tal como se emplea en el presente documento, significa al menos una de las siguientes:

a. Hiperpolarización del potencial de membrana en reposo de una neurona, de manera que se aleje aún más del umbral para la generación de un potencial de acción.

b. Aumentar la magnitud del estímulo necesario para causar que las neuronas generen potenciales de acción (Reobase).

c. Disminuir el número de potenciales de acción generados por un estímulo eléctrico o estímulos químico (Frecuencia de AP).

d. Reducir o eliminar la generación persistente de potenciales de acción más allá del cese del estímulo responsable de iniciar la descarga.

e. Reducir la frecuencia de los potenciales de acción espontáneos en ausencia de estímulo.

f. Reducir la duración o la frecuencia de las ráfagas de potenciales de acción espontáneos (las ráfagas se definen como al menos 5 potenciales de acción consecutivos con intervalos de 100 ms o menos).

La reducción de la excitabilidad se puede medir usando la técnica de fijación en parche de célula completa en modo de pinzamiento de corriente, inyectando pulsos de corriente rectangulares de magnitud creciente y registrando la variación del voltaje y la frecuencia de generación de potenciales de acción durante la duración del pulso. El potencial de membrana en reposo se mide en condiciones estables, mientras no ocurra la inyección (por ejemplo, el voltaje no varía más de 1-2 mV). La reobase se mide como la corriente mínima de entrada, de una duración determinada, necesaria para generar al menos un potencial de acción. La frecuencia potencial de acción se mide como el número de potenciales de acción generados durante un periodo de

tiempo específico, usualmente igual a la duración del pulso de corriente inyectado en la neurona.

La reducción de la excitabilidad también puede medirse mediante una matriz de microelectrodos (MEA), que consiste en una placa de cavidades con varios contactos de electrodo ubicados en la superficie de la base de cada cavidad. Se cultiva una población de neuronas en contacto con los electrodos de cada cavidad, de manera que su actividad pueda registrarse como potenciales de acción extracelulares (espigas). Las neuronas cultivadas en MEA producen potenciales de acción espontáneos, a menudo denominados espigas. Cinco o más espigas consecutivas registradas con intervalos de 100 ms o menos se denominan ráfagas. La frecuencia de las espigas espontáneas y las características de las ráfagas (frecuencia, duración de ráfaga, etc.) pueden utilizarse para determinar la excitabilidad neuronal.

En algunas modalidades, la regulación positiva de la subunidad del canal iónico Kv7,3 por la neurona transducida reduce la actividad de las células inervadas por las terminaciones axónicas de la misma neurona. Por ejemplo, la actividad del músculo gastrocnemio, medida por electromiografía (EMG), como un resultado de la regulación positiva de las subunidades del canal iónico Kv7,3 en las neuronas motoras del gastrocnemio. La actividad muscular reducida se caracteriza por uno o más de los siguientes: amplitud reducida de la acción muscular del compuesto evocada por la estimulación de las fibras nerviosas aferentes periféricas; duración reducida de la actividad EMG persistente después de la estimulación de las fibras nerviosas aferentes; frecuencia reducida, duración reducida de la actividad EMG espontánea; amplitud reducida de la actividad EMG espontánea; rigidez reducida de un músculo determinado por un médico capacitado; y rango incrementado de movimiento en las articulaciones asociadas al músculo afectado, como se determinó por un médico capacitado.

La invención también comprende métodos de tratamiento que implican la inyección de vectores virales AAV que comprenden la secuencia de nucleótidos SEQ

ID.1 o SEQ ID.2 en el cerebro o la médula espinal de un sujeto, o mediante inyección intramuscular; estos vectores virales AAV pueden luego transducir las neuronas objetivo.

La expresión de la secuencia de nucleótidos conduce a la expresión positiva de los canales iónicos Kv7,3 en estas neuronas. De este modo, el sujeto puede entonces ser tratado con un fármaco neuromodulador a una dosis menor que la que anteriormente se requería para obtener un efecto terapéutico. Esta menor dosis, a su vez, reduce los efectos secundarios y la toxicidad. El objetivo de curar, aliviar los síntomas y/o mejorar la calidad de vida de los pacientes con enfermedades causadas por neuronas hiperexcitables se logra sin efectos secundarios indeseables (o con menos efectos secundarios y de menor gravedad).

Los fármacos con efectos secundarios prohibitivos pueden volverse utilizables empleando el método aquí descrito, simplemente permitiendo que una dosis menor que sea efectiva en células específicas.

En algunas modalidades, el método implica que el vector de terapia génica o la partícula viral AAV infecte retrógradamente las neuronas con el fin de suministrar material genético a las neuronas, con el propósito de tratar enfermedades causadas por, o asociadas con, la disfunción del canal iónico Kv7.

En el caso de A315T, un canal con alta tasa de inserción en la membrana y niveles elevados de conductancia, es probable que solo se requieran niveles bajos de expresión para que sea efectivo. Esto significa que el virus puede suministrarse en cantidades menores, lo que reduce la probabilidad de inmunogenicidad y los costos.

En una modalidad, el método es un método ex vivo.

En el contexto de esta especificación, "que comprende" debe interpretarse como "que incluye".

También se pretende que los aspectos de la invención que comprenden ciertos elementos se extiendan a modalidades alternativas que “consisten” o “consisten esencialmente” de los elementos relevantes.

Cuando sea técnicamente apropiado, las modalidades de la invención se podrán combinar.

Las modalidades descritas en el presente documento comprenden ciertas características/elementos. La descripción también abarca modalidades separadas que consisten o consisten esencialmente en tales características/elementos.

El término "aproximadamente", tal como se emplea en el presente documento, se refiere a ± 10%.

Las referencias técnicas, tales como patentes y solicitudes, se incorporan al presente documento a modo de referencia.

Cualquier modalidad específica y explícita aquí citada puede formar la base de una renuncia, ya sea por sí sola o en combinación con una o más modalidades adicionales.

EJEMPLOS

Materiales y métodos

Cultivo celular

Las células progenitoras de neuronas sensoriales derivadas de células iPSC humanas (Axol Bioscience) se descongelaron y se sembraron a baja densidad sobre una monocapa de astrocitos corticales de rata en cubreobjetos de vidrio recubiertos con poli-D-lisina. Los cultivos se realizaron en medio NbActiv 4 (Neurobasal/B 27 suplementado con MAXIMIZER, GDNF, NGF, BDNF y NT 3) para promover la maduración celular hacia un fenotipo neuronal diferenciado.

AAV

pAAV- CMV- EGFP, pAAV -CMV- hKCNQ3 - T2A-EGFP y pAAV -CMV- $hKCNQ3_{(A315T)}$-T2A-EGFP a cavidades que contenían cultivos neuronales a los 7 días in vitro y se realizaron registros de fijación en parche 1 (DIV15-16) o 2 semanas después (DIV21-23).

Electrofisiología de fijación en parche

Se emplearon métodos estándar de fijación en parche. La composición de la solución de registro externa fue de 140 mM NaCl, 25 mM KCl y 2 mM La solución de registro interna contenía $CaCl_2$, 3 mM $MgCl_2$, 10 mM glucosa, 10 mM HEPES, pH 7,3. La solución de registro interna contenía gluconato de potasio 120 mM, KCl 20 mM, $MgCl_2$ 3 mM, EGTA 2,5 mM, $CaCl_2$ 0,5 mM, Na_2 -ATP 2.4 mM, Li_2GTP 0,3 mM, HEPES 10 mM, pH 7,3. Tras establecer la configuración de célula completa, se midieron las propiedades pasivas de la membrana de cada célula (capacitancia y resistencia) en modo de fijación de voltaje. Todas las mediciones de excitabilidad se realizaron en modo de fijación de corriente.

Registros de matrices multielectrodo

Las neuronas se cultivaron como se describió anteriormente en placas de 24 cavidades con 16 electrodos por cavidad, de manera que las neuronas estuvieran en contacto directo con electrodos individuales. La actividad de disparo espontáneo entonces se registró después de 30 días. Los registros se realizaron en condiciones del valor de referencia (sin fármaco) y posteriormente con concentraciones incrementadas del fármaco activador.

Modelo de espasticidad del ratón

Se practicó una sección medular completa a ratones neonatos (día postnatal 0) entre los segmentos torácicos 9^{no} y 10^{mo} para inducir espasticidad. Durante la misma cirugía, se inyectaron AAV en ambos músculos gastrocnemios.

Registro EMG

Entre 10 y 14 días después de la lesión medular, se insertaron electrodos EMG en los músculos inyectados para registrar la actividad eléctrica antes y después de la administración intraperitoneal de un fármaco activador (retigabina). Se probaron

diferentes dosis en días distintos.

Electrofisiología de fijación en parche en cortes de médula espinal de ratón

La médula espinal se extrajo bajo anestesia terminal y se cortó en rodajas de 300 µm con un vibrátomo. Posteriormente, se realizaron registros de fijación en parche en neuronas motoras que expresan GFP, como se describió anteriormente (electrofisiología de fijación en parche).

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SECUENCIAS

SEQ ID 1 - tipo silvestre Kv7,3

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) canal de potasio

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SEQ ID 2 – Kv7,3 A315T

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SEQ ID 3 – Kv7,3 A315S

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) canal de potasio A315S

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SEQ ID 4 – Kv7,3 A315V

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SEQ ID 5 – Kv7,3 A315C

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SEQ ID 6 – Kv7,3 A315N

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SEQ ID 7 – Kv7,3 A315Q

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SEQ ID 8 – Kv7,3 A315Y

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CGGGGC
TGGGCGCTGCTTTACCACGCGTTGGTGTTCCTGATTGTCCTGGGGTGCTTGATTC
TGGCT

GTCCTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTAC
TGGAG
ACATTTGCTATTTTCATCTTTGGAGCCGAGTTTGCTTTGAGGATCTGGGCTGCTGG
ATGT
TGCTGCCGATACAAAGGCTGGCGGGGCCGACTGAAGTTTGCCAGGAAGCCCCTG
TGCATG
TTGGACATCTTTGTGCTGATTGCCTCTGTGCCAGTGGTTGCTGTGGGAAACCAAG
GCAAT
GTTCTGGCCACCTCCCTGCGAAGCCTGCGCTTCCTGCAGATCCTGCGCATGCTG
CGGATG
GACCGGAGAGGTGGCACCTGGAAGCTTCTGGGCTCAGCCATCTGTGCCCACAGC
AAAGAA
CTCATCACGGCCTGGTACATCGGTTTCCTGACACTCATCCTTTCTTCATTTCTTGT
CTAC
CTGGTTGAGAAAGACGTCCCAGAGGTGGATGCACAAGGAGAGGAGATGAAAGAG
GAGTTT
GAGACCTATGCAGATGCCCTGTGGTGGGGCCTGATCACACTGTACACCATTGGCT
ATGGA
GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAA
TTGGC
GTCTCCTTTTTTGCCCTTCCAGCGGGCATCCTGGGGTCCGGGCTGGCCCTCAAG
GTGCAG
GAGCAACACCGTCAGAAGCACTTTGAGAAAAGGAGGAAGCCAGCTGCTGAGCTC
ATTCAG
GCTGCCTGGAGGTATTATGCTACCAACCCCAACAGGATTGACCTGGTGGCGACAT
GGAGA
TTTTATGAATCAGTCGTCTCTTTTCCTTTCTTCAGGAAAGAACAGCTGGAGGCAGC
ATCC

AGCCAAAAGCTGGGTCTCTTGGATCGGGTTCGCCTTTCTAATCCTCGTGGTAGCA
ATACT
AAAGGAAAGCTATTTACCCCTCTGAATGTAGATGCCATAGAAGAAAGTCCTTCTAA
AGAA
CCAAAGCCTGTTGGCTTAAACAATAAAGAGCGTTTCCGCACGGCCTTCCGCATGA
AAGCC
TACGCTTTCTGGCAGAGTTCTGAAGATGCCGGGACAGGTGACCCCATGGCGGAA
GACAGG
GGCTATGGGAATGACTTCCCCATCGAAGACATGATCCCCACCCTGAAGGCCGCC
ATCCGA
GCCGTCAGAATTCTACAATTCCGTCTCTATAAAAAAAAAATTCAAGGAGACTTTGAG
GCCT
TACGATGTGAAGGATGTGATTGAGCAGTATTCTGCCGGGCATCTCGACATGCTTT
CCAGG
ATAAAGTACCTTCAGACGAGAATAGATATGATTTTCACCCCTGGACCTCCCTCCAC
GCCA
AAACACAAGAAGTCTCAGAAAGGGTCAGCATTCACCTTCCCATCCCAGCAATCTC
CCAGG
AATGAACCATATGTAGCCAGACCATCCACATCAGAAATCGAAGACCAAAGCATGAT
GGGG
AAGTTTGTAAAAGTTGAAAGACAGGTTCAGGACATGGGGAAGAAGCTGGACTTCC
TCGTG
GATATGCACATGCAACACATGGAACGGTTGCAGGTGCAGGTCACGGAGTATTACC
CAACC
AAGGGCACCTCCTCGCCAGCTGAAGCAGAGAAGAAGGAGGACAACAGGTATTCC
GATTTG
AAAACCATCATCTGCAACTATTCTGAGACAGGCCCCCCGGAACCACCCTACAGCT
TCCAC

CAGGTGACCATTGACAAAGTCAGCCCCTATGGGTTTTTTGCACATGACCCTGTGA
ACCTG
CCCCGAGGGGGACCCAGTTCTGGAAAGGTTCAGGCAACTCCTCCTTCCTCAGCA
ACAACG
TATGTGGAGAGGCCCACGGTCCTGCCTATCTTGACTCTTCTCGACTCCCGAGTGA
GCTGC
CACTCCCAGGCTGACCTGCAGGGCCCCTACTCGGACCGAATCTCCCCCCGGCAG
AGACGT
AGCATCACGCGAGACAGTGACACACCTCTGTCCCTGATGTCGGTCAACCACGAG
GAGCTG
GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCG
GCCCC
AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGAC
GGACACA
GACACGGACCCCTTCACGCCCAGCGGCTCCATGCCTCTGTCGTCCACAGGGGAT
GGGATT
TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 9 – tipo silvestre Kv7,3

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC

CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLATIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 10 – Kv7,3 A315T

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315T

MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLTTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 11 – Kv7,3 A315S

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315S

MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLSTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR

SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 12 – Kv7,3 A315V

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315V
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLVTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT

KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 13 – Kv7,3 A315C

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315C
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLCTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP

YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 14 – Kv7,3 A315N

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315N
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLNTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS

SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 15 – Kv7,3 A315Q

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Q
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM

DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLQTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKV
Q
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 16 – Kv7,3 A315Y

>sp|O43525|KCNQ3_HUMAN canal dependiente de voltaje de potasio miembro 3 OS de subfamilia KQT=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Y
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVT
LALGAG

ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERP
RG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRML
RM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEE
F
ETYADALWWGLITLYTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLE
AAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRM
KA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETL
RP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQS
PR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVT
EYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPV
NL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQ
RR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGE
TDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

REIVINDICACIONES

1. Un método para incrementar la eficacia de un fármaco neuromodulador, caracterizado que comprende los pasos:

a. Introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forme canales iónicos funcionales, y

c. administrar una dosis menor del fármaco neuromodulador.

2. Un método para reducir los efectos secundarios de un fármaco neuromodulador, caracterizado por que comprende los pasos:

a. Introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. administrar una dosis menor del fármaco neuromodulador.

3. Un método para incrementar el índice terapéutico de un fármaco neuromodulador, caracterizado que comprende los pasos:

a. Introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16 a una neurona objetivo mediante un vector,

b. expresa positivamente la subunidad del canal iónico en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. administrar el fármaco neuromodulador.

4. Un método para reducir la excitabilidad de una neurona, caracterizado por que comprende los pasos:

a. Introducir una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16 a la neurona objetivo mediante un vector,

b. Expresa positivamente la subunidad del canal iónico Kv7.3 en la neurona objetivo de manera que forma canales iónicos funcionales, y

c. Opcionalmente administrar un fármaco neuromodulador.

5. Un método para tratar un trastorno neurológico, caracterizado por que comprende los pasos:

a. administrar a un sujeto en necesidad del mismo, una cantidad terapéuticamente efectiva del vector de terapia génica que comprende una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16;

b. regula positivamente la expresión de la subunidad del canal iónico Kv7.3 en una neurona objetivo de forma canales iónicos funcionales, y

c. administrar una dosis inferior de un fármaco neuromodulador conocido para canales iónicos Kv7.3.

6. Un vector de terapia génica, caracterizado que comprende una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia

de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16, en donde, cuando el vector transduce una neurona objetivo, la expresión de la subunidad del canal iónico Kv7.3 y se regula positivamente y se forman canales iónicos funcionales.

7. Un vector de terapia génica de conformidad con la reivindicación 6, caracterizado porque el vector transduce selectivamente la neurona objetivo.

8. Un vector de terapia génica de partículas virales AAV, caracterizado por que comprende:

a. una cápside de AAV que transduce selectivamente las neuronas objetivo, y

b. una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16, en donde el vector transduce las neuronas objetivo, se regula positivamente la expresión de la subunidad del canal iónico Kv7.3 y se forman canales iónicos funcionales.

9. Un vector de terapia génica, caracterizado por que comprende una cápside AAV que transduce selectivamente las neuronas objetivo y una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16, para su uso en la regulación positiva de la expresión de la subunidad del canal iónico Kv7.3 por la neurona objetivo de manera que forma canales iónicos funcionales en un sujeto que tiene un trastorno neurológico asociado con una mayor excitabilidad neuronal.

10. Un vector de terapia génica, caracterizado por que comprende una cápside de AAV que transduce selectivamente las neuronas objetivo y una secuencia de nucleótidos que codifica una subunidad del canal iónico Kv7.3 que comprende la

secuencia de cualquiera de las SEC ID.1 a SEC ID.8 o una secuencia de nucleótidos que codifica la secuencia de aminoácidos de la subunidad del canal iónico Kv7.3 de cualquiera de las SEC ID.9 a SEC ID.16, para su uso en el tratamiento de un sujeto que padece un trastorno neurológico, en donde, una vez que el vector ha transducido la neurona objetivo, la expresión del canal iónico Kv7.3 por la neurona objetivo se regula positivamente, de modo que se puede administrar efectivamente una dosis menor de un fármaco neuromodulador al sujeto.

11. El método de conformidad con cualquiera de las reivindicaciones 1 a 5 o el vector de terapia génica de conformidad con la reivindicación 10, en donde el fármaco neuromodulador se selecciona de Retigabina/Ezogabina y derivados del mismo, BHV-7000 y Xen496, Xen 1101, flupirtina, diclofenaco, BMS-204352, ácido meclofenámico, ETX-123 y linopirdina.

12. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque el canal iónico Kv7.3 se expresa como un homómero Kv7.3, un heterómero Kv7.2/7.3 o un heterómero Kv7.3/7.5.

13. El método o vector de terapia génica de conformidad con la reivindicación 12, caracterizado porque el canal iónico Kv7.3 se expresa funcionalmente como un homómero.

14. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque las neuronas objetivo son neuronas motoras.

15. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones 5 o 9 a 14, caracterizado porque el trastorno neurológico está asociado con una mayor excitabilidad de las neuronas.

16. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones 5 o 9 a 15, caracterizado porque el trastorno neurológico se selecciona del grupo que consiste en: epilepsia, espasticidad, BNFS, enfermedad de

Parkinson, dolor nociceptivo y dolor no nociceptivo.

17. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque la regulación positiva de la subunidad del canal iónico Kv7.3 reduce la reobase de la neurona objetivo en presencia del fármaco neuromodulador.

18. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque la regulación positiva de la subunidad del canal iónico Kv7.3 desplaza el potencial de membrana en reposo de la neurona objetivo alejándolo del umbral en presencia del fármaco neuromodulador.

19. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado por que la regulación positiva de la subunidad del canal iónico Kv7.3 reduce la excitabilidad de la neurona objetivo.

20. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque la regulación positiva de la subunidad del canal iónico Kv7.3 reduce la excitabilidad de una pluralidad de neuronas objetivo, como se registró usando un dispositivo de matriz de microelectrodos (MEA).

21. El método o el vector de terapia génica de conformidad con cualquiera de las reivindicaciones anteriores, caracterizado porque la regulación positiva de la subunidad del canal iónico Kv7.3 por la neurona objetivo reduce la actividad de las células inervadas por las terminales axónicas de la neurona objetivo.

22. El método de las reivindicaciones 19 a 21, en donde la reducción de la excitabilidad de la neurona objetivo, o la reducción de la actividad de las células inervadas por las terminales axónicas de la neurona objetivo, se produce o aumenta en presencia del fármaco neuromodulador.

23. Uso del vector de terapia génica de conformidad con las reivindicaciones 6 a 10 en la preparación de un medicamento para su uso en el tratamiento de condiciones mejoradas mediante la regulación positiva de la expresión de los canales iónicos Kv7.3.

24. Uso del vector de terapia génica de conformidad con las reivindicaciones 6 a 10 en la preparación de un medicamento para su uso en el tratamiento de un trastorno neurológico asociado con una excitabilidad incrementada de las neuronas, tal como, en donde el trastorno neurológico se selecciona del grupo que consiste en: epilepsia, espasticidad, BNFS, enfermedad de Parkinson, ELA, dolor nociceptivo y dolor no nociceptivo.

25. Uso de un vector de terapia génica de conformidad con las reivindicaciones 6 a 10 en la preparación de un medicamento para uso como antiespástico.

RESUMEN DE LA INVENCIÓN

La presente descripción se refiere a un método quimiogenético que permite administrar dosis más bajas de fármacos mediante la expresión positiva de su objetivo y a vectores para suministrar una secuencia de nucleótidos a las células objetivo para permitir la expresión positiva de la misma.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 November 2024 (14.11.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/231830 A1

(51) International Patent Classification:
C12N 15/86 (2006.01) *A61K 48/00* (2006.01)

(21) International Application Number:
PCT/IB2024/054426

(22) International Filing Date:
07 May 2024 (07.05.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2306914.9 10 May 2023 (10.05.2023) GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *of inventorship (Rule 4.17(iv))*

(54) Title: METHOD

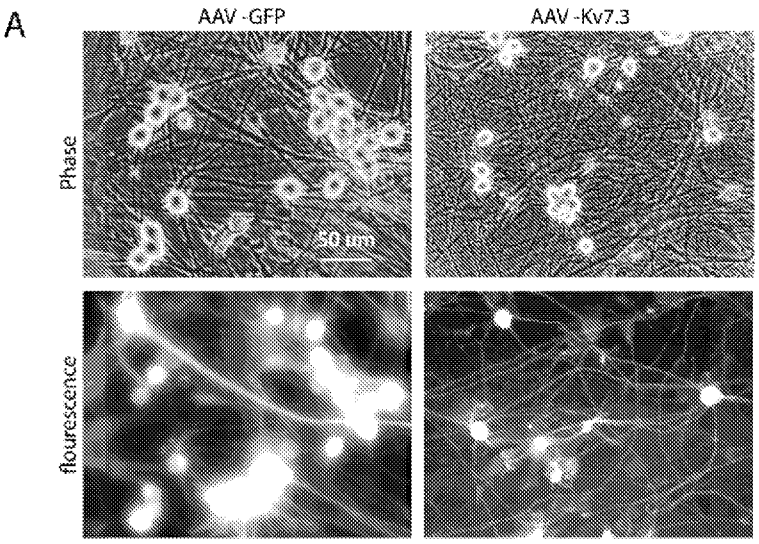

Figure 2

(57) Abstract: The present disclosure relates to a chemogenetic method of enabling lower doses of drugs to be administered by over-expressing their target and to vectors to deliver a nucleotide sequence to the target cells to enable overexpression thereof.

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[Continued on next page]

Published:

— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

METHOD

Field of the Invention

The present invention relates to methods of reducing the side effects of neuromodulatory drugs, to increasing the efficacy of neuromodulatory drugs and to methods of reducing the excitability of neurons. The invention also relates to gene therapy vectors useful in carrying out the methods of the invention.

Background to the Invention

Neurological disorders are characterised by abnormal activity in neurons and circuits within the central nervous system (CNS). Often, this abnormal activity is conveyed to the output neurons of the CNS, motor neurons, leading to inappropriate muscle contractions and sensory and motor dysfunction.

Pharmaceutical therapies have been developed aimed at ameliorating the abnormal activity typically by modulating ion channels that carry excitatory and inhibitory currents. However, drug delivery to the nervous system is difficult and existing therapies often have undesirable side effects due to low specificity.

Thus, although neuromodulatory drugs developed for neurological disorders have shown some therapeutic benefit, this has been limited by off-target effects on non-dysfunctional neurons and other systems expressing the target (cardiovascular, respiratory, gastrointestinal etc).

Potassium (K^+) channels are membrane proteins that allow rapid and selective flow of K^+ ions across the cell membrane, and thus generate electrical signals in cells. Voltage-gated K^+ channels (Kv channels), present in all excitable animal cells, open and close upon changes in the transmembrane potential. Kv channels are one of the key components in generation and propagation of electrical impulses in nervous system. Upon changes in transmembrane potential, these channels open and allow passive flow of ions from the cell to restore the resting membrane potential. Activation of Kv channels is therefore considered inhibitory to action potential generation.

There are 40 human voltage-gated potassium channel genes belonging to 12 subfamilies (Kv1–Kv12). The subfamilies are typically restricted to specific locations or cell/tissue types and are observed in excitable and non-excitable tissues. Remarkable diversity of Kv channels may be achieved due to the mix and match of Kv channel subunits. Within each of the Kv1, Kv2, Kv3, Kv4 and Kv7 families, homomeric and heteromeric channels may form with a range of functional properties. Kv2 family members may also assemble with Kv5, Kv6, Kv8 or Kv9 family members with more restricted expression patterns in the nervous system and smooth muscles.

Voltage-gated potassium channels, in neurons, are targeted to various subcellular compartments, and channels of different subunit compositions may be present in different subpopulations of neurons.

The tetrameric structure of Kv channels is made of two functionally and structurally independent domains: an ion conduction pore, and voltage-sensor domains. The ion conduction pore is made of four subunits which are arranged symmetrically around the conduction pathway. Voltage-sensor domains are positioned at the periphery of the channel and consist of four transmembrane segments (S1-S4).

Structural rearrangement of the voltage-sensor domains in response to changes in the membrane potential, and in particular S4, which includes positively charged amino acids at every third position, results in conformational changes in the conduction pore, which could open or occlude the ion conduction pathway. The mechanism of voltage-gating is not fully understood.

The Kv7 family of low threshold voltage-gated potassium (K^+) channels consists of five members (Kv7.1-7.5) encoded for by the KCNQ1-5 genes, respectively. Kv7 channels assemble as tetramers of identical or compatible α subunits, with each α subunit consisting of six transmembrane segments (S1–S6) and cytoplasmic N- and C-termini. Kv7 is widely expressed in both the CNS and other tissues, with Kv7.2 and 7.3, in particular, being known to control excitability of neurons.

Kv7.2 and 7.3 channels normally form heteromers with each other to increase conductance and are rarely expressed as low conductance homomers. Kv7.2 and 7.3 are expressed in most neuronal subtypes and tend to be localised to the initial segment of the axon where action potentials (spikes) are initiated. Kv7.3 and Kv7.2 conductances are therefore considered particularly potent inhibitors of action potential generation and activity in the same neuron as well as its targets (for example, motor neuron inhibition reduces muscle activity).

Neuromodulatory drugs targeting Kv7 channels have been developed to treat disorders associated with neuronal hyperexcitability. Retigabine is an anticonvulsant that acts mainly as a Kv7.2-Kv7.5 potassium channel opener, and GABA positive allosteric modulator at higher concentrations. The term "channel opener" refers to a left shift in the voltage dependence for channel opening, towards more negative potentials. In the presence of Retigabine, Kv7 channels open at more negative potentials, thus contributing to membrane hyperpolarisation and voltage stabilisation.

It has also been shown that Retigabine stabilises the open Kv7.2/7.3 (heteromeric) channel, making deactivation slower with little change in voltage dependence of deactivation. The overall effect is greater potassium conductance, hyperpolarisation of the membrane potential, and therefore inhibition of action potential generation. This effect of Retigabine is observed at concentrations below 10 µM, *in vitro* (Corbin-Leftwich et al 2016; Villalba-Galea 2020). Orally administered Retigabine at 600-1200 mg per day, which corresponds to a mean plasma concentration of 0.83 µM (Gunthorpe et al., 2012), has been shown to have up to 45% clinical efficacy for seizure reduction in humans with epilepsy (Brodie et al., 2010; French et al., 2011). Of note, while the EC50 of Retigabine is 0.6 µM for Kv7.3 channels, most channels in the nervous system are Kv7.2/7.3 heteromers, for which the EC50 is 1.6 µM. For some patients, these doses resulted in eye and skin discolouration that led to restriction of the indication. Retigabine was later voluntarily withdrawn for commercial reasons (Brickel et al., 2020).

Pharmaceutical interest in Kv7 as a therapeutic target remains. At least 3 activators with increased potency, increased selectivity for Kv7.2/7.3 and a lack of cosmetic side effects are in clinical trials and have shown favourable outcomes. However, due to the ubiquity of Kv7.2/Kv7.3 expression in the nervous system, neuronal side effects (somnolence, fatigue etc) are currently unavoidable. Although new Kv7.2/7.3 activators have improved safety profiles, neural side effects can only be reduced by increasing selectivity for dysfunctional neurons only.

Chemogenetics is a method by which proteins are engineered to interact with previously unrecognised small molecule chemical actuators. Since the 1990s, a large number of chemogenetic platforms have

been developed that have been particularly useful for neuroscientists wishing to modulate neural activity in specific populations. Several classes of proteins have been engineered in this way, including kinases, non-kinase enzymes, ligand-gated ion channels and G-protein-coupled receptors (GPCRs). The most widely used of these are known as Designer Receptors Activated by Designer Drugs (DREADDs).

DREADDS are engineered GPCRs that respond to synthetic ligands that cross the blood brain barrier (such as clozapine-N-oxide and olanzapine), but not their natural ligand, acetylcholine.

In use, a viral vector inserts the gene that encodes the DREADD protein into the cell to be studied. Various viral serotypes, promoters, and administration routes can be used to help select the target cells. Once transduced, the infected cell takes two or three weeks to express the engineered receptor protein which can then be activated using a DREADD ligand. Thus, activity can be modulated specifically in the targeted neurons with minimal influence on off target cells.

Described herein is a method similar to that employed by DREADDs, in which an ion channel responsive to specific drugs is expressed in neurons in order to modulate activity. Importantly, the method flips the existing concept of designing specifically paired, de novo receptor and drug and repurposes wild type and mutant ion channels to reduce or eliminate side effects of drugs acting on the channels. The method overexpresses wild type and/or mutated channels.

Furthermore, the method described beneficially uses channels that can be either opened (agonists) or closed (antagonists) depending on the drug used. In direct contrast, DREADD activation induces unidirectional excitability changes (either excitatory or inhibitory).

Summary of the Invention

The present disclosure relates to a chemogenetic method that allows modulation of activity in targeted neurons, thus increasing drug efficacy while reducing side effects, at least in part, due to the ability to use a lower dose. The method involves using gene therapy methodology via a delivery vector (e.g. AAV) to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses of specific activating and inhibiting drugs. That is, the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs.

We have shown that AAV-mediated overexpression of the voltage gated potassium channel Kv7.3 (encoded by KCNQ3) enables a Kv7 activator, such as retigabine, to significantly reduce excitability of human neurons at the individual neuron and population level. At low concentrations (0.3-3μM), retigabine reduced action potential number in individual hIPSC-derived sensory and motor neurons . At the same doses, retigabine has minimal effect on control neurons expressing green fluorescent protein only. Using multielctrode arrays, we have shown that retigabine was also effective at selectively reducing spontaneous bursting activity from a network of thousands of MNs overexpressing Kv7.3, but not GFP expressing neurons. The in vitro results translated to a mouse model of spasticity, in which hyperactive MNs cause excessive muscle contractions (muscle spasms). In this model, overexpression of Kv7.3 in motor neurons via an intramuscular injection of an AAV-Kv7 construct enabled a low dose of retigabine (2mg/kg) to reduce the intensity and duration of muscle spasms. However, the same dose did not significantly reduce spasticity in mice injected with an AAV-GFP construct. The reduction in

excitability of MNs was confirmed as the mechanism for the reduction in spasticity as 3μM retigabine reduced AP firing in Kv7.3 expressing mouse motor neurons, but not those expressing GFP. Additionally, we have shown that introducing a point mutation at amino acid residue 315 (Zaika et al., 2008; Gómez-Posada et al., 2010) of the Kv7.3 protein reduces motor neuron excitability at baseline in vitro and in vivo and further increases the effect sizes of retigabine at the tested concentrations in vitro.

The therapy will use AAV to overexpress Kv7.3 ion channel subunits and variations thereof, either as homomers or Kv7.2/7.3 heteromers, in hyperexcitable neurons of patients with neurological disorders, and use low doses of channel modulators to normalise activity.

According to a first aspect there is provided a method of increasing the efficacy of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering a lower dose of the neuromodulatory drug.

Advantageously, by increasing the efficacy of the drug, a lower dose can be administered to obtain the same therapeutic effect. This, beneficially, means that the occurrence of side effects can be reduced.

Thus, in a second aspect there is provided a method of reducing the side effects of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering a lower dose of the neuromodulatory drug.

Side effects of neuromodulatory drugs can range from minor to serious and can affect a subject's quality of life. For example, for Retigabine the NIH lists the most common adverse effects, occurring in greater than 10% of subjects in the clinical trials, include abnormal gait, confusion, dizziness, fatigue, headache, nausea, somnolence, speech disorder, tremor, urinary tract infection, and blurred vision (Harris and Murphy, 2011).

In some cases, side effects (adverse effects) can lead to drugs being removed from the market because the dosage needed leads to unacceptable adverse effects. If the dose can be lowered, adverse effects can be reduced and use of the drug can potentially be restored.

In a third aspect there is provided a method of increasing the therapeutic index of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering the neuromodulatory drug.

A fourth aspect provides a gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

Advantageously, delivery of the nucleotide sequence to a population of specific target neurons results in the nucleotide payload only being transduced into the neurons that exhibit increased excitability. These neurons then overexpress the ion channels, presenting more targets for the neuromodulatory drugs which, in turn, allows a lower dosage of the drug to be effective, resulting in fewer side effects. Targeting neurons in this way also means that off target activity is less likely.

In a fifth aspect there is provided an AAV viral particle gene therapy vector comprising:

a. an AAV capsid that selectively transduces target neurons, and
b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

In a further aspect there is provided a gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in upregulating expression of the Kv7.3 ion channel subunit by the target neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased neuronal excitability.

In a yet further aspect there is provided a gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

In another aspect there is provided the use of a gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a

nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain, ALS.

Brief Description of the Drawings

For a better understanding of the invention and to show how the same may be carried into effect, there will now be described by way of example only, specific embodiments, methods and processes according to the present invention with reference to the accompanying drawings in which:

Figure 1 shows the AAV constructs used to transduce neurons with either Kv7.3 (upper construct) or Kv7.3 A315T (lower construct). Note that a GFP sequence was included downstream of Kv7.3 and Kv7.3 A315T via T2A linkers, meaning GFP fluorescence is indicative of Kv7.3 expression.

Figure 2 shows that AAV transduction of hIPSC neurons leads to expression of proteins encoded by transgenes packaged into the AAV constructs (A). Specifically, in the presented example, hIPSC neurons were transduced with AAVs to induce expression of either green fluorescent protein (AAV-GFP) or human Kv7.3 channels and GFP (AAV-Kv7.3).

Figure 2 also shows the endpoints assessed using patch clamp electrophysiology at baseline (expression of Kv7.3 or GFP without activator drug (B), and following exposure to activator (retigabine, C). The endpoints were: rheobase (minimum current input to evoke 1 action potential), number of action potentials at 3x rheobase, and resting membrane potential. In the presence of retigabine, the excitability of the neurons is reduced as shown by an increase in the rheobase, a decrease in the number of action potentials at 3x rheobase and a hyperpolarisation of the resting membrane potential.

Figure 3 shows that baseline overexpression of Kv7.3 does not significantly alter the excitability of hIPSC sensory neurons compared to control neurons transduced with AAV -GFP. Panel (A) shows the mean number of action potentials generated at each current input value for AAV-GFP (dashed line) and AAV-Kv7.3 (solid line) transduced neurons. Shaded areas show 95% confidence intervals. Excitability levels were determined to be similar due to the extremely small effect sizes measured for APs/ 500ms (B), RMP (C) and Rheobase (D). In these Cummings estimation plots, individual points represent number of action potentials generated at 3x rheobase for neurons transduced with AAV-GFP (light grey) or AAV-Kv7.3 (dark grey). On the right hand side of the plot, Hedges G effect sizes and 95 % confidence intervals are plotted for bootstrap resampled (1000 repeats) data.

Figure 4 shows that retigabine delivered at concentrations of 0.3 μM, 1 μM, and 3 μM had limited effect on excitability of hIPSC sensory neurons transduced with AAV-GFP as determined by measuring the number of action potentials at 3x rheobase. However, large and very large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3. In each of the estimation plots (A, B and C), the upper graphs are line plots representing the change in AP number between recordings made before and after exposure to retigabine. Lower plots show the Hedges G effect sizes and confidence intervals.

Figure 5 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM had limited effects on excitability of hIPSC sensory neurons transduced with the AAV-GFP as determined by measuring the rheobase (minimum current to generate 1 action potential). However, large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3.

Figure 6 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM had small and medium effects on excitability of hIPSC sensory neurons transduced with the AAV-GFP as determined by measuring the resting membrane potential (RMP). However, large and very large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3.

Figure 7 shows that baseline expression of $Kv7.3_{A315T}$ (dashed line) significantly reduces the excitability of hIPSC sensory neurons. Excitability levels were shown to be decreased due to the inability of AAV-$Kv7.3_{A315T}$ transduced neurons to generate more than 3 action potentials (A, B). RMP was also hyperpolarised at baseline (C) but Rheobase was not substantially changed (D).

Figure 8 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM caused very large increases in the rheobase of AAV- $Kv7.3_{A315T}$ transduced neurons. Effect sizes were at least 2-3 times larger for AAV-$Kv7.3_{A315T}$ as compared with AAV-GFP. Note the differences in scale for both rheobase (primary axis), and Paired Hedges g effect size (secondary axis)

Figure 9 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM caused very large effects on hyperpolarisations of the RMP in hIPSC sensory neurons transduced with AAV- $Kv7.3_{A315T}$. Effect sizes were also at least 2-3 times larger than hIPSC neurons transduced with AAV-GFP.

Figure 10 shows Kv7.3 overexpression in hIPSC motor neurons by western blot (A) and ELISA (B). Note the strong bands at around 80 kDa.

Figure 11 shows that overexpression of Kv7.3 in hIPSC derived motor neurons induces a small baseline reduction in the frequency-current relationship (A-B), however action potential firing at 3 x rheobase (C) and rheobase (D) were unaffected. (E-H) Kv7.3 overexpression increased the sensitivity of hIPSC MNs to retigabine. Concentrations under 3uM had no effect on the excitability of AAV-GFP transduced neurons but reduced AP firing by up to 80% in AAV-Kv7.3 transduced neurons. Retigabine efficacy was also increased by Kv7.3 overexpression as demonstrated by the increase in the maximal effects on AP frequency (G), and FI slope (H).

Figure 12 shows that Kv7.3 overexpression increases the sensitivity of MNs to RTG at the population level, as measured using a multielectrode array (MEA) plate. hIPSC MNs are grown in a well plate with electrodes etched into the base so that spontaneous activity can be recorded. 50% of the wells are treated with AAV-GFP and 50% with AAV-Kv7.3. After sufficient transduction time, spontaneous activity is recorded at baseline (0 µM) and then increasing concentrations of RTG. RTG does not affect the viability of motor neurons, assessed using electrode impedance (A). However, MNs treated with RTG show greater sensitivity to RTG as evidenced by the leftward shift in the dose response for retigabine and spontaneous action potential burst frequency (B), burst duration (C) or total number of spikes. E-F show representative examples of activity recorded in wells treated with AAV-GFP (E) or AAV-Kv7.3 (F) after adding 1.8 µM (RTG). Vertical lines in top panels show network burst frequency. Below are raster plots of action potential firing recorded by each of the 16 electrodes (rows). G-H show representative

examples of individual bursts from MNs in wells treated with AAV-GFP (G) or AAV-Kv7.3 (H). Each row is spiking activity recorded by a single electrode. The the right of each plot is a heat legend indicating the change in firing frequency.

Figure 13 shows that in a neonatal mouse model of spasticity, induced by spinal cord injury, Kv7.3 can be successfully over expressed in motor neurons following intramuscular injection of AAV-Kv7.3. (A) shows a Kv7.3 western blot of spinal cords taken 2 weeks after injection and spinal cord injury. Note the strong bands at the expected molecular weight for Kv7.3 in the spinal cord samples from AAV-Kv7.3 treated mice but not the AAV-GFP treated mice. (B) shows Kv7.3 expression specifically in MNs using immunohistochemistry. (C-D) show changes in the duration of muscle spasms after administration of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. E-F shows the change spasm intensity (number of action potentials per spasm) after administration of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. G-H are representative electromyography (EMG) traces showing the effect of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. Top traces are pre RTG and lower traces are 20 minutes after RTG.

Figure 14 shows that Kv7.3 overexpression in motor neurons via an intramuscular injection of AAV-Kv7.3 does not significantly affect the baseline electrophysiological properties of motor neurons in a neonatal mouse model of spasticity. Recordings were made from spinal cord slices taken from mice with spasticity. (A) shows that the excitability of MNs was not reduced as the number of action potentials evoked at 2 x rheobase not different between AAV-GFP controls and AAV-SRx-C490 transduced MNs. (B) shows that the threshold for evoking a single action potential was not different in mice AAV-SRx-C490 transduced MNs. (C) shows that the resting membrane potential (RMP) was not different between the groups. (D) shows that MN size was not different between the groups. (E-H) shows that 3 µM retigabine significantly reduced excitability in MNs from AAV-SRxC490 treated mice but not AAV-GFP treated mice, as assessed by the change in number of action potentials at 2 x threshold and the rheobase.

Figure 15 shows that motor neurons in mice with spasticity treated with AAV-GFP fire repetitive trains of action potentials (A), however motor neurons from mice injected with a viral construct to overexpress a mutated form of Kv7 (A315T) are incapable of firing repetitively. This suggests a significant and permanent reduction in excitability of these motor neurons.

Detailed Description

As employed herein "increasing the efficacy" means an increase in the ability to produce the desired result. This may be achieved by improving the response to a drug rather than improving the effectiveness of the drug. Improved efficacy means that the same level of response can be achieved using less of the drug.

Therefore, increasing the efficacy means that a lower dosage can be used.

As employed herein neuromodulatory drug refers to a voltage gated potassium channel-targeting drug. Known drugs include, but are not limited to, Retigabine and derivatives thereof (see Musella et al., 2022 for examples), BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123, linopirdine.

In one embodiment the neuromodulatory drug is a channel opener.

In one embodiment the neuromodulatory drug is a channel blocker.

In one embodiment the neuromodulatory drug is selected from: Retigabine or a derivative thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123, linopirdine.

As employed herein introducing a nucleotide sequence refers to the transduction or transfection of a cell, such as a neuron, with one or more nucleotide sequences to either supplement existing genetic material with more of what is already present, or to provide new nucleotide sequences.

Kv7.3 ion channel subunit is a monomer of a voltage-gated potassium channel which assembled to form a tetrameric function ion channel. Kv7.3 can assemble as a homomer or as a heteromer with other Kv7 subunits, including Kv7.2 and Kv7.5, or other auxiliary subunits (e.g. KCNE).

Kv7.3 ion channels as employed herein is intended to refer to both Kv7.3 homomer and Kv7.3-containing heteromers.

As employed herein a nucleotide sequence encoding a Kv7.3 ion channel subunit refers to a nucleotide sequence (RNA or DNA) encoding the Kv7.3 ion channel subunit.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.1 or a variant of SEQ ID.1 encoding the same amino acid sequence encoded by SEQ ID.1.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.2 or a variant of SEQ ID.2 encoding the same amino acid sequence encoded by SEQ ID.2.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.3 or a variant of SEQ ID.3 encoding the same amino acid sequence encoded by SEQ ID.3.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.4 or a variant of SEQ ID.4 encoding the same amino acid sequence encoded by SEQ ID.4.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.5 or a variant of SEQ ID.5 encoding the same amino acid sequence encoded by SEQ ID.5.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.6 or a variant of SEQ ID.6 encoding the same amino acid sequence encoded by SEQ ID.6.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.7 or a variant of SEQ ID.7 encoding the same amino acid sequence encoded by SEQ ID.7.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.8 or a variant of SEQ ID.8 encoding the same amino acid sequence encoded by SEQ ID.8.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.9.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.10.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.11.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.12.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.13.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.14.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.15.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.16.

In one embodiment the nucleotide sequence encodes the Kv7.3 ion channel subunit according to any one of SEQ ID 9 to 16.

In certain embodiments the Kv7.3 ion channel subunit may comprise a mutation at amino acid residue 315 of SEQ ID.9. For example, A315T, A315S, A315V, A315C, A315N, A315Q or A315Y.

In one embodiment the ion channel subunit is wild type Kv7.3.

In one embodiment the ion channel subunit is not wild type Kv7.3.

Functional ion channel as employed herein means that the ion channel subunit has assembled into a tetramer, either as a homomer or as a heteromer, and is located at the neuron membrane in a position suitable to act as a voltage-gated potassium channel.

As employed herein, the term neuron includes a neuron and a portion or portions thereof (e.g. the neuron cell body, an axon and/or a dendrite). The term neuron denotes nervous system cells that are electrically active and include a cell body (or soma) and up to two types of extensions or projections: dendrites, by which the majority of neuronal signals are conveyed to the cell body, and axons, by which the majority of neuronal signals are conveyed from the cell body to effector cells, such as target neurons or muscle. Neurons can convey information from tissues and organs into the central nervous system (afferent or sensory neurons) and transmit signals from the central nervous systems to effector cells (efferent or motor neurons). Other neurons, designated interneurons, connect neurons within the central nervous system (the brain and spinal cord). Yet more neurons, designated “projection” neurons, extend their axons from one region of the nervous system to another.

In some embodiments the neuron is involved in sensory-motor activity.

In some embodiments the neuron is a motor neuron (motoneuron).

In some embodiments the neuron is a sensory neuron.

In some embodiments the neuron is an autonomic efferent neuron.

In some embodiments the neuron is an autonomic sensory neuron.

In some embodiments the neuron is subthalamic nucleus neuron.

As employed herein a vector refers to any suitable gene delivery vector as known in the art. Including, but not limited to, viral vectors (including adeno-associated virus, adenovirus and lentivirus) and non-viral methods (such as naked DNA or chemically assisted delivery methods). Typically, the vector is a gene therapy vector.

Overexpressing or overexpression as employed herein is synonymous with upregulation of a gene and intended to refer to an increase in the expression of a gene relative to a cell (neuron) that has not been transfected or transduced. To be effective, the neuron should be expressing higher levels of assembled Kv7.3-containing ion channels, either as Kv7.3 homomers or as heteromers, for example Kv7.2/7.3 heteromers.

As employed herein "administering a lower dose" refers to the subsequent exposure of a neuron that is overexpressing the introduced gene to a lower dose of a drug than would normally be administered to a patient in order to be therapeutically effective. For example, to treat epilepsy, Retigabine was typically administered at a dose of 600-1200 mg per day, which corresponds to a mean plasma concentration of 0.83 µM. Using the current method, we have shown that concentrations lower than 0.5 µM are effective in human neurons *in vitro*. Without wishing to be bound by theory, the present inventors consider that this would enable an effective dose lower than 0.5 µM to be administered *in vivo*.

In one embodiment the therapeutically effective dose of the neuromodulatory drug is reduced by up to 100%. For example, approximately 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 90%, 95%, 90% or 95%.

Whilst not wishing to be bound by theory, the inventors believe that overexpression of Kv7.3 may have a baseline effect that reduces excitability of neurons even in the absence of a neuromodulatory drug. Thus, in some embodiment administration of a neuromodulatory drug is not required.

It is expected that the reduction in therapeutically effective dose is sufficient to reduce side effects of the neuromodulatory drug to clinically acceptable levels. Clinically acceptable levels will be understood by those skilled in the art.

As employed herein "increasing drug efficacy" refers to the increased therapeutic effect of a given drug dose in transduced neurons that are overexpressing the transgene relative to non-transduced neurons.

As employed herein "reducing the side effects" refers to the lowering or elimination of undesirable side effects related to administration of a drug to a subject at a given dose.

For example, the neuromodulatory drug retigabine (ezogabine), which was marketed as an anticonvulsant, was found to cause drowsiness, dizziness, tinnitus and vertigo, confusion, and slurred speech at therapeutic doses (600-1200 mg). Less common side effects included tremor, memory loss, gait disturbances, and double vision. In 2013 the FDA warned the public that ezogabine can cause blue skin discoloration and eye abnormalities characterised by pigment changes in the retina. Whilst not wishing to be bound by theory, the inventors consider that increasing Kv7.3 expression in hyperexcitable neurons associated with epileptic seizures would be expected to shift the therapeutic range for retigabine to include lower doses, thereby reducing or eliminating above side effects. That is, the therapeutic index for retigabine would be increased. Indeed, trials including lower doses of retigabine (300-1200 mg) reported fewer dermatological, ophthalmological, and neurological adverse effects (Lerche et al., 2015; Brickel et al., 2020).

As employed herein, gene therapy vector is a vector suitable for gene therapy methods. Although AAV capsids are described herein, it will be appreciated that other vectors may be suitable and used without deviating from the scope of the present invention.

Typically, the gene therapy will be used to treat a neurological disorder or condition. One example of a neurological disorder or condition that can be treated by the gene therapy is spasticity. Spasticity is a neurological symptom suffered by people with a variety of neurological disorders, including but not limited to multiple sclerosis, stroke, traumatic brain injury, spinal cord injury, motor neuron disease and cerebral palsy. Spasticity results from excessive excitation of muscle by motor neurons, which, because of the disease, become “hyperexcitable.”

The excitability of neurons is determined by the frequency of action potentials generated in response to a given stimulus. Increased excitability is indicated by one or more of the measures described below.

Increased excitability as employed herein is defined as a neuron displaying at least one of the following:

a. An increase (depolarisation) of a neuron’s resting voltage (Resting membrane potential-RMP) such that it is closer to the threshold for generation of an action potential.
b. A decrease in the stimulus magnitude required to cause neurons to generate action potentials (Rheobase).
c. An increase in the number of action potentials generated by a given stimulus (Frequency) compared to a neuron with “normal” levels of excitability.
d. Persistent generation of action potentials beyond the cessation of a stimulus responsible for initiating firing.
e. An increase in the frequency of action potentials in a population of neurons (2-100,000) recorded using an array of microelectrodes (microelectrode array-MEA)
f. An increase in the number or duration of action potential bursts recorded by an MEA, defined as at least 5 consecutive action potentials with intervals of 100ms or less between each action potential.

For example, a hyperexcitable neuron may have a RMP of -55 mV which is roughly 15 mV more positive than is expected of neurons, and closer to the threshold for action potential generation (~ -40 mV). If a stimulus magnitude of 50 picoamperes (pA) causes a normal neuron to generate an action potential (Rheobase = 50 pA), 25 pA may be sufficient in a hyperexcitable neuron (Rheobase = 25 pA). Similarly, if

a stimulus magnitude of 100 pA causes a normal neuron to generate 10 action potentials in 1 second (Frequency= 10Hz), a hyperexcitable neuron may generate 20 at the same stimulus magnitude (Frequency= 20Hz).

Additionally, action potential firing is often sustained beyond the cessation of the stimulus in hyperexcitable neurons but not normal neurons. This is the case in spasticity, whereby the neurons fire more action potentials for longer time periods, and often in response to a lower stimulus.

Typically, the gene therapy vector is an AAV vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit. The ion channel subunit may be a wild type as encoded by SEQ ID.1 or a mutant as encoded by SEQ ID.2, or equivalent sequence thereof that encodes the amino acid sequences of SEQ ID.3 or SEQ ID.4, respectively.

AAV viral particles, as employed herein, are adeno-associated virus viral particles comprising a single stranded DNA genome packaged within a viral envelope that is capable of tranducing a cell, e.g. a target neuron. In particular, the AAV viral particles employed herein include the nucleotide sequence according to SEQ ID.1 or SEQ ID.2. In some embodiments the viral particles may be devoid of replication-encoding nucleotides, that is, they are replication deficient.

AAVs are small viruses belonging to the genus dependoparvovirus containing a single strand of DNA, up to ~4.9 Kb. The AAV genome contains three capsids proteins VP1, VP2 and VP3, all of which are translated from one mRNA via alternate splicing. In the wild, multiple serotypes of AAV have been identified each with unique sequences of capsid gene, and hence distinct tropisms, although wild serotypes tend to be able to infect multiple tissue and cell types. These serotypes are denoted by numbers: AAV1 , AAV2, etc. It has been shown that modification of capsid sequences via DNA recombination methods can generate non-native sequences with tailored properties and tropism directed towards (or against) particular cells or tissues, and that evade the immune system (Vandenberghe et al., 2009). As employed herein, the AAV viral particles selectively transduce target neurons, such as motor neurons.

AAV capsid as employed herein refers to the capsid of adeno-associated viruses. It is known that the AAV capsid is capable of remarkable selectivity due to its make up (the AAV capsid is composed of a mixture of VP1, VP2, and VP3 totalling 60 monomers arranged in icosahedral symmetry in a ratio of 1:1:10) and post-translational modifications. AAV capsid proteins contain 12 hypervariable surface regions, but the genome, in general, presents highly conserved replication and structural genes across serotypes.

The AAV capsid employed herein can selectively transduce a particular cell type such as neurons rather than muscle tissue and may selectively transduce target neurons over other neurons, even specific parts of neurons, such as the axon.

In use, the AAV capsid containing the nucleotide sequence according to any one of SEQ ID Nos: 1 to 8 (the viral particle) is delivered to a subject, the viral particle infects target neurons which become transduced neurons. The transduced neurons overexpress the amino acid sequences encoded by the nucleotide sequences, which assemble as functional Kv7.3-containing ion channels (either homomers or heteromers). In general, the time from delivery of the viral particles to the subject to overexpression of

the function ion channels is approximately 3 weeks. Once a suitable period of time has elapsed for the transduced neurons to over express the ion channels, the subject can be treated with a neuromodulatory drug, such a Retigabine, at a lower dose.

Selectively transduces as employed herein refers to the ability of the AAV capsid to selectively transduce one cell type in preference to another. The specificity may be neurons in preference to muscle cells, for example. Alternatively, it may be motor neurons in preference to sensory neurons, for example.

A subject as employed herein means a human or non-human mammal. Typically, the subject has a neurological disorder associated with increased excitability of neurons.

In some embodiments the subject has a neurological disorder associated with dysfunction of Kv7.3 ion channels.

In some embodiments the subject has symptoms characterised by neuronal hyperexcitability that may or may not be associated with dysfunction of Kv7.3 ion channels.

In some embodiments the subject has a neurological disorder and/or symptoms characterised by neuronal hyperexcitability that may or may not be associated with dysfunction of Kv channels.

Neurological disorder associated with increased neuronal excitability as employed herein refers to any condition in which excess neuronal activity leads to symptoms associated with the same neurological disorder. For example, epilepsy, spasticity, benign familial neonatal seizures (BNFS), nociceptive pain, non-nociceptive pain, Parkinson's disease, multiple sclerosis.

In one embodiment the neurological disorder is associated with hyperexcitability of neurons, such as motor neurons.

In one embodiment the neurological disorder is associated with increased excitability of neurons.

In one embodiment the neurological disorder is selected from epilepsy, spasticity, BNFS, nociceptive pain, non-nociceptive pain, Parkinson's disease, multiple sclerosis.

Treating or treatment as employed herein refers to the reversal of a condition, amelioration or relief of symptoms associated with a condition or prevention of further development/worsening of a condition.

The term "treatment" includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously. For example, the vector may be administered a day or more before the neuromodulatory drug is administered to the subject.

Lower dose as employed herein refers to a dose lower than that considered to be efficacious in a subject (or in the lower portion of an efficacious range), and therefore does not cause, or reduces severity of, side effects whilst maintaining therapeutic efficacy.

Therapeutically effective amount as employed herein means an amount of the compound, which is effective in treating the named disorder or condition.

In some embodiments the overexpressed Kv7.3 channels may assemble as heteromers, such as Kv7.2/7.3 or Kv7.3/7.5 heteromers. The subunit proportions of these heteromers may differ and allow refinement of the method described herein.

Similarly, in some embodiments the proportion of wild type to mutant Kv7.3 subunits may differ to allow refinement of the method. This could be true of both heteromers and homomers.

In some embodiments the overexpressed Kv7.3 channels may assemble as homomers.

Homomeric Kv7.3 channels may be minimally conducting such that they have minimal effect on baseline excitability of the transduced neuron, but still allow the neuromodulatory drug to act on the transduced neuron at a lower dose.

Rheobase as employed herein refers to the minimum current injection value required to depolarise neuronal membrane potential sufficiently to generate a single action potential.

Resting membrane potential as employed herein refers to the potential voltage difference recorded between the intracellular and extracellular neuronal compartments in the absence of any current injection. This voltage value is recorded using electrophysiology techniques such as whole cell patch clamp.

As employed herein "shifts the resting membrane potential (RMP) of the target neuron away from threshold" means that the voltage value in the neurons resting state is more negative (hyperpolarised) and therefore a greater stimulus magnitude is required to depolarise the voltage to a level at which action potentials are generated. For example, a typical RMP of a neuron may be -65 mV, meaning 500 pA of current (stimulus) is needed to depolarise the neuron sufficiently to reach the threshold membrane potential for generating action potentials (-45 mV). If the RMP becomes more negative (-75 mV), then it is further away from threshold and therefore requires more current injection to reach threshold and generate action potentials.

Reduces the excitability of the target neuron as employed herein means at least one of the following :

a. Hyperpolarising of a neuron's resting membrane potential such that it is further away from the threshold for generation of an action potential.
b. Increasing the stimulus magnitude required to cause neurons to generate action potentials (Rheobase).
c. Decreasing the number of action potentials generated by a given electrical or chemical stimulus (AP Frequency).
d. Reducing or eliminating persistent generation of action potentials beyond the cessation of a stimulus responsible for initiating firing.
e. Reducing the frequency of spontaneous action potentials in the absence of a stimulus.
f. Reducing the duration or frequency of spontaneous action potential bursts (bursts are defined as at least 5 consecutive action potentials with intervals of 100 ms or less).

Reductions in excitability can be measured using whole cell patch clamp electrophysiology in current clamp mode by injecting rectangular current pulses of increasing magnitude and recording the change in voltage and frequency of action potential generation during the duration of the current pulse. Resting

membrane potential is measured under stable conditions, while there is no current injection (e.g. the voltage does not vary by more than (1-2 mV). Rheobase is measured as the minimum current input of a designated duration necessary to generate at least one action potential. Action potential frequency is measured as the number of action potentials generated over a specified time period, usually equal to the duration of a current pulse injected into a neuron.

Reductions in excitability can also be measured using a microelectrode array (MEA) consisting of a well plate with several electrode contacts located at the surface of the base of each well. A population of neurons is grown in contact with the electrodes in each well such that their activity can be recorded as extracellular action potentials (spikes). Neurons grown on MEAs produce spontaneous action potentials often referred to as spikes. Five or more consecutive spikes recorded with intervals of 100 ms or less are referred to as bursts. Spontaneous spike frequency and bursting characteristics (burst frequency, duration etc) may be used to determine neuronal excitability.

In some embodiments the upregulation of the Kv7.3 ion channel subunit by the transduced neuron reduces activity of cells innervated by axon terminals of the same transduced neuron. For example, reduced gastrocnemius muscle activity measured by electromyography (EMG) as a result of upregulation of Kv7.3 ion channel subunits in gastrocnemius motor neurons. Reduced muscle activity is characterised by 1 or more of the following: reduced amplitude of a compound muscle action evoked by stimulation of peripheral afferent nerve fibres; reduced duration of persistent EMG activity following stimulation of afferent nerve fibres; reduced frequency of spontaneous EMG activity; reduced duration of spontaneous EMG activity; reduced amplitude of spontaneous EMG activity; reduced stiffness of a muscle as determined by a trained clinician; and increased range of motion in the joints associated with the targeted muscle as determined by a trained clinician.

The invention also comprises methods of treatment which involves injecting AAV viral vectors comprising the nucleotide sequence of SEQ ID.1 or SEQ ID.2 into the brain or spinal cord of a subject, or by intramuscular injection; these AAV viral vectors can then transduce the target neurons.

Expression of the nucleotide sequence leads to overexpression of Kv7.3 ion channels in these neurons. The subject can then be treated with a neuromodulatory drug at a lower dose than would previously have been necessary to obtain a therapeutic effect. The lower dose, in turn, causes fewer side effects and has reduces toxicity. The goal of curing, alleviating symptoms, and/or improving the quality of life of patients with diseases caused by hyperexcitable neurons is achieved without undesirable (or with fewer, less severe) side effects.

Prohibitively high side effect drugs may become usable by employing the method disclosed herein, simply by permitting a lower dose to be efficacious in specific cells.

In some embodiments, the method involves the gene therapy vector or AAV viral particle retrogradely infecting neurons for the purposes of delivering genetic material to neurons, with the purpose of treating diseases caused by, or associated with Kv7 ion channel dysfunction.

In the case of A315T, a channel with high membrane insertion rate and conductance levels, it is likely that only low expression levels are needed to be effective. This means the virus can be delivered at lower quantities, reducing the probability of immunogenicity and reducing costs.

In one embodiment the method is an ex vivo method.

In the context of this specification "comprising" is to be interpreted as "including".

Aspects of the invention comprising certain elements are also intended to extend to alternative embodiments "consisting" or "consisting essentially" of the relevant elements.

Where technically appropriate, embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Approximately as employed herein is intended to mean ±10%.

Technical references such as patents and applications are incorporated herein by reference.

Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

Examples

Materials and methods

Cell culture

Human iPSC sensory neuron progenitor cells (Axol Bioscience) were thawed and plated at low density on a monolayer of rat cortical astrocytes on poly D lysine coated glass coverslips. Cultures were grown in NbActiv 4 (Neurobasal/B 27 supplemented with MAXIMIZER, GDNF, NGF, BDNF, and NT 3) to promote the maturation of the cells to a differentiated neuronal phenotype.

AAVs

pAAV-CMV-EGFP, pAAV-CMV-hKCNQ3-T2A-EGFP, pAAV-CMV-hKCNQ3$_{(A315T)}$-T2A-EGFP, were added to wells containing neuronal cultures at 7 days in vitro and patch clamp recordings were made either 1 (DIV15-16) or 2 weeks later (DIV21-23).

Patch clamp electrophysiology

Standard patch clamp methods were employed. The external recording solution composition was 140 mM NaCl, 2 5 mM KCl, 2mM CaCl2, 3 mM MgCl2, 10 mM glucose, 10 mM HEPES, pH 7.3. The internal recording solution composition was 120 mM K gluconate, 20 mM KCl , 3 mM MgCl, 2.5 mM EGTA, 0.5 mM CaCl, 2.4 mM Na2- ATP, 0.3 mM Li GTP, 10 mM HEPES, pH 7.3. Upon establishing the whole cell configuration, passive membrane properties for every cell (capacitance and resistance) were measured in voltage clamp mode. All excitability measurements were made in current clamp.

Multielectrode array recordings

Neurons were grown as above in 24 well plates containing 16 electrodes per well, such that neurons were in direct contact with individual electrodes. Spontaneous firing activity was then recorded after 30 days. Recordings were made at baseline (no drug) and then at increasing concentrations of the activator drug.

Mouse spasticity model

Neonatal mice (Post natal day 0) received a complete spinal cord transection between the 9th-10th thoracic segment to induce spasticity. In the same surgery, AAVs were injected into both gastrocnemius muscles.

EMG recording

10-14 days after spinal cord injury, EMG electrodes were inserted into the injected muscles to record electrical activity before and after intraperitoneal administration of an activator drug (retigabine). Different doses were tested on different days.

Patch clamp electrophysiology from mouse spinal cord slices

The spinal cord was harvested under terminal anaesthesia and sliced at 300 um on a vibratome. Patch clamp recordings were then made from motor neurons expressing GFP as described above (patch clamp electrophysiology).

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Sequences

SEQ ID 1 - wild type Kv7.3

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel
ATGGGGCTCAAGGCGCGCAGGGCGGCGGGGGCGGCTGGCGGCGGCGGCGACGGGGGCGGC
GGAGGCGGCGGGGCGGCTAACCCAGCCGGAGGGGACGCGGCGGCGGCCGGCGACGAGGAG
CGGAAAGTGGGGCTGGCGCCCGGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGA
GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG
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SEQ ID 2 – Kv7.3 A315T

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315T
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SEQ ID 3 – Kv7.3 A315S

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315S
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SEQ ID 4 – Kv7.3 A315V

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315V
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SEQ ID 5 – Kv7.3 A315C

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315C
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SEQ ID 6 – Kv7.3 A315N

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SEQ ID 7 – Kv7.3 A315Q

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GCCGTCAGAATTCTACAATTCCGTCTCTATAAAAAAAAATTCAAGGAGACTTTGAGGCCT
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AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
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SEQ ID 8 – Kv7.3 A315Y

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315Y
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GGAGGCGGCGGGGCGGCTAACCCAGCCGGAGGGGACGCGGCGGCGGCCGGCGACGAGGAG
CGGAAAGTGGGGCTGGCGCCCGGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGA
GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG
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AACGCCAAGTACCGGCGCATCCAAACTTTGATCTACGACGCCCTGGAGAGACCGCGGGGC
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ACATTTGCTATTTTCATCTTTGGAGCCGAGTTTGCTTTGAGGATCTGGGCTGCTGGATGT
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GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGC
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CAGGTGACCATTGACAAAGTCAGCCCCTATGGGTTTTTTGCACATGACCCTGTGAACCTG
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TATGTGGAGAGGCCCACGGTCCTGCCTATCTTGACTCTTCTCGACTCCCGAGTGAGCTGC
CACTCCCAGGCTGACCTGCAGGGCCCCTACTCGGACCGAATCTCCCCCCGGCAGAGACGT
AGCATCACGCGAGACAGTGACACACCTCTGTCCCTGATGTCGGTCAACCACGAGGAGCTG
GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCGGCCCC
AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
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SEQ ID 9 – wild type Kv7.3

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLATIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 10 – Kv7.3 A315T

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315T
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF

ETYADALWWGLITLTTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 11 – Kv7.3 A315S

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315S
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLSTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 12 – Kv7.3 A315V

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315V
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLVTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 13 – Kv7.3 A315C

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315C
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
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DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLCTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
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SEQ ID 14 – Kv7.3 A315N

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315N
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ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLNTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
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DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 15 – Kv7.3 A315Q

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Q
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLQTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 16 – Kv7.3 A315Y

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Y

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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLYTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

Claims

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug.

2. A method of reducing the side effects of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug.

3. A method of increasing the therapeutic index of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering the neuromodulatory drug.

4. A method of reducing the excitability of a neuron comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to the target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. optionally administering a neuromodulatory drug.

5. A method of treating a neurological disorder comprising the steps:
    a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,

b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and
c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels.

6. A gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

7. A gene therapy vector according to claim 6, wherein the vector selectively transduces the target neuron.

8. An AAV viral particle gene therapy vector comprising:
   a. an AAV capsid that selectively transduces target neurons, and
   b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

9. A gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in upregulating expression of the Kv7.3 ion channel subunit by the target neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased neuronal excitability.

10. A gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

11. The method according to any one of claims 1 to 5 or the gene therapy vector according to claim 10, wherein the neuromodulatory drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

12. The method or the gene therapy vector according to any preceding claim, wherein the Kv7.3 ion channel is expressed as a Kv7.3 homomer, a Kv7.2/7.3 heteromer, or a Kv7.3/7.5 heteromer.

13. The method or gene therapy vector according to claim 12, wherein the Kv7.3 ion channel is functionally expressed as a homomer.

14. The method or the gene therapy vector according to any preceding claim, wherein the target neurons are motor neurons.

15. The method or the gene therapy vector according to any one of claims 5 or 9 to 14, wherein the neurological disorder is associated with increased excitability of neurons.

16. The method or the gene therapy vector according to any one of claims 5 or 9 to 15, wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain.

17. The method or the gene therapy vector according to any preceding claim, wherein the upregulation of the Kv7.3 ion channel subunit reduces the rheobase of the target neuron in the presence of the neuromodulatory drug.

18. The method or the gene therapy vector according to any preceding claim, wherein the upregulation of the Kv7.3 ion channel subunit shifts the resting membrane potential of the target neuron away from threshold in the presence of the neuromodulatory drug.

19. The method or the gene therapy vector according to any preceding claim, wherein the upregulation of the Kv7.3 ion channel subunit reduces the excitability of the target neuron.

20. The method or the gene therapy vector according to any preceding claim, wherein the upregulation of the Kv7.3 ion channel subunit reduces the excitability of a plurality of target neurons, as recorded using a microelectrode array (MEA) device.

21. The method or the gene therapy vector according to any preceding claim, wherein the upregulation of the Kv7.3 ion channel subunit by the target neuron reduces activity of cells innervated by axon terminals of the target neuron.

22. The method of claim 19 to 21 wherein the reduction of the excitability of the target neuron, or the reduction in activity of cells innervated by axon terminals of the target neuron, occurs or is increased in the presence of the neuromodulatory drug.

23. Use of the gene therapy vector according to claim 6 to 10 in the preparation of a medicament for use in the treatment of conditions ameliorated by upregulating expression of Kv7.3 ion channels.

24. Use of the gene therapy vector according to claim 6 to 10 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, ALS, nociceptive pain and non-nociceptive pain.

25. Use of a gene therapy vector according to claim 6 to 10 in the preparation of a medicament for use as an anti-spasticity.

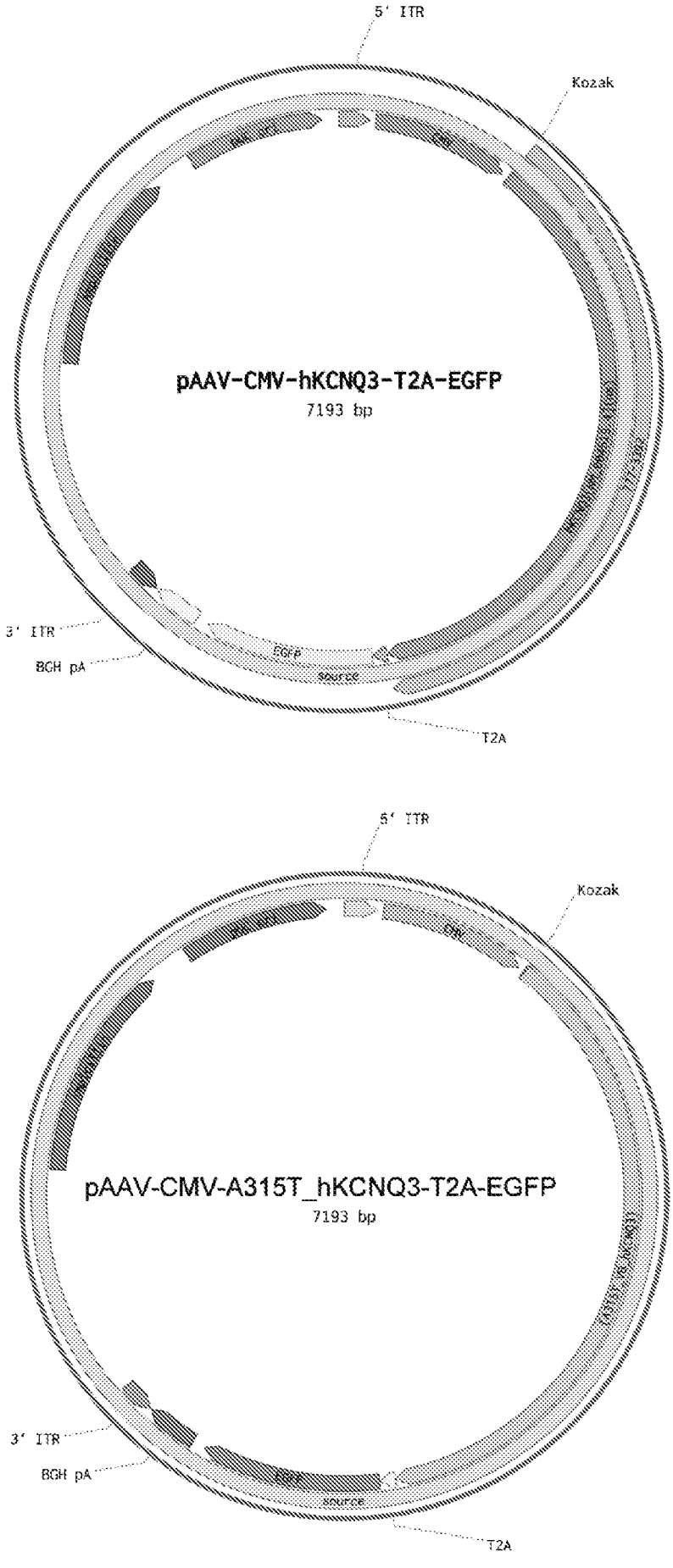

5' ITR
Kozak
pAAV-CMV-hKCNQ3-T2A-EGFP
7193 bp
3' ITR
BGH pA
EGFP
source
T2A
5' ITR
Kozak
pAAV-CMV-A315T_hKCNQ3-T2A-EGFP
7193 bp
3' ITR
BGH pA
source
T2A

Figure 1

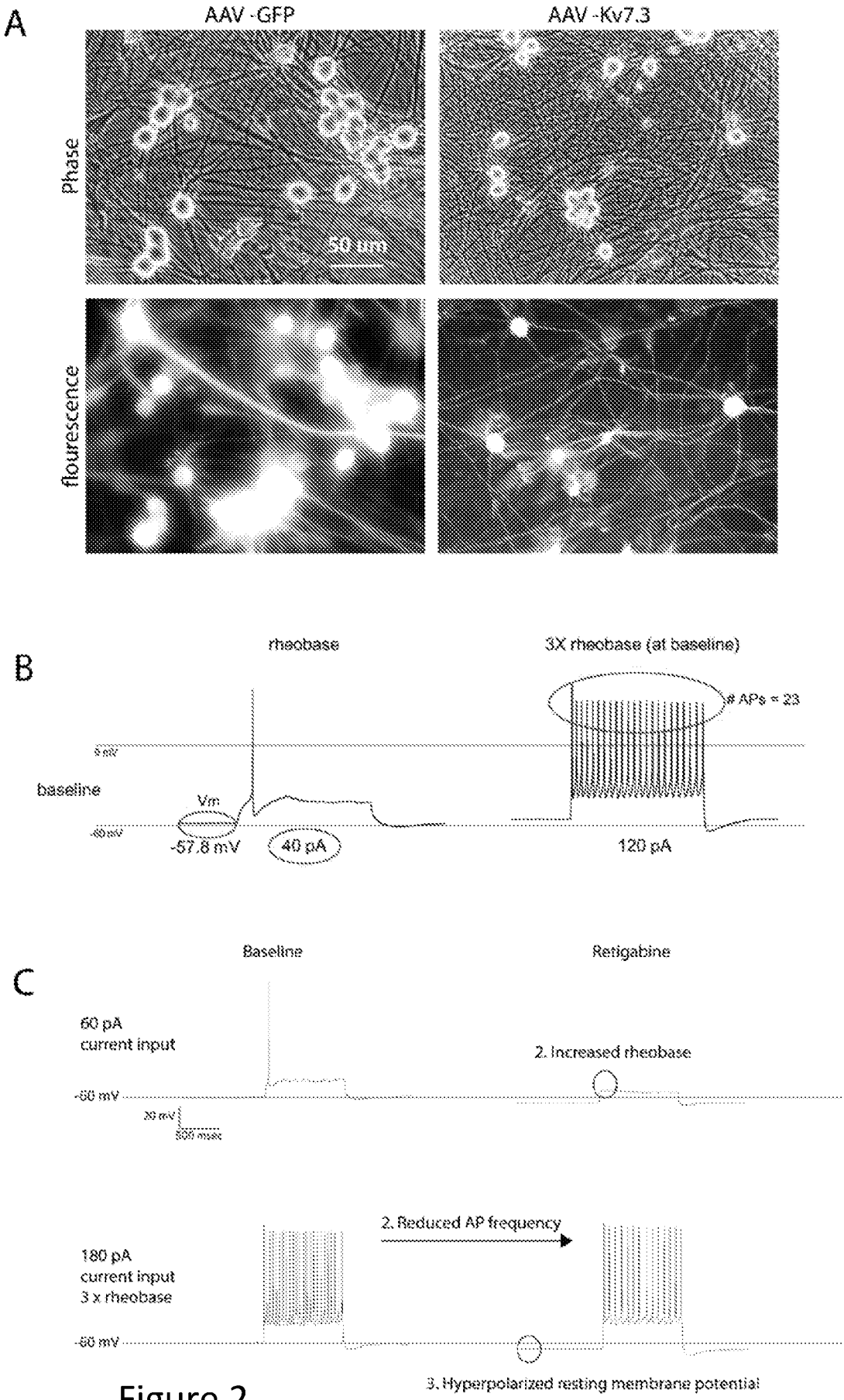

A
AAV -GFP
AAV -Kv7.3
Phase
flourescence
50 um
B
rheobase
3X rheobase (at baseline)
APs = 23
baseline
Vm
-57.8 mV
40 pA
120 pA
C
Baseline
Retigabine
60 pA
current input
2. Increased rheobase
2. Reduced AP frequency
180 pA
current input
3 x rheobase
3. Hyperpolarized resting membrane potential

Figure 2

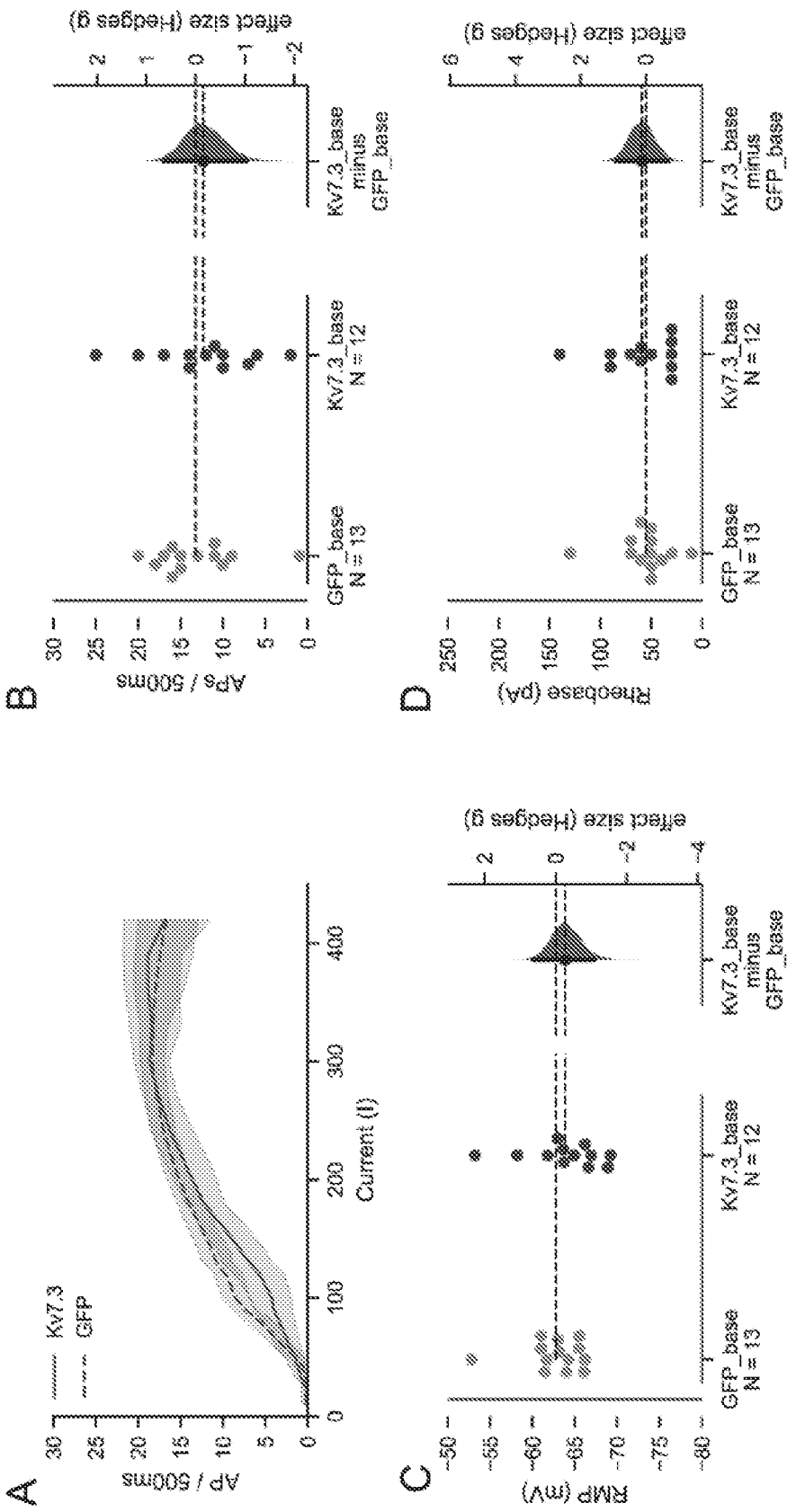

A
Kv7.3
GFP
AP / 500ms
Current (I)
B
APs / 500ms
GFP_base
N = 13
Kv7.3_base
N = 12
Kv7.3_base
minus
GFP_base
effect size (Hedges g)
C
RMP (mV)
GFP_base
N = 13
Kv7.3_base
N = 12
Kv7.3_base
minus
GFP_base
effect size (Hedges g)
D
Rheobase (pA)
GFP_base
N = 13
Kv7.3_base
N = 12
Kv7.3_base
minus
GFP_base
effect size (Hedges g)

Figure 3

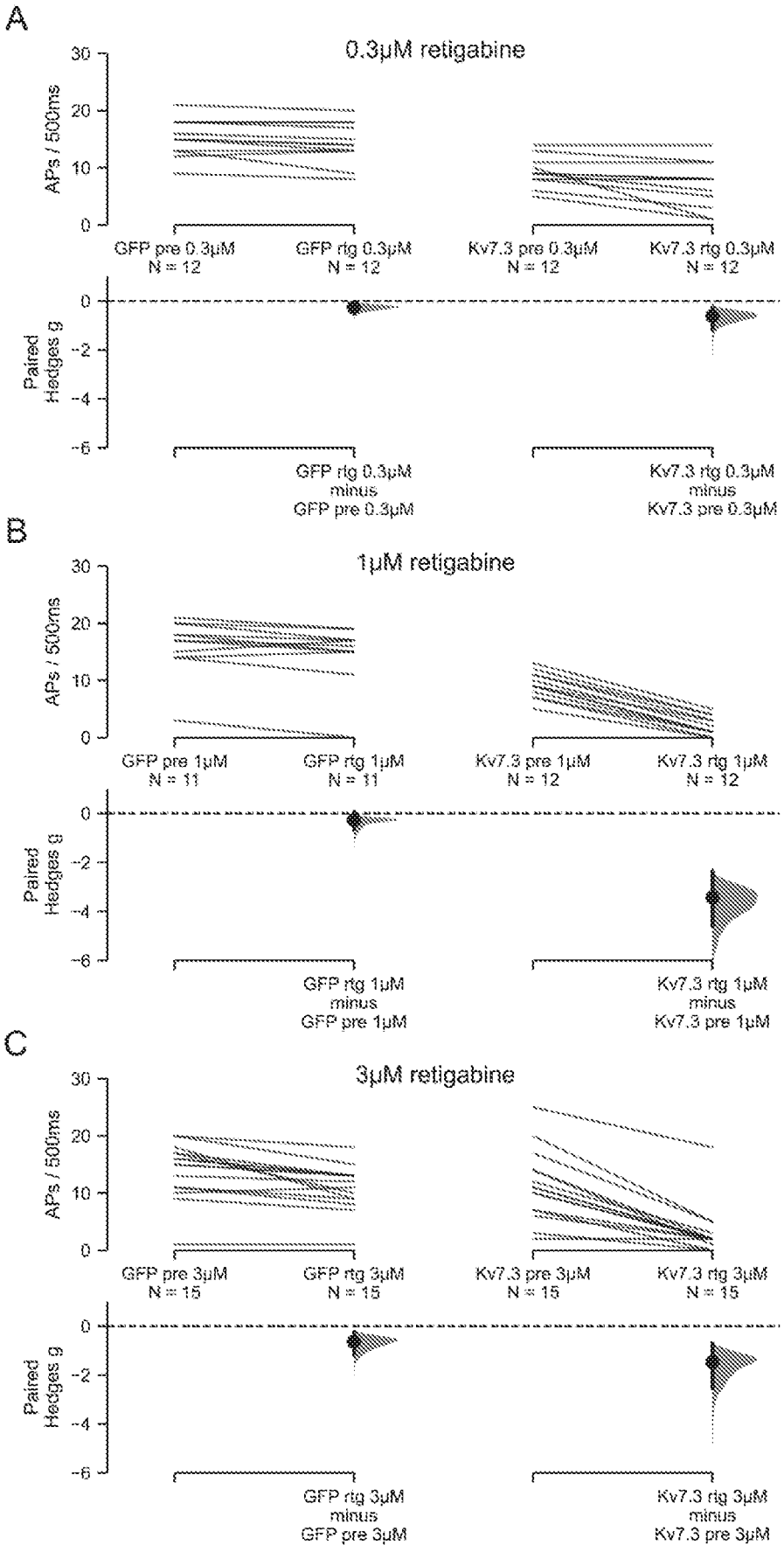

A
0.3µM retigabine
APs / 500ms
30
20
10
0
GFP pre 0.3µM
N = 12
GFP rtg 0.3µM
N = 12
Kv7.3 pre 0.3µM
N = 12
Kv7.3 rtg 0.3µM
N = 12
Paired Hedges g
0
-2
-4
-6
GFP rtg 0.3µM
minus
GFP pre 0.3µM
Kv7.3 rtg 0.3µM
minus
Kv7.3 pre 0.3µM
B
1µM retigabine
APs / 500ms
GFP pre 1µM
N = 11
GFP rtg 1µM
N = 11
Kv7.3 pre 1µM
N = 12
Kv7.3 rtg 1µM
N = 12
Paired Hedges g
GFP rtg 1µM
minus
GFP pre 1µM
Kv7.3 rtg 1µM
minus
Kv7.3 pre 1µM
C
3µM retigabine
APs / 500ms
GFP pre 3µM
N = 15
GFP rtg 3µM
N = 15
Kv7.3 pre 3µM
N = 15
Kv7.3 rtg 3µM
N = 15
Paired Hedges g
GFP rtg 3µM
minus
GFP pre 3µM
Kv7.3 rtg 3µM
minus
Kv7.3 pre 3µM

Fig. 4

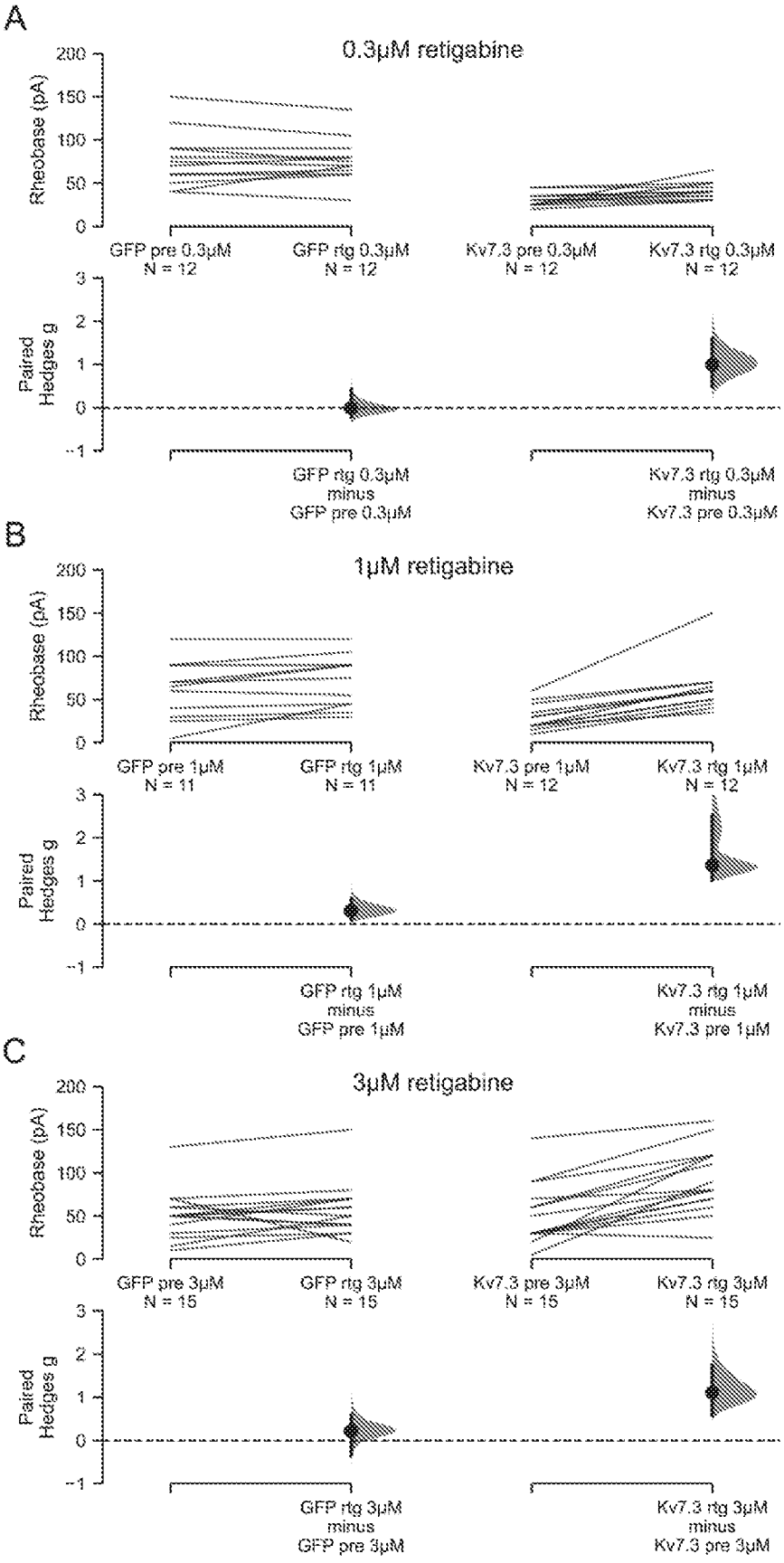

A
0.3μM retigabine
Rheobase (pA)
200
150
100
50
0
GFP pre 0.3μM
N = 12
GFP rtg 0.3μM
N = 12
Kv7.3 pre 0.3μM
N = 12
Kv7.3 rtg 0.3μM
N = 12
Paired Hedges g
3
2
1
0
−1
GFP rtg 0.3μM
minus
GFP pre 0.3μM
Kv7.3 rtg 0.3μM
minus
Kv7.3 pre 0.3μM
B
1μM retigabine
Rheobase (pA)
GFP pre 1μM
N = 11
GFP rtg 1μM
N = 11
Kv7.3 pre 1μM
N = 12
Kv7.3 rtg 1μM
N = 12
Paired Hedges g
GFP rtg 1μM
minus
GFP pre 1μM
Kv7.3 rtg 1μM
minus
Kv7.3 pre 1μM
C
3μM retigabine
Rheobase (pA)
GFP pre 3μM
N = 15
GFP rtg 3μM
N = 15
Kv7.3 pre 3μM
N = 15
Kv7.3 rtg 3μM
N = 15
Paired Hedges g
GFP rtg 3μM
minus
GFP pre 3μM
Kv7.3 rtg 3μM
minus
Kv7.3 pre 3μM

Fig. 5

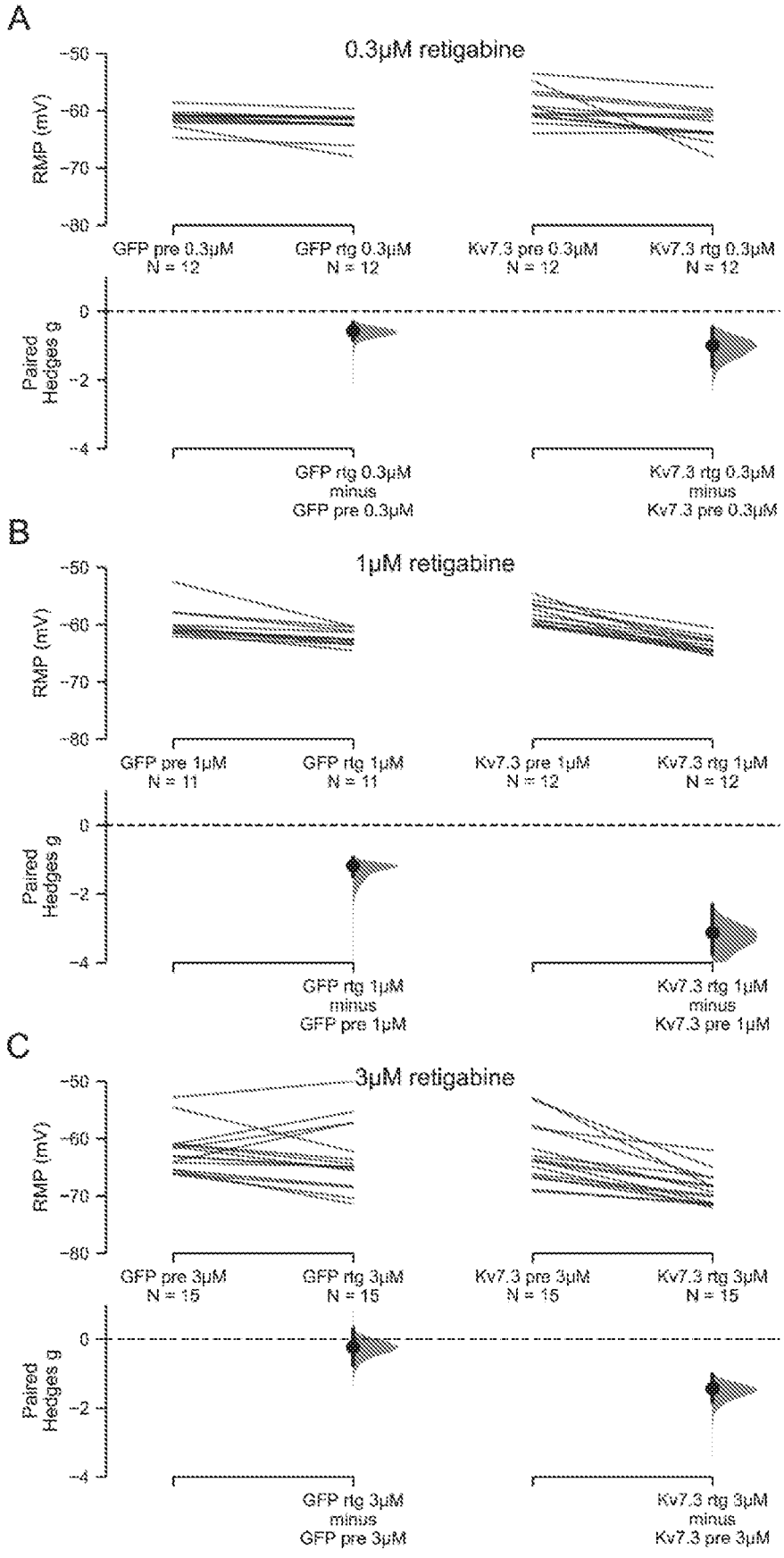

A
0.3μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 0.3μM
N = 12
GFP rtg 0.3μM
N = 12
Kv7.3 pre 0.3μM
N = 12
Kv7.3 rtg 0.3μM
N = 12
Paired Hedges g
0
−2
−4
GFP rtg 0.3μM
minus
GFP pre 0.3μM
Kv7.3 rtg 0.3μM
minus
Kv7.3 pre 0.3μM
B
1μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 1μM
N = 11
GFP rtg 1μM
N = 11
Kv7.3 pre 1μM
N = 12
Kv7.3 rtg 1μM
N = 12
Paired Hedges g
0
−2
−4
GFP rtg 1μM
minus
GFP pre 1μM
Kv7.3 rtg 1μM
minus
Kv7.3 pre 1μM
C
3μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 3μM
N = 15
GFP rtg 3μM
N = 15
Kv7.3 pre 3μM
N = 15
Kv7.3 rtg 3μM
N = 15
Paired Hedges g
0
−2
−4
GFP rtg 3μM
minus
GFP pre 3μM
Kv7.3 rtg 3μM
minus
Kv7.3 pre 3μM

Fig. 6

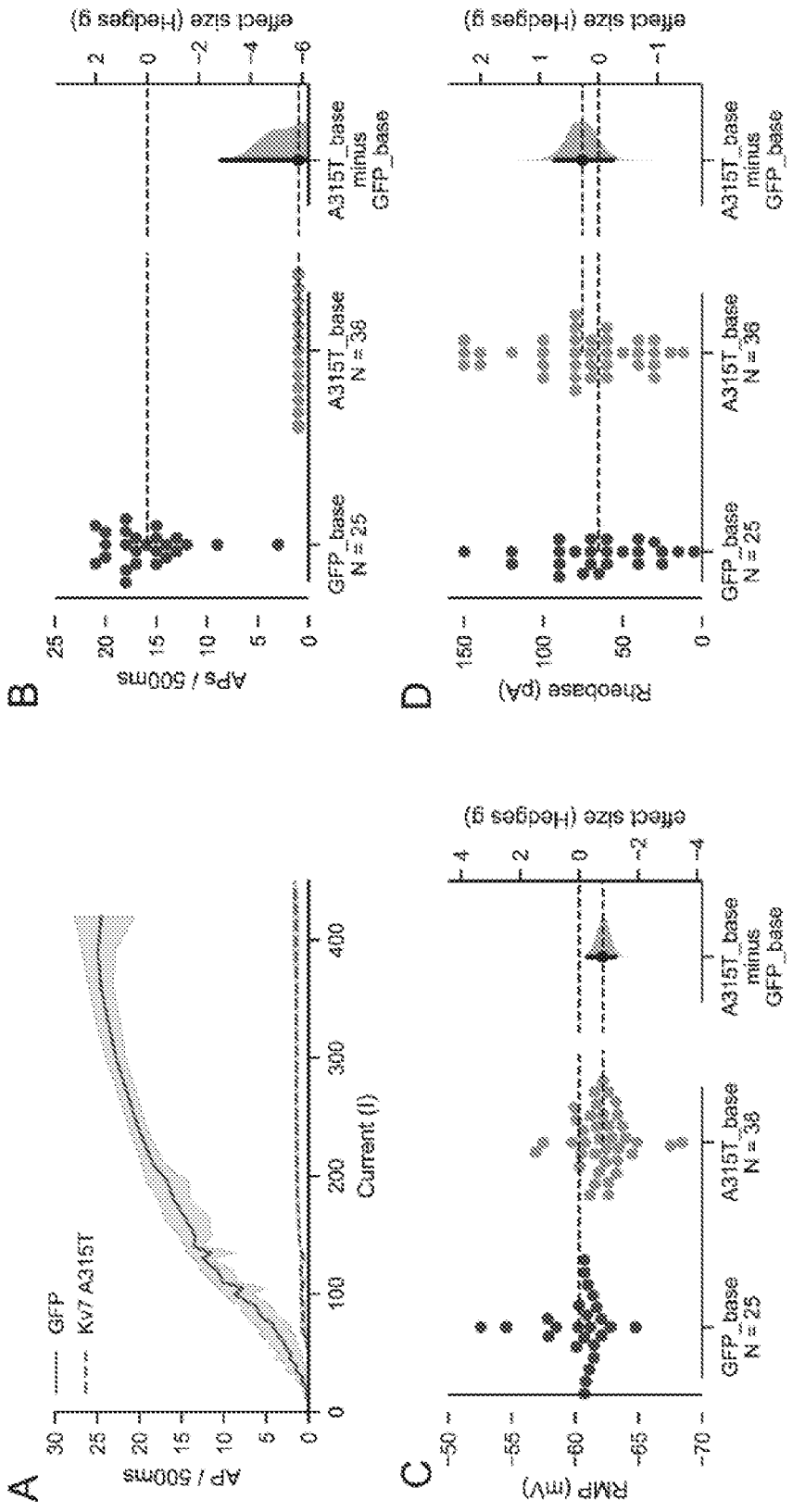

A
AP / 500ms
GFP
Kv7 A315T
Current (I)
B
APs / 500ms
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 36
A315T_base minus GFP_base
C
RMP (mV)
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 36
A315T_base minus GFP_base
D
Rheobase (pA)
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 36
A315T_base minus GFP_base

Figure 7

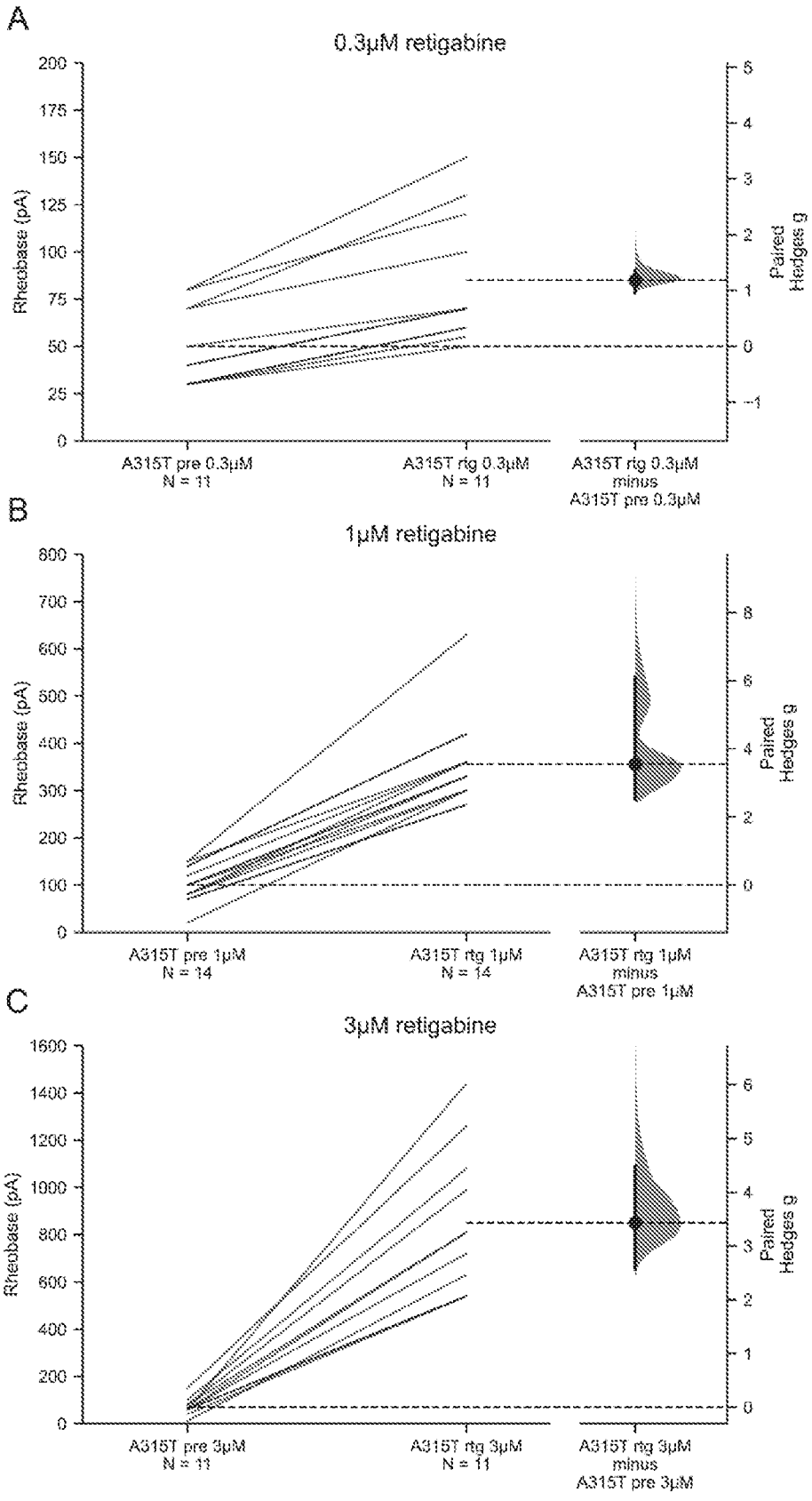

A
0.3µM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 0.3µM
N = 11
A315T rtg 0.3µM
N = 11
A315T rtg 0.3µM
minus
A315T pre 0.3µM
B
1µM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 1µM
N = 14
A315T rtg 1µM
N = 14
A315T rtg 1µM
minus
A315T pre 1µM
C
3µM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 3µM
N = 11
A315T rtg 3µM
N = 11
A315T rtg 3µM
minus
A315T pre 3µM

Fig. 8

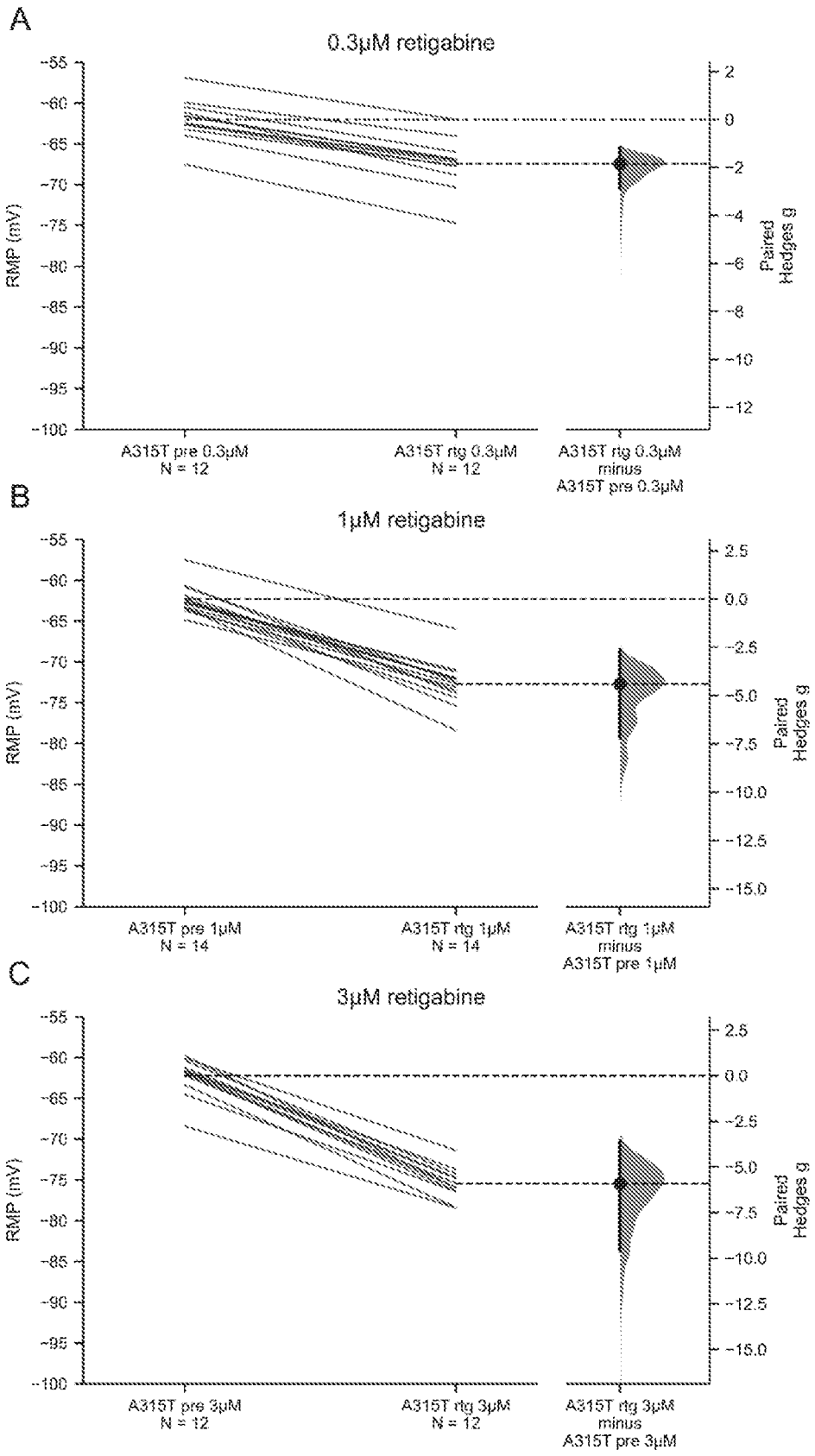

A
0.3μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 0.3μM
N = 12
A315T rtg 0.3μM
N = 12
A315T rtg 0.3μM
minus
A315T pre 0.3μM
B
1μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 1μM
N = 14
A315T rtg 1μM
N = 14
A315T rtg 1μM
minus
A315T pre 1μM
C
3μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 3μM
N = 12
A315T rtg 3μM
N = 12
A315T rtg 3μM
minus
A315T pre 3μM

Fig. 9

Figure 10

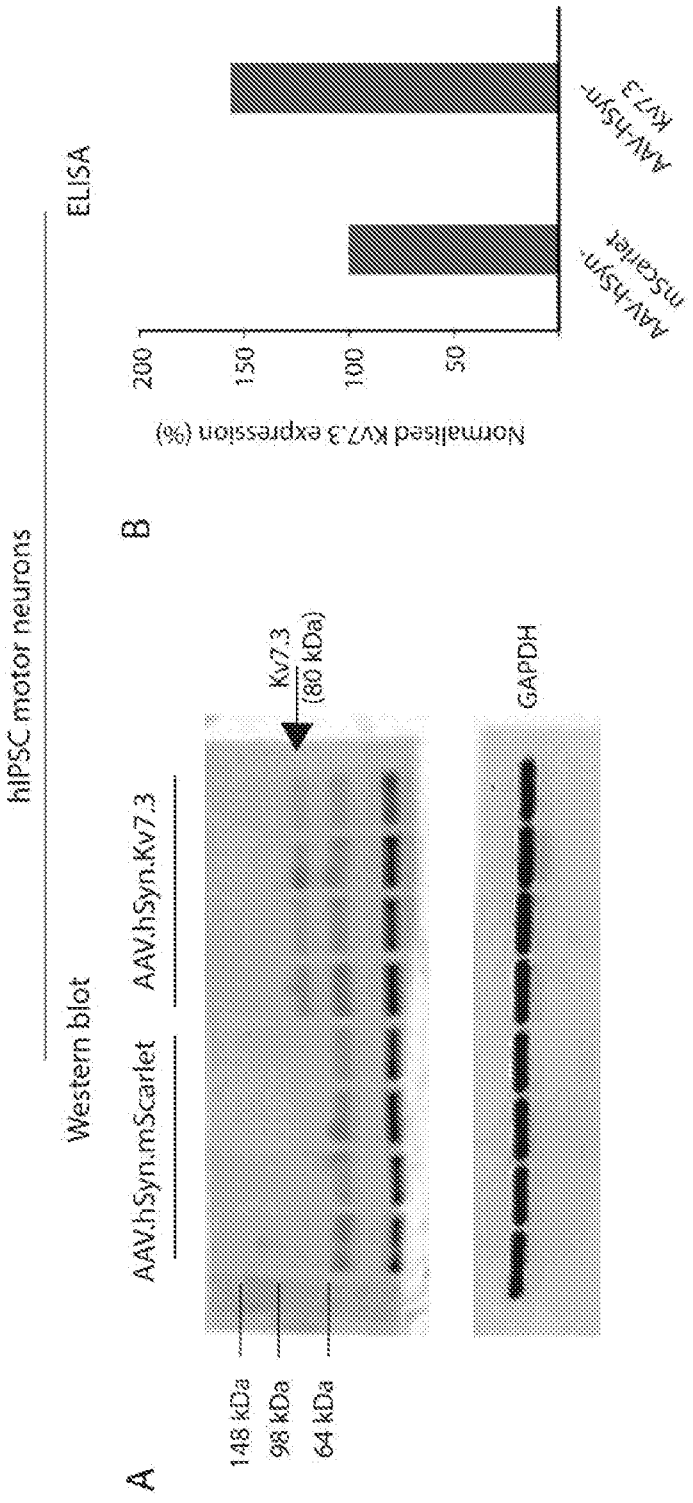
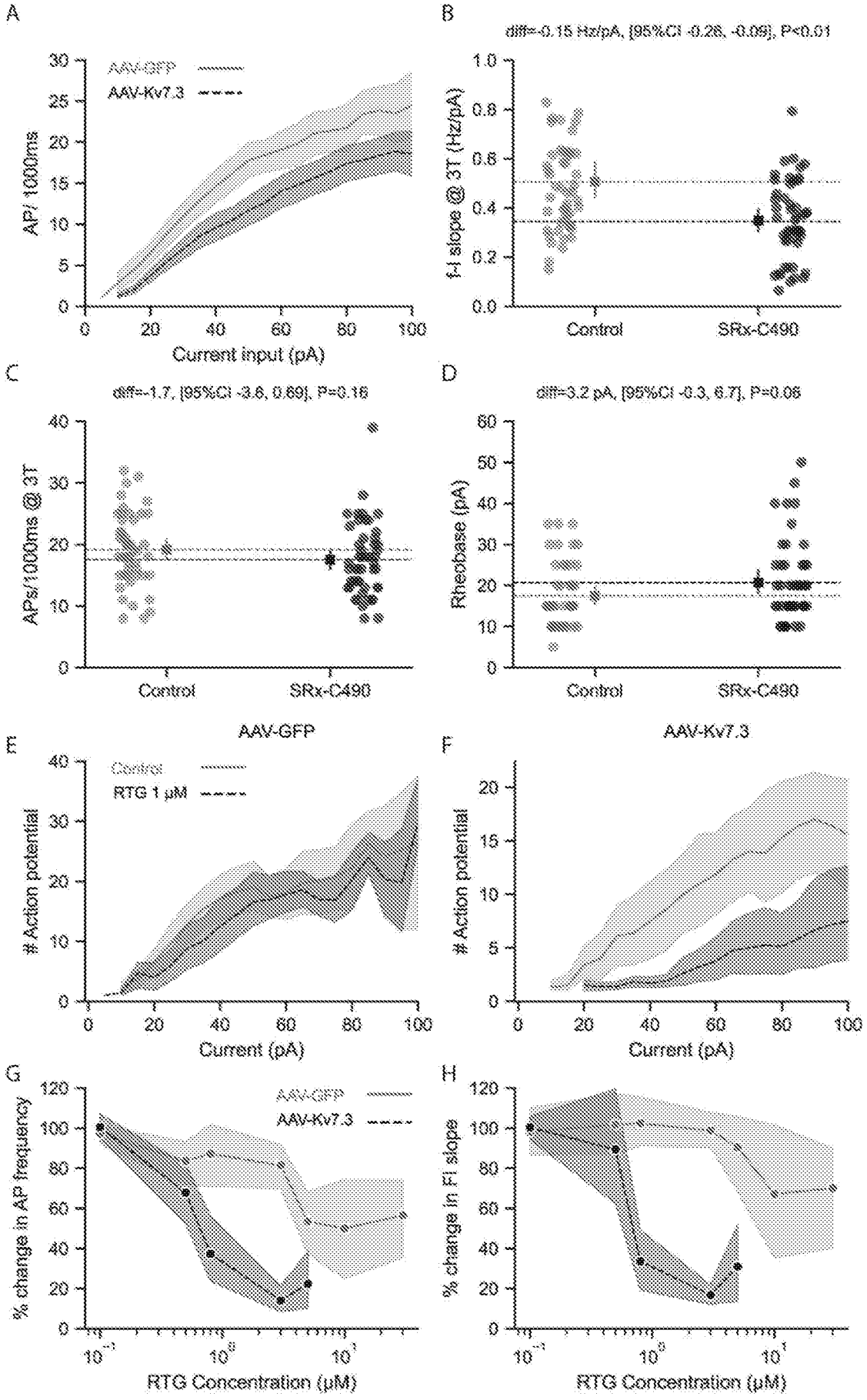

hIPSC motor neurons
Western blot
ELISA
A
B
AAV.hSyn.mScarlet
AAV.hSyn.Kv7.3
148 kDa
98 kDa
64 kDa
Kv7.3
(80 kDa)
GAPDH
Normalised Kv7.3 expression (%)
200
150
100
50
AAV.hSyn.mScarlet
AAV.hSyn.Kv7.3

Figure 11


A
AAV-GFP
AAV-Kv7.3
AP/ 1000ms
Current input (pA)
B
diff=-0.15 Hz/pA, [95%CI -0.26, -0.09], P<0.01
f-I slope @ 3T (Hz/pA)
Control
SRx-C490
C
diff=-1.7, [95%CI -3.6, 0.69], P=0.16
APs/1000ms @ 3T
Control
SRx-C490
D
diff=3.2 pA, [95%CI -0.3, 6.7], P=0.06
Rheobase (pA)
Control
SRx-C490
E
AAV-GFP
Control
RTG 1 µM
Action potential
Current (pA)
F
AAV-Kv7.3
Action potential
Current (pA)
G
AAV-GFP
AAV-Kv7.3
% change in AP frequency
RTG Concentration (µM)
H
% change in FI slope
RTG Concentration (µM)

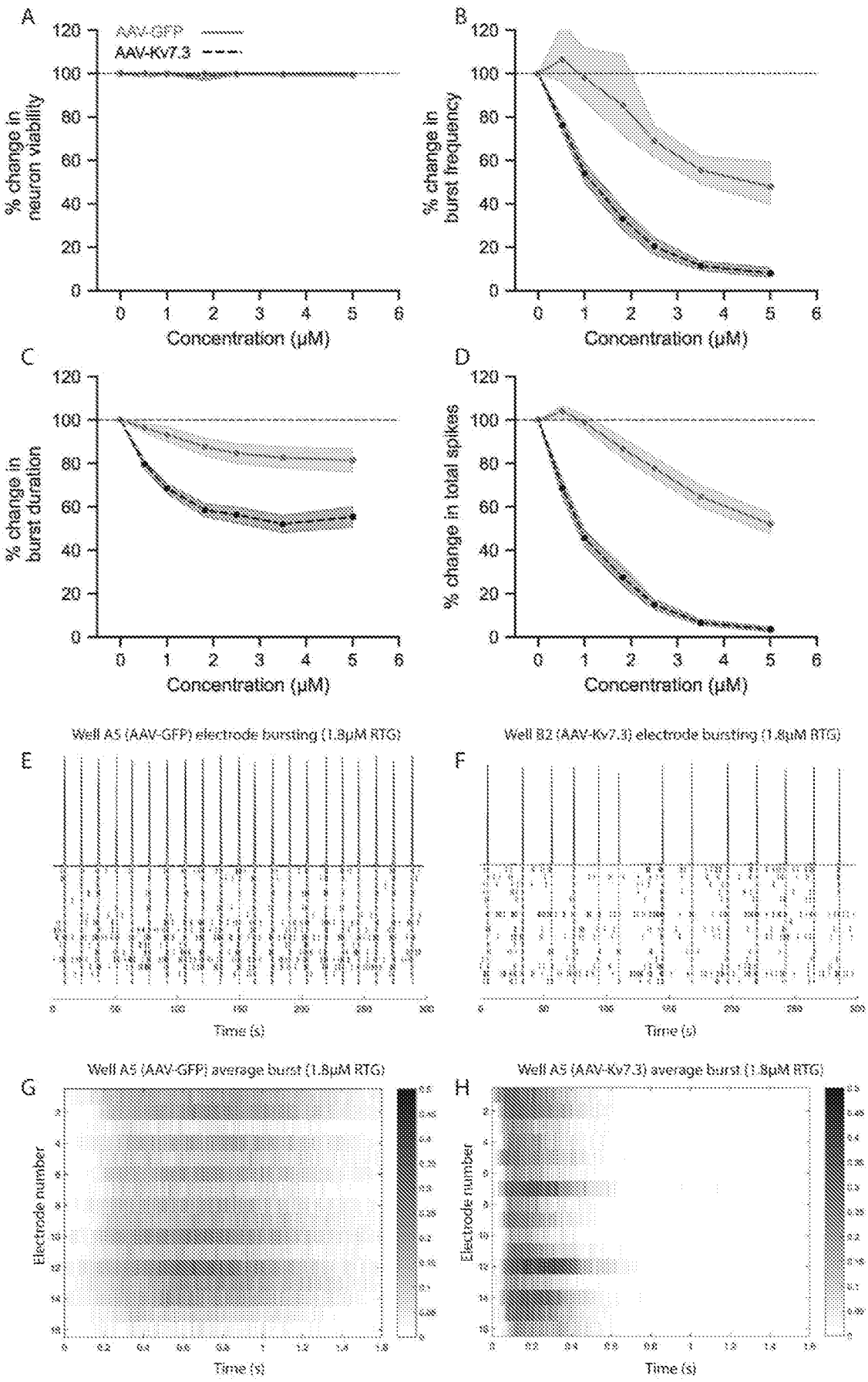

A
AAV-GFP
AAV-Kv7.3
% change in neuron viability
Concentration (µM)
B
% change in burst frequency
Concentration (µM)
C
% change in burst duration
Concentration (µM)
D
% change in total spikes
Concentration (µM)
Well A5 (AAV-GFP) electrode bursting (1.8µM RTG)
E
Time (s)
Well B2 (AAV-Kv7.3) electrode bursting (1.8µM RTG)
F
Time (s)
Well A5 (AAV-GFP) average burst (1.8µM RTG)
G
Electrode number
Time (s)
Well A5 (AAV-Kv7.3) average burst (1.8µM RTG)
H
Electrode number
Time (s)

Figure 12

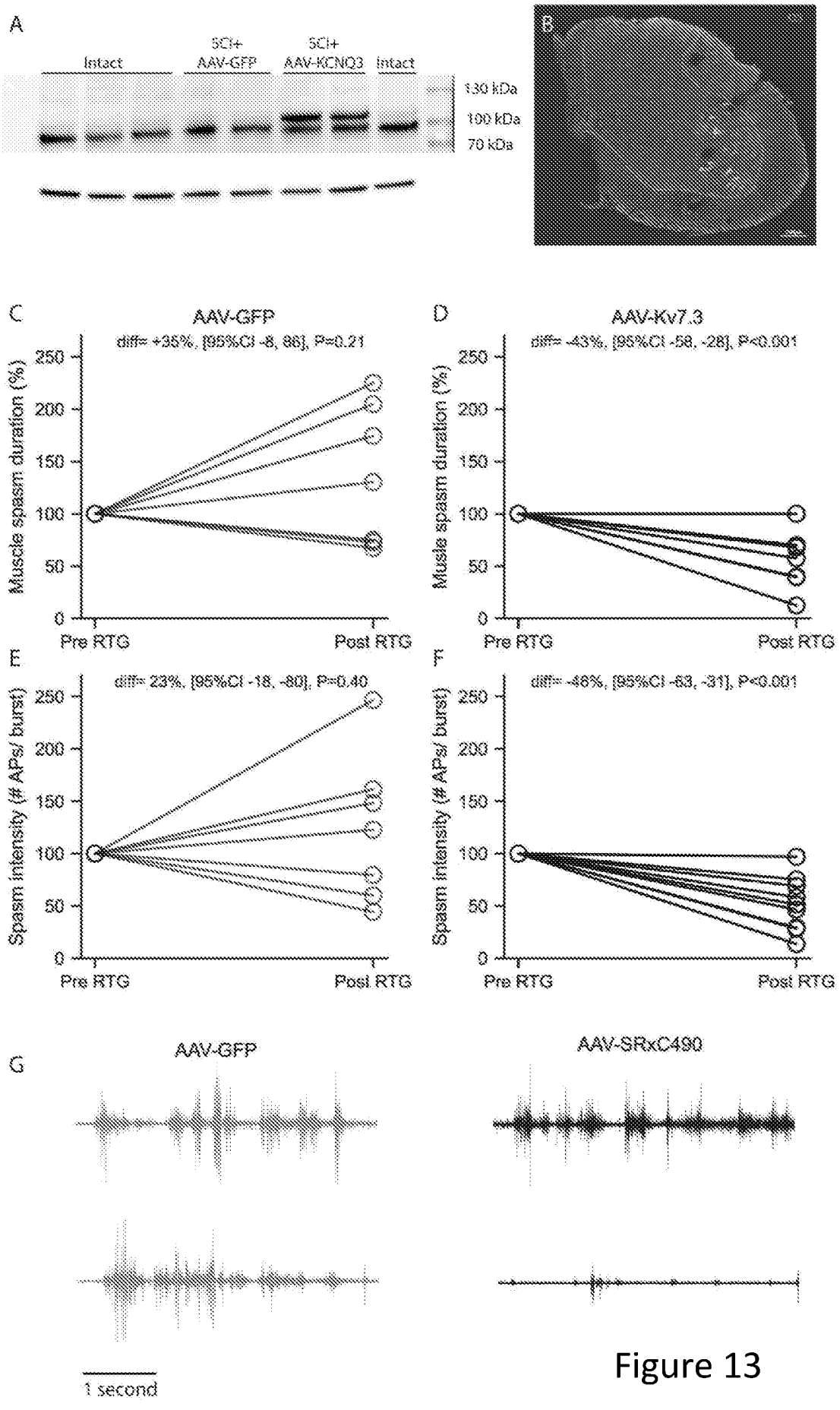

A
Intact
SCI+ AAV-GFP
SCI+ AAV-KCNQ3
Intact
130 kDa
100 kDa
70 kDa
B
C
AAV-GFP
diff= +35%, [95%CI -8, 86], P=0.21
Muscle spasm duration (%)
Pre RTG
Post RTG
D
AAV-Kv7.3
diff= -43%, [95%CI -58, -28], P<0.001
Muscle spasm duration (%)
Pre RTG
Post RTG
E
diff= 23%, [95%CI -18, -80], P=0.40
Spasm intensity (# APs/ burst)
Pre RTG
Post RTG
F
diff= -46%, [95%CI -63, -31], P<0.001
Spasm intensity (# APs/ burst)
Pre RTG
Post RTG
G
AAV-GFP
AAV-SRxC490
1 second

Figure 13

Figure 14

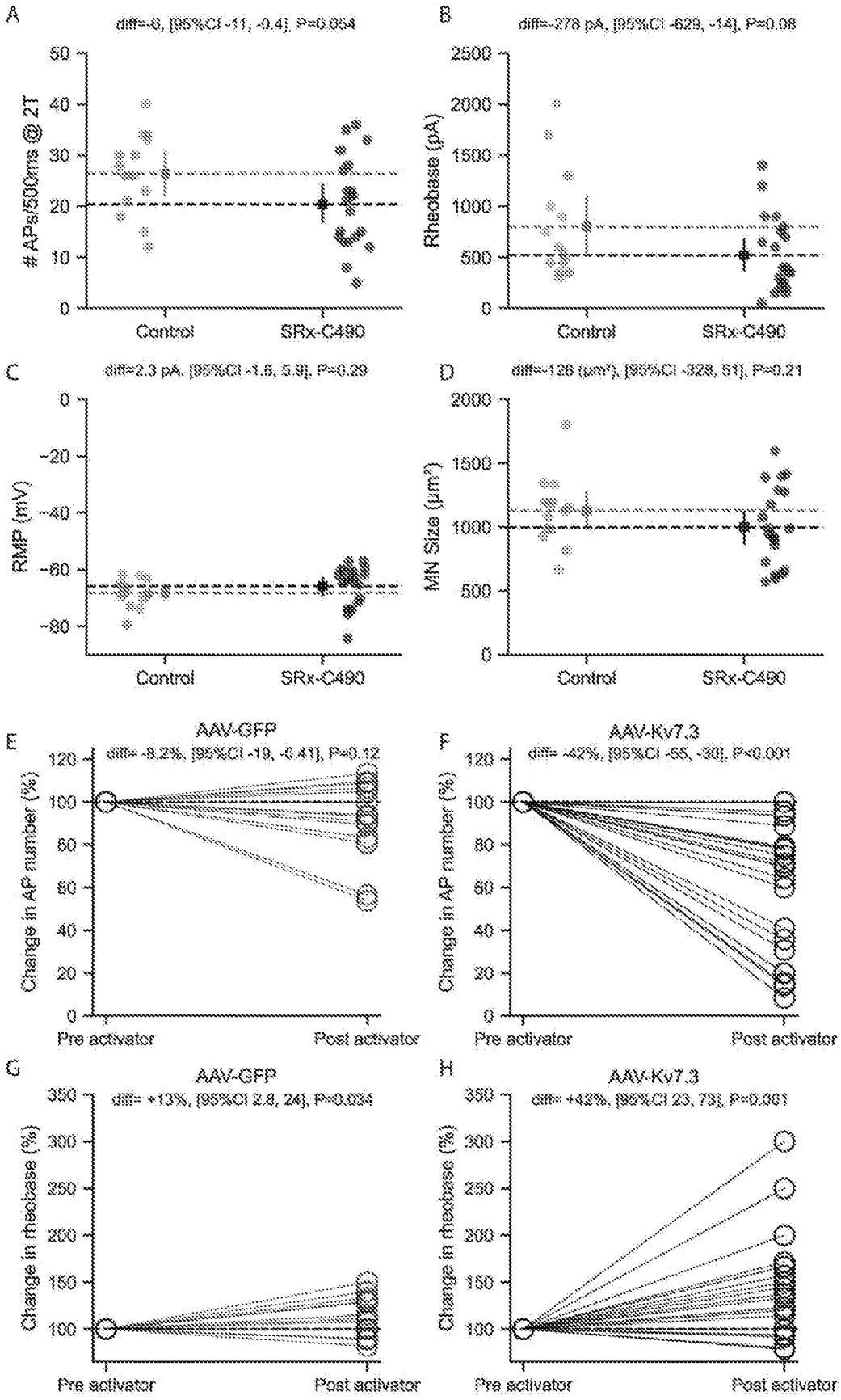
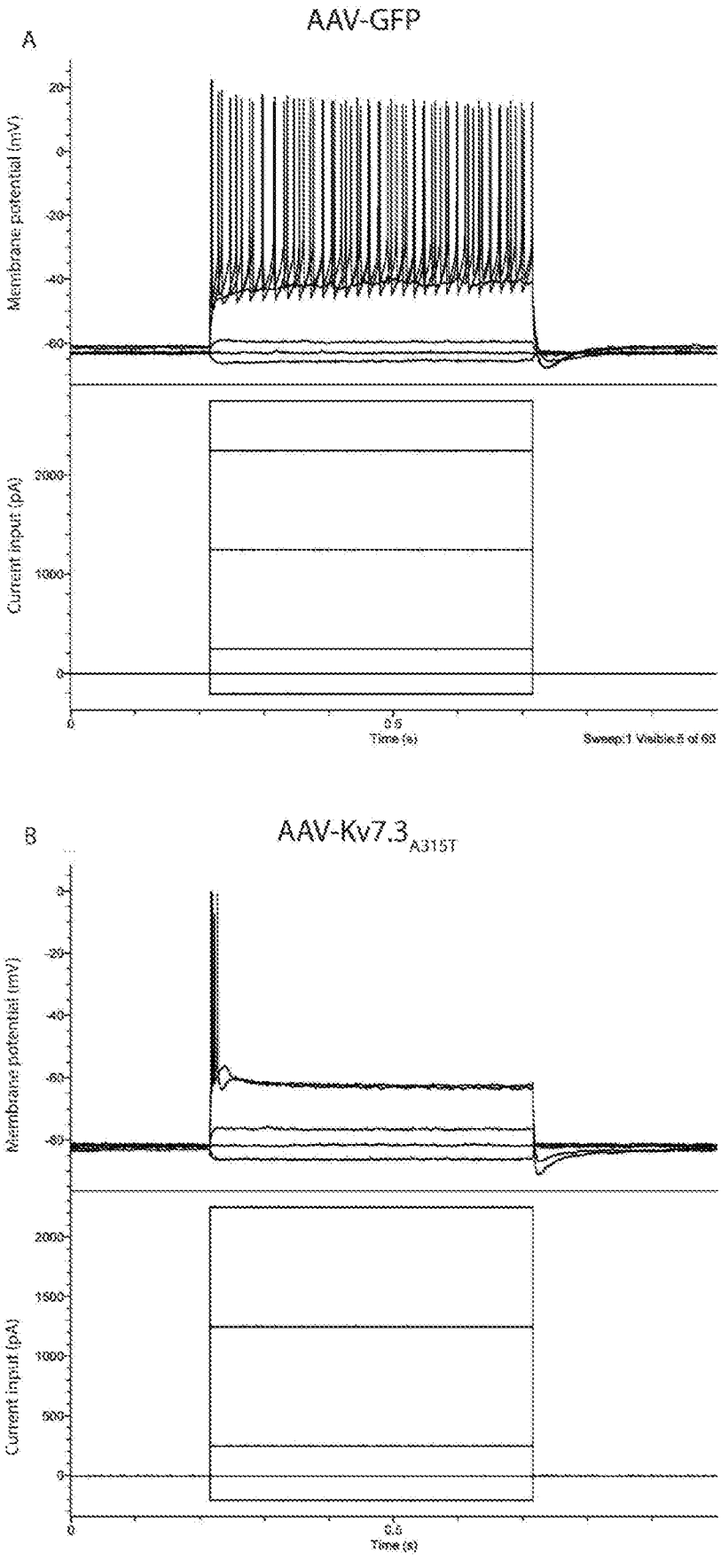

A
diff=-6, [95%CI -11, -0.4], P=0.054
# APs/500ms @ 2T
Control
SRx-C490
B
diff=-278 pA, [95%CI -629, -14], P=0.08
Rheobase (pA)
C
diff=2.3 pA, [95%CI -1.6, 5.9], P=0.29
RMP (mV)
D
diff=-126 (µm²), [95%CI -328, 61], P=0.21
MN Size (µm²)
E
AAV-GFP
diff= -8.2%, [95%CI -19, -0.41], P=0.12
Change in AP number (%)
Pre activator
Post activator
F
AAV-Kv7.3
diff= -42%, [95%CI -55, -30], P<0.001
G
AAV-GFP
diff= +13%, [95%CI 2.8, 24], P=0.034
Change in rheobase (%)
H
AAV-Kv7.3
diff= +42%, [95%CI 23, 73], P=0.001

Figure 15


A
AAV-GFP
Membrane potential (mV)
Current input (pA)
Time (s)
B
AAV-Kv7.3A315T
Membrane potential (mV)
Current input (pA)
Time (s)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/054426

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86 A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIU MIN ET AL: "Inactivation of the Lateral Hypothalamus Attenuates Methamphetamine-Induced Conditioned Place Preference through Regulation of Kcnq3 Expression", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 23, no. 13, 30 June 2022 (2022-06-30) , page 7305, XP093171968, Basel, CH ISSN: 1422-0067, DOI: 10.3390/ijms23137305 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9266452/pdf/ijms-23-07305.pdf> page 2, 1st para;section 4.3; page 7 ----- -/--	1-25

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2024	20/08/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merkl, Philipp

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/054426

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 883 812 B2 (CLAFFEY MICHELLE MARIE [US]; DAVOREN JENNIFER ELIZABETH [US] ET AL.) 11 November 2014 (2014-11-11) background section; col 11	6

X	FRIEDMAN ALLYSON K. ET AL: "KCNQ channel openers reverse depressive symptoms via an active resilience mechanism", NATURE COMMUNICATIONS, vol. 7, no. 1, 24 May 2016 (2016-05-24), XP055775444, DOI: 10.1038/ncomms11671 Retrieved from the Internet: URL:http://www.nature.com/articles/ncomms11671>	1-24
Y	abstract, Fig 1	25

A	WO 2021/119018 A1 (UNIV LELAND STANFORD JUNIOR [US]) 17 June 2021 (2021-06-17) 0008, 0052	1-25

Y	FLOETER MARY KAY ET AL: "Motor Neuron Firing Dysfunction in Spastic Patients With Primary Lateral Sclerosis", JOURNAL OF NEUROPHYSIOLOGY, vol. 94, no. 2, 1 August 2005 (2005-08-01) , pages 919-927, XP093191940, US ISSN: 0022-3077, DOI: 10.1152/jn.00185.2005 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360205/pdf/nihms7360.pdf> abstract	25

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/054426

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/054426

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8883812 B2	11-11-2014	AU 2011275393 A1	10-01-2013
		CA 2804165 A1	12-01-2012
		CN 102971307 A	13-03-2013
		EP 2590961 A1	15-05-2013
		JP 5763758 B2	12-08-2015
		JP 2013531006 A	01-08-2013
		KR 20130031323 A	28-03-2013
		SG 186409 A1	28-02-2013
		US 2013150391 A1	13-06-2013
		WO 2012004698 A1	12-01-2012
		ZA 201300499 B	25-09-2013
WO 2021119018 A1	17-06-2021	NONE	

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Acorn IP LLP
Colin Sanders Business Innovation Centre
Mewburn Road
Banbury
Oxfordshire OX16 9PA
ROYAUME UNI

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05.08.2025

Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
SANI3WO	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2024/054426	07.05.2024	10.05.2023

Applicant
SANIA TX LTD

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

   The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040

Authorized Officer

Torenvliet-Berruti

Tel. +31 70 340-4939

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SANI3WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IB2024/054426	International filing date *(day/month/year)* 07.05.2024	Priority date *(day/month/year)* 10.05.2023
International Patent Classification (IPC) or national classification and IPC INV. C12N15/86		
Applicant SANIA TX LTD		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ a total of 8 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ a separate electronic file containing a sequence listing *(sent to the International Bureau only)*

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.03.2025	05.08.2025

Name and mailing address of the international preliminary examining authority:

European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040

Authorized officer

Merkl, Philipp

Telephone No. +49 89 2399-1623

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2024/054426

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-32	filed on	03-03-2025

Sequence listings, SEQ ID NO

1-16	as originally filed

Claims, Numbers

1-18	filed on	30-05-2025

Drawings, Sheets

1/15-15/15	as originally filed

☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):
   - ☐ the description, pages
   - ☒ the claims, Nos. 2
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).

6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
   - ☒ A top-up search was carried out by this Authority on 24.03.2025 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
     - ☒ Additional relevant documents have been discovered during the top-up search.
   - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.

7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded".*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1, 3-6, 8, 10, 13-18
	No:	Claims	7, 9, 11, 12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1, 3-6, 8, 10, 13-18
Industrial applicability (IA)	Yes:	Claims	1, 3-18
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination,

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

   c. ☐ furnished to this Authority as an amendment* under PCT Article 34 on .

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established to the extent that a meaningful opinion referred to in Article 33(1) could be formed without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

* *If item 4 in Box No. I applies, the sequence listing, which forms part of the basis of the report, may be marked "superseded."*

Re Item I

Basis of the report

Amendments under Article 34 PCT

Pertaining to 20.10 ISPE, the international preliminary examination report must be established as if such amendment had not been made if the applicant seeks to amend the description (other than references to the prior art), the drawings, or the claims in such a way that subject matter which extends beyond the content of the application as filed.

1 For present claim 2, basis is indicated as page 3, lines 21-27, page 4, lines 18-19, page 7, lines 22-35, page11, lines 15-24 and 28-30, page 12, lines 5-9, page 14, lines 33-34 and page 16, lines 26-29. None of the above passages provide a direct and unambiguous disclosure of a method of reducing the therapeutically effective dose of a voltage gated potassium channel targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage gated potassium channel targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID 10 to SEQ ID 16.

The passage on page 3, lines 21-27 does not disclose a Kv7.3 potassium gated voltage channel. The passage on page 12 lines 5-9 is restricted to retigabine. Combining the two passages results in an intermediate generalization. Overall, if one would argue that the passage on page 3, lines 21-27 represents the other side of a coin, then a claim directed at such subject matter could as well cite the passage in a verbatim fashion. If a verbatim use is not possible, it appears that the subject matter of the claim is different from its indicated basis. All in all, this authority is not convinced despite making a serious effort.

2 In sum, amended claim 2 does not meet the requirements of Art. 34 PCT. According to 20.10 ISPE and Rule 70(2) PCT, the examination is being carried out on the basis of claims 1, 3-18 as filed on 30-05-2025. Present claim 2 is not examined because it was not part of the original set of claims.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3 Summary of the application

The application is concerned with provision of methods which reduce the side effects of neuromodulatory drugs/increases their efficacy and respective gene therapy vectors.

In the example, neuronal cells derived from iPSCs were transduced with AAV harboring a hKCNQ3 gene sequence (encoding Kv7.3). In combination with addition of the drug retigabine, less action potentials were observed in Kv7.3 transduced cells (Fig 4, 5, 6) and excitability is reduced (Fig 7) and rheobase is increased (Fig 8). Fig. 9-12 are dedicated to further effects of the Kv7.3 expression.

In addition, mice from a model of spasticity were treated with AAV Kv7.3 and shorter spasm duration and intensity was observed in the treated mice. Further mice were treated with Kv7.3 A315T. Such treated neurons were found not to be able to fire repetitively.

4 Citations

Reference is made to the following documents:

D1 LIU MIN ET AL: "Inactivation of the Lateral Hypothalamus Attenuates Methamphetamine-Induced Conditioned Place Preference through Regulation of Kcnq3 Expression",
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES,
vol. 23, no. 13, 30 June 2022 (2022-06-30), page 7305, XP093171968,
Basel, CH
ISSN: 1422-0067, DOI: 10.3390/ijms23137305
Retrieved from the Internet:
URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9266452/pdf/ijms-23-07305.pdf

D2 US 8 883 812 B2 (CLAFFEY MICHELLE MARIE [US]; DAVOREN JENNIFER ELIZABETH [US] ET AL.) 11 November 2014 (2014-11-11)

D3 FRIEDMAN ALLYSON K. ET AL: "KCNQ channel openers reverse depressive symptoms via an active resilience mechanism",
NATURE COMMUNICATIONS,
vol. 7, no. 1, 24 May 2016 (2016-05-24), XP055775444,

DOI: 10.1038/ncomms11671
Retrieved from the Internet:
URL:http://www.nature.com/articles/ncomms11671

D4 WO 2021/119018 A1 (UNIV LELAND STANFORD JUNIOR [US]) 17 June 2021 (2021-06-17)

D5 FLOETER MARY KAY ET AL: "Motor Neuron Firing Dysfunction in Spastic Patients With Primary Lateral Sclerosis",
JOURNAL OF NEUROPHYSIOLOGY,
vol. 94, no. 2, 1 August 2005 (2005-08-01), pages 919-927, XP093191940, US
ISSN: 0022-3077, DOI: 10.1152/jn.00185.2005
Retrieved from the Internet:
URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360205/pdf/nihms7360.pdf

The following documents were not cited in the international search report.

D6 LIU E ET AL: "Activation of Kv7 channels normalizes hyperactivity of the VTA-NAcLat circuit and attenuates methamphetamine-induced conditioned place preference and sensitization in mice",
MOLECULAR PSYCHIATRY,
vol. 28, no. 12, 21 August 2023 (2023-08-21), pages 5183-5194, XP093191537,
London
ISSN: 1359-4184, DOI: 10.1038/s41380-023-02218-5
Retrieved from the Internet:
URL:https://www.nature.com/articles/s41380-023-02218-5

D7 WANG JUN ET AL: "Effect of Methamphetamine on the Microglial Damage: Role of Potassium Channel Kv1.3",
PLOS ONE,
vol. 9, no. 2, 12 February 2014 (2014-02-12), page e88642, XP093258658, US
ISSN: 1932-6203, DOI: 10.1371/journal.pone.0088642
Retrieved from the Internet:
URL:https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0088642&type=printable

D8 WANG YA-JEAN ET AL: "Methamphetamine inhibits voltage-gated potassium currents in NG108-15 cells: Possible contribution of large-conductance calcium-activated potassium channels", TOXICOLOGY LETTERS, vol. 223, no. 2 , pages 139-145, XP028765857, ISSN: 0378-4274, DOI: 10.1016/J.TOXLET.2013.08.021

D9 ZAIKA OLEG ET AL: "Determinants within the Turret and Pore-Loop Domains of KCNQ3 K+ Channels Governing Functional Activity", BIOPHYSICAL JOURNAL, vol. 95, no. 11, 1 December 2008 (2008-12-01), pages 5121-5137, XP093262664, AMSTERDAM, NL ISSN: 0006-3495, DOI: 10.1529/biophysj.108.137604 Retrieved from the Internet: URL:https://www.sciencedirect.com/science/article/pii/S0006349508789392

D10 YANG NIEN-DU ET AL: "Electro-mechanical coupling of KCNQ channels is a target of epilepsy-associated mutations and retigabine", SCIENCE ADVANCES, vol. 8, no. 29, 22 July 2022 (2022-07-22), XP093262677, US ISSN: 2375-2548, DOI: 10.1126/sciadv.abo3625

Summary of D1 see below.

D2 is concerned with dimethoxy-pyrimidine amides and their use in treatment of CNS disorders, including epilepsy. Said compounds are openers of the Kv7.2/7.3 potassium channels. Channel opening is known do decrease neuronal excitability and retigabine is named as a known drug for channel opening (background section). The document discloses transfection vectors harboring human Kv7.2 and 7.3 channel subunits (col 11).

D3 discloses that overexpression of KCNQ (HSV mediated) channels in the VTA dopaminergic neurons and administration of retigabine normalizes neuronal hyperactivity and depressive behaviour. Overexpression resulted in lover neuron firing activity (abstract, Fig 1).

D4 aims at improving the sleep quality of elderly patients. It states that aged Hcrt neurons are more excitable which leads to sleep fragmentation [0008]. It proposes as a solution to manipulate the Kv7 channel, including Kv7.3 or 7.2 by administration of agents which open the channel. The document further discloses that retigabine is a KCNQ (=Kv7) channel opener (052). Further presented are experiments wherein the KCNQ 2/3 genes are disrupted via AAV mediated Cas/CRISPR action. This was found to lead to hyperexcitability of the Hcrt neurons.

D6 is a P document. However, this document is merely used to back up the role of methamphetamin. D6 (abstract) shows that methamphetamine does not directly act on Kv7.3 ion channels, but it does influence their activity indirectly. Research suggests that methamphetamine can downregulate KCNQ genes, which encode Kv7 channel proteins, including Kv7.3. These channels play a role in modulating neuronal excitability. Studies have shown that increasing Kv7.3 activity in specific brain circuits can reduce methamphetamine-induced behaviors, such as reward-seeking and hyperactivity.

In addition, D7 (abstract) and D8 (abstract, title) show that methamphetamine does interact with voltage-gated potassium channels. Research indicates that methamphetamine can influence the activity of certain potassium channels, such as Kv1.3, which are involved in neuronal and microglial functions. For example, methamphetamine has been shown to increase the expression of Kv1.3 channels, leading to microglial damage. Additionally, methamphetamine may inhibit potassium currents in specific cell types, such as NG108-15 cells.

D9 discloses KCNQ3 mutations A315T, A315S, A315V and reveals that the A315T mutation increases the current density. The same is reported for the A315S mutation. For the A315V mutation, a very small current density is reported (abstract, pages 5129 left col, table 1).

D10 discloses injection of cRNA expressing KCNQ3 A315T into Xenopus oocytes followed by retagabine treatment (material & methods, Fig 6, 7, page 8, right col). The document reports a large hyperpolarizing shift upon 100microM RTG treatment.

5 **Article 33(2) PCT**

The present application does not meet the requirements of Article 33(1) PCT because the subject-matter of claims 7, 9, 11, 12 not new within the meaning of Article 33(2) PCT.

5.1 D1 aims at modulating excitability of neurons by Kcnq (Kv7) channels (page 2, 1st para). In this regard, an AAV CMV Kcnq3 WPRE is described (section 4.3), which expresses Kv7.3. Section 2.6 describes a method in which a nucleotide sequence encoding a Kv7.3 ion channel subunit is overexpressed in a neuronal cell and a neuromodulatory drug (metamphetamine) is administered. The document teaches that the frequency of excitatory postsynaptic currents was reduced in AAV Kv7.3 infected neurons (page 7).

In view of the many desiderata in the claims (see Item VIII), the following is noted: The effect that expression of a transgene has is an inherent feature of the transgene and the cell type in which it is expressed. Hence, either all the alleged effects are inherently present and thus not new or they are not present and it is not disclosed how the effects would be achieved (Art. 5 PCT).

It is noted that SEQ IDs 9-16 encompass the WT Kv7.3 AA sequence which is thus deemed implicitly disclosed.

In view of D6-D8 (summary see above), it is clear that methamphetamine falls under the definition of being a "voltage gated potassium channel targeting drug", more specifically that it acts also on Kv7.3 expression.

Thus, in view of D1, the subject-matter of claims 7, 9, 11, 12 does not meet the requirements of Art. 33(2) PCT.

5.2 The subject-matter of claims 1, 3-6, 8, 10, 13-18 is not described in any of D1-D10 and thus novel over the prior art (Article 33(2) PCT).

6 **Article 33(3) PCT**

The present application does not meet the requirements of Article 33(3) PCT because the subject-matter of claims 1, 3-6, 8, 10, 13-18 does not involve an inventive step.

6.1 Claim 3:

D1 is considered the closest prior art. Summary of D1 see above.

The subject-matter of claim 3 differs from D1 in the AA sequence of the Kv7.3 channel. The addition of a drug is optional in claim 3.

The technical effect is reduced neuronal excitability.

The problem to be solved can thus be considered as provision of a method to reduce excitability of neurons.

D10 discloses injection of cRNA expressing KCNQ3 A315T into Xenopus oocytes followed by retigabine treatment (material & methods, Fig 6, 7, page 8, right col). The document reports a large hyperpolarizing shift upon 100microM RTG treatment. Hence, D10 discloses the principle of elevation of the current density and thus reduction of excitability in neurons via expression of KCNQ A315T channels (in conjunction with retigabine). It is implicitly disclosed in D10 that the A315T mutation already reduces excitability in view of the teaching of D9 (see above). Thus, the subject-matter of claim 3 does not meet the requirements of Art. 33(3) PCT.

6.2 The same argumentation applies to claim 1.

6.3 The same argumentation applies in essence for claim 5. D1 discloses an AAV KCNQ3 vector and a person skilled in the art would produce such AAV vector with the mutants disclosed in D9 with reasonable expectation of success.

6.4 The same applies to claim 12, retigabine is already mentioned in D10.

7 **Article 33(4) PCT**

Claims 1-18 are industrially applicable.

8 The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Patentability, in particular novelty and inventive step, of claims 1-4, 8-18 has been assessed on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

XP093191537 (D6)

Re Item VIII

Certain observations on the international application

9 **Article 6 PCT**

The application does not meet the requirements of Article 6 PCT because claims 1-18 are not clear.

9.1 Claims 1-4 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("increasing efficacy, reducing side effects, increasing therapeutic index, reducing excitability, such that it forms functional ion channels, selectively transduces, is upregulated etc."). Such a definition is only allowable under the conditions elaborated in the Guidelines EPO/PCT F-IV, 4.10. In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

9.2 In conjunction with the above, most of the terms cited above are relative terms in addition which lack a point of reference. E.g. claim 1 increasing efficacy against what baseline? Overexpressing compared to what? Lower dose as compared to what? It is noted that the wording "than would be normally administered" is still a relative term. The application does not disclose in how far there is a reduction in the amount of a drug.

9.3 The application contains 18 claims of which 10 are independent claims. The application thus lacks conciseness and the set of claims needs to be consolidated. Specific attention should be paid to exemplary claims 1-3 which describe the same method but differ merely in the alleged desired effect, which arises when carrying out the method, hence is not limiting (GL F-IV 4-13-3)

9.4 Claim 1 refers to a derivative of retigabine which is virtually any compound and thus rendering the scope of the claim unclear.

acorn
INTELLECTUAL PROPERTY

Colin Sanders Business
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OX16 9PA

International Preliminary Examination Authority
European Patent Office
80331 Munich
Germany

Our ref: SANI.3WO
30 May 2025

Dear Colleagues,

PCT Application Number: PCT/IB2024/154426
Applicant: Sania Rx Ltd
Title: Method

Thank you for the WO-IPEA, dated 31.03.2025. The Applicant hereby provides the following comments and amended claims in reply to the opinion.

Amendments

Claims 1 and 4 have been limited to include the list of neuromodulatory drugs of claim 12 (original claim 11, now claim 10).

Claim 8 has been amended to make it dependent only on claims 6 or 7, thereby rectifying the accidental removal of the AAV capsid from original claim 10, on which claim 8 (filed 3 March) was based.

Claims 9 and 11 have been deleted. Remaining claims and dependencies have corrected accordingly.

The amendments do not add subject matter.

Admissibility of the claims filed 03 March 2025

The Examiner has indicated that claims 2, 6, 8, 9 and 11 extend beyond the content of the application as filed. We provide the following comments in reply.

Claim 2

Claim 2 was newly added and reads:

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

The present invention is directed to a chemogenetics method in which increasing the number of Kv7.3-containing ion channels in target neurons permits a lower dose of an ion channel-targeting drug to be efficacious. In essence, presenting more targets for the drug to bind to allows a lower dose of the drug to be administered. In other words, the drugs efficacy is increased. This lower dose then leads to fewer side effects.

This description is apparent throughout the application as filed, particularly so at page 3, lines 21 to 27. Which states:

Summary of the Invention

The present disclosure relates to a chemogenetic method that allows modulation of activity in targeted neurons, thus increasing drug efficacy while reducing side effects, at least in part, due to the ability to use a lower dose. The method involves using gene therapy methodology via a delivery vector (e.g. AAV) to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses of specific activating and inhibiting drugs. That is, the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs.

Consequently, the Applicant submits that claim 2 is directly and unambiguously disclosed in the application as filed at page 3, lines 21 to 27. Breaking claim 2 down into its component parts:

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step:

increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

The method of reducing the therapeutically effective dose is found at line 22-23 which states "increasing drug efficacy [...] due to the ability to use a lower dose. This is clearly a statement that the drugs efficacy is increased which is the same as reducing the therapeutically effective dose. We respectfully submit that the skilled person would understand that these two concepts are opposite sides of the same coin and go hand in hand. This is further supported by page 4, lines 18-19.

The second underlined part of the claim relates to increasing the sensitivity of a target neuron to the drug by overexpressing Kv7.3. This concept is clearly disclosed at lines 26-27 which state "the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs", and at lines 24-25 which state "...to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses...".

Therefore, we submit that new claims 2 is fully, directly, and unambiguously disclosed in the application as filed and request removal of this objection based on the above reasoning.

Claim 6

Claim 6 is essentially original claim 8 which read:

> 8. An AAV viral particle gene therapy vector comprising:
> a. an AAV capsid that selectively transduces target neurons, and
> b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

However, to reduce the number of independent claims, claim 6 (former claim 8) has been made dependent on claim 5 (former claim 6), which read:

> 6. A gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

Part b. of claim 8 is, word for word, claim 6. Thus, claim 8 is the gene therapy vector of claim 6, plus an AAV capsid to create an AAV viral particle. The term "AAV viral particle" has been deleted from new claim 6 but removal of this term is not adding matter – the features of the claims are all still present and the product of claim 6 would be an AAV viral particle gene therapy vector as originally claimed in claim 8.

Therefore, we respectfully request removal of this objection.

Claim 8

Amended claim 8 is original claim 10 made dependent on claims 6 or 7. As discussed above, claim 6 finds basis in original claims 6 and 8. Thus, claim 8 is based on original claim 10, dependent on original claims 6 and 8 (not original claims 4 and 5 as indicated in the report). Amended claim 8 reads:

> (new) 8. The gene therapy vector according to any one of claims 6 or 7 for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective can be effectively administered to the subject.

Each of claims 6 and 7 comprises the feature (Kv7.3 sequences) struck out below in original claim 10. Thus, amended claim 8 also includes the sequences by virtue of its dependence on claim 6 or 7. As is the AAV capsid that selectively transduces target neurons. Hence, when we look at original claim 10 (shown below for ease of reference) the section that is now struck out, can be disregarded. Leaving "A gene therapy vector for use in treating [...] administered to the subject". These features are entirely present in amended claim 8.

> (original) 10. A gene therapy ~~vector comprising an AAV capsid that selectively transduces target neurons~~, ~~and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16~~, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

The only remaining difference between original claim 10 and amended claim 8 are the "lower dose of a neuromodulatory drug" of original claim 10 now reads "lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective". Amendment to voltage-gated potassium channel-targeting drug is a limitation that is entirely permissible and has been accepted in other claims. The addition of clarification of the lower dose than would normally be administered is similarly admissible elsewhere. Thus, the claim finds full basis in claims 10, 6 and 8 as originally filed.

We trust the removal of dependence on claim 5, which does not comprise the AAV capsid of original claim 10, resolves the objections.

Claim 9 and claim 11

Claims 9 and 11 have been deleted, therefore the objections are moot.

Novelty

Claims 1 and 4 are now limited to the list of neuromodulatory drugs of original claim 11. These drugs are not disclosed in D1, hence claims 1 and 4 are novel in view of D1.

We request reconsideration of claim 2 based on the above explanation that the claim does not add matter and reiterate the argumentation presented previously.

Claim 2 discloses a method of reducing the therapeutically effective dose of a voltage-gated potassium channel targeting drug, by increasing the sensitivity of a target neuron by overexpressing a Kv7.3 A315 mutant. D1 to D3 do not disclose mutant Kv7.3 channels as claimed in claim 2. Thus, claim 2 is novel in view of D1 to D3.

We thank the Examiner for the positive opinion of the novelty of claims 3, 5, 12 (now claims 3, 5, 10) and request that this be reflected in the summary of the report in Box No. V on page 3.

We therefore submit that independent claims 1, 2, 3, 4, 5, and 7 are novel and that, consequently, all dependent claims are also novel.

Inventive Step

Given the points above, we will defer commenting further on inventive step until a clear picture on the admissibility of the claims, and the novelty thereof, has been established.

Clarity

Given the divergence of local practice, we will defer commenting further on the clarity of the pending claims until the national phase.

We submit that the amended claims are now at least novel and do not add matter. We look forward to receipt of the International Preliminary Examination Report in due course.

Yours sincerely,

S Kaye

Dr Samantha Kaye CPA EPA
Representative for the Applicant

Enclosed: amended claims and specification – clean and marked up format

Claims

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug than would normally be administered to a subject in order to be therapeutically effective

    wherein the neuromodulatory drug is a voltage gated potassium channel-targeting drug,

    wherein the voltage-gated potassium channel-targeting drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

3. A method of reducing the excitability of a neuron comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 to the target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. optionally administering a voltage-gated potassium channel-targeting drug.

4. A method of treating a neurological disorder comprising the steps:
    a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,
    b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and
    c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels than would normally be administered to the subject in order to be therapeutically effective,

    wherein the neuromodulatory drug is a voltage-gated potassium channel-targeting drug selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

5. A gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 wherein, when the vector

transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

6. The gene therapy vector according to claim 5, further comprising an AAV capsid that selectively transduces target neurons.

7. A gene therapy vector comprising an AAV capsid that selectively transduces target sensory-motor neurons, and a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16.

8. The gene therapy vector according to any one of claims 6 or 7 for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective can be effectively administered to the subject.

9. The gene therapy vector according to claim 7, for use in upregulating expression of the Kv7.3 ion channel subunit by the target sensory-motor neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased sensory-motor neuron excitability.

10. The method according to any one of claims 2 or 3 or the gene therapy vector according to claim 8 wherein the voltage-gated potassium channel-targeting drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

11. The method or the gene therapy vector according to any preceding claim, wherein the Kv7.3 ion channel is expressed as a Kv7.3 homomer, a Kv7.2/7.3 heteromer, or a Kv7.3/7.5 heteromer.

12. The method or gene therapy vector according to claim 11, wherein the Kv7.3 ion channel is functionally expressed as a homomer.

13. The method or the gene therapy vector according to any preceding claim, wherein the target neurons are motor neurons.

14. The method according to claim 4 or the gene therapy vector according to claim 8, wherein the neurological disorder is associated with increased excitability of neurons.

15. The method according to claim 4 or the gene therapy vector according to claim 8, wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain.

16. Use of the gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use in the treatment of conditions ameliorated by upregulating expression of Kv7.3 ion channels.

17. Use of the gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, ALS, nociceptive pain and non-nociceptive pain.

18. Use of a gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use as an anti-spasticity.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

Acorn IP LLP
Colin Sanders Business Innovation Centre
Mewburn Road
Banbury
Oxfordshire OX16 9PA
ROYAUME UNI

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 20 August 2024 (20-08-2024)
Applicant's or agent's file reference SANI3WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IB2024/054426	International filing date *(day/month/year)* 7 May 2024 (07-05-2024)
Applicant SANIA RX LTD	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   How? Directly to the International Bureau preferably through ePCT, or on paper to:
   The International Bureau of WIPO, 34 chemin des Colombettes, 1211 Geneva 20, Switzerland

   For more detailed instructions, see the *PCT Applicant's Guide,* International Phase, paragraphs 9.004 - 9.011.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months f**rom the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040	Authorized officer HAHN, Catriona Tel: +49 (0)89 2399-7713

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference SANI3WO	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/IB2024/054426	International filing date *(day/month/year)* 7 May 2024 (07-05-2024)	(Earliest) Priority Date *(day/month/year)* 10 May 2023 (10-05-2023)
Applicant SANIA RX LTD		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 2A
      [X] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/054426

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/054426

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86 A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIU MIN ET AL: "Inactivation of the Lateral Hypothalamus Attenuates Methamphetamine-Induced Conditioned Place Preference through Regulation of Kcnq3 Expression", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 23, no. 13, 30 June 2022 (2022-06-30), page 7305, XP093171968, Basel, CH ISSN: 1422-0067, DOI: 10.3390/ijms23137305 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9266452/pdf/ijms-23-07305.pdf> page 2, 1st para;section 4.3; page 7 ----- -/--	1-25

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2024	20/08/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merkl, Philipp

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/054426

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 883 812 B2 (CLAFFEY MICHELLE MARIE [US]; DAVOREN JENNIFER ELIZABETH [US] ET AL.) 11 November 2014 (2014-11-11) background section; col 11	6

X	FRIEDMAN ALLYSON K. ET AL: "KCNQ channel openers reverse depressive symptoms via an active resilience mechanism", NATURE COMMUNICATIONS, vol. 7, no. 1, 24 May 2016 (2016-05-24), XP055775444, DOI: 10.1038/ncomms11671 Retrieved from the Internet: URL:http://www.nature.com/articles/ncomms11671>	1-24
Y	abstract, Fig 1	25

A	WO 2021/119018 A1 (UNIV LELAND STANFORD JUNIOR [US]) 17 June 2021 (2021-06-17) 0008, 0052	1-25

Y	FLOETER MARY KAY ET AL: "Motor Neuron Firing Dysfunction in Spastic Patients With Primary Lateral Sclerosis", JOURNAL OF NEUROPHYSIOLOGY, vol. 94, no. 2, 1 August 2005 (2005-08-01), pages 919-927, XP093191940, US ISSN: 0022-3077, DOI: 10.1152/jn.00185.2005 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360205/pdf/nihms7360.pdf> abstract	25

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/054426

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 8883812 B2	11-11-2014	AU 2011275393 A1	10-01-2013
		CA 2804165 A1	12-01-2012
		CN 102971307 A	13-03-2013
		EP 2590961 A1	15-05-2013
		JP 5763758 B2	12-08-2015
		JP 2013531006 A	01-08-2013
		KR 20130031323 A	28-03-2013
		SG 186409 A1	28-02-2013
		US 2013150391 A1	13-06-2013
		WO 2012004698 A1	12-01-2012
		ZA 201300499 B	25-09-2013
WO 2021119018 A1	17-06-2021	NONE	

Information on Search Strategy

The type of information contained in this sheet may change during the pilot for improving the usefulness of this new service.

Application Number

PCT/IB2024/054426

TITLE: METHOD

APPLICANT: SANIA RX LTD

IPC CLASSIFICATION: C12N15/86, A61K48/00

EXAMINER: Merkl, Philipp

CONSULTED DATABASES: NPL, INET

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC: C12N15/86, A61K48/005, C12N2750/14143

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:
gene therapy vectors harboring a coding sequence for the ion channel Kv7.3 and uses thereof in modulating firing activity of neurons, particularly in combination with drugs

XS CPRTENFRDE

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2024/054426	07.05.2024	10.05.2023

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/86 A61K48/00

Applicant
SANIA RX LTD

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0	see form PCT/ISA/210	Merkl, Philipp Telephone No. -0

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2024/054426

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	25
	No:	Claims	1-24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-25
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Summary of the application

The application is concerned with provision of methods which reduce the side effects of neuromodulatory drugs/increases their efficacy and respective gene therapy vectors.

In the example, neuronal cells derived from iPSCs were transduced with AAV harboring a hKCNQ3 gene sequence (encoding Kv7.3). In combination with addition of the drug retigabine, less action potentials were observed in Kv7.3 transduced cells (Fig 4, 5, 6) and excitability is reduced (Fig 7) and rheobase is increased (Fig 8). Fig. 9-12 are dedicated to further effects of the Kv7.3 expression.

In addition, mice from a model of spasticity were treated with AAV Kv7.3 and shorter spasm duration and intensity was observed in the treated mice. Further mice were treated with Kv7.3 A315T. Such treated neurons were found not to be able to fire repetitively.

2 Citations

Reference is made to the following documents:

D1 LIU MIN ET AL: "Inactivation of the Lateral Hypothalamus Attenuates Methamphetamine-Induced Conditioned Place Preference through Regulation of Kcnq3 Expression",
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES,
vol. 23, no. 13, 30 June 2022 (2022-06-30), page 7305, XP093171968,
Basel, CH
ISSN: 1422-0067, DOI: 10.3390/ijms23137305
Retrieved from the Internet:
URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9266452/pdf/ijms-23-07305.pdf

D2 US 8 883 812 B2 (CLAFFEY MICHELLE MARIE [US]; DAVOREN JENNIFER ELIZABETH [US] ET AL.) 11 November 2014 (2014-11-11)

D3 FRIEDMAN ALLYSON K. ET AL: "KCNQ channel openers reverse depressive symptoms via an active resilience mechanism", NATURE COMMUNICATIONS, vol. 7, no. 1, 24 May 2016 (2016-05-24), XP055775444, DOI: 10.1038/ncomms11671 Retrieved from the Internet: URL:http://www.nature.com/articles/ncomms11671

D4 WO 2021/119018 A1 (UNIV LELAND STANFORD JUNIOR [US]) 17 June 2021 (2021-06-17)

D5 FLOETER MARY KAY ET AL: "Motor Neuron Firing Dysfunction in Spastic Patients With Primary Lateral Sclerosis", JOURNAL OF NEUROPHYSIOLOGY, vol. 94, no. 2, 1 August 2005 (2005-08-01), pages 919-927, XP093191940, US ISSN: 0022-3077, DOI: 10.1152/jn.00185.2005 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360205/pdf/nihms7360.pdf

Summary of D1-D5 see below.

D4 aims at improving the sleep quality of elderly patients. It states that aged Hcrt neurons are more excitable which leads to sleep fragmentation [0008]. It proposes as a solution to manipulate the Kv7 channel, including Kv7.3 or 7.2 by administration of agents which open the channel. The document further discloses that retigabine is a KCNQ (=Kv7) channel opener (052). Further presented are experiments wherein the KCNQ 2/3 genes are disrupted via AAV mediated Cas/CRISPR action. This was found to lead to hyperexcitability of the Hcrt neurons.

3 **Article 33(2) PCT**

The present application does not meet the requirements of Article 33(1) PCT because the subject-matter of claims 1-24 is not new within the meaning of Article 33(2) PCT.

3.1 D1 aims at modulating excitability of neurons by Kcnq (Kv7) channels (page 2, 1st para). In this regard, an AAV CMV Kcnq3 WPRE is described (section 4.3), which expresses Kv7.3. Section 2.6 describes a method in which a nucleotide sequence encoding a Kv7.3 ion channel subunit is overexpressed in a neuronal cell and a neuromodulatory drug (metamphetamine) is administered. The document teaches that the frequency of excitatory postsynaptic currents was reduced in AAV Kv7.3 infected neurons (page 7).

In view of the many desiderata in the claims (see Item VIII), the following is noted: The effect that expression of a transgene has is an inherent feature of the transgene and the cell type in which it is expressed. Hence, either all the alleged effects are inherently present and thus not new or they are not present and it is not disclosed how the effects would be achieved (Art. 5 PCT).

It is noted that SEQ IDs 1-8 encompass the WT Kv7.3 coding sequence which is thus deemed implicitly disclosed.

Thus, in view of D1, the subject-matter of claims 1-10, 12-22 does not meet the requirements of Art. 33(2) PCT.

3.2 D2 is concerned with dimethoxy-pyrimidine amides and their use in treatment of CNS disorders, including epilepsy. Said compounds are openers of the Kv7.2/7.3 potassium channels. Channel opening is known do decrease neuronal excitability and retigabine is named as a known drug for channel opening (background section). The document discloses transfection vectors harboring human Kv7.2 and 7.3 channel subunits (col 11).

Thus, in view of D2, the subject-matter of claim 6 does not meet the requirements of Art. 33(2) PCT.

3.3 D3 discloses that overexpression of KCNQ (HSV mediated) channels in the VTA dopaminergic neurons and administration of retigabine normalizes neuronal hyperactivity and depressive behaviour. Overexpression resulted in lover neuron firing activity (abstract, Fig 1).

Thus, in view of D3, the subject-matter of claims 1-7, 11-24 does not meet the requirements of Art. 33(2) PCT.

3.4 The subject-matter of claim 25 is not described in any of D1-D4 and thus novel over the prior art (Article 33(2) PCT).

4 Article 33(3) PCT

The present application does not meet the requirements of Article 33(3) PCT because the subject-matter of claim 25 does not involve an inventive step.

4.1 Either of D1-D4 can be considered closest prior art for claim 25. For the purpose of this opinion, D3 is used. Summary see above.

The subject-matter of claim 25 therefore differs from the disclosed use of the gene therapy vector in that it is not declared as for use as an anti spasticity.

No technical effect can be ascribed to this technical difference as per the application.

Therefore, the problem needs to be reformulated in a less ambitious way (GL G-VII, 5.2, T 87/08 headnote)

The problem to be solved by the present application therefore may be regarded as provision of an alternative gene therapy vector.

D5 discloses that spasticity is due to elevated firing of the respective neurons. Hence, a person skilled in the art looking for a therapy for spasticity knows that the firing must be reduced. D3 discloses gene therapy vectors which do exactly this in combination with retigabine.

A person skilled in the art would have come to the conclusion to combine the teachings of D3 and D5 to arrive at the solution proposed by the application.

Thus, in view of D3 and D5, the subject-matter of claim 25 does not meet the requirements of Article 33(3) PCT.

5 Article 33(4) PCT

Claims 1-25 are industrially applicable.

6 The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Patentability, in particular novelty and inventive step, of claims 1-5, 11-25 has been assessed on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

Re Item VIII

Certain observations on the international application

7 **Article 6 PCT**

The application does not meet the requirements of Article 6 PCT because claims 1-10, 15-23 are not clear.

7.1 Claims 1-10, 15-23 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("increasing efficacy, reducing side effects, increasing therapeutic index, reducing excitability, such that it forms functional ion channels, selectively transduces, is upregulated etc."). Such a definition is only allowable under the conditions elaborated in the Guidelines EPO/PCT F-IV, 4.10. In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

7.2 In this regard, most of the terms cited above are relative terms in addition which lack a point of reference. E.g. claim 1 increasing efficacy against what baseline? Overexpressing compared to what? Lower dose as compared to what?

Hence, claims 1-10, 15-23 comprise ambiguous terms which are not suitable to clearly define the subject-matter of the claim.

7.3 The application contains 25 claims of which 13 are independent claims. The application thus lacks conciseness and the set of claims needs to be consolidated. Specific attention should be paid to exemplary claims 1-5 which describe the same method but differ merely in the alleged desired effect. Furthermore, it should suffice to claim only the nucleotide sequence or the protein sequence in an allowable independent claim.

7.4 Claim 1 further uses the word "neuromodulatory drug", which is an ambiguous term. Any substance can be defined as neuromodulatory, even water, as dehydration will lead to neuromodulatory effects.

7.5 Hence, if construed in its broadest sense, claim 1 refers to a method comprising introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit into a neuronal cell, expressing the Kv7.3 ion channel subunit, followed by administration of a drug, wherein the nucleotide sequences are either of SEQ ID 1-8.

7.6 Several claims refer to a method or a gene therapy vector. A claim must have one single category (product or method) and hence the category of the respective claims is not clear.

Claims

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug than would normally be administered to a subject in order to be therapeutically effective

    wherein the neuromodulatory drug is a voltage gated potassium channel-targeting drug.

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

3. A method of reducing the excitability of a neuron comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 to the target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. optionally administering a voltage-gated potassium channel-targeting drug.

4. A method of treating a neurological disorder comprising the steps:
    a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,
    b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and
    c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels than would normally be administered to the subject in order to be therapeutically effective.

5. A gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

6. The gene therapy vector according to claim 5, further comprising an AAV capsid that selectively transduces target neurons.

7. A gene therapy vector comprising an AAV capsid that selectively transduces target sensory-motor neurons, and a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16.

8. The gene therapy vector according to any one of claims 5 to 7 for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective can be effectively administered to the subject.

9. The gene therapy vector according to any one of claims 5 to 7 for use in reducing adverse effects of a voltage-gated potassium channel-targeting drug in a subject being treated therewith, relative to a subject not receiving the gene therapy vector, by increasing the subject's sensitivity to the voltage-gated potassium channel-targeting drug, and thereby lowering the dose of voltage-gated potassium channel-targeting drug required to be therapeutically effective.

10. The gene therapy vector according to claim 7, for use in upregulating expression of the Kv7.3 ion channel subunit by the target sensory-motor neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased sensory-motor neuron excitability.

11. The gene therapy vector according to any one of claims 5 to 7 for use in the treatment of a neurological disorder associated with increased neuronal excitability to be administered to a subject a day or more before administration of a voltage-gated potassium channel-targeting drug.

12. The method according to any one of claims 1 to 4 or the gene therapy vector according to claim 8, claim 9, or claim 11 wherein the voltage-gated potassium channel-targeting drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

13. The method or the gene therapy vector according to any preceding claim, wherein the Kv7.3 ion channel is expressed as a Kv7.3 homomer, a Kv7.2/7.3 heteromer, or a Kv7.3/7.5 heteromer.

14. The method or gene therapy vector according to claim 13, wherein the Kv7.3 ion channel is functionally expressed as a homomer.

15. The method or the gene therapy vector according to any preceding claim, wherein the target neurons are motor neurons.

16. The method according to claim 4 or the gene therapy vector according to any one of claims 8, 10, or 11, wherein the neurological disorder is associated with increased excitability of neurons.

17. The method according to claim 4 or the gene therapy vector according to any one of claims 8, 10, or 11, wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain.

18. Use of the gene therapy vector according to any one of claims 5 to 17 in the preparation of a medicament for use in the treatment of conditions ameliorated by upregulating expression of Kv7.3 ion channels.

19. Use of the gene therapy vector according to any one of claims 5 to 17 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, ALS, nociceptive pain and non-nociceptive pain.

20. Use of a gene therapy vector according to any one of claims 5 to 17 in the preparation of a medicament for use as an anti-spasticity.

METHOD

Field of the Invention

The present invention relates to methods of reducing the side effects of neuromodulatory drugs, to increasing the efficacy of neuromodulatory drugs and to methods of reducing the excitability of neurons. The invention also relates to gene therapy vectors useful in carrying out the methods of the invention.

Background to the Invention

Neurological disorders are characterised by abnormal activity in neurons and circuits within the central nervous system (CNS). Often, this abnormal activity is conveyed to the output neurons of the CNS, motor neurons, leading to inappropriate muscle contractions and sensory and motor dysfunction.

Pharmaceutical therapies have been developed aimed at ameliorating the abnormal activity typically by modulating ion channels that carry excitatory and inhibitory currents. However, drug delivery to the nervous system is difficult and existing therapies often have undesirable side effects due to low specificity.

Thus, although neuromodulatory drugs developed for neurological disorders have shown some therapeutic benefit, this has been limited by off-target effects on non-dysfunctional neurons and other systems expressing the target (cardiovascular, respiratory, gastrointestinal etc).

Potassium (K^+) channels are membrane proteins that allow rapid and selective flow of K^+ ions across the cell membrane, and thus generate electrical signals in cells. Voltage-gated K^+ channels (Kv channels), present in all excitable animal cells, open and close upon changes in the transmembrane potential. Kv channels are one of the key components in generation and propagation of electrical impulses in nervous system. Upon changes in transmembrane potential, these channels open and allow passive flow of ions from the cell to restore the resting membrane potential. Activation of Kv channels is therefore considered inhibitory to action potential generation.

There are 40 human voltage-gated potassium channel genes belonging to 12 subfamilies (Kv1–Kv12). The subfamilies are typically restricted to specific locations or cell/tissue types and are observed in excitable and non-excitable tissues. Remarkable diversity of Kv channels may be achieved due to the mix and match of Kv channel subunits. Within each of the Kv1, Kv2, Kv3, Kv4 and Kv7 families, homomeric and heteromeric channels may form with a range of functional properties. Kv2 family members may also assemble with Kv5, Kv6, Kv8 or Kv9 family members with more restricted expression patterns in the nervous system and smooth muscles.

Voltage-gated potassium channels, in neurons, are targeted to various subcellular compartments, and channels of different subunit compositions may be present in different subpopulations of neurons.

The tetrameric structure of Kv channels is made of two functionally and structurally independent domains: an ion conduction pore, and voltage-sensor domains. The ion conduction pore is made of four subunits which are arranged symmetrically around the conduction pathway. Voltage-sensor domains are positioned at the periphery of the channel and consist of four transmembrane segments (S1-S4).

Structural rearrangement of the voltage-sensor domains in response to changes in the membrane potential, and in particular S4, which includes positively charged amino acids at every third position, results in conformational changes in the conduction pore, which could open or occlude the ion conduction pathway. The mechanism of voltage-gating is not fully understood.

The Kv7 family of low threshold voltage-gated potassium (K^+) channels consists of five members (Kv7.1-7.5) encoded for by the KCNQ1-5 genes, respectively. Kv7 channels assemble as tetramers of identical or compatible α subunits, with each α subunit consisting of six transmembrane segments (S1–S6) and cytoplasmic N- and C-termini. Kv7 is widely expressed in both the CNS and other tissues, with Kv7.2 and 7.3, in particular, being known to control excitability of neurons.

Kv7.2 and 7.3 channels normally form heteromers with each other to increase conductance and are rarely expressed as low conductance homomers. Kv7.2 and 7.3 are expressed in most neuronal subtypes and tend to be localised to the initial segment of the axon where action potentials (spikes) are initiated. Kv7.3 and Kv7.2 conductances are therefore considered particularly potent inhibitors of action potential generation and activity in the same neuron as well as its targets (for example, motor neuron inhibition reduces muscle activity).

Neuromodulatory drugs targeting Kv7 channels have been developed to treat disorders associated with neuronal hyperexcitability. Retigabine is an anticonvulsant that acts mainly as a Kv7.2-Kv7.5 potassium channel opener, and GABA positive allosteric modulator at higher concentrations. The term "channel opener" refers to a left shift in the voltage dependence for channel opening, towards more negative potentials. In the presence of Retigabine, Kv7 channels open at more negative potentials, thus contributing to membrane hyperpolarisation and voltage stabilisation.

It has also been shown that Retigabine stabilises the open Kv7.2/7.3 (heteromeric) channel, making deactivation slower with little change in voltage dependence of deactivation. The overall effect is greater potassium conductance, hyperpolarisation of the membrane potential, and therefore inhibition of action potential generation. This effect of Retigabine is observed at concentrations below 10 µM, *in vitro* (Corbin-Leftwich et al 2016; Villalba-Galea 2020). Orally administered Retigabine at 600-1200 mg per day, which corresponds to a mean plasma concentration of 0.83 µM (Gunthorpe et al., 2012), has been shown to have up to 45% clinical efficacy for seizure reduction in humans with epilepsy (Brodie et al., 2010; French et al., 2011). Of note, while the EC50 of Retigabine is 0.6 µM for Kv7.3 channels, most channels in the nervous system are Kv7.2/7.3 heteromers, for which the EC50 is 1.6 µM. For some patients, these doses resulted in eye and skin discolouration that led to restriction of the indication. Retigabine was later voluntarily withdrawn for commercial reasons (Brickel et al., 2020).

Pharmaceutical interest in Kv7 as a therapeutic target remains. At least 3 activators with increased potency, increased selectivity for Kv7.2/7.3 and a lack of cosmetic side effects are in clinical trials and have shown favourable outcomes. However, due to the ubiquity of Kv7.2/Kv7.3 expression in the nervous system, neuronal side effects (somnolence, fatigue etc) are currently unavoidable. Although new Kv7.2/7.3 activators have improved safety profiles, neural side effects can only be reduced by increasing selectivity for dysfunctional neurons only.

Chemogenetics is a method by which proteins are engineered to interact with previously unrecognised small molecule chemical actuators. Since the 1990s, a large number of chemogenetic platforms have

been developed that have been particularly useful for neuroscientists wishing to modulate neural activity in specific populations. Several classes of proteins have been engineered in this way, including kinases, non-kinase enzymes, ligand-gated ion channels and G-protein-coupled receptors (GPCRs). The most widely used of these are known as Designer Receptors Activated by Designer Drugs (DREADDs).

DREADDS are engineered GPCRs that respond to synthetic ligands that cross the blood brain barrier (such as clozapine-N-oxide and olanzapine), but not their natural ligand, acetylcholine.

In use, a viral vector inserts the gene that encodes the DREADD protein into the cell to be studied. Various viral serotypes, promoters, and administration routes can be used to help select the target cells. Once transduced, the infected cell takes two or three weeks to express the engineered receptor protein which can then be activated using a DREADD ligand. Thus, activity can be modulated specifically in the targeted neurons with minimal influence on off target cells.

Described herein is a method similar to that employed by DREADDs, in which an ion channel responsive to specific drugs is expressed in neurons in order to modulate activity. Importantly, the method flips the existing concept of designing specifically paired, de novo receptor and drug and repurposes wild type and mutant ion channels to reduce or eliminate side effects of drugs acting on the channels. The method overexpresses wild type and/or mutated channels.

Furthermore, the method described beneficially uses channels that can be either opened (agonists) or closed (antagonists) depending on the drug used. In direct contrast, DREADD activation induces unidirectional excitability changes (either excitatory or inhibitory).

Summary of the Invention

The present disclosure relates to a chemogenetic method that allows modulation of activity in targeted neurons, thus increasing drug efficacy while reducing side effects, at least in part, due to the ability to use a lower dose. The method involves using gene therapy methodology via a delivery vector (e.g. AAV) to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses of specific activating and inhibiting drugs. That is, the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs.

We have shown that AAV-mediated overexpression of the voltage gated potassium channel Kv7.3 (encoded by KCNQ3) enables a Kv7 activator, such as retigabine, to significantly reduce excitability of human neurons at the individual neuron and population level. At low concentrations (0.3-3µM), retigabine reduced action potential number in individual hIPSC-derived sensory and motor neurons. At the same doses, retigabine has minimal effect on control neurons expressing green fluorescent protein only. Using multielectrode arrays, we have shown that retigabine was also effective at selectively reducing spontaneous bursting activity from a network of thousands of MNs overexpressing Kv7.3, but not GFP expressing neurons. The in vitro results translated to a mouse model of spasticity, in which hyperactive MNs cause excessive muscle contractions (muscle spasms). In this model, overexpression of Kv7.3 in motor neurons via an intramuscular injection of an AAV-Kv7 construct enabled a low dose of retigabine (2mg/kg) to reduce the intensity and duration of muscle spasms. However, the same dose did not significantly reduce spasticity in mice injected with an AAV-GFP construct. The reduction in

excitability of MNs was confirmed as the mechanism for the reduction in spasticity as 3µM retigabine reduced AP firing in Kv7.3 expressing mouse motor neurons, but not those expressing GFP. Additionally, we have shown that introducing a point mutation at amino acid residue 315 (Zaika et al., 2008; Gómez-Posada et al., 2010) of the Kv7.3 protein reduces motor neuron excitability at baseline in vitro and in vivo and further increases the effect sizes of retigabine at the tested concentrations in vitro.

The therapy will use AAV to overexpress Kv7.3 ion channel subunits and variations thereof, either as homomers or Kv7.2/7.3 heteromers, in hyperexcitable neurons of patients with neurological disorders, and use low doses of channel modulators to normalise activity.

According to a first aspect there is provided a method of increasing the efficacy of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering a lower dose of the neuromodulatory drug.

Advantageously, by increasing the efficacy of the drug, a lower dose can be administered to obtain the same therapeutic effect. This, beneficially, means that the occurrence of side effects can be reduced.

Thus, in a second aspect there is provided a method of reducing the side effects of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering a lower dose of the neuromodulatory drug.

Side effects of neuromodulatory drugs can range from minor to serious and can affect a subject's quality of life. For example, for Retigabine the NIH lists the most common adverse effects, occurring in greater than 10% of subjects in the clinical trials, include abnormal gait, confusion, dizziness, fatigue, headache, nausea, somnolence, speech disorder, tremor, urinary tract infection, and blurred vision (Harris and Murphy, 2011).

In some cases, side effects (adverse effects) can lead to drugs being removed from the market because the dosage needed leads to unacceptable adverse effects. If the dose can be lowered, adverse effects can be reduced and use of the drug can potentially be restored.

In a third aspect there is provided a method of increasing the therapeutic index of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. administering the neuromodulatory drug.

A fourth aspect provides a gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

Advantageously, delivery of the nucleotide sequence to a population of specific target neurons results in the nucleotide payload only being transduced into the neurons that exhibit increased excitability. These neurons then overexpress the ion channels, presenting more targets for the neuromodulatory drugs which, in turn, allows a lower dosage of the drug to be effective, resulting in fewer side effects. Targeting neurons in this way also means that off target activity is less likely.

In a fifth aspect there is provided an AAV viral particle gene therapy vector comprising:

a. an AAV capsid that selectively transduces target neurons, and
b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

In a further aspect there is provided a gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in upregulating expression of the Kv7.3 ion channel subunit by the target neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased neuronal excitability.

In a yet further aspect there is provided a gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

In another aspect there is provided the use of a gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a

nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain, ALS.

Brief Description of the Drawings

For a better understanding of the invention and to show how the same may be carried into effect, there will now be described by way of example only, specific embodiments, methods and processes according to the present invention with reference to the accompanying drawings in which:

Figure 1 shows the AAV constructs used to transduce neurons with either Kv7.3 (upper construct) or Kv7.3 A315T (lower construct). Note that a GFP sequence was included downstream of Kv7.3 and Kv7.3 A315T via T2A linkers, meaning GFP fluorescence is indicative of Kv7.3 expression.

Figure 2 shows that AAV transduction of hIPSC neurons leads to expression of proteins encoded by transgenes packaged into the AAV constructs (A). Specifically, in the presented example, hIPSC neurons were transduced with AAVs to induce expression of either green fluorescent protein (AAV-GFP) or human Kv7.3 channels and GFP (AAV-Kv7.3).

Figure 2 also shows the endpoints assessed using patch clamp electrophysiology at baseline (expression of Kv7.3 or GFP without activator drug (B), and following exposure to activator (retigabine, C). The endpoints were: rheobase (minimum current input to evoke 1 action potential), number of action potentials at 3x rheobase, and resting membrane potential. In the presence of retigabine, the excitability of the neurons is reduced as shown by an increase in the rheobase, a decrease in the number of action potentials at 3x rheobase and a hyperpolarisation of the resting membrane potential.

Figure 3 shows that baseline overexpression of Kv7.3 does not significantly alter the excitability of hIPSC sensory neurons compared to control neurons transduced with AAV -GFP. Panel (A) shows the mean number of action potentials generated at each current input value for AAV-GFP (dashed line) and AAV-Kv7.3 (solid line) transduced neurons. Shaded areas show 95% confidence intervals. Excitability levels were determined to be similar due to the extremely small effect sizes measured for APs/ 500ms (B), RMP (C) and Rheobase (D). In these Cummings estimation plots, individual points represent number of action potentials generated at 3x rheobase for neurons transduced with AAV-GFP (light grey) or AAV-Kv7.3 (dark grey). On the right hand side of the plot, Hedges G effect sizes and 95 % confidence intervals are plotted for bootstrap resampled (1000 repeats) data.

Figure 4 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM had limited effect on excitability of hIPSC sensory neurons transduced with AAV-GFP as determined by measuring the number of action potentials at 3x rheobase. However, large and very large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3. In each of the estimation plots (A, B and C), the upper graphs are line plots representing the change in AP number between recordings made before and after exposure to retigabine. Lower plots show the Hedges G effect sizes and confidence intervals.

Figure 5 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM had limited effects on excitability of hIPSC sensory neurons transduced with the AAV-GFP as determined by measuring the rheobase (minimum current to generate 1 action potential). However, large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3.

Figure 6 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM had small and medium effects on excitability of hIPSC sensory neurons transduced with the AAV-GFP as determined by measuring the resting membrane potential (RMP). However, large and very large effect sizes were measured at the same concentrations for neurons transduced with AAV-Kv7.3.

Figure 7 shows that baseline expression of $Kv7.3_{A315T}$ (dashed line) significantly reduces the excitability of hIPSC sensory neurons. Excitability levels were shown to be decreased due to the inability of AAV-$Kv7.3_{A315T}$ transduced neurons to generate more than 3 action potentials (A, B). RMP was also hyperpolarised at baseline (C) but Rheobase was not substantially changed (D).

Figure 8 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM caused very large increases in the rheobase of AAV- $Kv7.3_{A315T}$ transduced neurons. Effect sizes were at least 2-3 times larger for AAV-$Kv7.3_{A315T}$ as compared with AAV-GFP. Note the differences in scale for both rheobase (primary axis), and Paired Hedges g effect size (secondary axis)

Figure 9 shows that retigabine delivered at concentrations of 0.3 µM, 1 µM, and 3 µM caused very large effects on hyperpolarisations of the RMP in hIPSC sensory neurons transduced with AAV- $Kv7.3_{A315T}$. Effect sizes were also at least 2-3 times larger than hIPSC neurons transduced with AAV-GFP.

Figure 10 shows Kv7.3 overexpression in hIPSC motor neurons by western blot (A) and ELISA (B). Note the strong bands at around 80 kDa.

Figure 11 shows that overexpression of Kv7.3 in hIPSC derived motor neurons induces a small baseline reduction in the frequency-current relationship (A-B), however action potential firing at 3 x rheobase (C) and rheobase (D) were unaffected. (E-H) Kv7.3 overexpression increased the sensitivity of hIPSC MNs to retigabine. Concentrations under 3uM had no effect on the excitability of AAV-GFP transduced neurons but reduced AP firing by up to 80% in AAV-Kv7.3 transduced neurons. Retigabine efficacy was also increased by Kv7.3 overexpression as demonstrated by the increase in the maximal effects on AP frequency (G), and FI slope (H).

Figure 12 shows that Kv7.3 overexpression increases the sensitivity of MNs to RTG at the population level, as measured using a multielectrode array (MEA) plate. hIPSC MNs are grown in a well plate with electrodes etched into the base so that spontaneous activity can be recorded. 50% of the wells are treated with AAV-GFP and 50% with AAV-Kv7.3. After sufficient transduction time, spontaneous activity is recorded at baseline (0 µM) and then increasing concentrations of RTG. RTG does not affect the viability of motor neurons, assessed using electrode impedance (A). However, MNs treated with RTG show greater sensitivity to RTG as evidenced by the leftward shift in the dose response for retigabine and spontaneous action potential burst frequency (B), burst duration (C) or total number of spikes. E-F show representative examples of activity recorded in wells treated with AAV-GFP (E) or AAV-Kv7.3 (F) after adding 1.8 µM (RTG). Vertical lines in top panels show network burst frequency. Below are raster plots of action potential firing recorded by each of the 16 electrodes (rows). G-H show representative

examples of individual bursts from MNs in wells treated with AAV-GFP (G) or AAV-Kv7.3 (H). Each row is spiking activity recorded by a single electrode. The right of each plot is a heat legend indicating the change in firing frequency.

Figure 13 shows that in a neonatal mouse model of spasticity, induced by spinal cord injury, Kv7.3 can be successfully over expressed in motor neurons following intramuscular injection of AAV-Kv7.3. (A) shows a Kv7.3 western blot of spinal cords taken 2 weeks after injection and spinal cord injury. Note the strong bands at the expected molecular weight for Kv7.3 in the spinal cord samples from AAV-Kv7.3 treated mice but not the AAV-GFP treated mice. (B) shows Kv7.3 expression specifically in MNs using immunohistochemistry. (C-D) show changes in the duration of muscle spasms after administration of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. E-F shows the change spasm intensity (number of action potentials per spasm) after administration of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. G-H are representative electromyography (EMG) traces showing the effect of 2mg/kg RTG in AAV-GFP treated and AAV-Kv7.3 treated mice. Top traces are pre RTG and lower traces are 20 minutes after RTG.

Figure 14 shows that Kv7.3 overexpression in motor neurons via an intramuscular injection of AAV-Kv7.3 does not significantly affect the baseline electrophysiological properties of motor neurons in a neonatal mouse model of spasticity. Recordings were made from spinal cord slices taken from mice with spasticity. (A) shows that the excitability of MNs was not reduced as the number of action potentials evoked at 2 x rheobase not different between AAV-GFP controls and AAV-SRx-C490 transduced MNs. (B) shows that the threshold for evoking a single action potential was not different in mice AAV-SRx-C490 transduced MNs. (C) shows that the resting membrane potential (RMP) was not different between the groups. (D) shows that MN size was not different between the groups. (E-H) shows that 3 µM retigabine significantly reduced excitability in MNs from AAV-SRxC490 treated mice but not AAV-GFP treated mice, as assessed by the change in number of action potentials at 2 x threshold and the rheobase.

Figure 15 shows that motor neurons in mice with spasticity treated with AAV-GFP fire repetitive trains of action potentials (A), however motor neurons from mice injected with a viral construct to overexpress a mutated form of Kv7 (A315T) are incapable of firing repetitively. This suggests a significant and permanent reduction in excitability of these motor neurons.

Detailed Description

As employed herein "increasing the efficacy" means an increase in the ability to produce the desired result. This may be achieved by improving the response to a drug rather than improving the effectiveness of the drug. Improved efficacy means that the same level of response can be achieved using less of the drug.

Therefore, increasing the efficacy means that a lower dosage can be used.

As employed herein neuromodulatory drug refers to a voltage gated potassium channel-targeting drug. Known drugs include, but are not limited to, Retigabine and derivatives thereof (see Musella et al., 2022 for examples), BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123, linopirdine.

In one embodiment the neuromodulatory drug is a channel opener.

In one embodiment the neuromodulatory drug is a channel blocker.

In one embodiment the neuromodulatory drug is selected from: Retigabine or a derivative thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123, linopirdine.

As employed herein introducing a nucleotide sequence refers to the transduction or transfection of a cell, such as a neuron, with one or more nucleotide sequences to either supplement existing genetic material with more of what is already present, or to provide new nucleotide sequences.

Kv7.3 ion channel subunit is a monomer of a voltage-gated potassium channel which assembled to form a tetrameric function ion channel. Kv7.3 can assemble as a homomer or as a heteromer with other Kv7 subunits, including Kv7.2 and Kv7.5, or other auxiliary subunits (e.g. KCNE).

Kv7.3 ion channels as employed herein is intended to refer to both Kv7.3 homomer and Kv7.3-containing heteromers.

As employed herein a nucleotide sequence encoding a Kv7.3 ion channel subunit refers to a nucleotide sequence (RNA or DNA) encoding the Kv7.3 ion channel subunit.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.1 or a variant of SEQ ID.1 encoding the same amino acid sequence encoded by SEQ ID.1.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.2 or a variant of SEQ ID.2 encoding the same amino acid sequence encoded by SEQ ID.2.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.3 or a variant of SEQ ID.3 encoding the same amino acid sequence encoded by SEQ ID.3.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.4 or a variant of SEQ ID.4 encoding the same amino acid sequence encoded by SEQ ID.4.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.5 or a variant of SEQ ID.5 encoding the same amino acid sequence encoded by SEQ ID.5.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.6 or a variant of SEQ ID.6 encoding the same amino acid sequence encoded by SEQ ID.6.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.7 or a variant of SEQ ID.7 encoding the same amino acid sequence encoded by SEQ ID.7.

In one embodiment the nucleotide sequence comprises the sequence of SEQ ID.8 or a variant of SEQ ID.8 encoding the same amino acid sequence encoded by SEQ ID.8.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.9.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.10.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.11.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.12.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.13.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.14.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.15.

In one embodiment the Kv7.3 ion channel subunit comprises or consists of the amino acid sequence according to SEQ ID.16.

In one embodiment the nucleotide sequence encodes the Kv7.3 ion channel subunit according to any one of SEQ ID 9 to 16.

In certain embodiments the Kv7.3 ion channel subunit may comprise a mutation at amino acid residue 315 of SEQ ID.9. For example, A315T, A315S, A315V, A315C, A315N, A315Q or A315Y.

In one embodiment the ion channel subunit is wild type Kv7.3.

In one embodiment the ion channel subunit is not wild type Kv7.3.

Functional ion channel as employed herein means that the ion channel subunit has assembled into a tetramer, either as a homomer or as a heteromer, and is located at the neuron membrane in a position suitable to act as a voltage-gated potassium channel.

As employed herein, the term neuron includes a neuron and a portion or portions thereof (e.g. the neuron cell body, an axon and/or a dendrite). The term neuron denotes nervous system cells that are electrically active and include a cell body (or soma) and up to two types of extensions or projections: dendrites, by which the majority of neuronal signals are conveyed to the cell body, and axons, by which the majority of neuronal signals are conveyed from the cell body to effector cells, such as target neurons or muscle. Neurons can convey information from tissues and organs into the central nervous system (afferent or sensory neurons) and transmit signals from the central nervous systems to effector cells (efferent or motor neurons). Other neurons, designated interneurons, connect neurons within the central nervous system (the brain and spinal cord). Yet more neurons, designated “projection” neurons, extend their axons from one region of the nervous system to another.

In some embodiments the neuron is involved in sensory-motor activity.

In some embodiments the neuron is a motor neuron (motoneuron).

In some embodiments the neuron is a sensory neuron.

In some embodiments the neuron is an autonomic efferent neuron.

In some embodiments the neuron is an autonomic sensory neuron.

In some embodiments the neuron is subthalamic nucleus neuron.

As employed herein a vector refers to any suitable gene delivery vector as known in the art. Including, but not limited to, viral vectors (including adeno-associated virus, adenovirus and lentivirus) and non-viral methods (such as naked DNA or chemically assisted delivery methods). Typically, the vector is a gene therapy vector.

Overexpressing or overexpression as employed herein is synonymous with upregulation of a gene and intended to refer to an increase in the expression of a gene relative to a cell (neuron) that has not been transfected or transduced. To be effective, the neuron should be expressing higher levels of assembled Kv7.3-containing ion channels, either as Kv7.3 homomers or as heteromers, for example Kv7.2/7.3 heteromers.

As employed herein "administering a lower dose" refers to the subsequent exposure of a neuron that is overexpressing the introduced gene to a lower dose of a drug than would normally be administered to a patient in order to be therapeutically effective. For example, to treat epilepsy, Retigabine was typically administered at a dose of 600-1200 mg per day, which corresponds to a mean plasma concentration of 0.83 µM. Using the current method, we have shown that concentrations lower than 0.5 µM are effective in human neurons *in vitro*. Without wishing to be bound by theory, the present inventors consider that this would enable an effective dose lower than 0.5 µM to be administered *in vivo*.

In one embodiment the therapeutically effective dose of the neuromodulatory drug is reduced by up to 100%. For example, approximately 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 90%, 95%, 90% or 95%.

Whilst not wishing to be bound by theory, the inventors believe that overexpression of Kv7.3 may have a baseline effect that reduces excitability of neurons even in the absence of a neuromodulatory drug. Thus, in some embodiment administration of a neuromodulatory drug is not required.

It is expected that the reduction in therapeutically effective dose is sufficient to reduce side effects of the neuromodulatory drug to clinically acceptable levels. Clinically acceptable levels will be understood by those skilled in the art.

As employed herein "increasing drug efficacy" refers to the increased therapeutic effect of a given drug dose in transduced neurons that are overexpressing the transgene relative to non-transduced neurons.

As employed herein "reducing the side effects" refers to the lowering or elimination of undesirable side effects related to administration of a drug to a subject at a given dose.

For example, the neuromodulatory drug retigabine (ezogabine), which was marketed as an anticonvulsant, was found to cause drowsiness, dizziness, tinnitus and vertigo, confusion, and slurred speech at therapeutic doses (600-1200 mg). Less common side effects included tremor, memory loss, gait disturbances, and double vision. In 2013 the FDA warned the public that ezogabine can cause blue skin discoloration and eye abnormalities characterised by pigment changes in the retina. Whilst not wishing to be bound by theory, the inventors consider that increasing Kv7.3 expression in hyperexcitable neurons associated with epileptic seizures would be expected to shift the therapeutic range for retigabine to include lower doses, thereby reducing or eliminating above side effects. That is, the therapeutic index for retigabine would be increased. Indeed, trials including lower doses of retigabine (300-1200 mg) reported fewer dermatological, ophthalmological, and neurological adverse effects (Lerche et al., 2015; Brickel et al., 2020).

As employed herein, gene therapy vector is a vector suitable for gene therapy methods. Although AAV capsids are described herein, it will be appreciated that other vectors may be suitable and used without deviating from the scope of the present invention.

Typically, the gene therapy will be used to treat a neurological disorder or condition. One example of a neurological disorder or condition that can be treated by the gene therapy is spasticity. Spasticity is a neurological symptom suffered by people with a variety of neurological disorders, including but not limited to multiple sclerosis, stroke, traumatic brain injury, spinal cord injury, motor neuron disease and cerebral palsy. Spasticity results from excessive excitation of muscle by motor neurons, which, because of the disease, become “hyperexcitable.”

The excitability of neurons is determined by the frequency of action potentials generated in response to a given stimulus. Increased excitability is indicated by one or more of the measures described below.

Increased excitability as employed herein is defined as a neuron displaying at least one of the following:

a. An increase (depolarisation) of a neuron’s resting voltage (Resting membrane potential-RMP) such that it is closer to the threshold for generation of an action potential.
b. A decrease in the stimulus magnitude required to cause neurons to generate action potentials (Rheobase).
c. An increase in the number of action potentials generated by a given stimulus (Frequency) compared to a neuron with “normal” levels of excitability.
d. Persistent generation of action potentials beyond the cessation of a stimulus responsible for initiating firing.
e. An increase in the frequency of action potentials in a population of neurons (2-100,000) recorded using an array of microelectrodes (microelectrode array-MEA)
f. An increase in the number or duration of action potential bursts recorded by an MEA, defined as at least 5 consecutive action potentials with intervals of 100ms or less between each action potential.

For example, a hyperexcitable neuron may have a RMP of -55 mV which is roughly 15 mV more positive than is expected of neurons, and closer to the threshold for action potential generation (~ -40 mV). If a stimulus magnitude of 50 picoamperes (pA) causes a normal neuron to generate an action potential (Rheobase = 50 pA), 25 pA may be sufficient in a hyperexcitable neuron (Rheobase = 25 pA). Similarly, if

a stimulus magnitude of 100 pA causes a normal neuron to generate 10 action potentials in 1 second (Frequency= 10Hz), a hyperexcitable neuron may generate 20 at the same stimulus magnitude (Frequency= 20Hz).

Additionally, action potential firing is often sustained beyond the cessation of the stimulus in hyperexcitable neurons but not normal neurons. This is the case in spasticity, whereby the neurons fire more action potentials for longer time periods, and often in response to a lower stimulus.

Typically, the gene therapy vector is an AAV vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit. The ion channel subunit may be a wild type as encoded by SEQ ID.1 or a mutant as encoded by SEQ ID.2, or equivalent sequence thereof that encodes the amino acid sequences of SEQ ID.3 or SEQ ID.4, respectively.

AAV viral particles, as employed herein, are adeno-associated virus viral particles comprising a single stranded DNA genome packaged within a viral envelope that is capable of tranducing a cell, e.g. a target neuron. In particular, the AAV viral particles employed herein include the nucleotide sequence according to SEQ ID.1 or SEQ ID.2. In some embodiments the viral particles may be devoid of replication-encoding nucleotides, that is, they are replication deficient.

AAVs are small viruses belonging to the genus dependoparvovirus containing a single strand of DNA, up to ~4.9 Kb. The AAV genome contains three capsid proteins VP1, VP2 and VP3, all of which are translated from one mRNA via alternate splicing. In the wild, multiple serotypes of AAV have been identified each with unique sequences of capsid gene, and hence distinct tropisms, although wild serotypes tend to be able to infect multiple tissue and cell types. These serotypes are denoted by numbers: AAV1, AAV2, etc. It has been shown that modification of capsid sequences via DNA recombination methods can generate non-native sequences with tailored properties and tropism directed towards (or against) particular cells or tissues, and that evade the immune system (Vandenberghe et al., 2009). As employed herein, the AAV viral particles selectively transduce target neurons, such as motor neurons.

AAV capsid as employed herein refers to the capsid of adeno-associated viruses. It is known that the AAV capsid is capable of remarkable selectivity due to its make up (the AAV capsid is composed of a mixture of VP1, VP2, and VP3 totalling 60 monomers arranged in icosahedral symmetry in a ratio of 1:1:10) and post-translational modifications. AAV capsid proteins contain 12 hypervariable surface regions, but the genome, in general, presents highly conserved replication and structural genes across serotypes.

The AAV capsid employed herein can selectively transduce a particular cell type such as neurons rather than muscle tissue and may selectively transduce target neurons over other neurons, even specific parts of neurons, such as the axon.

In use, the AAV capsid containing the nucleotide sequence according to any one of SEQ ID Nos: 1 to 8 (the viral particle) is delivered to a subject, the viral particle infects target neurons which become transduced neurons. The transduced neurons overexpress the amino acid sequences encoded by the nucleotide sequences, which assemble as functional Kv7.3-containing ion channels (either homomers or heteromers). In general, the time from delivery of the viral particles to the subject to overexpression of

the function ion channels is approximately 3 weeks. Once a suitable period of time has elapsed for the transduced neurons to over express the ion channels, the subject can be treated with a neuromodulatory drug, such a Retigabine, at a lower dose.

Selectively transduces as employed herein refers to the ability of the AAV capsid to selectively transduce one cell type in preference to another. The specificity may be neurons in preference to muscle cells, for example. Alternatively, it may be motor neurons in preference to sensory neurons, for example.

A subject as employed herein means a human or non-human mammal. Typically, the subject has a neurological disorder associated with increased excitability of neurons.

In some embodiments the subject has a neurological disorder associated with dysfunction of Kv7.3 ion channels.

In some embodiments the subject has symptoms characterised by neuronal hyperexcitability that may or may not be associated with dysfunction of Kv7.3 ion channels.

In some embodiments the subject has a neurological disorder and/or symptoms characterised by neuronal hyperexcitability that may or may not be associated with dysfunction of Kv channels.

Neurological disorder associated with increased neuronal excitability as employed herein refers to any condition in which excess neuronal activity leads to symptoms associated with the same neurological disorder. For example, epilepsy, spasticity, benign familial neonatal seizures (BNFS), nociceptive pain, non-nociceptive pain, Parkinson's disease, multiple sclerosis.

In one embodiment the neurological disorder is associated with hyperexcitability of neurons, such as motor neurons.

In one embodiment the neurological disorder is associated with increased excitability of neurons.

In one embodiment the neurological disorder is selected from epilepsy, spasticity, BNFS, nociceptive pain, non-nociceptive pain, Parkinson's disease, multiple sclerosis.

Treating or treatment as employed herein refers to the reversal of a condition, amelioration or relief of symptoms associated with a condition or prevention of further development/worsening of a condition.

The term "treatment" includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously. For example, the vector may be administered a day or more before the neuromodulatory drug is administered to the subject.

Lower dose as employed herein refers to a dose lower than that considered to be efficacious in a subject (or in the lower portion of an efficacious range), and therefore does not cause, or reduces severity of, side effects whilst maintaining therapeutic efficacy.

Therapeutically effective amount as employed herein means an amount of the compound, which is effective in treating the named disorder or condition.

In some embodiments the overexpressed Kv7.3 channels may assemble as heteromers, such as Kv7.2/7.3 or Kv7.3/7.5 heteromers. The subunit proportions of these heteromers may differ and allow refinement of the method described herein.

Similarly, in some embodiments the proportion of wild type to mutant Kv7.3 subunits may differ to allow refinement of the method. This could be true of both heteromers and homomers.

In some embodiments the overexpressed Kv7.3 channels may assemble as homomers.

Homomeric Kv7.3 channels may be minimally conducting such that they have minimal effect on baseline excitability of the transduced neuron, but still allow the neuromodulatory drug to act on the transduced neuron at a lower dose.

Rheobase as employed herein refers to the minimum current injection value required to depolarise neuronal membrane potential sufficiently to generate a single action potential.

Resting membrane potential as employed herein refers to the potential voltage difference recorded between the intracellular and extracellular neuronal compartments in the absence of any current injection. This voltage value is recorded using electrophysiology techniques such as whole cell patch clamp.

As employed herein "shifts the resting membrane potential (RMP) of the target neuron away from threshold" means that the voltage value in the neuron's resting state is more negative (hyperpolarised) and therefore a greater stimulus magnitude is required to depolarise the voltage to a level at which action potentials are generated. For example, a typical RMP of a neuron may be -65 mV, meaning 500 pA of current (stimulus) is needed to depolarise the neuron sufficiently to reach the threshold membrane potential for generating action potentials (-45 mV). If the RMP becomes more negative (-75 mV), then it is further away from threshold and therefore requires more current injection to reach threshold and generate action potentials.

Reduces the excitability of the target neuron as employed herein means at least one of the following :

a. Hyperpolarising of a neuron's resting membrane potential such that it is further away from the threshold for generation of an action potential.
b. Increasing the stimulus magnitude required to cause neurons to generate action potentials (Rheobase).
c. Decreasing the number of action potentials generated by a given electrical or chemical stimulus (AP Frequency).
d. Reducing or eliminating persistent generation of action potentials beyond the cessation of a stimulus responsible for initiating firing.
e. Reducing the frequency of spontaneous action potentials in the absence of a stimulus.
f. Reducing the duration or frequency of spontaneous action potential bursts (bursts are defined as at least 5 consecutive action potentials with intervals of 100 ms or less).

Reductions in excitability can be measured using whole cell patch clamp electrophysiology in current clamp mode by injecting rectangular current pulses of increasing magnitude and recording the change in voltage and frequency of action potential generation during the duration of the current pulse. Resting

membrane potential is measured under stable conditions, while there is no current injection (e.g. the voltage does not vary by more than (1-2 mV). Rheobase is measured as the minimum current input of a designated duration necessary to generate at least one action potential. Action potential frequency is measured as the number of action potentials generated over a specified time period, usually equal to the duration of a current pulse injected into a neuron.

Reductions in excitability can also be measured using a microelectrode array (MEA) consisting of a well plate with several electrode contacts located at the surface of the base of each well. A population of neurons is grown in contact with the electrodes in each well such that their activity can be recorded as extracellular action potentials (spikes). Neurons grown on MEAs produce spontaneous action potentials often referred to as spikes. Five or more consecutive spikes recorded with intervals of 100 ms or less are referred to as bursts. Spontaneous spike frequency and bursting characteristics (burst frequency, duration etc) may be used to determine neuronal excitability.

In some embodiments the upregulation of the Kv7.3 ion channel subunit by the transduced neuron reduces activity of cells innervated by axon terminals of the same transduced neuron. For example, reduced gastrocnemius muscle activity measured by electromyography (EMG) as a result of upregulation of Kv7.3 ion channel subunits in gastrocnemius motor neurons. Reduced muscle activity is characterised by 1 or more of the following: reduced amplitude of a compound muscle action evoked by stimulation of peripheral afferent nerve fibres; reduced duration of persistent EMG activity following stimulation of afferent nerve fibres; reduced frequency of spontaneous EMG activity; reduced duration of spontaneous EMG activity; reduced amplitude of spontaneous EMG activity; reduced stiffness of a muscle as determined by a trained clinician; and increased range of motion in the joints associated with the targeted muscle as determined by a trained clinician.

The invention also comprises methods of treatment which involves injecting AAV viral vectors comprising the nucleotide sequence of SEQ ID.1 or SEQ ID.2 into the brain or spinal cord of a subject, or by intramuscular injection; these AAV viral vectors can then transduce the target neurons.

Expression of the nucleotide sequence leads to overexpression of Kv7.3 ion channels in these neurons. The subject can then be treated with a neuromodulatory drug at a lower dose than would previously have been necessary to obtain a therapeutic effect. The lower dose, in turn, causes fewer side effects and has reduces toxicity. The goal of curing, alleviating symptoms, and/or improving the quality of life of patients with diseases caused by hyperexcitable neurons is achieved without undesirable (or with fewer, less severe) side effects.

Prohibitively high side effect drugs may become usable by employing the method disclosed herein, simply by permitting a lower dose to be efficacious in specific cells.

In some embodiments, the method involves the gene therapy vector or AAV viral particle retrogradely infecting neurons for the purposes of delivering genetic material to neurons, with the purpose of treating diseases caused by, or associated with Kv7 ion channel dysfunction.

In the case of A315T, a channel with high membrane insertion rate and conductance levels, it is likely that only low expression levels are needed to be effective. This means the virus can be delivered at lower quantities, reducing the probability of immunogenicity and reducing costs.

In one embodiment the method is an ex vivo method.

In the context of this specification "comprising" is to be interpreted as "including".

Aspects of the invention comprising certain elements are also intended to extend to alternative embodiments "consisting" or "consisting essentially" of the relevant elements.

Where technically appropriate, embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Approximately as employed herein is intended to mean ±10%.

Technical references such as patents and applications are incorporated herein by reference.

Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

Examples

Materials and methods

Cell culture

Human iPSC sensory neuron progenitor cells (Axol Bioscience) were thawed and plated at low density on a monolayer of rat cortical astrocytes on poly D lysine coated glass coverslips. Cultures were grown in NbActiv 4 (Neurobasal/B 27 supplemented with MAXIMIZER, GDNF, NGF, BDNF, and NT 3) to promote the maturation of the cells to a differentiated neuronal phenotype.

AAVs

pAAV-CMV-EGFP, pAAV-CMV-hKCNQ3-T2A-EGFP, pAAV-CMV-hKCNQ3$_{(A315T)}$-T2A-EGFP, were added to wells containing neuronal cultures at 7 days in vitro and patch clamp recordings were made either 1 (DIV15-16) or 2 weeks later (DIV21-23).

Patch clamp electrophysiology

Standard patch clamp methods were employed. The external recording solution composition was 140 mM NaCl, 2 5 mM KCl, 2mM CaCl2, 3 mM MgCl2, 10 mM glucose, 10 mM HEPES, pH 7.3. The internal recording solution composition was 120 mM K gluconate, 20 mM KCl , 3 mM MgCl, 2.5 mM EGTA, 0.5 mM CaCl, 2.4 mM Na2- ATP, 0.3 mM Li GTP, 10 mM HEPES, pH 7.3. Upon establishing the whole cell configuration, passive membrane properties for every cell (capacitance and resistance) were measured in voltage clamp mode. All excitability measurements were made in current clamp.

Multielectrode array recordings

Neurons were grown as above in 24 well plates containing 16 electrodes per well, such that neurons were in direct contact with individual electrodes. Spontaneous firing activity was then recorded after 30 days. Recordings were made at baseline (no drug) and then at increasing concentrations of the activator drug.

Mouse spasticity model

Neonatal mice (Post natal day 0) received a complete spinal cord transection between the 9th-10th thoracic segment to induce spasticity. In the same surgery, AAVs were injected into both gastrocnemius muscles.

EMG recording

10-14 days after spinal cord injury, EMG electrodes were inserted into the injected muscles to record electrical activity before and after intraperitoneal administration of an activator drug (retigabine). Different doses were tested on different days.

Patch clamp electrophysiology from mouse spinal cord slices

The spinal cord was harvested under terminal anaesthesia and sliced at 300 um on a vibratome. Patch clamp recordings were then made from motor neurons expressing GFP as described above (patch clamp electrophysiology).

Paragraphs

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug.

2. A method of reducing the side effects of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug.

3. A method of increasing the therapeutic index of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering the neuromodulatory drug.

4. A method of reducing the excitability of a neuron comprising the steps:

a. introducing a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to the target neuron via a vector,
b. overexpressing the ion channel subunit in the target neuron such that it forms functional ion channels, and
c. optionally administering a neuromodulatory drug.

5. A method of treating a neurological disorder comprising the steps:
   a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,
   b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and
   c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels.

6. A gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

7. A gene therapy vector according to paragraph 6, wherein the vector selectively transduces the target neuron.

8. An AAV viral particle gene therapy vector comprising:
   a. an AAV capsid that selectively transduces target neurons, and
   b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

9. A gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in upregulating expression of the Kv7.3 ion channel subunit by the target neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased neuronal excitability.

10. A gene therapy vector comprising an AAV capsid that selectively transduces target neurons, and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the

sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

11. The method according to any one of paragraphs 1 to 5 or the gene therapy vector according to paragraph 10, wherein the neuromodulatory drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

12. The method or the gene therapy vector according to any preceding paragraph, wherein the Kv7.3 ion channel is expressed as a Kv7.3 homomer, a Kv7.2/7.3 heteromer, or a Kv7.3/7.5 heteromer.

13. The method or gene therapy vector according to paragraph 12, wherein the Kv7.3 ion channel is functionally expressed as a homomer.

14. The method or the gene therapy vector according to any preceding paragraph, wherein the target neurons are motor neurons.

15. The method or the gene therapy vector according to any one of paragraphs 5 or 9 to 14, wherein the neurological disorder is associated with increased excitability of neurons.

16. The method or the gene therapy vector according to any one of paragraphs 5 or 9 to 15, wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain.

17. The method or the gene therapy vector according to any preceding paragraph, wherein the upregulation of the Kv7.3 ion channel subunit reduces the rheobase of the target neuron in the presence of the neuromodulatory drug.

18. The method or the gene therapy vector according to any preceding paragraph, wherein the upregulation of the Kv7.3 ion channel subunit shifts the resting membrane potential of the target neuron away from threshold in the presence of the neuromodulatory drug.

19. The method or the gene therapy vector according to any preceding paragraph, wherein the upregulation of the Kv7.3 ion channel subunit reduces the excitability of the target neuron.

20. The method or the gene therapy vector according to any preceding paragraph, wherein the upregulation of the Kv7.3 ion channel subunit reduces the excitability of a plurality of target neurons, as recorded using a microelectrode array (MEA) device.

21. The method or the gene therapy vector according to any preceding paragraph, wherein the upregulation of the Kv7.3 ion channel subunit by the target neuron reduces activity of cells innervated by axon terminals of the target neuron.

22. The method of paragraph 19 to 21 wherein the reduction of the excitability of the target neuron, or the reduction in activity of cells innervated by axon terminals of the target neuron, occurs or is increased in the presence of the neuromodulatory drug.

23. Use of the gene therapy vector according to paragraph 6 to 10 in the preparation of a medicament for use in the treatment of conditions ameliorated by upregulating expression of Kv7.3 ion channels.

24. Use of the gene therapy vector according to paragraph 6 to 10 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, ALS, nociceptive pain and non-nociceptive pain.

25. Use of a gene therapy vector according to paragraph 6 to 10 in the preparation of a medicament for use as an anti-spasticity.

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Sequences

SEQ ID 1 - wild type Kv7.3

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SEQ ID 2 – Kv7.3 A315T

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SEQ ID 3 – Kv7.3 A315S

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SEQ ID 4 – Kv7.3 A315V

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AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
GACACGGACCCCTTCACGCCCAGCGGCTCCATGCCTCTGTCGTCCACAGGGGATGGGATT
TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 5 – Kv7.3 A315C

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315C
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GGAGGCGGCGGGGCGGCTAACCCAGCCGGAGGGGACGCGGCGGCGGCCGGCGACGAGGAG
CGGAAAGTGGGGCTGGCGCCCGGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGA
GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG
ACCCCGCAGGGCATCGGGCTCCTGGCCAAGACCCCGCTGAGCCGCCCAGTCAAGAGAAAC
AACGCCAAGTACCGGCGCATCCAAACTTTGATCTACGACGCCCTGGAGAGACCGCGGGGC
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GTCCTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGGAG
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GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGC
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CAGGTGACCATTGACAAAGTCAGCCCCTATGGGTTTTTTGCACATGACCCTGTGAACCTG
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GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCGGCCCC
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TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 6 – Kv7.3 A315N

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315N
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GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG
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AACGCCAAGTACCGGCGCATCCAAACTTTGATCTACGACGCCCTGGAGAGACCGCGGGGC
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GTCCTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGGAG
ACATTTGCTATTTTCATCTTTGGAGCCGAGTTTGCTTTGAGGATCTGGGCTGCTGGATGT
TGCTGCCGATACAAAGGCTGGCGGGGCCGACTGAAGTTTGCCAGGAAGCCCCTGTGCATG
TTGGACATCTTTGTGCTGATTGCCTCTGTGCCAGTGGTTGCTGTGGGAAACCAAGGCAAT
GTTCTGGCCACCTCCCTGCGAAGCCTGCGCTTCCTGCAGATCCTGCGCATGCTGCGGATG
GACCGGAGAGGTGGCACCTGGAAGCTTCTGGGCTCAGCCATCTGTGCCCACAGCAAAGAA
CTCATCACGGCCTGGTACATCGGTTTCCTGACACTCATCCTTTCTTCATTTCTTGTCTAC
CTGGTTGAGAAAGACGTCCCAGAGGTGGATGCACAAGGAGAGGAGATGAAAGAGGAGTTT
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GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGC
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GCCGTCAGAATTCTACAATTCCGTCTCTATAAAAAAAAATTCAAGGAGACTTTGAGGCCT
TACGATGTGAAGGATGTGATTGAGCAGTATTCTGCCGGGCATCTCGACATGCTTTCCAGG
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GATATGCACATGCAACACATGGAACGGTTGCAGGTGCAGGTCACGGAGTATTACCCAACC
AAGGGCACCTCCTCGCCAGCTGAAGCAGAGAAGAAGGAGGACAACAGGTATTCCGATTTG
AAAACCATCATCTGCAACTATTCTGAGACAGGCCCCCCGGAACCACCCTACAGCTTCCAC
CAGGTGACCATTGACAAAGTCAGCCCCTATGGGTTTTTTGCACATGACCCTGTGAACCTG
CCCCGAGGGGGACCCAGTTCTGGAAAGGTTCAGGCAACTCCTCCTTCCTCAGCAACAACG
TATGTGGAGAGGCCCACGGTCCTGCCTATCTTGACTCTTCTCGACTCCCGAGTGAGCTGC
CACTCCCAGGCTGACCTGCAGGGCCCCTACTCGGACCGAATCTCCCCCCGGCAGAGACGT
AGCATCACGCGAGACAGTGACACACCTCTGTCCCTGATGTCGGTCAACCACGAGGAGCTG
GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCGGCCCC
AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
GACACGGACCCCTTCACGCCCAGCGGCTCCATGCCTCTGTCGTCCACAGGGGATGGGATT
TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 7 – Kv7.3 A315Q

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315Q
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CGGAAAGTGGGGCTGGCGCCCGGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGA
GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG

ACCCCGCAGGGCATCGGGCTCCTGGCCAAGACCCCGCTGAGCCGCCCAGTCAAGAGAAAC
AACGCCAAGTACCGGCGCATCCAAACTTTGATCTACGACGCCCTGGAGAGACCGCGGGGC
TGGGCGCTGCTTTACCACGCGTTGGTGTTCCTGATTGTCCTGGGGTGCTTGATTCTGGCT
GTCCTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGGAG
ACATTTGCTATTTTCATCTTTGGAGCCGAGTTTGCTTTGAGGATCTGGGCTGCTGGATGT
TGCTGCCGATACAAAGGCTGGCGGGGCCGACTGAAGTTTGCCAGGAAGCCCCTGTGCATG
TTGGACATCTTTGTGCTGATTGCCTCTGTGCCAGTGGTTGCTGTGGGAAACCAAGGCAAT
GTTCTGGCCACCTCCCTGCGAAGCCTGCGCTTCCTGCAGATCCTGCGCATGCTGCGGATG
GACCGGAGAGGTGGCACCTGGAAGCTTCTGGGCTCAGCCATCTGTGCCCACAGCAAAGAA
CTCATCACGGCCTGGTACATCGGTTTCCTGACACTCATCCTTTCTTCATTTCTTGTCTAC
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GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGC
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AAAGGAAAGCTATTTACCCCTCTGAATGTAGATGCCATAGAAGAAAGTCCTTCTAAAGAA
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GCCGTCAGAATTCTACAATTCCGTCTCTATAAAAAAAAATTCAAGGAGACTTTGAGGCCT
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AAGGGCACCTCCTCGCCAGCTGAAGCAGAGAAGAAGGAGGACAACAGGTATTCCGATTTG
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TATGTGGAGAGGCCCACGGTCCTGCCTATCTTGACTCTTCTCGACTCCCGAGTGAGCTGC
CACTCCCAGGCTGACCTGCAGGGCCCCTACTCGGACCGAATCTCCCCCCGGCAGAGACGT
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GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCGGCCCC
AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
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TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 8 – Kv7.3 A315Y

>ENA|AAC96101|AAC96101.1 Homo sapiens (human) potassium channel A315Y
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CGGAAAGTGGGGCTGGCGCCCGGCGACGTGGAGCAAGTCACCTTGGCGCTCGGGGCCGGA
GCCGACAAAGACGGGACCCTGCTGCTGGAGGGCGGCGGCCGCGACGAGGGGCAGCGGAGG
ACCCCGCAGGGCATCGGGCTCCTGGCCAAGACCCCGCTGAGCCGCCCAGTCAAGAGAAAC
AACGCCAAGTACCGGCGCATCCAAACTTTGATCTACGACGCCCTGGAGAGACCGCGGGGC
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GTCCTGACCACATTCAAGGAGTATGAGACTGTCTCGGGAGACTGGCTTCTGTTACTGGAG
ACATTTGCTATTTTCATCTTTGGAGCCGAGTTTGCTTTGAGGATCTGGGCTGCTGGATGT
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TTGGACATCTTTGTGCTGATTGCCTCTGTGCCAGTGGTTGCTGTGGGAAACCAAGGCAAT
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CTGGTTGAGAAAGACGTCCCAGAGGTGGATGCACAAGGAGAGGAGATGAAAGAGGAGTTT
GAGACCTATGCAGATGCCCTGTGGTGGGGCCTGATCACACTGTACACCATTGGCTATGGA

GACAAGACACCCAAAACGTGGGAAGGCCGTCTGATTGCCGCCACCTTTTCCTTAATTGGC
GTCTCCTTTTTTGCCCTTCCAGCGGGCATCCTGGGGTCCGGGCTGGCCCTCAAGGTGCAG
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GCCGTCAGAATTCTACAATTCCGTCTCTATAAAAAAAAATTCAAGGAGACTTTGAGGCCT
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AAGGGCACCTCCTCGCCAGCTGAAGCAGAGAAGAAGGAGGACAACAGGTATTCCGATTTG
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GAGAGGTCTCCAAGTGGCTTCAGCATCTCCCAGGACAGAGATGATTATGTGTTCGGCCCC
AATGGGGGGTCGAGCTGGATGAGGGAGAAGCGGTACCTCGCCGAGGGTGAGACGGACACA
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TCTGATTCAGTATGGACCCCTTCCAATAAGCCCATTTAA

SEQ ID 9 – wild type Kv7.3

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2
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ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLATIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
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SEQ ID 10 – Kv7.3 A315T

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315T
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF

ETYADALWWGLITLTTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
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NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
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SEQ ID 11 – Kv7.3 A315S

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315S
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
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EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
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SEQ ID 12 – Kv7.3 A315V

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315V
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WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLVTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
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SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
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SEQ ID 13 – Kv7.3 A315C

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315C
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DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLCTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
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SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 14 – Kv7.3 A315N

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315N
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVTLALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLNTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 15 – Kv7.3 A315Q

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Q
MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVTLALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLQTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

SEQ ID 16 – Kv7.3 A315Y

>sp|O43525|KCNQ3_HUMAN Potassium voltage-gated channel subfamily KQT member 3 OS=Homo sapiens OX=9606 GN=KCNQ3 PE=1 SV=2 A315Y

MGLKARRAAGAAGGGGDGGGGGGGAANPAGGDAAAAGDEERKVGLAPGDVEQVTLALGAG
ADKDGTLLLEGGGRDEGQRRTPQGIGLLAKTPLSRPVKRNNAKYRRIQTLIYDALERPRG
WALLYHALVFLIVLGCLILAVLTTFKEYETVSGDWLLLLETFAIFIFGAEFALRIWAAGC
CCRYKGWRGRLKFARKPLCMLDIFVLIASVPVVAVGNQGNVLATSLRSLRFLQILRMLRM
DRRGGTWKLLGSAICAHSKELITAWYIGFLTLILSSFLVYLVEKDVPEVDAQGEEMKEEF
ETYADALWWGLITLYTIGYGDKTPKTWEGRLIAATFSLIGVSFFALPAGILGSGLALKVQ
EQHRQKHFEKRRKPAAELIQAAWRYYATNPNRIDLVATWRFYESVVSFPFFRKEQLEAAS
SQKLGLLDRVRLSNPRGSNTKGKLFTPLNVDAIEESPSKEPKPVGLNNKERFRTAFRMKA
YAFWQSSEDAGTGDPMAEDRGYGNDFPIEDMIPTLKAAIRAVRILQFRLYKKKFKETLRP
YDVKDVIEQYSAGHLDMLSRIKYLQTRIDMIFTPGPPSTPKHKKSQKGSAFTFPSQQSPR
NEPYVARPSTSEIEDQSMMGKFVKVERQVQDMGKKLDFLVDMHMQHMERLQVQVTEYYPT
KGTSSPAEAEKKEDNRYSDLKTIICNYSETGPPEPPYSFHQVTIDKVSPYGFFAHDPVNL
PRGGPSSGKVQATPPSSATTYVERPTVLPILTLLDSRVSCHSQADLQGPYSDRISPRQRR
SITRDSDTPLSLMSVNHEELERSPSGFSISQDRDDYVFGPNGGSSWMREKRYLAEGETDT
DTDPFTPSGSMPLSSTGDGISDSVWTPSNKPI

Claims

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. administering a lower dose of the neuromodulatory drug than would normally be administered to a subject in order to be therapeutically effective

    wherein the neuromodulatory drug is a voltage gated potassium channel-targeting drug,

    wherein the voltage-gated potassium channel-targeting drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

3. A method of reducing the excitability of a neuron comprising the steps:
    a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 to the target neuron via a vector,
    b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and
    c. optionally administering a voltage-gated potassium channel-targeting drug.

4. A method of treating a neurological disorder comprising the steps:
    a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,
    b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and
    c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels than would normally be administered to the subject in order to be therapeutically effective,

    wherein the neuromodulatory drug is a voltage-gated potassium channel-targeting drug selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

5. A gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.10 to SEQ ID.16 wherein, when the vector

transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

6. The gene therapy vector according to claim 5, further comprising an AAV capsid that selectively transduces target neurons.

7. A gene therapy vector comprising an AAV capsid that selectively transduces target sensory-motor neurons, and a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16.

8. The gene therapy vector according to any one of claims 6 or 7 for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective can be effectively administered to the subject.

9. The gene therapy vector according to claim 7, for use in upregulating expression of the Kv7.3 ion channel subunit by the target sensory-motor neuron such that it forms functional ion channels in a subject having a neurological disorder associated with increased sensory-motor neuron excitability.

10. The method according to any one of claims 2 or 3 or the gene therapy vector according to claim 8 wherein the voltage-gated potassium channel-targeting drug is selected from Retigabine/Ezogabine and derivatives thereof, BHV-7000 and Xen496, Xen 1101, flupirtine, diclofenac, BMS-204352, meclofenamic acid, ETX-123 and linopirdine.

11. The method or the gene therapy vector according to any preceding claim, wherein the Kv7.3 ion channel is expressed as a Kv7.3 homomer, a Kv7.2/7.3 heteromer, or a Kv7.3/7.5 heteromer.

12. The method or gene therapy vector according to claim 11, wherein the Kv7.3 ion channel is functionally expressed as a homomer.

13. The method or the gene therapy vector according to any preceding claim, wherein the target neurons are motor neurons.

14. The method according to claim 4 or the gene therapy vector according to claim 8, wherein the neurological disorder is associated with increased excitability of neurons.

15. The method according to claim 4 or the gene therapy vector according to claim 8, wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, nociceptive pain and non-nociceptive pain.

16. Use of the gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use in the treatment of conditions ameliorated by upregulating expression of Kv7.3 ion channels.

17. Use of the gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use in the treatment of a neurological disorder associated with increased excitability of neurons, such as wherein the neurological disorder is selected from the group consisting: epilepsy, spasticity, BNFS, Parkinson's disease, ALS, nociceptive pain and non-nociceptive pain.

18. Use of a gene therapy vector according to any one of claims 5 to 15 in the preparation of a medicament for use as an anti-spasticity.

Colin Sanders Business
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OX16 9PA

International Preliminary Examination Authority
European Patent Office
80331 Munich
Germany

Our ref: SANI.3WO
30 May 2025

Dear Colleagues,

PCT Application Number: PCT/IB2024/154426
Applicant: Sania Rx Ltd
Title: Method

Thank you for the WO-IPEA, dated 31.03.2025. The Applicant hereby provides the following comments and amended claims in reply to the opinion.

Amendments

Claims 1 and 4 have been limited to include the list of neuromodulatory drugs of claim 12 (original claim 11, now claim 10).

Claim 8 has been amended to make it dependent only on claims 6 or 7, thereby rectifying the accidental removal of the AAV capsid from original claim 10, on which claim 8 (filed 3 March) was based.

Claims 9 and 11 have been deleted. Remaining claims and dependencies have corrected accordingly.

The amendments do not add subject matter.

Admissibility of the claims filed 03 March 2025

The Examiner has indicated that claims 2, 6, 8, 9 and 11 extend beyond the content of the application as filed. We provide the following comments in reply.

Claim 2

Claim 2 was newly added and reads:

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

The present invention is directed to a chemogenetics method in which increasing the number of Kv7.3-containing ion channels in target neurons permits a lower dose of an ion channel-targeting drug to be efficacious. In essence, presenting more targets for the drug to bind to allows a lower dose of the drug to be administered. In other words, the drugs efficacy is increased. This lower dose then leads to fewer side effects.

This description is apparent throughout the application as filed, particularly so at page 3, lines 21 to 27. Which states:

Summary of the Invention

The present disclosure relates to a chemogenetic method that allows modulation of activity in targeted neurons, thus increasing drug efficacy while reducing side effects, at least in part, due to the ability to use a lower dose. The method involves using gene therapy methodology via a delivery vector (e.g. AAV) to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses of specific activating and inhibiting drugs. That is, the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs.

Consequently, the Applicant submits that claim 2 is directly and unambiguously disclosed in the application as filed at page 3, lines 21 to 27. Breaking claim 2 down into its component parts:

2. A method of reducing the therapeutically effective dose of a voltage-gated potassium channel-targeting drug comprising the step:

increasing the sensitivity of a target neuron to the voltage-gated potassium channel-targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID.10 to SEQ ID.16.

The method of reducing the therapeutically effective dose is found at line 22-23 which states "increasing drug efficacy [...] due to the ability to use a lower dose. This is clearly a statement that the drugs efficacy is increased which is the same as reducing the therapeutically effective dose. We respectfully submit that the skilled person would understand that these two concepts are opposite sides of the same coin and go hand in hand. This is further supported by page 4, lines 18-19.

The second underlined part of the claim relates to increasing the sensitivity of a target neuron to the drug by overexpressing Kv7.3. This concept is clearly disclosed at lines 26-27 which state "the method specifically increases the sensitivity of dysfunctional neurons to modulatory drugs", and at lines 24-25 which state "...to overexpress (upregulate) a therapeutic ion channel in targeted neurons, so that the channel's conductance (and therefore neuronal activity) can be modulated by low doses...".

Therefore, we submit that new claims 2 is fully, directly, and unambiguously disclosed in the application as filed and request removal of this objection based on the above reasoning.

Claim 6

Claim 6 is essentially original claim 8 which read:

> 8. An AAV viral particle gene therapy vector comprising:
> a. an AAV capsid that selectively transduces target neurons, and
> b. a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16, wherein, when the vector transduces the target neurons, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

However, to reduce the number of independent claims, claim 6 (former claim 8) has been made dependent on claim 5 (former claim 6), which read:

> 6. A gene therapy vector comprising a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 wherein, when the vector transduces a target neuron, expression of the Kv7.3 ion channel subunit is upregulated and functional ion channels are formed.

Part b. of claim 8 is, word for word, claim 6. Thus, claim 8 is the gene therapy vector of claim 6, plus an AAV capsid to create an AAV viral particle. The term "AAV viral particle" has been deleted from new claim 6 but removal of this term is not adding matter – the features of the claims are all still present and the product of claim 6 would be an AAV viral particle gene therapy vector as originally claimed in claim 8.

Therefore, we respectfully request removal of this objection.

Claim 8

Amended claim 8 is original claim 10 made dependent on claims 6 or 7. As discussed above, claim 6 finds basis in original claims 6 and 8. Thus, claim 8 is based on original claim 10, dependent on original claims 6 and 8 (not original claims 4 and 5 as indicated in the report). Amended claim 8 reads:

> (new) 8. The gene therapy vector according to any one of claims 6 or 7 for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective can be effectively administered to the subject.

Each of claims 6 and 7 comprises the feature (Kv7.3 sequences) struck out below in original claim 10. Thus, amended claim 8 also includes the sequences by virtue of its dependence on claim 6 or 7. As is the AAV capsid that selectively transduces target neurons. Hence, when we look at original claim 10 (shown below for ease of reference) the section that is now struck out, can be disregarded. Leaving "A gene therapy vector for use in treating [...] administered to the subject". These features are entirely present in amended claim 8.

> (original) 10. A gene therapy ~~vector comprising an AAV capsid that selectively transduces target neurons~~, ~~and a nucleotide sequence encoding a Kv7.3 ion channel subunit comprising the sequence of any of SEQ ID.1 to SEQ ID.8 or a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16~~, for use in treating a subject having a neurological disorder, wherein, once the vector has transduced the target neuron, expression of the Kv7.3 ion channel by the target neuron is upregulated, such that a lower dose of a neuromodulatory drug can be effectively administered to the subject.

The only remaining difference between original claim 10 and amended claim 8 are the "lower dose of a neuromodulatory drug" of original claim 10 now reads "lower dose of a voltage-gated potassium channel-targeting drug than would normally be administered to the subject in order to be therapeutically effective". Amendment to voltage-gated potassium channel-targeting drug is a limitation that is entirely permissible and has been accepted in other claims. The addition of clarification of the lower dose than would normally be administered is similarly admissible elsewhere. Thus, the claim finds full basis in claims 10, 6 and 8 as originally filed.

We trust the removal of dependence on claim 5, which does not comprise the AAV capsid of original claim 10, resolves the objections.

Claim 9 and claim 11

Claims 9 and 11 have been deleted, therefore the objections are moot.

Novelty

Claims 1 and 4 are now limited to the list of neuromodulatory drugs of original claim 11. These drugs are not disclosed in D1, hence claims 1 and 4 are novel in view of D1.

We request reconsideration of claim 2 based on the above explanation that the claim does not add matter and reiterate the argumentation presented previously.

Claim 2 discloses a method of reducing the therapeutically effective dose of a voltage-gated potassium channel targeting drug, by increasing the sensitivity of a target neuron by overexpressing a Kv7.3 A315 mutant. D1 to D3 do not disclose mutant Kv7.3 channels as claimed in claim 2. Thus, claim 2 is novel in view of D1 to D3.

We thank the Examiner for the positive opinion of the novelty of claims 3, 5, 12 (now claims 3, 5, 10) and request that this be reflected in the summary of the report in Box No. V on page 3.

We therefore submit that independent claims 1, 2, 3, 4, 5, and 7 are novel and that, consequently, all dependent claims are also novel.

Inventive Step

Given the points above, we will defer commenting further on inventive step until a clear picture on the admissibility of the claims, and the novelty thereof, has been established.

Clarity

Given the divergence of local practice, we will defer commenting further on the clarity of the pending claims until the national phase.

We submit that the amended claims are now at least novel and do not add matter. We look forward to receipt of the International Preliminary Examination Report in due course.

Yours sincerely,

S Kaye

Dr Samantha Kaye CPA EPA
Representative for the Applicant

Enclosed: amended claims and specification – clean and marked up format

FILE COPY

CERTIFICATE OF INCORPORATION ON CHANGE OF NAME

Company Number **13756774**

The Registrar of Companies for England and Wales hereby certifies that under the Companies Act 2006:

SANIA RX LTD

a company incorporated as private limited by shares, having its registered office situated in England and Wales, has changed its name to:

SANIA TX LTD

Given at Companies House on **29th April 2025**

* N13756774R *

The above information was communicated by electronic means and authenticated by the Registrar of Companies under section 1115 of the Companies Act 2006

Companies House

THE OFFICIAL SEAL OF THE REGISTRAR OF COMPANIES

Companies House

NM01 (ef)

Notice of Change of Name by Resolution

Company Number: **13756774**

Company Name: **SANIA RX LTD**

Received for filing in Electronic Format on the:**28/04/2025**

Notice is hereby given that the company has changed its name as set out in the attached resolution

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Authorisation

Authenticated

This form was authorised by one of the following:

Director, Secretary, Person Authorised, Administrator, Administrative Receiver, Receiver, Receiver manager, Charity Commission Receiver and Manager, CIC Manager

COMPANIES ACT 2006
SPECIAL RESOLUTION ON CHANGE OF NAME

Company number: 13756774

Existing company name:
SANIA RX LTD

The following special resolution to change the name of the company was agreed and passed by the members.

On the 24th April 2025

That the name of the company be changed to:
SANIA TX LTD

Colin Sanders Business
Innovation Centre
Mewburn Road
Banbury
Oxfordshire, UK
OX16 9PA

International Preliminary Examination Authority
European Patent Office
80331 Munich
Germany

Our ref: SANI.3WO
03 March 2025

Dear Colleagues,

PCT Application Number: PCT/IB2024/154426
Applicant: Sania Rx Ltd
Title: Method

Thank you for the ISR-WO, dated 20.08.2024. The Applicant hereby petitions for international preliminary examination under Chapter II PCT. Appended form PCT/IPEA/401 comprises the Demand. We respectfully request examination of the application on the basis of the enclosed amended claims and the comments below.

Amendments

Claims 2, 3, 7, 17 to 22 have been deleted.

New claims 2, 7, 8, 9 and 11 have been added.

Remaining claims have been renumbered, and dependencies corrected, accordingly.

Claims 1-5 (formerly 1, 2, 4, 5, 6) have been amended to remove SEQ ID 1 to SEQ ID 8, we consider these sequences to be encompassed by "a nucleotide sequence encoding the amino acid sequence of SEQ IDs 9 to 16".

Claims 1 and 4 (formerly 1 and 5) have also been amended to include the baseline against which "lower dose" is measured. Basis for the amendment is found on page 11, lines 16-17 and page 14, lines 30-33 of the PCT as published.

Claims 1, 3 and 12 have also been limited to specify that the neuromodulatory drug is a voltage gated potassium channel-targeting drug. Basis for this amendment is found on page 8, line 36.

Claim 3 (formerly claim 4) has been limited to SEQ IDs 10 to 16.

Claim 6 (formerly claim 8) has been made dependent on claim 5.

New claim 2 has been added. Basis for the claim is found throughout the application, examples of which include page 3, lines 21-27, page 4, lines 18-19, page 7, lines 22-35, page 11, lines 15-24 and 28-30, page 12, lines 5-9, page 14, lines 33-34 and page 16, lines 26-29.

New claim 7 has been added. Basis for the claim is found in original second medical use claim 9, reformulated as a product claim.

Claim 8 is former claim 10 made dependent on claims 5 to 7 and with the addition of the baseline definition for "lower dose" added, as described above. The amendment finds additional basis on page 5, lines 13-17.

Claim 9 is new and finds basis in original claim 2. Further basis is found at page 3, lines 18, 19, 21-27, page 4, lines 34-36, page 5, lines 13-17, page 11, lines 28-30, 34, 35, and page 16, lines 26-31.

Claim 10 is former claim 9 made dependent on claim 7 and with the added limitation that the target neurons are sensory-motor neurons. Basis for this amendment is found on page 10, line 34.

Claim 11 is new and finds basis at page 14, lines 28-29.

The amendments do not add subject matter.

Novelty

Claims 1-5 and 7 are now independent claims, claims 1-4 covering various methods of using the vector of the invention, which is the subject of claims 5 and 7.

Claims 1 and 4 broadly disclose methods of administering a vector to boost expression of Kv7.3 ion channels (wild type and A315 mutants), followed by a subsequent administration of a neuromodulatory drug. The method is a chemogenetic approach which enables a lower dose of a drug, relative to the dose needed without the vector, to be administered.

D1 (Liu et al, 2022) discloses a disease model that is induced by methamphetamine. Methamphetamine is an indirect agonist of dopamine, norepinephrine, and serotonin. It competitively binds to monoamine transporters, thus causing sustained release of dopamine, norepinephrine, and serotonin. Methamphetamine is not a voltage-gated potassium channel targeting drug and does not target Kv7.3 ion channels. D1 does not disclose a voltage-gated potassium channel targeting drug, nor a Kv7.3 ion channel targeting drug and thus, claims 1 and 4 are novel in view of D1.

D3 discloses overexpression of KCNQ3 (Kv7.3) which reduced depressive behaviour. D3 also separately discloses that an infusion of retigabine reduced depressive behaviour. D3 does not disclose overexpressing Kv7.3 with subsequent administration of a voltage-gated potassium channel targeting drug. They are described as two separate experiments. Thus, claims 1 and 4 are novel in view of D3.

Claim 2 discloses a method of reducing the therapeutically effective dose of a voltage-gated potassium channel targeting drug, by increasing the sensitivity of a target neuron by overexpressing a Kv7.3 A315 mutant. D1 to D3 do not disclose mutant Kv7.3 channels as claimed in claim 2. Thus claim 2 is novel in view of D1 to D3.

Claim 3 discloses a method of reducing the excitability of a neuron by overexpressing a Kv7.3 A315 mutant. D1 to D3 do not disclose mutant Kv7.3 channels as claimed in claim 3. Thus claim 3 is novel in view of D1 to D3.

Claim 5 discloses a gene therapy vector comprising a Kv7.3 A315 mutant. D1 to D3 do not disclose mutant Kv7.3 channels. Thus claim 5 is novel in view of D1 to D3.

Claim 7 is now limited to sensory-motor target neurons. None of the prior art documents D1 to D3 disclose a gene therapy vector that selectively targets sensory-motor neurons. D1 relates to the lateral hypothalamus and D3 relates to the ventral tegmental area (VTA), part of the mid-brain, therefore both relate to the CNS, not the peripheral nervous system.

The field of invention in D2 states that it relates to compositions and "their use in the treatment of central nervous system disorders". Furthermore, D2 discloses expression of Kv7.2/3 channels in Cho-K1 cells, not in neurons. Thus, claim 7 is novel in view of D1 to D3.

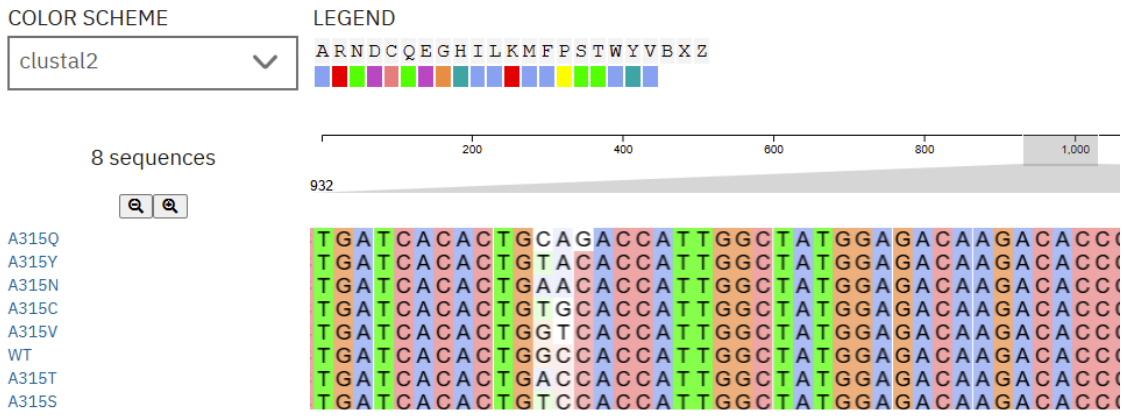
Claims 6 and 8 to 20 are novel at least by their dependence on one or more of claims 1-5 or 7. Therefore, all claims in the amended claim set are now novel.

Inventive Step

Claims 2, 3 and 5

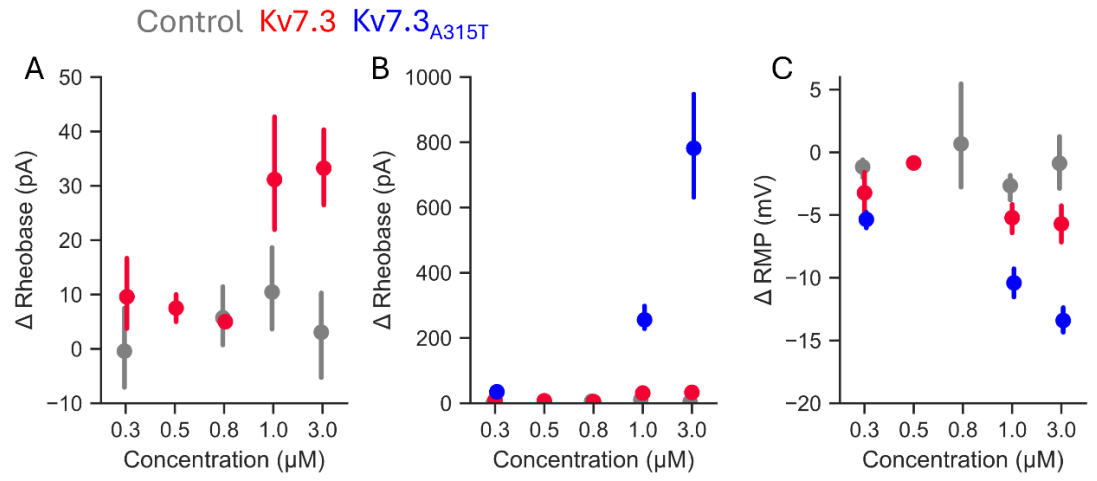
The Examiner has indicated that SEQ IDs 1-8 encompass the WT Kv7.3 coding sequence. Only SEQ ID 1 (and SEQ ID 9) is wild type Kv7.3. Therefore, although the general group of SEQ ID 1-8 includes WT, SEQ IDs 2-8 (coding sequence taken from GenBank) and SEQ IDs 10-16 do not encompass the WT sequence and are not implicitly disclosed.

The following alignment shows the position of the mutation in each of the nucleotide sequences SEQ IDs 1-8. It is apparent that the codon at position 943-945 is mutated and are not wild type.



Claims 2, 3 and 5 are limited to mutated Kv7.3 of SEQ IDs. 10 to 16. None of the cited prior art documents teach or suggest a mutation at position 315. Thus, the skilled person would not consider using anything other than WT KCNQ3 based on the teaching of D1 to D3. In fact, there are no known variants acknowledged at A315 of the canonical Kv7.3 peptide sequence according to UniProt entry OC43525, even though currently 1,114 variants are known https://www.uniprot.org/uniprotkb/O43525/variant-viewer.

Advantageously, the present inventors have observed that expression of Kv7.3-containing ion channels with mutations at position 315 (see figs 7 to 9, for example) significantly reduces excitability of sensory neurons relative to WT Kv7.3. The following figure, directly comparing data provided in the application as filed, is shown to highlight this (note the scale difference in the graphs):



Retigabine (as an example neuromodulatory drug) was seen to cause a very large increase in the rheobase of 315 mutant Kv7.3-transduced neurons, and on hyperpolarisation of the resting membrane potential.

Nothing in the teaching of D1 to D3 would motivate a skilled person to mutate the ion channels. The mutations at A315 have a proven advantage over the WT, thus amended claims 2, 3 and 5 are inventive in view of D1 to D3.

Claims 1, 4 and 7

D1 (Liu et al, 2022) discloses a disease model that is induced by methamphetamine which remodels the neurons of the lateral hypothalamus (LH) synaptic plasticity, increases intracellular calcium levels, and enhances spontaneous current and evoked potentials of neurons relative to a (saline) control group. This leads to conditioned place preference (CPP) which is linked to addiction. This disease state leads to increased excitability of the neurons. The researchers then overexpressed Kcnq3 (Kv7.3) to reduce the excitability of the neurons, reversing CPP and alleviating addictive behaviours. There was no subsequent administration of a neuromodulatory drug.

The methodology of D1 is the very different to the one disclosed in the present application. D1 uses a neuromodulatory drug to induce increased excitability. D1 teaches that methamphetamine (MA) reduces expression of Kcnq3 at mRNA and protein levels, and that restoring expression via use of an AAV vector reduces MA-induced CPP. In doing so, D1 hypothesises that overexpression of Kcnq3 may alleviate addictive behaviours.

Following this through to its natural conclusion, overexpression of Kcnq3 would require a larger dose of MA to achieve the same effect as seen without the overexpression (in the absence of treatment with the vector). Thus, D1 actually teaches away from the presently claimed invention which aims to reduce the dosage of neuromodulatory drug required to have a therapeutic effect.

D1 differs from the presently claimed invention in that:

- the neuromodulatory drug (MA) is used to induce disease state, not treat it
- MA is not voltage gated potassium channel-targeting drug
- there is no subsequent administration of MA after Kcnq3 is overexpressed
- MA is used/shown to lead to reduced expression of Kcnq3
- the AAV vector is used to restore Kcnq3, not increase levels relative to a normal cell
- teaches only in relation to lateral hypothalamus (CNS (brain)), not peripheral nervous system
- overexpression of Kcnq3 results in a larger dose of MA being needed, not lower
- Kcnq3 is wild type only
- overexpression of Kcnq3 does not increase the efficacy or reduce the therapeutically effective dose of MA
- no suggestion of using the neuromodulatory drug therapeutically

Thus, all claims are inventive in view of D1.

D2 (US8,883,812) discloses compositions, including retigabine, for use in treating central nervous system disorders. As part of the process of testing the compounds disclosed in D2, they produced cells expressing Kv7.2/3 channels in a controlled fashion by using a plasmid vector to transfect CHO-K1 cells. CHO-K1 cells are hamster ovarian cells, often used in studies of ion channels because they have low levels of endogenous ion channels. These cells are not neurons. D2 simply does not disclose overexpression of Kv7.3 in neurons. Furthermore, D2 does not disclose any mutant Kv7.3 sequences.

Although D2 presents a laundry list of conditions characterised by "dysfunction of neuronal excitability" for which the compositions may be useful at columns 7 and 8, at no point does D2 teach overexpression of Kv7.3 in a neuron followed by subsequent administration of the compositions. Thus, D2 provides no teaching in relation to the vectors or chemogenetic approach disclosed in the present invention. As such, all claims are inventive in view of D2.

D3 (Friedman et al, 2016) discloses that overexpression of KCNQ3 normalises depressive phenotypes. The researchers took this as evidence that increasing KCNQ3 channel activity reduces depression and looked at other ways to increase activity that do not require overexpressing KCNQ3. They therefore tested KCNQ channel openers (e.g. retigabine) to determine if they have the same effect. Thus, D3 also, separately, discloses that KCNQ openers reduce depressive behaviour.

D3 does not disclose combining the two experiments in order to increase the sensitivity of neurons to retigabine. Furthermore, even if that had been suggested, a teaching in relation to a wild type Kv7.3 and its effect in the CNS on depression does not provide any teaching in relation to mutant Kv7.3, nor about the effect of Kv7.3 and retigabine outside of the CNS. Therefore all claims are inventive in view of D3.

D4 (WO2021/119018) discloses CRISPR-mediated gene disruption (knock-down) of kcnq2/3 in Hcrt neurons. It does not disclose overexpression of Kv7.3. Therefore, all claims are inventive in view of D4.

We respectfully reject the Examiner's opinion that any one of D1 to D4 could be considered the closest prior art. As discussed above, D1, is the opposite to the present invention, D2 does not express Kv7.3 in neurons, and D4 (which is listed as an A citation) does not overexpress Kv7.3, it knocks it down using CRISPR.

Claim 1

1. A method of increasing the efficacy of a neuromodulatory drug comprising the steps:

a. introducing a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16 to a target neuron via a vector,

b. overexpressing the Kv7.3 ion channel subunit in the target neuron such that it forms functional ion channels, and

c. administering a lower dose of the neuromodulatory drug than would normally be administered to a subject in order to be therapeutically effective wherein the neuromodulatory drug is a voltage gated potassium channel-targeting drug.

Taking D3 as the closest prior art, the difference between D3 and claim 1 is that the method of claim 1 requires the overexpression of Kv7.3 ion channels in a target neuron, followed by the administration of a lower dose of a voltage gated potassium channel-targeting drug than would normally be therapeutically effective.

The technical effect of the difference is that the neurons are made more sensitive to the drug by presenting more targets, thereby allowing a lower dose of the drug to be used. As such, the efficacy of the drug is improved by the method. Thus, the objective technical problem can be considered to be how to improve the efficacy of a neuromodulatory drug.

Starting from D3, the skilled person understands that upregulation of KCNQ3 or administration of retigabine lead to reduction of depressive behaviours. The two, entirely separate, experiments achieve a similar end – the reduction of depressive behaviour. As such, there is no reason to combine the two experiments. D3 does not set out to increase the efficacy of retigabine, it aims to mimic the effect of upregulating KCNQ3. Since one or the other method achieve their end, there is no motivation to try both.

Faced with the problem of how to improve the efficacy of retigabine (as an example of a voltage gated potassium channel-targeting drug), the skilled person would find no motivation to combine the individual experiments in D3 because the overexpression of KCNQ3 in D3 is used solely to prove a hypothesis that K+ channels are linked to depression. The ONLY therapeutic option discussed in D3 is the use of KCNQ channel openers. Furthermore, there is no indication that overexpression of KCNQ3 would permit a lower dose of retigabine to be used.

In order to arrive at the invention of claim 1, starting from D3, the skilled person would need to combine the different experiments of D3 AND simultaneously lower the dose of retigabine used. In the absence of any implicit prompting to do so in D3, we submit that the

skilled person would not have combined the two experimental methods and, further would not have lowered the dose of retigabine. The Examiner is reminded that the guidelines state that "*it is not whether the skilled person could have arrived at the invention by adapting or modifying the closest prior art but whether the skilled person* ***would have done*** *so because the prior art provided motivation to do so in the expectation of some improvement or advantage*".

Thus claim 1 is inventive in view of D3.

Combining D3 with D1 would not remedy the deficiencies because, as discussed above, D1 uses an opposite methodology in which MA causes a disease state, it is not used as a therapeutic. The two disclosures are simply not compatible.

Combining D3 with D2 would also not render claim 1 obvious because D2 also does not combine upregulation of Kcnq3 with administration of a lower dose of neuromodulatory drug.

Finally, combining D1 with D4 would be incompatible because D4 knocks down kcnq3, whilst D3 overexpresses it.

Thus claim 1 is inventive in view of D3 in combination with any of D1, D2 or D4.

Claim 4

4. A method of treating a neurological disorder comprising the steps:

a. administering to a subject in need thereof, a therapeutically effective amount of the gene therapy vector comprising a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16,

b. upregulating expression of the Kv7.3 ion channel subunit in a target neuron such that it forms functional ion channels, and

c. administering a lower dose of a neuromodulatory drug known to target Kv7.3 ion channels than would normally be administered to the subject in order to be therapeutically effective.

Taking D3 as the closest prior art, the difference between D3 and claim 4 is that the method of claim 4 requires the upregulation of Kv7.3 ion channel expression in a target neuron, followed by the administration of a lower dose of a voltage gated potassium channel-targeting drug than would normally be therapeutically effective.

As with claim 1, the technical effect of the difference is that the neurons are made more sensitive to the drug, thereby allowing a lower dose of the drug to be used. As such, the efficacy of the drug is improved by the method. Thus, the objective technical problem can be considered to be how to administer a lower dose of a neuromodulatory drug effectively.

The same arguments presented in relation to claim 1 apply here, thus claim 4 is inventive in view of D3.

Similarly, claim 4 is inventive in view of D3 in combination with any of D1, D2 or D4.

Claim 7

> 7. A gene therapy vector comprising an AAV capsid that selectively transduces target sensory-motor neurons, and a nucleotide sequence encoding the Kv7.3 ion channel subunit amino acid sequence of any of SEQ ID.9 to SEQ ID.16.

Taking D3 as the closest prior art, the difference between D3 and claim 7 is that the vector selectively targets sensory-motor neurons, that is, peripheral nervous system.

The technical effect of the difference is that the neurons transduced by the vector are physiologically different to those in the CNS. Thus, the objective technical problem can be considered to be how to provide a vector that overexpresses Kv7.3 in sensory-motor neurons.

Starting from D3, the skilled person understands that upregulation of KCNQ3 by VTA dopaminergic neurons, or systemic administration of retigabine each lead to reduction of depressive behaviours. There is no recognition of delivering Kv7.3 to sensory-motor neurons.

Faced with the problem of how to overexpress Kv7.3 in sensory motor neurons, the skilled person would simply not look to D3 because D3 is directed CNS neurons.

Thus claim 7 is inventive in view of D3.

Combining D3 with D1 would not remedy the deficiencies because, as discussed above, D1 is also directed to CNS neurons and D3 is incompatible with D1.

Combining D3 with D2 would also not help, because D2 does not express Kv7.3 in neurons at all.

Combining D3 with D4 is not possible because the disclosures are incompatible.

Thus claim 7 is inventive in view of D3 in combination with any of D1, D2 or D4.

All independent claims are therefore inventive in view of the cited prior art documents and all dependent claims are deemed inventive at least by virtue of the dependency.

Clarity

Claims using "lower dose" have now been amended to specify the baseline of "than would normally be administered to a subject to be therapeutically effective". The skilled person readily understands what a therapeutic dose is because such dosages are provided on the label of pharmaceuticals/drugs. Thus, the term is now clear.

The terms "increasing the efficacy" and "reducing therapeutically effective dose" are readily understandable by the skilled person. In the context of the present invention, it is an increase in the sensitivity of a neuron to the neuromodulatory drug that makes the drug more effective – the drug has more targets upon which to act. Hence, the efficacy is increased which is equivalent to being able to reduce the therapeutically effective dose. We submit that "effective dose" is commonly using in pharmaceutical and biotech patent descriptions and is simply part of the lexicon of the field, readily understood by all readers.

Claims reciting reduced side effects and increasing therapeutic index have been deleted, thus the objection is now moot.

Neuromodulatory drug has now been limited to voltage gated potassium channel-targeting drug throughout, therefore the objection is overcome.

The number of independent claims has been reduced to 6. Each of which relates to the same inventive concept, but various aspects or applications thereof. We submit that the claims are now clear and concise, particularly in view of the likelihood that some claims (e.g. method of treatment claims) will necessarily be deleted in some territories due to local practice.

Claims have been limited to nucleotide sequences that encode the amino acid sequence throughout, therefore the objection is overcome.

We have retained dependent claims that span two categories at present. We submit that these are not unclear, the skilled person can readily determine the scope of the claims with respect to their dependencies.

We submit that the amended claims are now novel and inventive and look forward to receipt of the International Preliminary Examination Report in due course.

Yours sincerely,

S Kaye

Dr Samantha Kaye CPA EPA
Representative for the Applicant

Enclosed: amended claims and specification – clean and marked up format

PCT

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA: EP	Date of receipt of DEMAND: 03 March 2025(03.03.2025)

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference SANI3WO
International application No. PCT/IB2024/054426	International filing date 07 May 2024(07.05.2024)	(Earliest) Priority date 10 May 2023(10.05.2023)
Title of invention METHOD		
Box No. II APPLICANT(S)		
Name and address: **SANIA RX LTD** **Waterfront Building** **Manbre Wharf** **Manbre Road** **London W6 9RH** **United Kingdom**		Telephone No. Facsimile No. Applicant's registration No. with the Office
Nationality: United Kingdom	Residence: United Kingdom	

E-mail authorization: Marking one of the check-boxes below authorizes the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application if those offices are willing to do so.

☐ as advance copies followed by paper notifications; or

☐ exclusively in electronic form (no paper notifications will be sent).

E-mail address:

☐ Further applicants are indicated on a continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☑ agent ☐ common representative

and ☑ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address:	Telephone No.
ACORN IP LLP **Colin Sanders Business Innovation Centre** **Mewburn Road** **Banbury, Oxfordshire OX16 9PA** **United Kingdom**	+44(0)7961241903
	Facsimile No.
	Agent's registration No. with the Office

E-mail authorization: Marking one of the check-boxes below authorizes the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send notifications issued in respect of this international application if those offices are willing to do so.

☐ as advance copies followed by paper notifications; or

☑ exclusively in electronic form (no paper notifications will be sent).

E-mail address: patents@acorn-ip.co.uk

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

the description	☐ as originally filed, or
	☑ as amended under Article 34
the sequence listing (if any)	☑ as originally filed, or
	☐ as amended under Article 34
the claims	☐ as originally filed, or
	☐ as amended under Article 19, and/or
	☑ as amended under Article 34
the drawings (if any)	☑ as originally filed, or
	☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.
3. ☐ Where the IPEA wishes to start the international preliminary examination at the same time as the international search in accordance with Rule 69.1(b), the applicant requests the IPEA **to postpone** the start of the international preliminary examination until the expiration of the applicable time limit under Rule 69.1(d).
4. ☐ The applicant expressly requests **to postpone** the start of the international preliminary examination until the expiration of the applicable time limit under Rule 54bis.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☑ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☑ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- Article 34 Amendments: 4 documents
- Letter accompanying amendments under Article 34: 1 document

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

/Samantha KAYE/

Name (LAST, First): **KAYE, Samantha**

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND: 03 March 2025(03.03.2025)	
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):	
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly. 4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5. 5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rules 82 or 82quater.	6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54bis.1(a) and item 7 or 8, below, does not apply. 7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54bis.1(a) as extended by virtue of Rule 80.5. 8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54bis.1(a), the delay in arrival is EXCUSED pursuant to Rules 82 or 82quater.

For International Bureau use only

Demand received from IPEA on:

Intellectual Property Office

Sania RX Limited
c/o Mishcon de Reya LLP
Africa House
70 Kingsway
London
WC2B 6AH

Patents Directorate

Concept House
Cardiff Road, Newport
South Wales, NP10 8QQ

Direct Line: 01633 811209
[t]**E-Mail:** Andrew.Guy@ipo.gov.uk
Switchboard: 0300 300 2000

Your Reference: 152472.7GBp
Application No: GB2306914.9

20 November 2023

Dear Recipient

Patents Act 1977:
Combined Search and Examination Report under Sections 17 and 18(3)

Latest date for reply: **12 May 2025**

I enclose a copy of my search and examination report and a copy of the cited non-patent literature. Please note that published patent documents mentioned in my report may be obtained for free on the internet and are usually freely available from http://worldwide.espacenet.com.

By the above date you should either file amendments to meet the objections in the report or make observations on them. If you do not, the application may be refused. I will consider your response and will reply in a timescale consistent with our current target: https://www.gov.uk/government/publications/timeliness-target-for-re-examination-of-patent-applications

Online e-filing

You may file such amendments or observations electronically if you wish, using the online patent filing services detailed in https://www.gov.uk/government/publications/how-to-file-documents-with-the-intellectual-property-office .

Other search results

As part of substantive examination, we routinely give due consideration to the search results and examination reports of equivalent applications filed at other patent offices. Consequently, there is no need to supply us with published reports on equivalent applications although we may ask you to provide us with copies of reports or citations if we are unable to obtain them ourselves.

However, sending us unpublished search or examination reports on equivalent patent applications enables us to consider these earlier in the examination process, leading to more efficient patent processing.

Publication

I estimate that preparations for publication of your application will be completed soon after **1 October 2024**. At this time you will receive a letter confirming the exact date of the completion of the preparations for publication. This letter will also tell you the publication number and date of publication of your application. However, it should **NOT** be relied upon as a reminder if you are intending to withdraw your application before publication, as it may not be issued in time for you to do so.

On the date of publication details of your application, including your name and address, will be entered in the Register of Patents and will become publicly available, including on our website. Some documents and correspondence from your application file will also be made publicly available on our website at http://www.ipo.gov.uk/p-ipsum.

Withdrawal

If you wish to withdraw your application to prevent publication you must withdraw it before the preparations for publication are complete. One way that you can withdraw your application is by emailing withdraw@ipo.gov.uk. Further details on withdrawal are available from https://www.gov.uk/patent-your-invention. **WARNING** – once preparations for publication are complete it will NOT be possible to prevent publication.

Amendment

If you wish to file amended claims for inclusion with the published application you must do so before the preparations for publication are completed.

Correspondence

If you write to the Office less than 3 weeks before 1 October 2024 please mark your letter prominently: **"URGENT - PUBLICATION IMMINENT"**.

Discussion with the examiner

A conversation with the examiner can often help to clarify objections and hence result in more efficient processing of an application. I am always happy to discuss an application at any point. To get the most from our conversation it would be helpful to arrange a mutually convenient time in advance. To arrange an audio/video call please contact me at the email address above indicating your availability and the subject you would like to discuss. This will give me a chance to review your application, leading to a more efficient discussion.

Yours sincerely

Dr Andrew Guy

Dr Andrew Guy
Examiner

Important information about combined search and examination

I also ask that you take note of the following points. These might have a bearing on the future stages of your application because the examination report has been sent to you before your application has been published.

(a) You may file voluntary amendments before making a full response to my examination report. We will publish with your application any new or amended claims you file voluntarily or as a full response, provided that they are received before preparations for publication are completed. It would help us when you file amendments before publication if you could **prominently indicate** in a covering letter whether or not the amendments are intended as a full response to the examination report.

(b) If you file a full response to the examination report before your application is published I will consider it as soon as possible. However, if this would disrupt the publication of your application, I would have to delay taking any action until the application had been published. This delay could be up to 3 months, depending upon when we receive your response.

(c) There is another situation when there might be a delay between you filing a full response and the Office responding to it. This would arise if you met all my objections but your application had not or had only recently been published. I could not report the outcome of my re-examination until I was satisfied that the search was complete for documents published before the priority date of your invention and that anybody interested in the application has had three months following publication of the application to make observations on the patentability of your invention.

(d) Provided that the requirements of the Act have been met, I can send your application to grant as early as three months after publication. Before doing so I will bring the original search up to date and raise with you any further objection that might result from this top-up search. However, there is a possibility that at that time I may not have access to all the patent applications published after the priority date of your invention and of possible relevance to your application. If this is the case I would have to complete the search after grant and if necessary raise any new found novelty objection then.

Intellectual Property Office

Your ref :	152472.7GBp	**Examiner :**	Dr Andrew Guy
Application No:	GB2306914.9	**Tel :**	01633 811209
Applicant :	Sania RX Limited	**Date of report :**	20 November 2023
Latest date for reply:	12 May 2025	Page 1/2	

Patents Act 1977
Combined Search and Examination Report under Sections 17 & 18(3)

Excluded subject matter

1. In their current format, claims 1-5 and their dependents at least in part define methods of treatment, and are excluded from patentability under section 4A of the Act.

Novelty

2. The invention as defined in claims 6 and 12-13 is not new because it has already been disclosed in the following documents:

US 8883812 B2	Claffey et al	See column 11 line 37-column 12 line 4

3. US 8883812 B2 describes at the listed passage the creation of Kv7.3 vectors for the transfection of cell lines. The scope of 'gene therapy vector' is not wholly clear; any vector capable of transfecting a mammalian cell is considered within its scope. Similarly, it is not wholly clear whether the sequence used in this document is identical to those of the current application, but it is human Kv7.3 and assumed therefore to be identical to SEQ ID NO 1, which appears to be the wildtype human sequence. The remaining features of claim 6 are essentially desired results rather than a technical feature of the vector, being defined by what happens when the vector is used – the document is considered an anticipation of claim 6 as it is otherwise a vector comprising a Kv7.3 subunit, presumed to be of SEQ ID NO 1.

4. The document is also considered to anticipate claims 12-13.

Inventive step

5. The invention as defined in claims 6-8, 12-13 and 19-21 is obvious in view of what has already been disclosed in the following documents:

Int J Mol Sci, vol. 23, 2022, 'Inactivation of the lateral hypothalamus attenuates…' art. 7305	Liu et al	See abstract; section 2.6 page 7; figure 6 page8

6. Liu et al describes the generation of AAV vectors comprising the murine homologue of KCNQ3, Kcnq3. The vectors are used in the context of reversing addictive behaviour in mice by additional expression of the Kv7.3 channel, with at least some implication that this

[Examination Report contd.]

would be a potential therapeutic strategy (e.g, section 3, final two sentences). Generation of a vector containing the human gene is therefore considered obvious in context as any therapeutic motivation for treating methamphetamine addiction is unlikely to be of benefit in mice. Additionally, considering a claim to a per se vector, there is little inventive skill in switching one homologue for another. Claim 6 is obvious in light of this document.

7. The AAV vector is also stated to transduce neurons and is not otherwise expressed alongside other channels; claims 7-8 and 12-13 are also obvious.

8. Claims 19-21 effectively define the invention by result; use of the vector in any context would be considered to have these effects. These claims are also obvious.

Clarity

9. Many of the claims define the invention by desired result rather than specifying a technical feature which gives rise to this result. Such claims do not clearly define the invention.

Intellectual Property Office

Application No:	GB2306914.9	**Examiner:**	Dr Andrew Guy
Claims searched:	1-22	**Date of search:**	17 November 2023

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	6-8, 12-13 and 19-21	Int J Mol Sci, vol. 23, 2022, Liu et al, 'Inactivation of the lateral hypothalamus attenuates...' art. 7305 See abstract; section 2.6 page 7; figure 6 page 8
X	6 and 12-13	US 8883812 B2 (CLAFFEY ET AL) See column 11 line 37-column 12 line 4
A	-	EP 1407768 A2 (GLAXO GROUP LTD) See abstract

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

The following online and other databases have been used in the preparation of this search report

SEARCH-PATENT, SEARCH-NPL

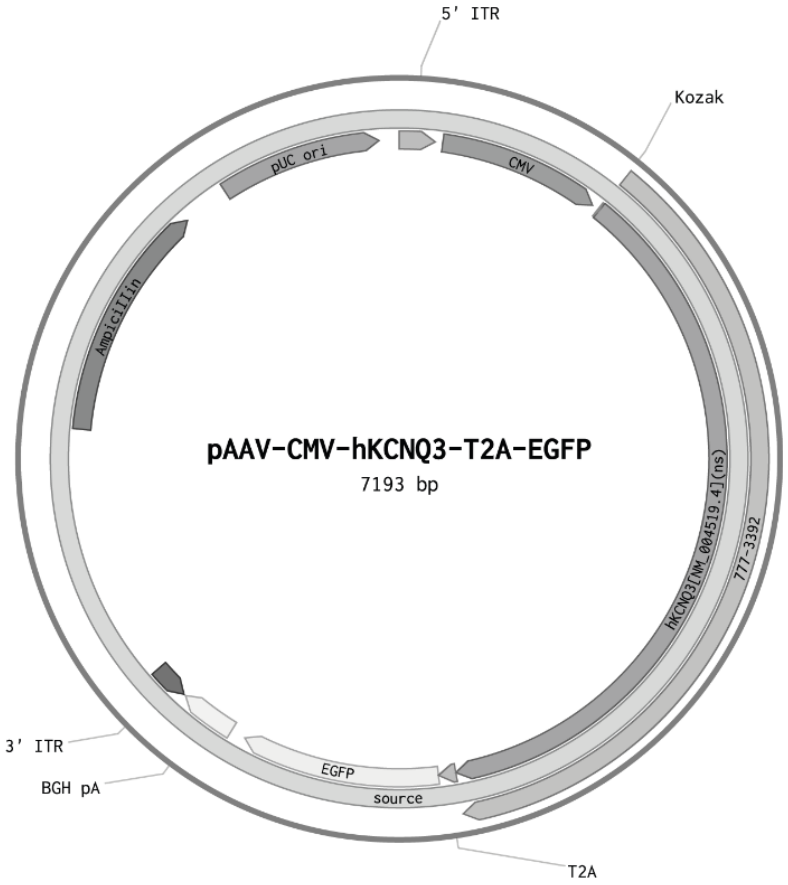
International Classification:

Subclass	Subgroup	Valid From
C12N	0015/864	01/01/2006
A61K	0048/00	01/01/2006

PCT

(Original in Electronic Form)

VIII-2-1	**Declaration: Entitlement to apply for and be granted a patent** Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51bis.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate: Name (LAST, First)	**In relation to this international application** **SANIA RX LTD is entitled to apply for and be granted a patent by virtue of the following:**
VIII-2-1 (ii)		**SANIA RX LTD is entitled as employer of the inventor, SMITH, Calvin**
VIII-2-1 (ii)		**SANIA RX LTD is entitled as employer of the inventor, BROWNSTONE, Robert**


5' ITR
Kozak
pUC ori
CMV
Ampicillin
pAAV-CMV-hKCNQ3-T2A-EGFP
7193 bp
hKCNQ3(NM_004519.4)(ns)
777-3392
3' ITR
BGH pA
EGFP
source
T2A

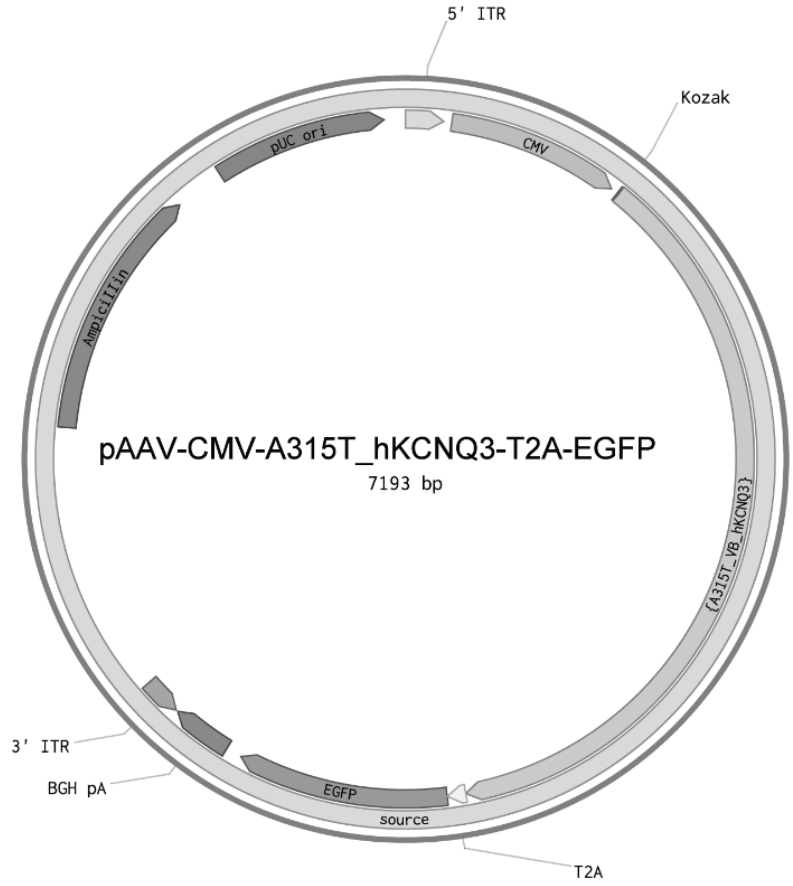
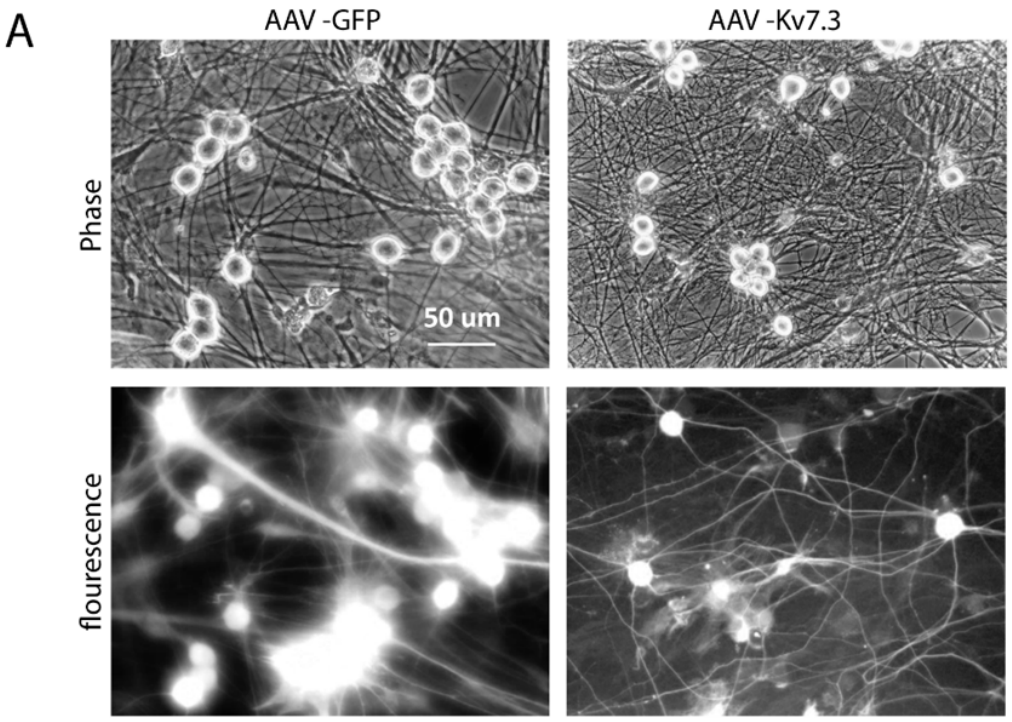
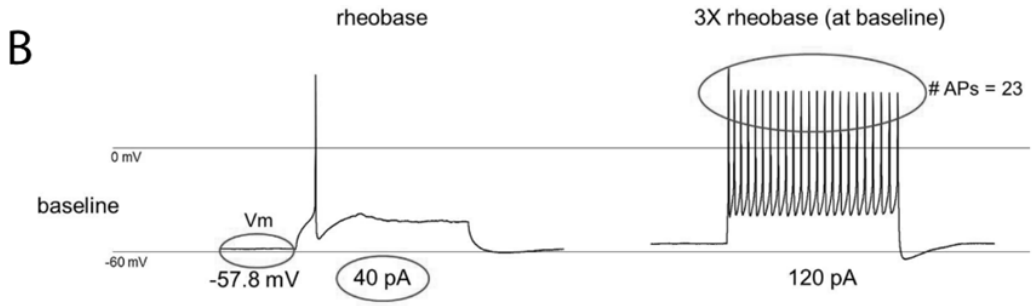

5' ITR
Kozak
pUC ori
CMV
Ampicillin
pAAV-CMV-A315T_hKCNQ3-T2A-EGFP
7193 bp
(A315T_VB_hKCNQ3)
3' ITR
BGH pA
EGFP
source
T2A

Figure 1


A
AAV -GFP
AAV -Kv7.3
Phase
flourescence
50 um


B
rheobase
3X rheobase (at baseline)
APs = 23
0 mV
baseline
Vm
-60 mV
-57.8 mV
40 pA
120 pA

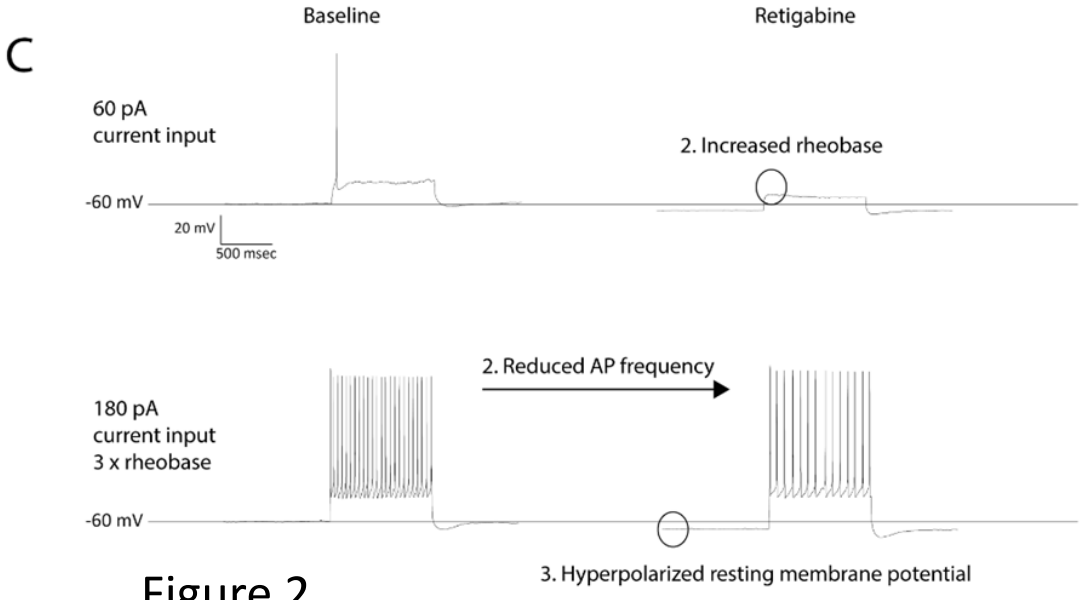

C
Baseline
Retigabine
60 pA
current input
2. Increased rheobase
-60 mV
20 mV
500 msec
2. Reduced AP frequency
180 pA
current input
3 x rheobase
-60 mV
3. Hyperpolarized resting membrane potential

Figure 2

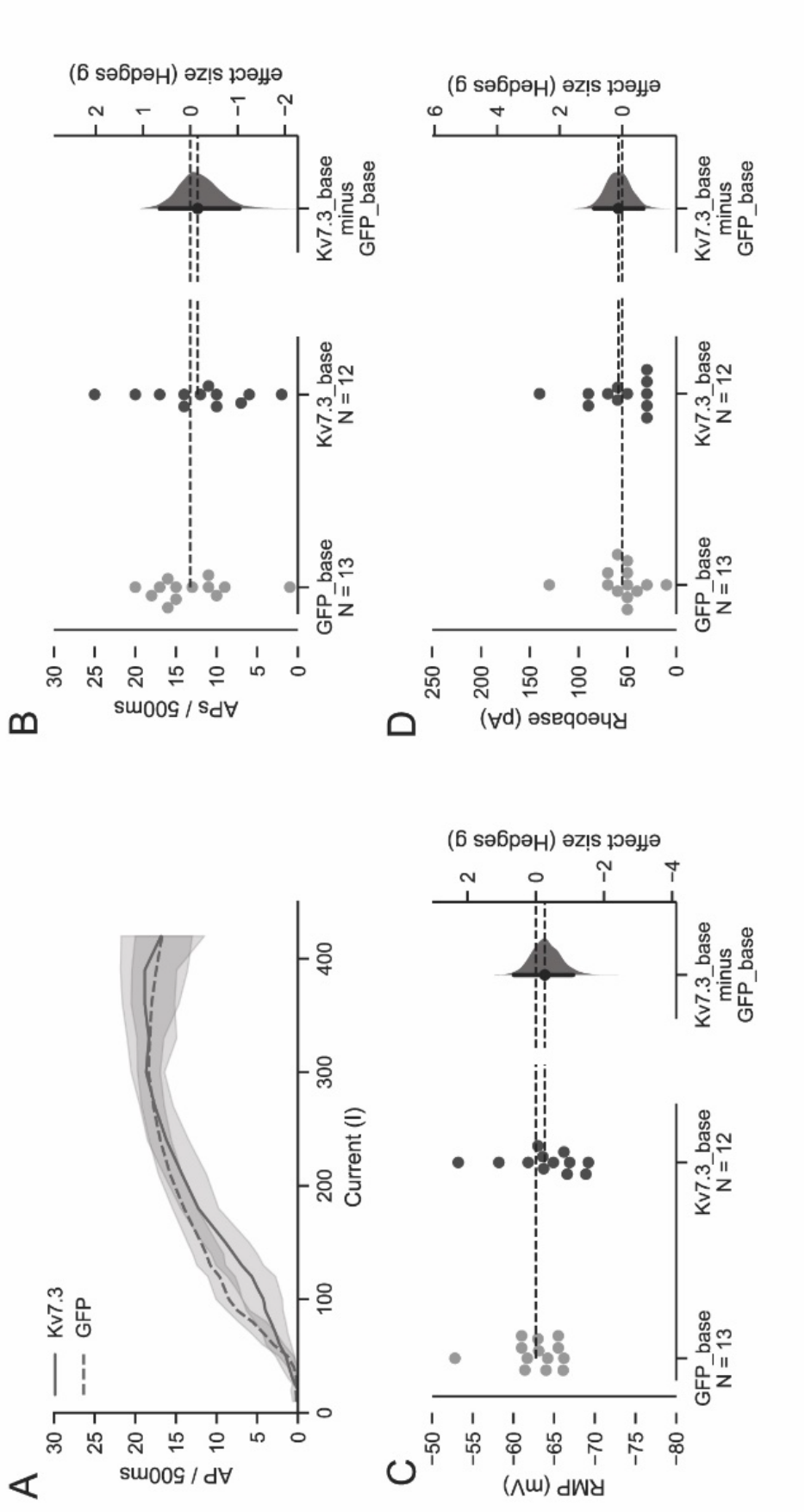

A
Kv7.3
GFP
APs / 500ms
Current (I)
B
APs / 500ms
effect size (Hedges g)
GFP_base
N = 13
Kv7.3_base
N = 12
Kv7.3_base
minus
GFP_base
C
RMP (mV)
effect size (Hedges g)
D
Rheobase (pA)
effect size (Hedges g)

Figure 3

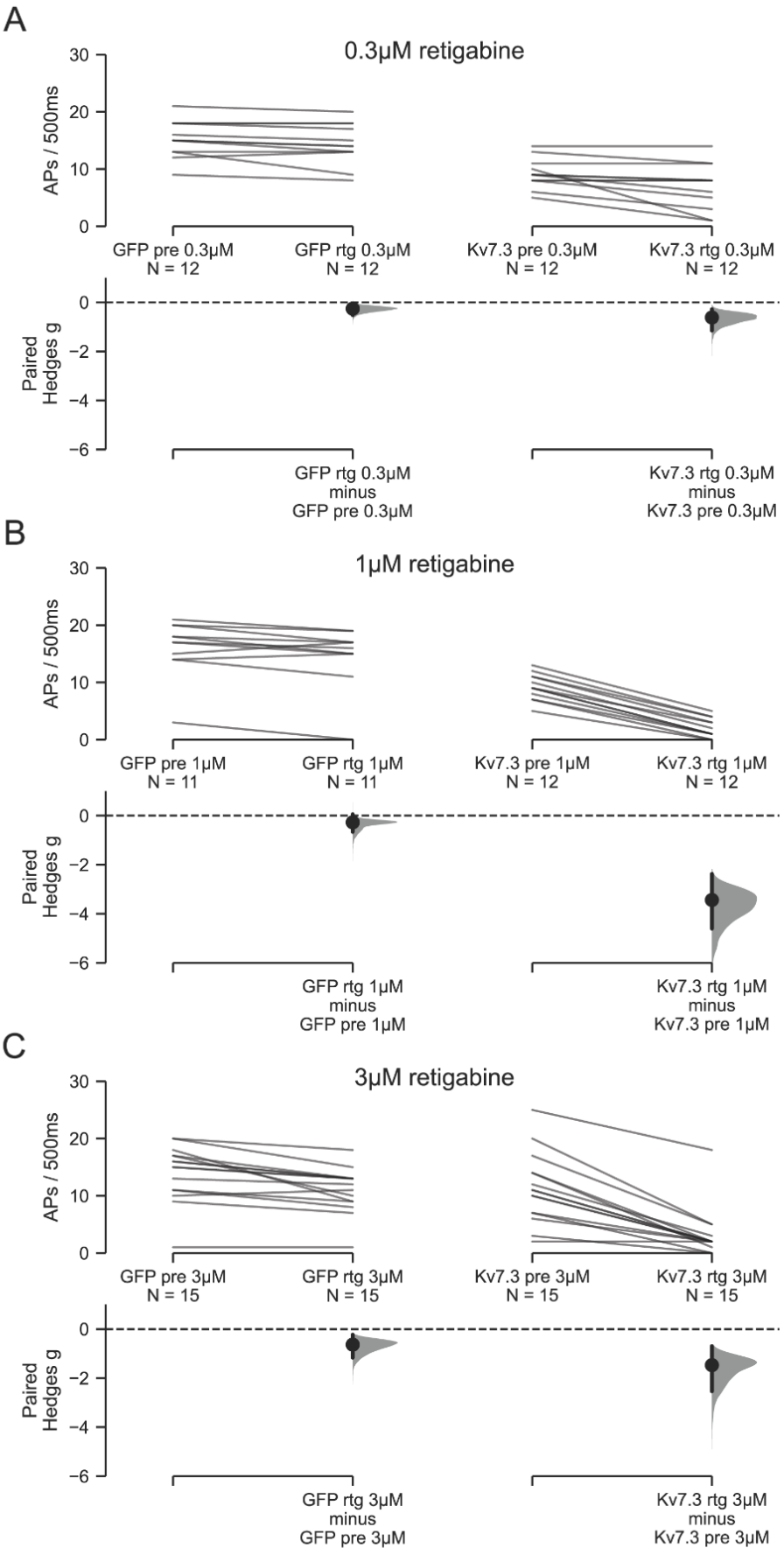

A
0.3μM retigabine
APs / 500ms
30
20
10
0
GFP pre 0.3μM
N = 12
GFP rtg 0.3μM
N = 12
Kv7.3 pre 0.3μM
N = 12
Kv7.3 rtg 0.3μM
N = 12
Paired
Hedges g
0
−2
−4
−6
GFP rtg 0.3μM
minus
GFP pre 0.3μM
Kv7.3 rtg 0.3μM
minus
Kv7.3 pre 0.3μM
B
1μM retigabine
APs / 500ms
GFP pre 1μM
N = 11
GFP rtg 1μM
N = 11
Kv7.3 pre 1μM
N = 12
Kv7.3 rtg 1μM
N = 12
Paired
Hedges g
GFP rtg 1μM
minus
GFP pre 1μM
Kv7.3 rtg 1μM
minus
Kv7.3 pre 1μM
C
3μM retigabine
APs / 500ms
GFP pre 3μM
N = 15
GFP rtg 3μM
N = 15
Kv7.3 pre 3μM
N = 15
Kv7.3 rtg 3μM
N = 15
Paired
Hedges g
GFP rtg 3μM
minus
GFP pre 3μM
Kv7.3 rtg 3μM
minus
Kv7.3 pre 3μM

Fig. 4

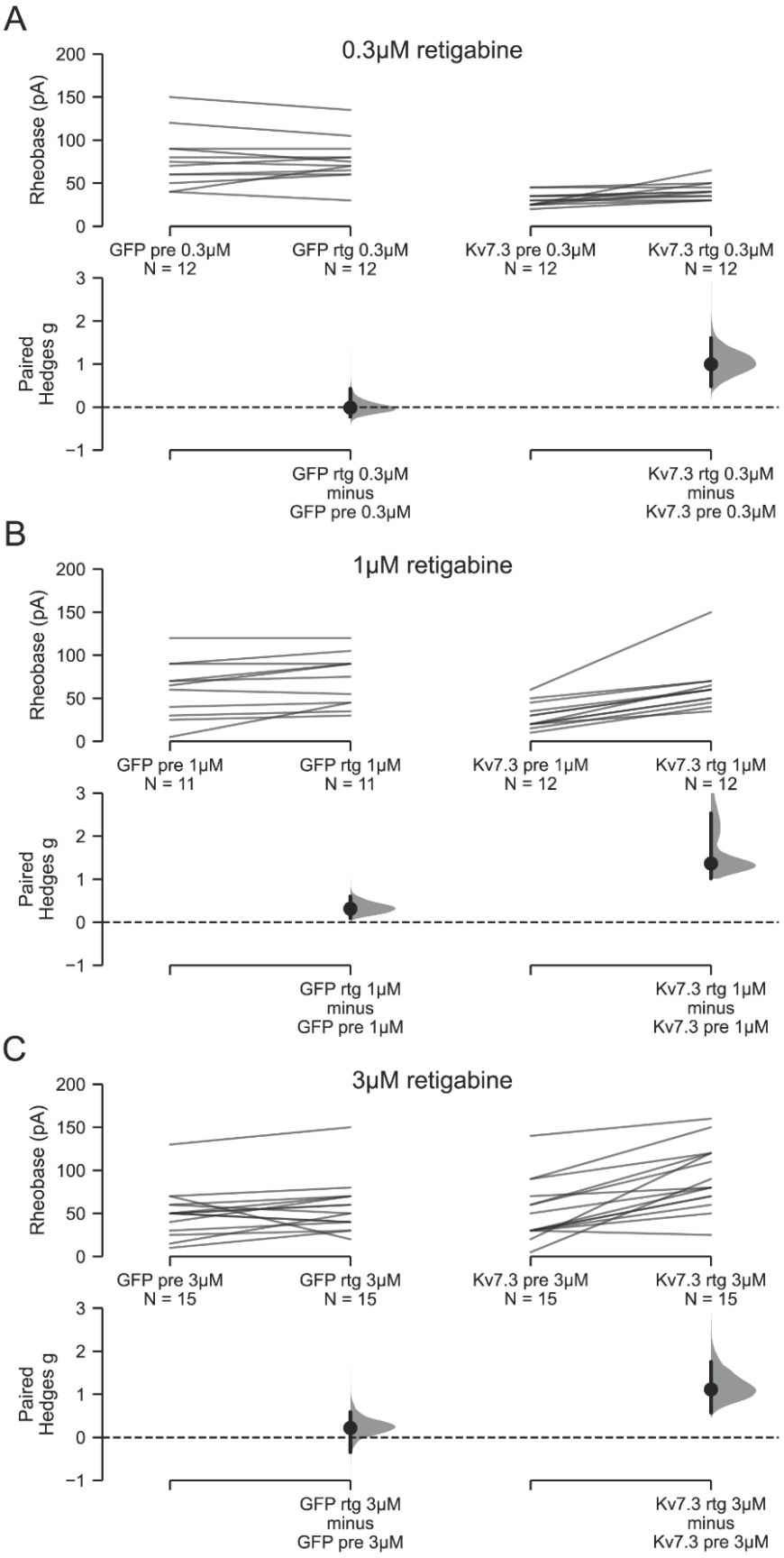

A
0.3μM retigabine
Rheobase (pA)
200
150
100
50
0
GFP pre 0.3μM
N = 12
GFP rtg 0.3μM
N = 12
Kv7.3 pre 0.3μM
N = 12
Kv7.3 rtg 0.3μM
N = 12
Paired
Hedges g
3
2
1
0
−1
GFP rtg 0.3μM
minus
GFP pre 0.3μM
Kv7.3 rtg 0.3μM
minus
Kv7.3 pre 0.3μM
B
1μM retigabine
Rheobase (pA)
GFP pre 1μM
N = 11
GFP rtg 1μM
N = 11
Kv7.3 pre 1μM
N = 12
Kv7.3 rtg 1μM
N = 12
Paired
Hedges g
GFP rtg 1μM
minus
GFP pre 1μM
Kv7.3 rtg 1μM
minus
Kv7.3 pre 1μM
C
3μM retigabine
Rheobase (pA)
GFP pre 3μM
N = 15
GFP rtg 3μM
N = 15
Kv7.3 pre 3μM
N = 15
Kv7.3 rtg 3μM
N = 15
Paired
Hedges g
GFP rtg 3μM
minus
GFP pre 3μM
Kv7.3 rtg 3μM
minus
Kv7.3 pre 3μM

Fig. 5

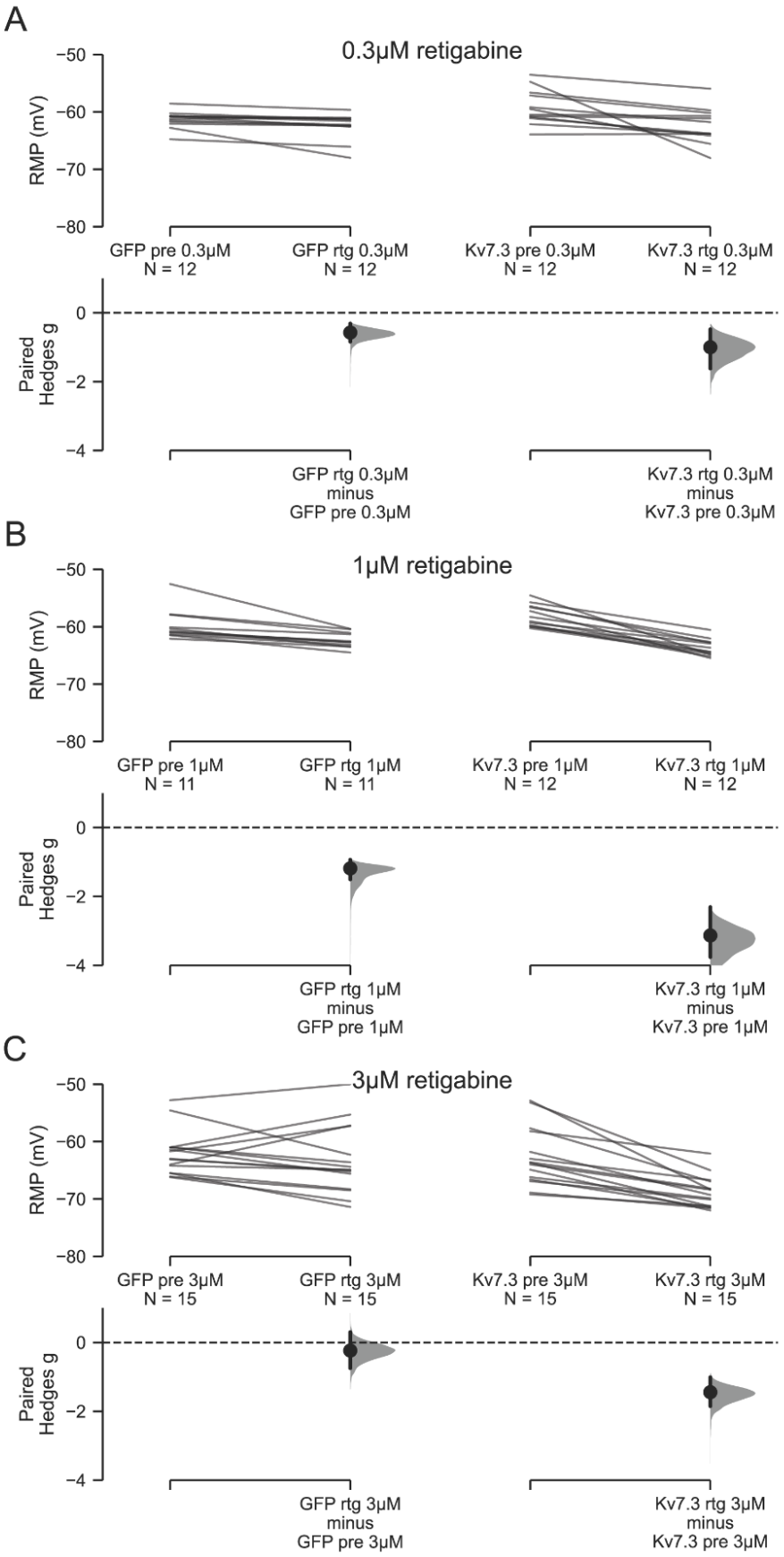

A
0.3μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 0.3μM
N = 12
GFP rtg 0.3μM
N = 12
Kv7.3 pre 0.3μM
N = 12
Kv7.3 rtg 0.3μM
N = 12
Paired Hedges g
0
−2
−4
GFP rtg 0.3μM
minus
GFP pre 0.3μM
Kv7.3 rtg 0.3μM
minus
Kv7.3 pre 0.3μM
B
1μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 1μM
N = 11
GFP rtg 1μM
N = 11
Kv7.3 pre 1μM
N = 12
Kv7.3 rtg 1μM
N = 12
Paired Hedges g
0
−2
−4
GFP rtg 1μM
minus
GFP pre 1μM
Kv7.3 rtg 1μM
minus
Kv7.3 pre 1μM
C
3μM retigabine
RMP (mV)
−50
−60
−70
−80
GFP pre 3μM
N = 15
GFP rtg 3μM
N = 15
Kv7.3 pre 3μM
N = 15
Kv7.3 rtg 3μM
N = 15
Paired Hedges g
0
−2
−4
GFP rtg 3μM
minus
GFP pre 3μM
Kv7.3 rtg 3μM
minus
Kv7.3 pre 3μM

Fig. 6

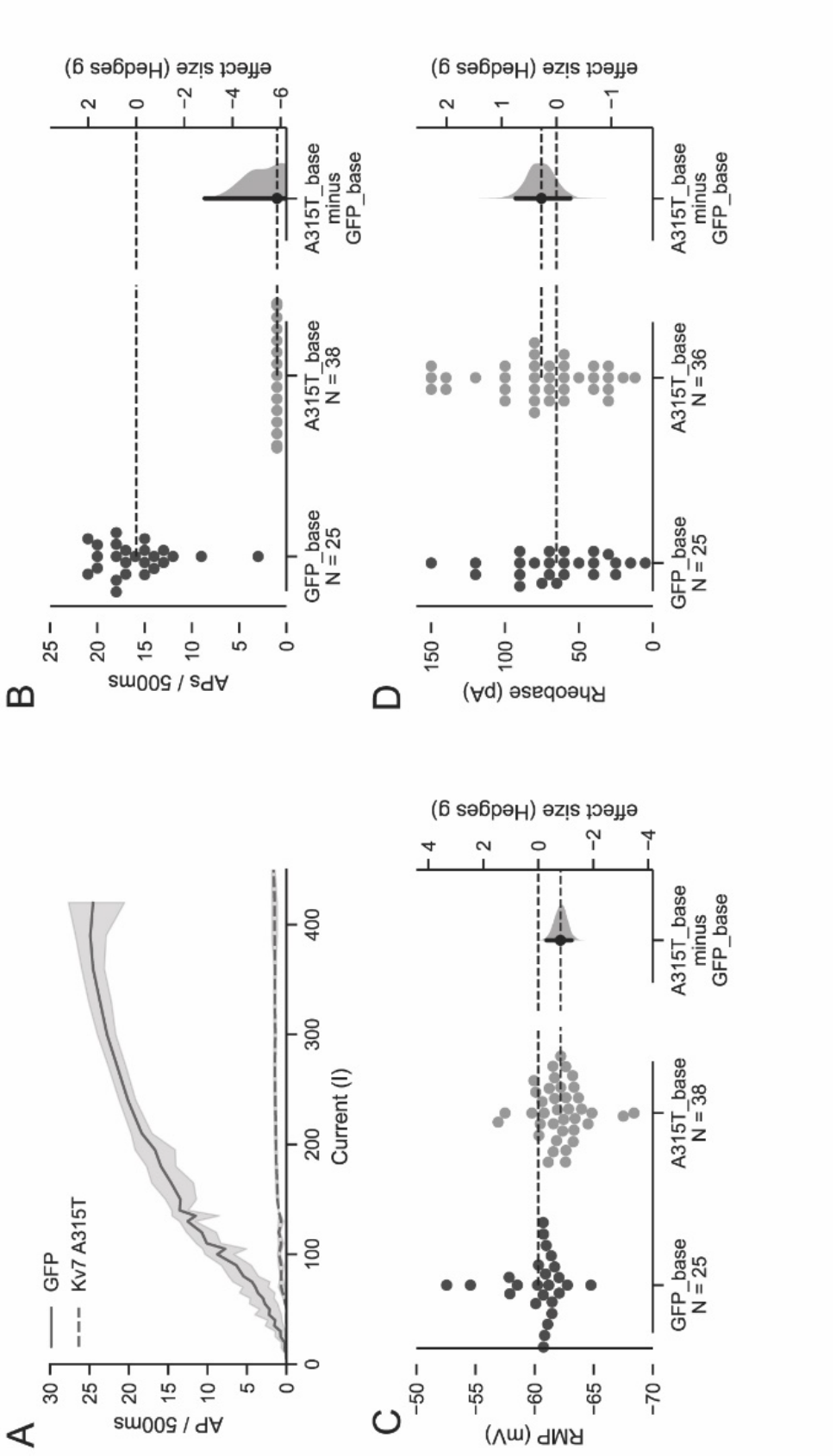

A
GFP
Kv7 A315T
AP / 500ms
Current (I)
B
APs / 500ms
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 38
A315T_base minus GFP_base
C
RMP (mV)
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 38
A315T_base minus GFP_base
D
Rheobase (pA)
effect size (Hedges g)
GFP_base N = 25
A315T_base N = 36
A315T_base minus GFP_base

Figure 7

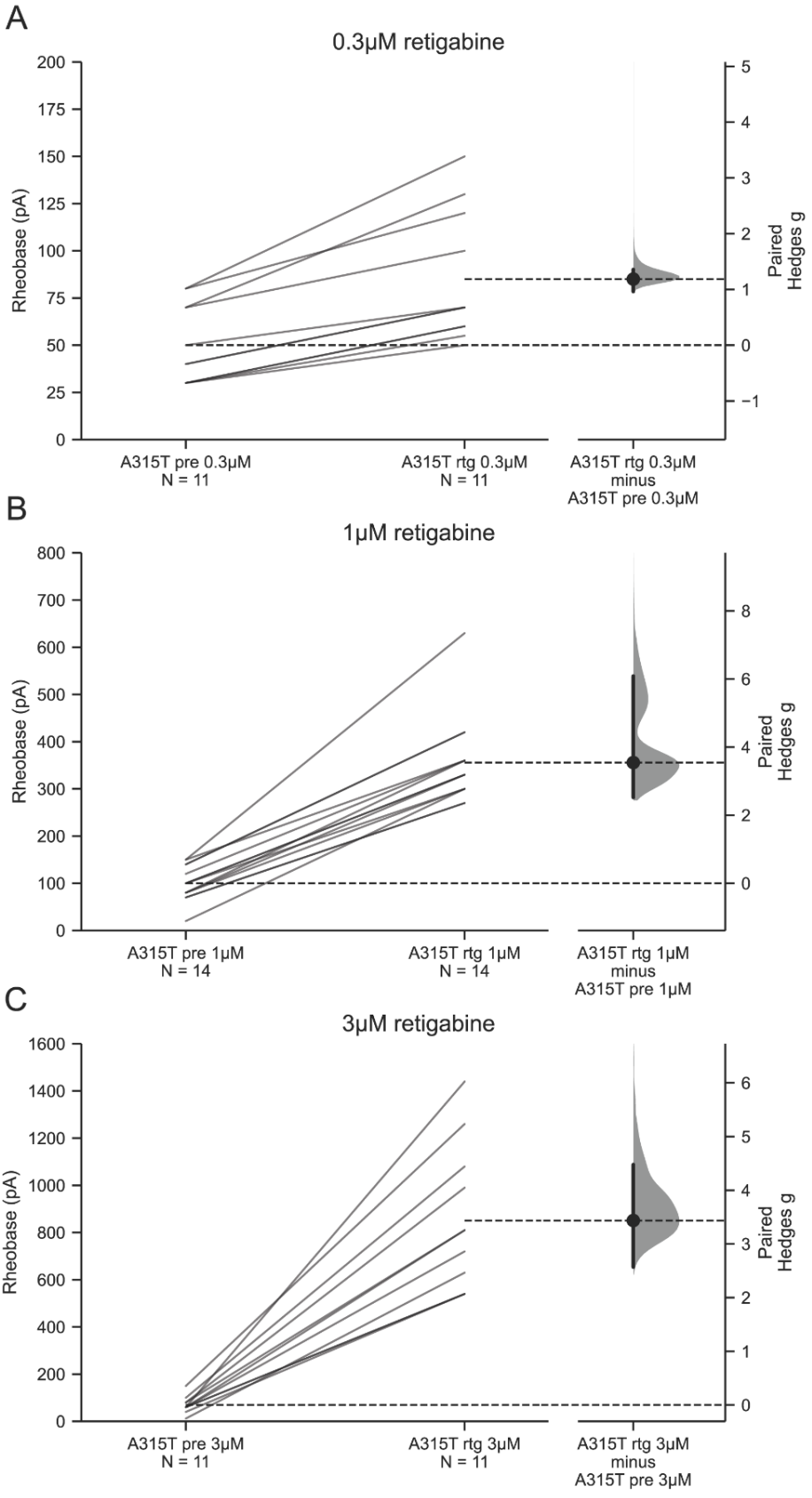

A
0.3μM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 0.3μM
N = 11
A315T rtg 0.3μM
N = 11
A315T rtg 0.3μM
minus
A315T pre 0.3μM
B
1μM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 1μM
N = 14
A315T rtg 1μM
N = 14
A315T rtg 1μM
minus
A315T pre 1μM
C
3μM retigabine
Rheobase (pA)
Paired Hedges g
A315T pre 3μM
N = 11
A315T rtg 3μM
N = 11
A315T rtg 3μM
minus
A315T pre 3μM

Fig. 8

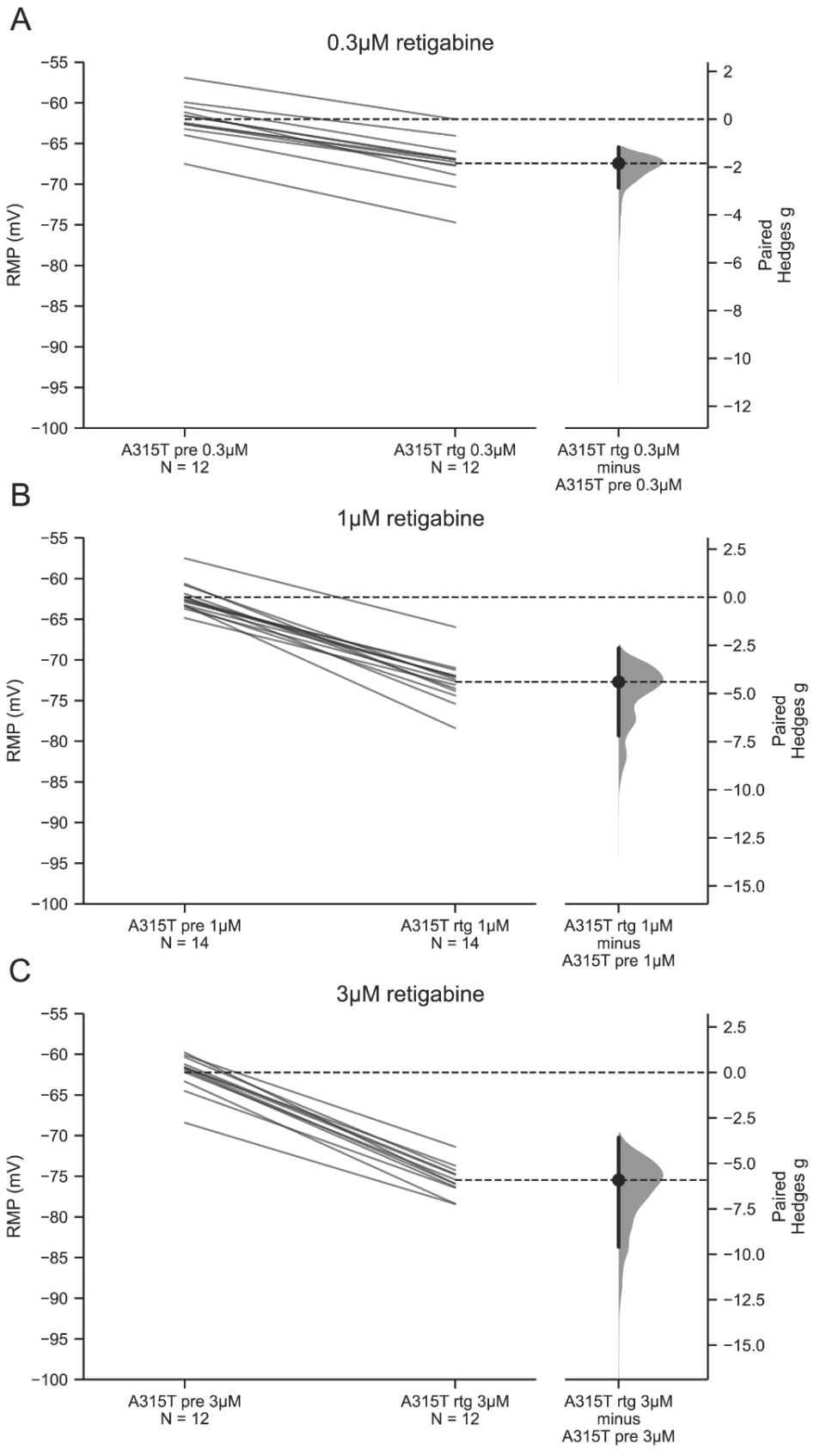

A
0.3μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 0.3μM
N = 12
A315T rtg 0.3μM
N = 12
A315T rtg 0.3μM
minus
A315T pre 0.3μM
B
1μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 1μM
N = 14
A315T rtg 1μM
N = 14
A315T rtg 1μM
minus
A315T pre 1μM
C
3μM retigabine
RMP (mV)
Paired Hedges g
A315T pre 3μM
N = 12
A315T rtg 3μM
N = 12
A315T rtg 3μM
minus
A315T pre 3μM

Fig. 9

Figure 10

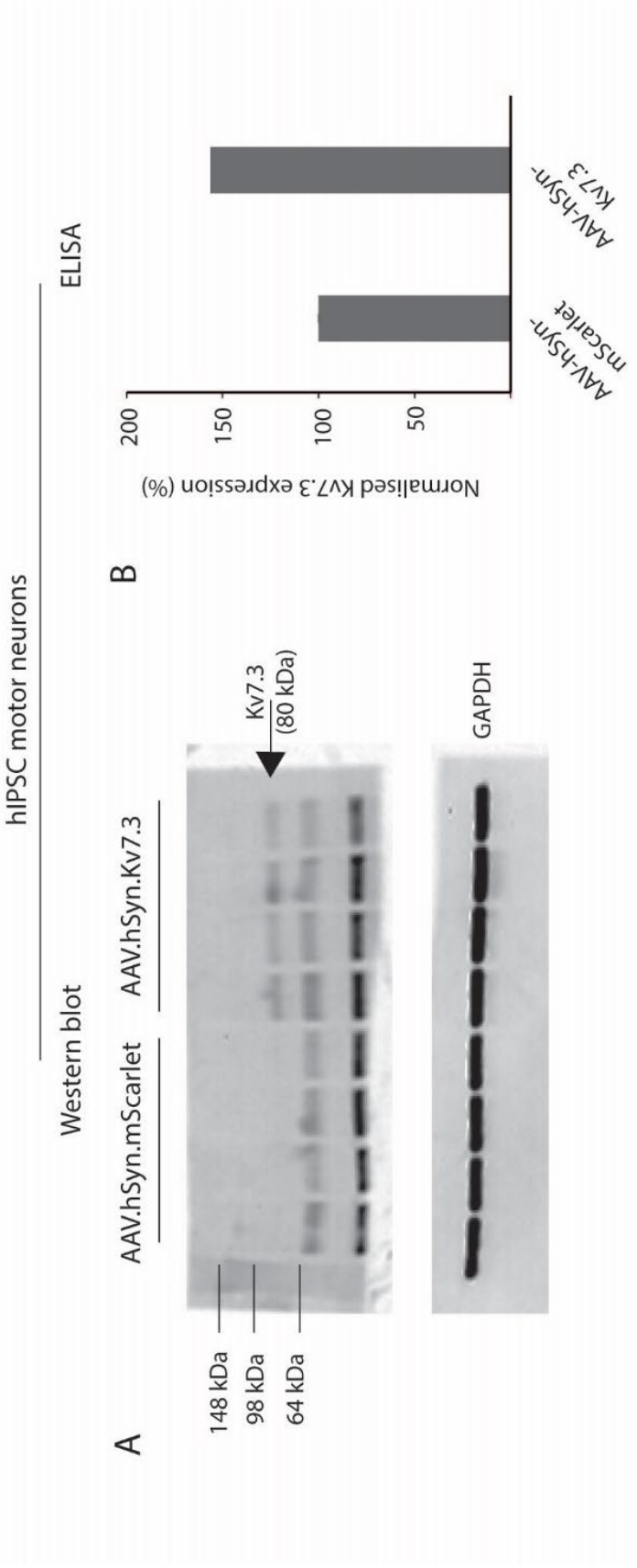
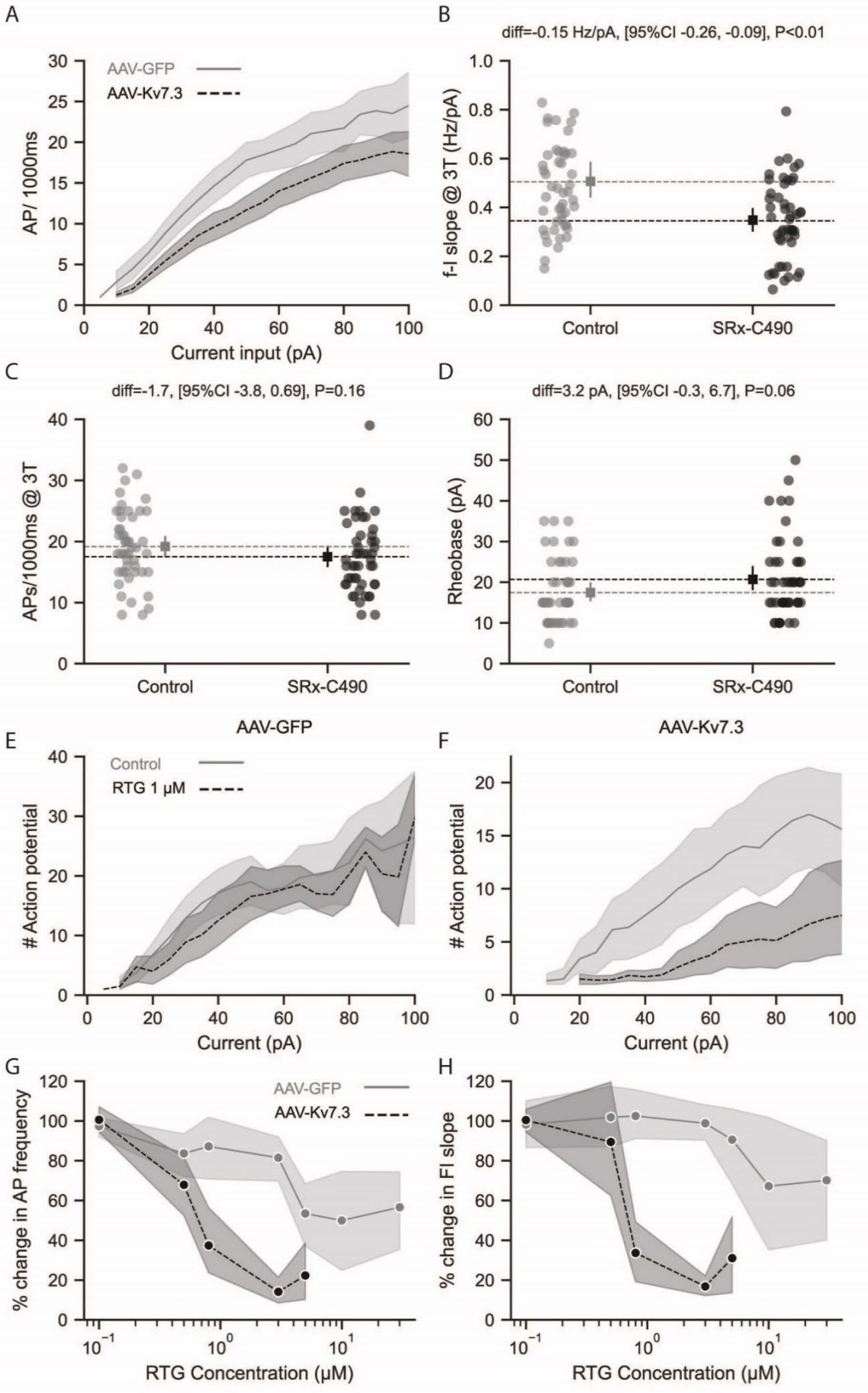

hIPSC motor neurons
Western blot
ELISA
A
B
AAV.hSyn.mScarlet
AAV.hSyn.Kv7.3
148 kDa
98 kDa
64 kDa
Kv7.3
(80 kDa)
GAPDH
Normalised Kv7.3 expression (%)
200
150
100
50
AAV-hSyn-mScarlet
AAV-hSyn-Kv7.3

Figure 11


A
AAV-GFP
AAV-Kv7.3
AP/ 1000ms
Current input (pA)
B
diff=-0.15 Hz/pA, [95%CI -0.26, -0.09], P<0.01
f-I slope @ 3T (Hz/pA)
Control
SRx-C490
C
diff=-1.7, [95%CI -3.8, 0.69], P=0.16
APs/1000ms @ 3T
Control
SRx-C490
D
diff=3.2 pA, [95%CI -0.3, 6.7], P=0.06
Rheobase (pA)
Control
SRx-C490
E
AAV-GFP
Control
RTG 1 µM
Action potential
Current (pA)
F
AAV-Kv7.3
Action potential
Current (pA)
G
AAV-GFP
AAV-Kv7.3
% change in AP frequency
RTG Concentration (µM)
H
% change in FI slope
RTG Concentration (µM)

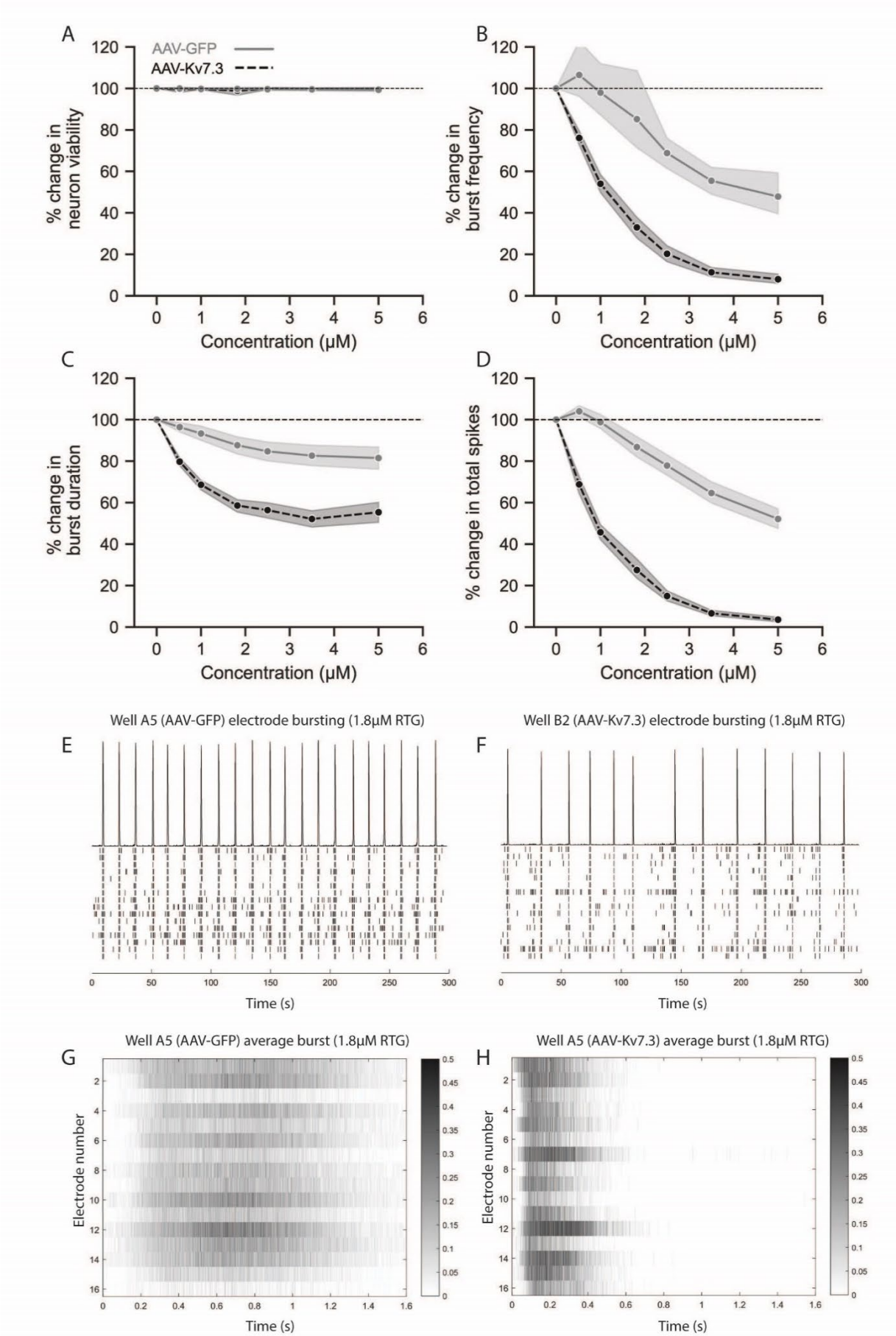

A
AAV-GFP
AAV-Kv7.3
% change in neuron viability
Concentration (μM)
B
% change in burst frequency
Concentration (μM)
C
% change in burst duration
Concentration (μM)
D
% change in total spikes
Concentration (μM)
E
Well A5 (AAV-GFP) electrode bursting (1.8μM RTG)
Time (s)
F
Well B2 (AAV-Kv7.3) electrode bursting (1.8μM RTG)
Time (s)
G
Well A5 (AAV-GFP) average burst (1.8μM RTG)
Electrode number
Time (s)
H
Well A5 (AAV-Kv7.3) average burst (1.8μM RTG)
Electrode number
Time (s)

Figure 12

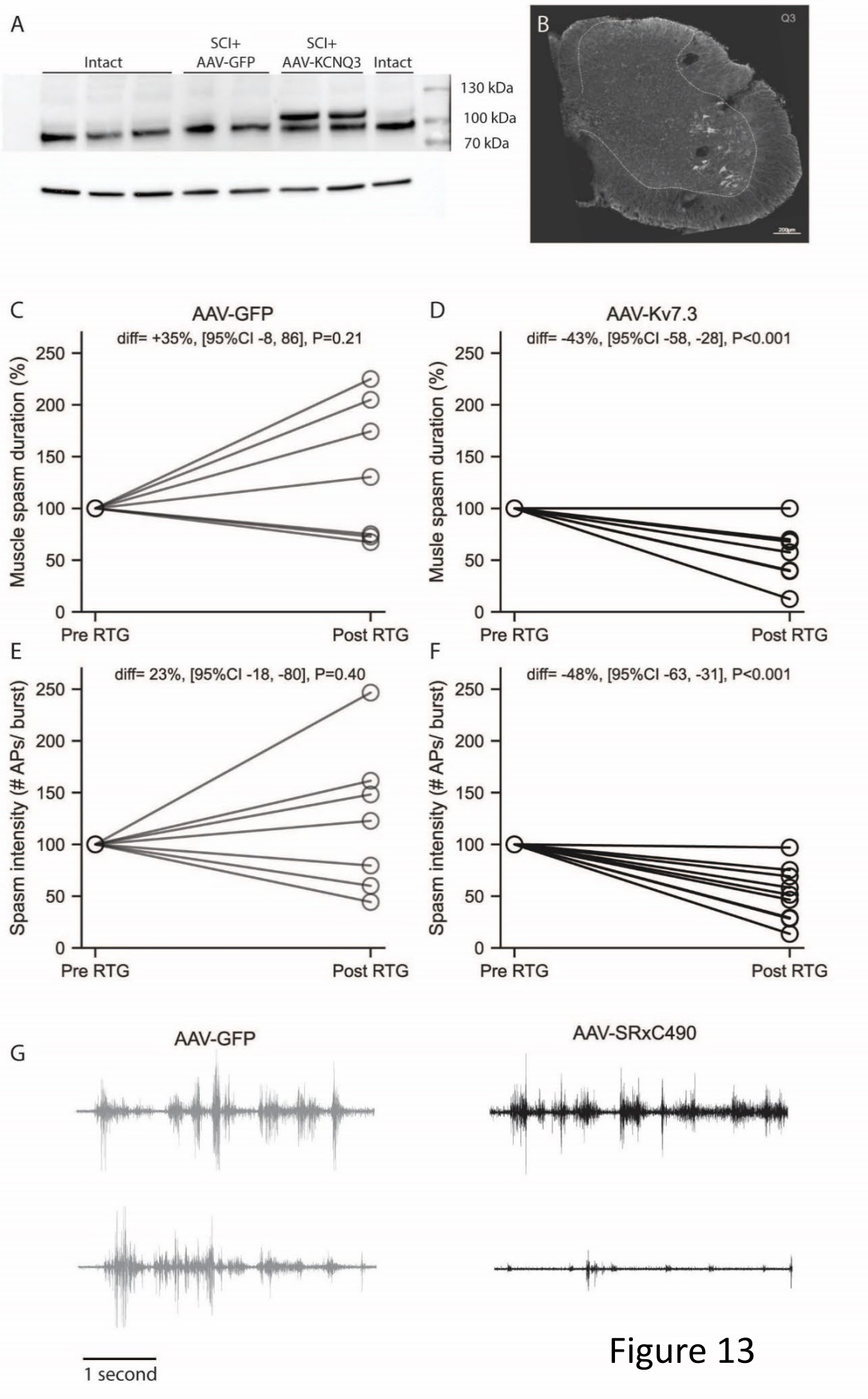

A
Intact
SCI+
AAV-GFP
SCI+
AAV-KCNQ3
Intact
130 kDa
100 kDa
70 kDa
B
Q3
C
AAV-GFP
diff= +35%, [95%CI -8, 86], P=0.21
Muscle spasm duration (%)
Pre RTG
Post RTG
D
AAV-Kv7.3
diff= -43%, [95%CI -58, -28], P<0.001
Musle spasm duration (%)
Pre RTG
Post RTG
E
diff= 23%, [95%CI -18, -80], P=0.40
Spasm intensity (# APs/ burst)
Pre RTG
Post RTG
F
diff= -48%, [95%CI -63, -31], P<0.001
Spasm intensity (# APs/ burst)
Pre RTG
Post RTG
G
AAV-GFP
AAV-SRxC490
1 second

Figure 13

Figure 14

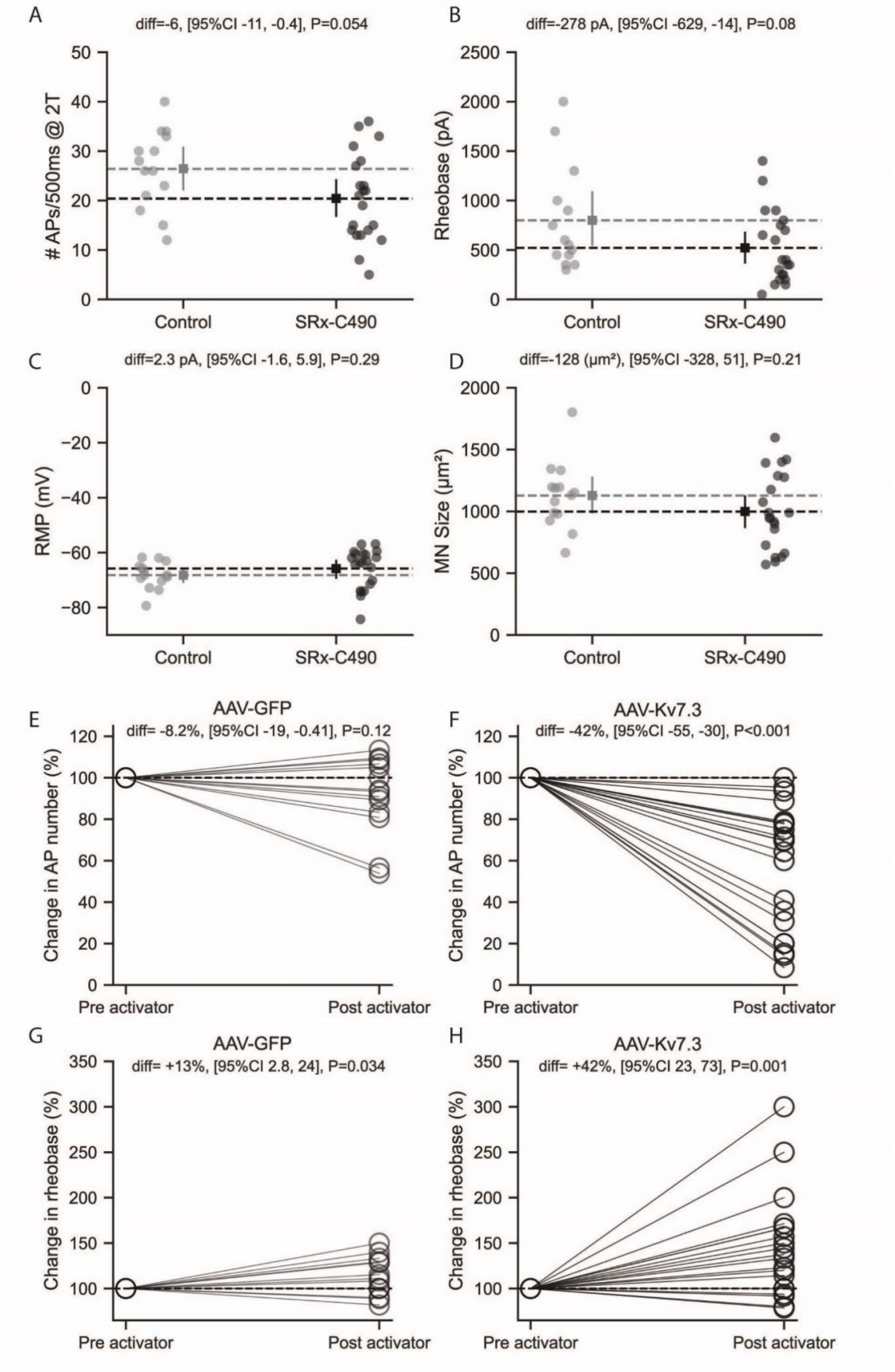
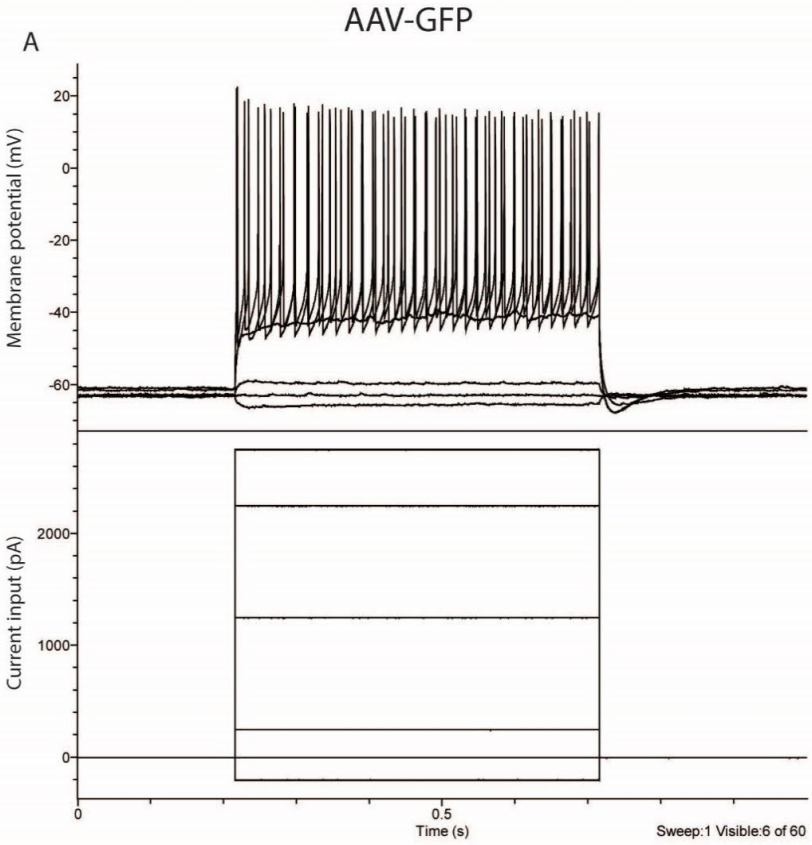
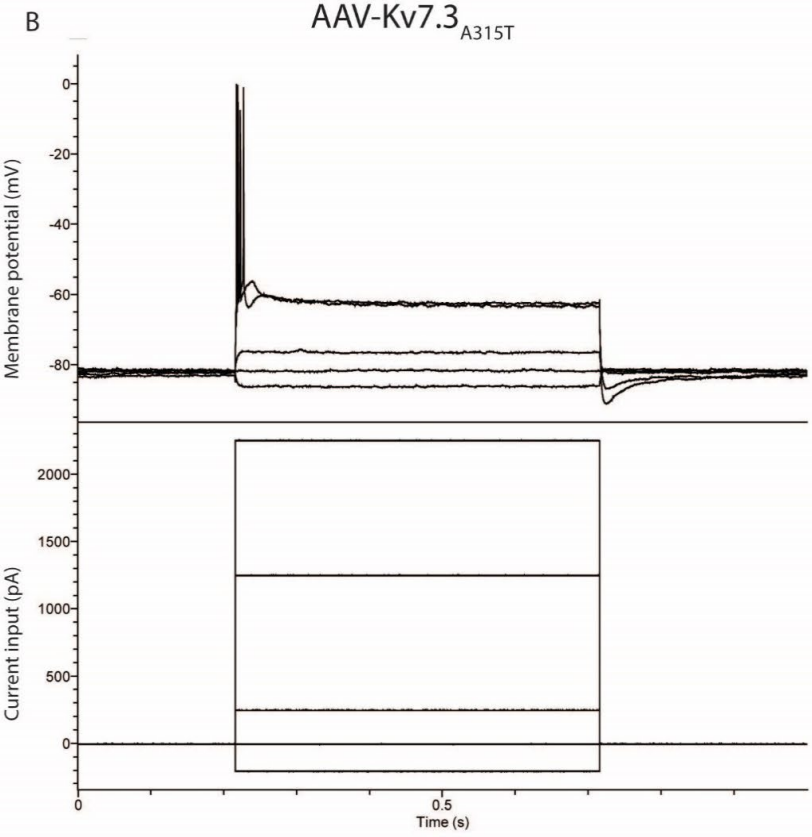

A
diff=-6, [95%CI -11, -0.4], P=0.054
# APs/500ms @ 2T
Control
SRx-C490
B
diff=-278 pA, [95%CI -629, -14], P=0.08
Rheobase (pA)
Control
SRx-C490
C
diff=2.3 pA, [95%CI -1.6, 5.9], P=0.29
RMP (mV)
Control
SRx-C490
D
diff=-128 (µm²), [95%CI -328, 51], P=0.21
MN Size (µm²)
Control
SRx-C490
E
AAV-GFP
diff= -8.2%, [95%CI -19, -0.41], P=0.12
Change in AP number (%)
Pre activator
Post activator
F
AAV-Kv7.3
diff= -42%, [95%CI -55, -30], P<0.001
Change in AP number (%)
Pre activator
Post activator
G
AAV-GFP
diff= +13%, [95%CI 2.8, 24], P=0.034
Change in rheobase (%)
Pre activator
Post activator
H
AAV-Kv7.3
diff= +42%, [95%CI 23, 73], P=0.001
Change in rheobase (%)
Pre activator
Post activator

Figure 15


AAV-GFP
A
Membrane potential (mV)
20
0
-20
-40
-60
Current input (pA)
2000
1000
0
0
0.5
Time (s)
Sweep:1 Visible:6 of 60


B
AAV-Kv7.3A315T
Membrane potential (mV)
0
-20
-40
-60
-80
Current input (pA)
2000
1500
1000
500
0
0
0.5
Time (s)

Intellectual Property Office

Sania RX Limited
c/o Acorn IP LLP
Colin Sanders Business Innovation Centre
Mewburn Road
Banbury
Oxfordshire
OX16 9PA

Patents Directorate

Concept House
Cardiff Road, Newport
South Wales, NP10 8QQ

Direct Line: 01633 811209
[t]**E-Mail:** Andrew.Guy@ipo.gov.uk
Switchboard: 0300 300 2000

Your Reference: SANI.3GB
Application No: GB2406311.7

27 March 2025

Dear Recipient

Patents Act 1977: Examination Report under Section 18(3)

Latest date for reply: **27 May 2025**

I have re-examined your application in response to your agent's letter of 11 October 2024 and enclose a copy of my further examination report and a copy of the cited non-patent literature. Please note that published patent documents mentioned in my report may be obtained for free on the internet and are usually freely available from http://worldwide.espacenet.com.

By the above date you should either file amendments to meet the objections in the enclosed report or make observations on them. If you do not, the application may be refused. I will consider your response and will reply in a timescale consistent with our current target: https://www.gov.uk/government/publications/timeliness-target-for-re-examination-of-patent-applications

Online e-filing

You may file such amendments or observations electronically if you wish, using the online patent filing services detailed in https://www.gov.uk/government/publications/how-to-file-documents-with-the-intellectual-property-office .

Discussion with the examiner

A conversation with the examiner can often help to clarify objections and hence result in more efficient processing of an application. I am always happy to discuss an application at any point. To get the most from our conversation it would be helpful to arrange a mutually convenient time in advance. To arrange an audio/video call please contact me at the email

address above indicating your availability and the subject you would like to discuss. This will give me a chance to review your application, leading to a more efficient discussion.

Yours sincerely

Dr Andrew Guy

Dr Andrew Guy
Examiner

Intellectual Property Office

Your ref :	SANI.3GB	**Examiner :**	Dr Andrew Guy
Application No:	GB2406311.7	**Tel :**	01633 811209
Applicant :	Sania RX Limited	**Date of report :**	27 March 2025
Latest date for reply:	27 May 2025	Page 1/3	

Patents Act 1977
Examination Report under Section 18(3)

Basis of the examination

1. My examination has taken account of the amendments filed with your agent's letter of 11 October 2024.

2. The search has been updated. The search has also been expanded to fully take into account the mutant variants at position 315 (SEQ ID Nos 2-8). The search was extended into the BLASTn database.

Clarity and claim construction

3. As noted previously, the term 'gene therapy vector' as per the current claim 1 and its dependents is of unclear scope. Without further clarification or mitigating argument I have assumed any vector can perform this function under particular circumstances.

4. In both the last report and this report I have construed claims 3 and 4 as second medical use claims. However, neither is clearly claimed.

5. Claim 3 is taken to be a medical use claim, in that in the first instance 'upregulating expression of the Kv7.3 ion channel' is assumed to be inherently therapeutic, but this is not immediately clear – if this is not inherently therapeutic, the claim is simply construed as a claim to the vector suitable for use in upregulating expression, i.e. the vector *per se*. The claim requires clarification for its intended scope.

6. Similarly the disease is 'a neurological disorder associated with increased neuronal excitability'. Based on the description this covers a large potential range of conditions, from MS to injuries to motor neuron disease, as well as pain-related or epileptic conditions. The skilled person is therefore potentially left uncertain as to what falls within the scope of the claim, as this term does not clearly limit the conditions.

7. Claim 4 is also unclear in that it defines the treatment partly by desired result ('wherein once the vector has transduced the target [...] such that a lower dose of a neuromodulatory drug can be effectively administered'). Whether the administration of the drug is part of the method is unclear, i.e. whether the claim seeks to protect coadministration of the vector with the drug. Alternatively, if it is defining the desired result, the claim is simply construed as the vector for use in treating a subject having a neurological disorder. The claim requires clarification.

[Examination Report contd.]

8. Similarly to the point raised in paragraph 7, the claims may be missing essential features where they relate to the medical use. The stated purpose of the invention is to overexpress Kv7.3 receptors, which in turn allows a lower dose of an agent to be administered. Strictly the agent is an essential feature for the purpose of effecting the medical use, if the administration of the vector does not in and of itself have a treatment effect.

Novelty

9. The invention as defined in claims 1 and 6-7 is not new because it has already been disclosed in the following documents:

Biophys J, vol. 95, 2008, "Determinants within the Turret and Pore-Loop Domains of KCNQ3 K+ Channels..." pp. 5121-5137	Zaika et al	See abstract; figure 6; 'cDNA constructs' section page 5122
Biophys J, vol. 110, 2016, "The Voltage Activation of Cortical KCNQ Channels Depends on Global PIP2 Levels" pp. 1089-1098	Kim et al	See abstract; figure 1 and caption; 'Constructs' section page 1090

10. The above documents were found during the updating of the search. They are considered examples only where they relate to the A315T mutant and vectors comprising it.

11. Zaika et al describes the generation and characterisation of KCNQ3 mutants at position 315, including A315T, A315S and A315V (equivalent to SEQ ID Nos 2, 3 and 4). The 'cDNA constructs' section describes the method used to generate the transfection vectors by cloning of the relevant gene into plasmid vectors. As noted above, this is considered to fall within the scope of 'gene therapy vector' as per claim 1, which is anticipated.

12. The document also anticipates claims 6-7.

13. Kim et al also describes vectors comprising the A315T mutant, and similarly anticipates claims 1 and 6-7.

Clarity and support

14. The scope of 'a lower dose' as per claim 4 is unclear as it is a purely relative term.

15. The scope of 'neuromodulatory drug' as per claim 4 is unclear and not of particularly limiting scope.

[Examination Report contd.]

16. The claimed mutants include point mutations known to increase Kv7.3 channel throughput (A315T, A315S) but also encompass those known to reduce throughput (A315V, which as per Zaika et al has a very low ionic current compared to wildtype). It is not therefore clear how such a channel could be used in the same method as one that increases ionic current and achieve the same results.

17. Similarly there is primarily data for the A315T mutant but several of the other proposed mutations do not appear to have been tested – these additional mutants may be unsupported, particularly for the medical use claims where evidence of effectiveness is required (*Prendergast's Applications* [2000] RPC 446). This is particularly true if they have substantially different ionic current profiles.

Conflict with a corresponding PCT patent application

18. This application appears to be similar to your international patent application published under number WO 2024/231830, having the same priority date and designating GB (European Patent). If patents granted on these two applications relate to the same invention, the Comptroller will in due course revoke the patent granted on the present application unless **either** you amend the present specification to remove the conflict **or**, before the date of grant of the present application under Section 25(1), you begin proceedings to surrender the European patent(UK). Of course if the GB designation is withdrawn before the grant of the European patent, no action will be required under Section 73(2).

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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Acorn IP LLP
Colin Sanders Business Innovation Centre
Mewburn Road
Banbury
Oxfordshire OX16 9PA
ROYAUME UNI

PCT

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 66)

Date of mailing *(day/month/year)* 31.03.2025

Applicant's or agent's file reference
SANI3WO

REPLY DUE
within 2 month(s) from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2024/054426	07.05.2024	10.05.2023

International Patent Classification (IPC) or both national classification and IPC
INV. C12N15/86

Applicant
Sania Rx Ltd

1. ☒ The written opinion established by the International Searching Authority:
   ☒ is ☐ is not
   considered to be a written opinion of the International Preliminary Examining Authority.

2. This second opinion contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.
   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(e).
   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 55.3 and 66.8.
   Also: For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6. For an additional opportunity to submit amendments, see Rule 66.4.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 10.09.2025

Name and mailing address of the international preliminary examining authority:
European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040

Authorized Officer
Merkl, Philipp
Telephone No. +49 89 2399-1623

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

International application No.
PCT/IB2024/054426

Box No. I Basis of the opinion

1. With regard to the **language** , this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into ,
   which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b)).
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the elements of the international application, this opinion has been established on the basis of *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-32	filed on	03-03-2025

Sequence listings, SEQ ID NO

1-16	as originally filed

Claims, Numbers

1-20	filed on	03-03-2025

Drawings, Sheets

1/15-15/15	as originally filed

☒ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☒ This opinion has been established as if (some of) the amendments listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):
   - ☐ the description, pages
   - ☒ the claims, Nos. 2, 6, 8, 9, 11
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This opinion has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 66.1(d-*bis*)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 66.4*bis*).

6. ☐ Supplementary international search report(s) from Authority(ies) have been received and taken into account in drawing up this opinion (Rule 45*bis*.8(b) and (c)).

Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	No:	Claims	1, 3-5, 7, 10, 13-20
Inventive step (IS)	No:	Claims	1-20
Industrial applicability (IA)	No:	Claims	

2. Citations and explanations:

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search and/or examination,

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

   c. ☐ furnished to this Authority as an amendment under PCT Article 34 on .

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established to the extent that a meaningful opinion referred to in Article 33(1) could be formed without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

Re Item I

Basis of the report

Amendments under Article 34 PCT

Pertaining to 20.10 ISPE, the international preliminary examination report must be established as if such amendment had not been made if the applicant seeks to amend the description (other than references to the prior art), the drawings, or the claims in such a way that subject matter which extends beyond the content of the application as filed.

1 For present claim 2, basis is indicated as page 3, lines 21-27, page 4, lines 18-19, page 7, lines 22-35, page11, lines 15-24 and 28-30, page 12, lines 5-9, page 14, lines 33-34 and page 16, lines 26-29. None of the above passages provide a direct and unambiguous disclosure of a method of reducing the therapeutically effective dose of a voltage gated potassium channel targeting drug comprising the step: increasing the sensitivity of a target neuron to the voltage gated potassium channel targeting drug by overexpressing a Kv7.3 ion channel subunit comprising the amino acid sequence of any of SEQ ID 10 to SEQ ID 16.

2 For present claim 6, no basis has been provided for the amendment "comprising an AAV capsid".

3 For newly added claim 8, basis has been indicated as oc 10 made dependent on (present) claims 5-7, with addition of the baseline definition for "lower dose" and page 5 lines 13-17.

This authority does not see that present claim 8 finds basis in oc 10, present claims 5-7 (= oc 4, 5, 9). The combination of features is not directly and unambiguously derivable from the application as filed.

4 The applicant states that newly added claim 9 finds basis in oc 2, page 3 lines 18, 19, 21-27, page 4 lines 34-36, page 5 lines 13-17, page 11 lines 28-30, 34, 35 and page 16 lines 26-31.

This authority cannot follow the argumentation. Claim 9 is medical use claim, whilst oc2 is a method claim. Whilst reformulation into the medical use format required by the EPO is allowable, the combination of features does not find direct and unambiguous basis in the cited passages and original claims.

5 Newly added claim 11 supposedly finds basis in page 14, lines 28-29. This passage discloses administration of "the vector" a day or more before "the neuromodulatory drug" is administered. Claim 11 however refers to specific gene therapy vectors (present claims 5-7) in conjunction with administration of a "voltage gated potassium channel targeting drug".

Thus, this authority cannot find a direct and unambiguous disclosure of the subject-matter of claim 11 in the indicated passages.

6 In sum, amended claims 2, 6, 8, 9, 11 do not meet the requirements of Art. 34 PCT. According to 20.10 ISPE and Rule 70(2) PCT, the examination is being carried out on the basis of the originally filed claims 2 and 6 and claims 1, 3-5, 7, 10, 12-20 as filed on 03-03-2025. Present claims 8, 9, 11 were newly added and are thus not examined.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

7 Summary of the application

The application is concerned with provision of methods which reduce the side effects of neuromodulatory drugs/increases their efficacy and respective gene therapy vectors.

In the example, neuronal cells derived from iPSCs were transduced with AAV harboring a hKCNQ3 gene sequence (encoding Kv7.3). In combination with addition of the drug retigabine, less action potentials were observed in Kv7.3 transduced cells (Fig 4, 5, 6) and excitability is reduced (Fig 7) and rheobase is increased (Fig 8). Fig. 9-12 are dedicated to further effects of the Kv7.3 expression.

In addition, mice from a model of spasticity were treated with AAV Kv7.3 and shorter spasm duration and intensity was observed in the treated mice. Further mice were treated with Kv7.3 A315T. Such treated neurons were found not to be able to fire repetitively.

8 Citations

Reference is made to the following documents:

D1 LIU MIN ET AL: "Inactivation of the Lateral Hypothalamus Attenuates Methamphetamine-Induced Conditioned Place Preference through Regulation of Kcnq3 Expression",
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES,
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Summary of D1 see below.

D2 is concerned with dimethoxy-pyrimidine amides and their use in treatment of CNS disorders, including epilepsy. Said compounds are openers of the Kv7.2/7.3 potassium channels. Channel opening is known do decrease neuronal excitability and retigabine is named as a known drug for channel opening (background section). The document discloses transfection vectors harboring human Kv7.2 and 7.3 channel subunits (col 11).

D3 discloses that overexpression of KCNQ (HSV mediated) channels in the VTA dopaminergic neurons and administration of retigabine normalizes neuronal hyperactivity and depressive behaviour. Overexpression resulted in lover neuron firing activity (abstract, Fig 1).

D4 aims at improving the sleep quality of elderly patients. It states that aged Hcrt neurons are more excitable which leads to sleep fragmentation [0008]. It proposes as a solution to manipulate the Kv7 channel, including Kv7.3 or 7.2 by administration of agents which open the channel. The document further discloses that retigabine is a KCNQ (=Kv7) channel opener (052). Further presented are experiments wherein the KCNQ 2/3 genes are disrupted via AAV mediated Cas/CRISPR action. This was found to lead to hyperexcitability of the Hcrt neurons.

D6 is a P document. However, this document is merely used to back up the role of methamphetamin. D6 (abstract) shows that methamphetamine does not directly act on Kv7.3 ion channels, but it does influence their activity indirectly. Research suggests that methamphetamine can downregulate KCNQ genes, which encode Kv7 channel proteins, including Kv7.3. These channels play a

role in modulating neuronal excitability. Studies have shown that increasing Kv7.3 activity in specific brain circuits can reduce methamphetamine-induced behaviors, such as reward-seeking and hyperactivity.

In addition, D7 (abstract) and D8 (abstract, title) show that methamphetamine does interact with voltage-gated potassium channels. Research indicates that methamphetamine can influence the activity of certain potassium channels, such as Kv1.3, which are involved in neuronal and microglial functions. For example, methamphetamine has been shown to increase the expression of Kv1.3 channels, leading to microglial damage. Additionally, methamphetamine may inhibit potassium currents in specific cell types, such as NG108-15 cells.

D9 discloses KCNQ3 mutations A315T, A315S, A315V and reveals that the A315T mutation increases the current density. The same is reported for the A315S mutation. For the A315V mutation, a very small current density is reported (abstract, pages 5129 left col, table 1).

D10 discloses injection of cRNA expressing KCNQ3 A315T into Xenopus oocytes followed by retagabine treatment (material & methods, Fig 6, 7, page 8, right col). The document reports a large hyperpolarizing shift upon 100microM RTG treatment.

9 **Article 33(2) PCT**

The present application does not meet the requirements of Article 33(1) PCT because the subject-matter of claims 1, 3-5, 7, 10, 13-20 not new within the meaning of Article 33(2) PCT.

9.1 D1 aims at modulating excitability of neurons by Kcnq (Kv7) channels (page 2, 1st para). In this regard, an AAV CMV Kcnq3 WPRE is described (section 4.3), which expresses Kv7.3. Section 2.6 describes a method in which a nucleotide sequence encoding a Kv7.3 ion channel subunit is overexpressed in a neuronal cell and a neuromodulatory drug (metamphetamine) is administered. The document teaches that the frequency of excitatory postsynaptic currents was reduced in AAV Kv7.3 infected neurons (page 7).

In view of the many desiderata in the claims (see Item VIII), the following is noted: The effect that expression of a transgene has is an inherent feature of the transgene and the cell type in which it is expressed. Hence, either all the alleged effects are inherently present and thus not new or they are not present and it is not disclosed how the effects would be achieved (Art. 5 PCT).

It is noted that SEQ IDs 9-16 encompass the WT Kv7.3 AA sequence which is thus deemed implicitly disclosed.

In view of D6-D8 (summary see above), it is clear that methamphetamine falls under the definition of being a "voltage gated potassium channel targeting drug", more specifically that it acts also on Kv7.3 expression.

Thus, in view of D1, the subject-matter of claims 1, 4, 7, 10, 13-20 does not meet the requirements of Art. 33(2) PCT.

In addition, in view of D1, the subject-matter of original claims 2, 6 does not meet the requirements of Art. 33(2) PCT.

9.2 The subject-matter of claims 3, 5, 12 is not described in any of D1-D10 and thus novel over the prior art (Article 33(2) PCT).

10 **Article 33(3) PCT**

The present application does not meet the requirements of Article 33(3) PCT because the subject-matter of claim s 3, 5, 12 does not involve an inventive step.

10.1 Claim 3:

D1 is considered the closest prior art. Summary of D1 see above.

The subject-matter of claim 3 differs from D1 in the AA sequence of the Kv7.3 channel. The addition of a drug is optional in claim 3.

The technical effect is reduced neuronal excitability.

The problem to be solved can thus be considered as provision of a method to reduce excitability of neurons.

D10 discloses injection of cRNA expressing KCNQ3 A315T into Xenopus oocytes followed by retigabine treatment (material & methods, Fig 6, 7, page 8, right col). The document reports a large hyperpolarizing shift upon 100microM RTG treatment. Hence, D10 discloses the principle of elevation of the current

density and thus reduction of excitability in neurons via expression of KCNQ A315T channels (in conjunction with retigabine). It is implicitly disclosed in D10 that the A315T mutation already reduces excitability in view of the teaching of D9 (see above). Thus, the subject-matter of claim 3 does not meet the requirements of Art. 33(3) PCT.

10.2 The same argumentation applies in essence for claim 5. D1 discloses an AAV KCNQ3 vector and a person skilled in the art would produce such AAV vector with the mutants disclosed in D9 with reasonable expectation of success.

10.3 The same applies to claim 12, retigabine is already mentioned in D10.

11 **Article 33(4) PCT**

Claims 1-20 are industrially applicable.

12 The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

Patentability, in particular novelty and inventive step, of claims 1-4, 8-20 has been assessed on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

XP093191537 (D6)

Re Item VIII

Certain observations on the international application

13 **Article 6 PCT**

The application does not meet the requirements of Article 6 PCT because claims 1-20 are not clear.

13.1 Claims 1-4 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("increasing efficacy, reducing side effects, increasing therapeutic index, reducing excitability, such that it forms functional ion channels, selectively transduces, is upregulated etc."). Such a definition is only allowable under the conditions elaborated in the Guidelines EPO/PCT F-IV, 4.10. In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, viz. in terms of how the effect is to be achieved.

13.2 In conjunction with the above, most of the terms cited above are relative terms in addition which lack a point of reference. E.g. claim 1 increasing efficacy against what baseline? Overexpressing compared to what? Lower dose as compared to what? It is noted that the wording "than would be normally administered" is still a relative term. The application does not disclose in how far there is a reduction in the amount of a drug.

13.3 The application contains 20 claims of which 6 are independent claims. The application thus lacks conciseness and the set of claims needs to be consolidated. Specific attention should be paid to exemplary claims 1-4 which describe the same method but differ merely in the alleged desired effect.

13.4 Hence, if construed in its broadest sense, claim 1 refers to a method comprising introducing a nucleotide sequence encoding a Kv7.3 A315mut ion channel subunit into a neuronal cell, expressing the Kv7.3 ion channel subunit, followed by administration of a voltage gated potassium channel-targeting drug wherein the nucleotide sequences are either of SEQ ID 1-8.