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EFECTO DE LA HIPNOSIS COMBINADA CON ESTIMULACIÓN TRANSCRANIANA DE CORRIENTE CONTINUA EN LA PERCEPCIÓN DEL DOLOR Y EN LA FUNCIÓN DEL SISTEMA DESCENDENTE EN SUJETOS SANOS: ENSAYO CLÍNICO RANDOMIZADO CIEGO CRUZADO CONTROLADO CON USO SIMULADO

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RESUMEN

Introducción:

El dolor es un problema de salud pública, asociado al sufrimiento y la incapacidad funcional. Sus consecuencias permanecen en la vida personal y social del paciente, llevando a cambios significativos en sus relaciones interpersonales, laborales, familiar y social, disminuyendo la capacidad para realizar las actividades diarias. Las técnicas farmacológicas tienes resultados parciales en los pacientes, muchos de ellos acaban por convertirse en pacientes polimedicados y refractarios al tratamiento. Las técnicas no farmacológicas son promisorias y tienen evidencia científica positivas en el efecto del dolor, teniendo mayores estudios en los últimos años con técnicas como electroacupuntura. estimulación magnética transcraneana, estimulación transcraneana de corriente continua, terapia cognitiva conductual y hipnosis. Entre las que se destacan es la estimulación transcraniana de corriente continua (ETCC) y la sugestion a la analgesia hipnótica. Considerando las limitaciones de las opciones farmacológicas en el tratamiento de dolores crónicos, nuevas investigaciones deben orientarse a propiciar el avance en el proceso y comprensión de los mecanismos del dolor y proporcionar nuevas posibilidades terapéuticas con el potencial de modificar los procesos de neuroplasticidad disfuncional asociados al dolor crónico. **Objetivo**: Los objetivos fueron dos: **(I)** Determinar si la sugerencia de analgesia hipnótica y la estimulación transcraniana de corriente continua (ETCC) tienen un efecto diferencial en la percepción del dolor. Planteamos la hipótesis de que la estimulación transcraniana de corriente continua sería más efectiva que la analgesia hipnótica para cambiar el sistema de modulación del dolor descendente, mientras que la sugestión hipnótica tendría un

mayor efecto en las pruebas sensoriales cuantitativas. (**ii**) Pretende comprender el efecto combinado de la estimulación de corriente continua transcraneal (a-ETCC) y la sugerencia de analgesia hipnótica sobre la percepción del dolor y el sistema de modulación del dolor descendente (SMDD). Esta investigación también pretende determinar si el efecto de a-ETCC y la sugerencia de analgesia hipnótica en las medidas psicofísicas *(CPM-task, HPT, HPTo y CPT)* podría asociarse con el nivel sérico de *BDNF*. Planteamos la hipótesis de que la terapia combinada (a-ETCC / sugerencia hipnótica) presentaría más efectividad en la percepción del dolor y SMDD que las intervenciones individuales (a-ETCC o sugerencia hipnótica). **Métodos:** Se incluyeron mujeres sanas de 18 a 45 años, con una alta susceptibilidad a la hipnosis, según la Escala de susceptibilidad hipnótica de Waterloo-Stanford Group, Forma C . Los sujetos recibieron una estimulación de corriente continua transcraneal anodal (a-ETCC) sobre DLPFC izquierdo (2 mA durante 20 min) y analgesia hipnótica (20 min). **En estudio I**: Se incluyeron 24 mujeres aleatorizados y asignados en uno de los dos grupos de intervención con una distribución cruzada para uno del los grupos: **(1)** a-ETCC (2mA, 20min) e **(2)** Sugerencia hipnótica (20min). **En estudio II**: Se incluyeron 48 mujeres aleatorizados y asignados en uno de los cuatro grupos de intervención: **(1)** a-ETCC (2mA, 20min), **(2)** Sugerencia hipnótica (20min), **(3)** a-ETCC / Sugerencia hipnótica (2mA, 20min) o **(4)** s-ETCC / Sugerencia hipnótica (0mA, 20min). Después de la primera intervención, los participantes regresaron para una segunda sesión experimental para recibir una intervención alternativa. El grupo 1 pasó a recibir la intervención del grupo 2 y viceversa. Lo mismo se aplicó entre el grupo 3 y 4. **Resultados:** Los resultados **del estudio I** revelaron que solo la sugerencia hipnótica produjo cambios que son estadísticamente significativos desde antes de la

intervención hasta después de la intervención en las siguientes medidas: umbral de dolor por calor, tolerancia al dolor por calor, prueba de presión en frío y factor Neurotrófico derivado del cerebro en suero. El análisis mostró un efecto principal significativo para el tratamiento (F = 4.32; P = 0.04) cuando comparamos la tarea delta- (Δ) de la modulación condicionada del dolor entre los grupos de estimulación transcraniana de corriente continua y de sugerencia hipnótica. Además, el cambio en el factor Neurotrófico derivado del cerebro se correlacionó positivamente con la tarea de modulación del dolor condicionada. Los resultados de **estudio II** demostraron que la sugerencia de hipnosis sola o combinada para a- o s-ETCC ha demostrado reducir la eficiencia del sistema de modulación del dolor descendente cuando se compara con a-ETCC solamente. El uso de a-ETCC mejoró el SMDD en un 53,70% en comparación con a-ETCC / Sugerencia hipnótica. La sugerencia hipnótica combinada con a-ETCC o s-ETCC aumentó el *HPTo* cuando se comparó con a-ETCC solo. Mientras que en s-ETCC / Sugerencia hipnótica aumentó *HPTo* casi 16 veces. La combinación de a-ETCC y la sugerencia hipnótica aumentó el *CPT* casi seis veces en comparación con a-ETCC solo y en un 156% en comparación con la sugerencia hipnótica solamente. Además, los niveles más altos de *BDNF* en la línea de base se correlacionaron positivamente con un cambio mayor en el valor de *CPTo* en el a-ETCC y en el *CPT* con a-ETCC / Sugerencia hipnótica.

Conclusiones: Los resultados confirman un efecto diferencial entre la sugerencia hipnótica y la estimulación de corriente directa transcraneal en las medidas del dolor. Sugieren que el impacto de las intervenciones tiene mecanismos neurales diferenciales, ya que la sugerencia hipnótica mejoró la percepción del dolor, mientras que la estimulación transcraniana de

corriente continua aumentó la inhibición del sistema de modulación del dolor descendente. Mientras que el efecto combinado no mejoró la eficiencia de la inhibición en el SMDD.

Registro del estudio: Clinical Trial Registration: identifier NCT03744897.

ABSTRACT

Introduction:

Pain is a public health problem, associated with suffering and functional disability. Its consequences remain in the personal and social life of the patient, leading to significant changes in their interpersonal, work, family and social relationships, decreasing the ability to perform daily activities. Pharmacological techniques present a poor therapeutic response in most patients, many of them end up becoming polymedicated and refractory to treatment. The non-pharmacological techniques are promising and have positive scientific evidence of its effects on pain, with an increase in the number of studies with neuromodulatory techniques such as acupuncture, electroacupuncture, TMS, tDCS and hypnosis. Among those that stand out most are the transcranial direct current stimulation (ETCC) and the hypnotic analgesia suggestion. Considering the limitations of pharmacological options in the treatment of chronic pain, new research should be oriented to promote progress in the process and understanding of the mechanism of pain, providing new therapeutic possibilities with the potential to modify the dysfunctional neuroplasticity processes associated with chronic pain, aiming at clinical improvement. **Objectives:** There were two objectives: **(I)** To determine whether the suggestion of hypnotic analgesia and the transcranial direct current stimulation (tDCS) have a differential effect on the perception of pain. We hypothesized that transcranial direct current stimulation would be more effective than hypnotic analgesia in changing the descending pain modulating system (DPMS), whereas hypnotic suggestion would have a greater effect on quantitative sensory tests. **(ii)** Understand the combined effect of transcranial direct current stimulation (a-

tDCS) and the hypnotic analgesia suggestion on pain perception and the descending pain modulating system. This research also aims to determine if the effect of a-tDCS and the suggestion of hypnotic analgesia in psychophysical measures (CPM-task, HPT, HPTo and CPT) could be associated with the serum level of BDNF. We hypothesized that the combined therapy (a-tDCS / hypnotic analgesia suggestion) would be more effective in pain perception and DPMS than individual interventions (a-tDCS or hypnotic analgesic suggestion). **Methods:** Healthy women aged 18 to 45 years were included, with a high susceptibility to hypnosis, according to the Waterloo-Stanford Group hypnotic susceptibility scale, Form C. Subjects received an anodal transcranial direct current stimulation (a-tDCS) on left DLPFC (2 mA for 20 min) and hypnotic analgesia (20 min). **In study I:** 24 randomized and assigned women were included in one of the two intervention groups with a cross-sectional distribution for one of the groups: (1) a-tDCS (2mA, 20min) or (2) Hypnotic suggestion (20min). **In study II:** 48 randomized and assigned women were included in one of the four intervention groups: (1) a-tDCS (2mA, 20min), (2) Hypnotic analgesia suggestion (20min), (3) a-tDCS / Hypnotic analgesia suggestion (2mA, 20min) or (4) s-tDCS / Hypnotic analgesia suggestion (0mA, 20min). After the first intervention, the participants returned for a second experimental session to receive an alternative intervention. Group 1 went on to receive group 2 intervention and vice versa. The same was applied between group 3 and 4. **Results:** The results of the **study I** revealed that only the hypnotic suggestion produced changes that are statistically significant from pre to pos intervention in the following measures: heat pain threshold, heat pain tolerance, cold pressure test and neurotrophic factor derived from the brain in serum. The analysis showed a significant main effect for the treatment ($F = 4.32$, $P = 0.04$) when we compared the delta-

(Δ) task of the conditioned pain modulation between the groups of transcranial direct current stimulation and hypnotic suggestion. In addition, the change in the neurotrophic factor derived from the brain correlated positively with the task of conditioned pain modulation. The results of **study II** demonstrated that hypnotic suggestion alone or combined with a- or s-tDCS has been shown to reduce the efficiency of the descending pain modulation system when compared to a-tDCS alone. The use of a-tDCS improved the DPMS by 53.70% compared to a-tDCS / Hypnotic suggestion. The hypnotic suggestion combined with a-TDCS or s-TDCS increased the HPTo when compared with a-TDCS alone. While in s-tDCS / Hypnotic suggestion HPTo increased almost 16 times. The combination of a-tDCS and the hypnotic suggestion increased the CPT by almost six times compared to a-tDCS alone and by 156% compared to the hypnotic suggestion alone. In addition, the highest levels of BDNF in the baseline correlated positively with a greater change in the CPTo value in the a-tDCS and in the CPT with a-tDCS / Hypnotic suggestion. **Conclusions:** The results confirm a differential effect between hypnotic suggestion and transcranial direct current stimulation in pain measurements. They suggest that the impact of the interventions has differential neural mechanisms, since the hypnotic suggestion improved the perception of pain, whereas the stimulation by transcranial direct current increased the inhibition of the descending pain modulating system. While the combined effect did not improve the efficiency of inhibition in the DPMS.

LISTA DE ABREVIATURAS

ACC	Corteza Anterior Cingulado
AMPA	α-Amino-3-hydroxy-5-metil-4-isoxazolepropionic acid
AMY	Amígdala
ANCOVA	Analysis of Covariance
a-tDCS	tDCS ativo
BBB	Blood-Brain Barrier
BDNF	Factor Neurotrófico Derivado del Cerebro
BDI-II	Beck Depression Inventory – II
BP-CSI	Central Sensitization Inventory
CPM-task	Conditioned Pain Modulation/ Tarea – Modulación del dolor condicionado
CPT	Cold Pressor Task
CS	Central Sensitization
CSS	Central Sensitization Syndrome
DC	Dolor crónico
DHSC	Cuerno Dorsal de la Medula Espinal
DIP	Differential element performance
DLPFC	Corteza dorsolateral prefrontal
DLPT	Tegmentum pontino dorsolateral
DNIC	Descending noxious inhibitory controls
EEG	Electroencefalograma
ES	Tamaño del efecto
ETCC	Estimulación de corriente directa transcraneal
ETCCa	ETCC ativa
ETCCs	ETCC sham o placebo
FM	Fibromialgia
fMRI	Functional Magnetic Ressonance Imaging
FMS	Fibromiálgicas
GABA	Ácido gama-amino butírico
GRD	Ganglios de la raíz dorsal
HCPA	Hospital de Clínicas de Porto Alegre
HPT	Teste de dolor por calor
HPTh	Umbral de dolor por calor
HPTo	Máxima tolerancia al calor
HT	Hipotálamo
HTTh	Umbral de calor
L-DLPFC	Left DLPFC
LTP	Potenció de larga duración
LTD	Depressión de larga duración
M1	Corteza motora primaria
mA	Miliamperio

NCF	Núcleo cuneiforme
MDS	Modulatory Descending System
NE	Neuro Electric
NIBS	Noninvasive Brain Stimulation
NMDA	N-Metil-D-Aspartato
NPS	Escala numérica del dolor
NS	Nociceptores específicos
PA	Potencial de acción
PAG	Sustancia gris periacuedutal
PCS	Pain Catastrophizing Scale
PET	Positron Emission Tomography
PPGCM	Programa de Postgrado em Ciencias Médicas
PRGC	Gen de la calcicomina
QST	Quantitative sensory test
R-III	Reflejo nociceptivo
RVM	Médula ventromedial rostral
sEPSC	Corriente post-sináptica excitatoria espontánea
SI	Corteza somatosensorial primaria
SII	Corteza somatosensorial secundaria
SD	Standard Deviation
SMD	Standardized Mean Difference
SN	Sistema Nervioso
SNC	Sistema Nervioso Central
SP	Sustancia P
SPSS	Statistical Package for the Social Sciences
SRQ-20	Self-report Questionaire
STAI	State-Trait Anxiety Inventory
s-tDCS	tDCS sham
STT	Tracto espinotalámico
tDCS	Transcranial Direct-Current Stimulation
TENS	Estimulación nerviosa eléctrica transcutánea
TH	Tálamo
TTS	Tracto espinotalámico
UCACUE	Universidad Catolica de Cuenca
VAS	Escala analógica visual
WDR	Wide Dynamic Range
WHO	World Health Organization
WSGC	Waterloo-Stanford Group C

LISTA DE FIGURAS DEL ARTÍCULO 1

LISTA DE FIGURAS DEL ARTÍCULO 2

LISTA DE TABLAS DE LA REVISIÓN DE LA LITERATURA

LISTA DE TABLAS DEL ARTÍCULO 1

LISTA DE TABLAS DEL ARTÍCULO 2

Índice

PRESENTACIÓN

Esta tesis está estructurada en seis capítulos:

Capítulo I - Introducción

Capítulo II - Revisión de la literatura

Capítulo III – Justificación y objetivos

Referencias de la revisión de la literatura

Capítulo IV - Artículos

Artículo 1
Artículo 2
Capítulo V - Consideraciones finales y perspectivas futuras

Anexos

Capítulo I

1. INTRODUCCIÓN

El dolor afecta la calidad de vida de las personas, sus familias y la propia sociedad. El dolor crónico tiene una prevalencia media de 9,6 % en la población [1] y es difícil de tratar, muchos de los pacientes suelen ser refractarios al tratamiento y en general poli-medicados. Además, es un problema de salud pública costoso, con una media de 9.573 dólares por año por cada pacientes en los Estados Unidos [2] y llevando a la pérdida de productividad. El tratamiento del dolor crónico es complejo de tratar y incluye tanto tratamientos farmacológicos y no farmacológicos, [3]Inclusive usando estas modalidades terapéuticas, la mayoría de los pacientes con dolor crónico no experimentan un alivio clínicamente significativo con solo el manejo de medicamentos. En un metanálisis que se evaluó la eficacia de los opiáceos, para el dolor crónico, concluyó que estos fármacos solo producen efectos comparables a los antidepresivos tricíclicos en comparación con el placebo.[4] Existen otras evidencias recientes que el uso excesivo de analgésicos como anti imflamatorios no esteroides debilita el sistema modulatorio endógeno, aumenta la vulnerabilidad al dolor y afecta la respuesta a la terapia [5].

La eficacia reducida de los tratamientos farmacológicos disponibles hace que los investigadores examinen cada vez más los beneficios potenciales de las intervenciones neuromodulatorias, psicológicas y eléctricas para el tratamiento de diversas afecciones dolorosas, rara vez se hace en una perspectiva que integra un marco biopsicosocial. Sin embargo, podrían lograrse mejoras sustanciales en el manejo del dolor crónico si un enfoque más estratégico y coordinado pudiera identificar el mecanismo específico del dolor sobre un

contexto biopsicosocial [6]. Por lo tanto, debido a los complejos mecanismos del dolor, las intervenciones innovadoras deben probarse en un paradigma experimental que nos permita caracterizar los componentes etiológicos del dolor (naturaleza, localización, intensidad, frecuencia y duración del desencadenante para provocar el dolor). Consecuentemente, los estímulos para provocar el dolor deberían ser adecuados para activar las vías del dolor, mientras que no son invasivos y permiten su aplicación repetida[7].

Los estudios desarrollados en el campo de la neuromodulación permiten evaluar el efecto terapéutico y clínico de procesos de neuroplasticidad, en las cuales una técnica neuromodulatoria bastante utilizada es la estimulación transcraneal de corriente continua (ETCC), cuyos efectos son prometedores para la terapia del dolor crónico, tratamiento de trastornos neuro-psiquiátricos y en la rehabilitación. Esta estimulación modula la excitabilidad cortical usando corriente continua de baja intensidad (1-2 mA) mediante electrodos de superficie [8]. La estimulación anódica aumenta la excitabilidad cortical, mientras que la estimulación catódica causa hiperpolarización, reduciendo la excitabilidad de la membrana neuronal. El efecto agudo de la ETCC resulta de alteraciones directas en el potencial de membrana de las neuronas, al igual que los efectos de duración surgen debido a la potenciación de larga duración (LTP) o de depresión de larga duración (LTD). La excitabilidad cortical aquí se define como la fuerza de la respuesta de las neuronas corticales a una estimulación, que refleja la reactividad neuronal a un estímulo específico, siendo, por lo tanto, un aspecto fundamental de la función cerebral. Su eficacia ha sido repetidamente demostrada en el tratamiento del dolor y progresivamente se viene construyendo conocimiento de su efecto bajo redes neurales, corticales y infracorticales, involucradas en el procesamiento del dolor y en los

procesos de neuroplasticidad. [9,10]. Aunque el efecto individual de la técnica ha mostrado beneficios, investigaciones recientes buscan comprender el efecto combinado con otras técnicas complementarias, como el tratamiento cognitivo , electroacupuntura , memoria de trabajo y realidad virtual, llevando a efectos potencializados en la percepción del dolor y en los síntomas de la fibromialgia [11–14].

La analgesia hipnótica puede ser incluida en este conjunto de técnicas complementarias, que mejoran el control del dolor por medio de la activación de áreas corticales involucradas en los mecanismos de dolor, la literatura informa diferentes respuestas a la percepción del dolor en sujetos altamente susceptibles. Sus efectos incluyen una disminución de la amplitud del reflejo H[15] y una disminución de la percepción subjetiva del dolor. Además, reduce la actividad neuronal de la corteza somatosensorial primaria (SI) (Jensen et al. 2008a). Aunque el alivio del dolor con la analgesia hipnótica es uno de los tratamientos más antiguos, el interés en los tratamientos hipnóticos para el dolor surgió sólo en la última década[17].

La razón más fuerte para considerar las intervenciones neuromodulatorias como tratamientos para el dolor es el abordaje que contempla las tres dimensiones: **sensorial-discriminativo:** hace referencia a cualidades estrictamente sensoriales del dolor, tales como su localización, calidad, intensidad y su característica temporo-espaciales. **cognitivo-evaluativo:** analiza e interpreta el dolor en función de lo que se está sintiendo y lo que puede ocurrir. **Afectivo-emocional:** por el que la sensación dolorosa se acompaña de ansiedad, depresión, temor, angustia etc. Respuestas en relación con experiencias dolorosas previas, a la personalidad del individuo y con factores **socioculturales** [18]. Se percibe que el dolor es el

resultado de un complejo proceso, el cual implica mecanismos a nivel cortical y infra-cortical. El cerebro es el responsable de permitir la experiencia del dolor; por lo tanto, las intervenciones relacionadas con el dolor presentan un gran potencial para influir en la actividad cortical. A partir de la construcción de este racional, se han desarrollado técnicas que apuntan a la modulación *top-down* del dolor. Destacándose de este modo, la analgesia hipnótica, que es una técnica que modula el dolor por medio de la sugestión, inducida por la interacción social, en la que una persona (el sujeto) responde a las sugerencias dadas por otra persona (el hipnotizador). El así llamado ¨transe hipnótico¨ es visto como una técnica eficaz para producir experiencias que involucran cambios tanto en la percepción, en la memoria y en el control voluntario. La sugestión puede ser directa (la hipnosis tradicional) o permisiva (hipnosis Ericksoniana) [19]. Ambas técnicas se destinan a promover cambios en la experiencia subjetiva, en alteraciones de la percepción, sensación, emoción, pensamiento o comportamiento del individuo[19].

El efecto de la analgesia hipnótica en las redes neuronales involucradas en el procesamiento del dolor tienen sido demostradas por medio de estudios de neuroimagen, en las cuales observaron que la analgesia hipnótica puede reducir la actividad en prácticamente todas las áreas supra-espinales que se identificaron como componentes de la matriz del dolor, incluyendo el tálamo, córtex sensorial, ínsula, córtex cingulado anterior (ACC) control frontal[20,21]. Las investigaciones sobre la analgesia hipnótica llegaron a conclusiones que las sugerencias a la analgesias hipnóticas afectan a todos los procesos neurofisiológicos subyacentes a la experiencia dolorosa, desde la región periférica hasta el tracto espino-talámico y áreas corticales [22–24]. Además, estudios por medio de electroencefalografía, mostraron que

la analgesia hipnótica se asoció a un aumento en la actividad de ondas alfa y gamma, alteraciones electrofisiológicas igualmente alcanzadas por las técnicas de relajación y meditación [25].

Además, los efectos específicos de la hipnosis en la actividad cerebral dependen del contenido de las sugerencias hipnóticas [26]. Hay evidencias que con una simple sugerencia ya se pueden percibir resultados deseados [27]. La activación de las áreas cerebrales correlacionadas puede ser mejor evaluada y medida sobre un estado más profundo de inducción hipnótica. Se sabe también que los individuos más propensos a responder a la sugerencia hipnótica son aquellos cuyo patrón de actividad cerebral era mayor en ondas theta y gamma [25].

A partir de lo mencionado anteriormente, elegimos dos técnicas terapéuticas (la ETCC y la analgesia hipnótica, que parecen ser una alternativa viable para mejorar la percepción del dolor y el proceso disfuncional en el dolor crónico. Esta elección se fundamenta en la fisiopatología del dolor crónico en donde involucra vías de procesamiento disfuncionales que aumentan la susceptibilidad a respuestas amplificadas al dolor.

La evidencia reciente ha demostrado también efectos promisorios con la combinación de la ETCC con otros tipos de intervención, ya sean: cognitivas, actividad física y programa de rehabilitación del dolor [11,12,28].Así, el ETCC puede modular los circuitos prefrontales con capacidad para mejorar la tolerancia y minimizar el componente emocional de la experiencia dolorosa y se espera que la combinación con la hipnosis analgésica pueda llevar a ganancias adicionales en este proceso de modulación. Considerando que existe limitaciones de las opciones farmacológicas en el tratamiento de dolores crónicos y que persisten una falta de

conocimiento, tanto en la comprensión de los procesos fisiopatológicos y terapéuticos esta tesis tienen dos objetivos principales, que originaron dos artículos que se presentan de acuerdo con las normas de los periódicos de sumisión, cuyos objetivos son: **primer artículo**; (i) evaluar si la hipnosis tendría un efecto más significativo que el ETCC anódico sobre la DLPFC izquierda y la DPLPC catódica sobre la percepción del dolor *(QST)* según lo evaluado por el umbral de dolor por calor *(-HPT)*, la tolerancia al dolor por calor *(*Δ- *HPTo)* y prueba de presión en frío *(*Δ-*CPT).* (ii) Comparar si el efecto anódico ETCC sobre DLPFC izquierdo y DPLPC catódico sería más efectivo que la hipnosis para cambiar la función SMDD evaluada por el valor delta (Δ) del cambio en NPS (0-10) durante una modulación de dolor condicionada *(CPM)*. Además, evaluar la influencia de la neuroplasticidad por el Δ-*BDNF* según el grupo de intervención en las medidas psicofísicas. **Segundo articulo**: Evaluamos la hipótesis de que la terapia combinada (a-ETCC / sugerencia hipnótica) sería más eficaz que las intervenciones simuladas (s-ETCC / sugerencia hipnótica) o intervenciones únicas (a-ETCC o sugerencia hipnótica). El efecto de estas intervenciones se evaluó dentro y entre los grupos de intervenciones en los siguientes resultados: sistema de modulación del dolor descendente según lo medido por el cambio en las escalas numéricas de dolor (NPS0-10) durante la tarea de modulación del dolor condicionada (*CPM*-tarea) por el delta (Δ) -valor desde la post-intervención hasta la pre-intervención (resultado primario). Además, evaluamos la efectividad de las intervenciones en el valor delta desde la post-intervención hasta la pre intervención en los resultados secundarios: umbral de dolor por calor *(HPT)*, tolerancia al dolor por calor *(*Δ-*HPTo)* y prueba de presión en frío *(CPT)*. Además, examinamos si el efecto de la ETCC y la sugerencia hipnótica en las

medidas psicofísicas *(CPM-tarea, HPT, HPTo y CPT*) podrían asociarse con el nivel sérico de *BDNF*.

La estructura de la presentación de esta tesis sigue las normas del Programa de Post-Graduación en Medicina: Ciencias Médicas de la Facultad de Medicina de la Universidad Federal de Rio Grande do Sul (UFRGS). El formato de los artículos fue realizado de acuerdo con las revistas.

Capítulo II

2. REVISIÓN DE LA LITERATURA

2.1 Estrategias para la búsqueda y selección sistematizada de la información

Esta revisión de la literatura se centra en investigar cómo la combinación de hipnosis analgésica y la estimulación transcraniana de corriente continua se relacionan con marcadores de neuroplasticidad como la modulación descendente del dolor, la prueba de presión en frio y marcadores de *BDNF*.

Para presentar el tema central de este estudio, se buscó apoyo en estudios experimentales, observacionales, ensayos clínicos aleatorizados y doble ciegos controlados y para no restringir la investigación, se utilizó comparaciones. La estrategia de búsqueda involucró las siguientes bases de datos: MEDLINE (PubMed), EMBASE, SciELO y Lilacs, sin período delimitado. Las búsquedas se realizaron por medio de descriptores [MeSH (MEDLINE / PubMed) y EMTREE (EMBASE)], y las palabras clave cuando se encontraron los términos:

(1) *Transcranial direct current stimulation (tDCS)*; (2) *hypn*osis, (3) Hypnotic analgesia, (4) Suggestion for hypnotic analgesia (5) *Nociceptive pain*; (6) *Pain perception*; (7) *Pain threshold*; (8) *Pain measurement*; (9) *Descending pain modulatory system*; (10) *Diffuse Noxious Inhibitory Control (DNIC)*; (11) *Conditional Pain Modulation (CPM)*; (12) *Brain-derived neurotrophic factor (BDNF)*. Los términos fueron agrupados en tres grupos: procesamientos de dolor, actividad cortical y neuromodulación.

La tabla 1 sintetiza la estrategia de búsqueda de las referencias bibliográficas usadas,

con base en los aspectos que estructuran el objetivo del estudio.

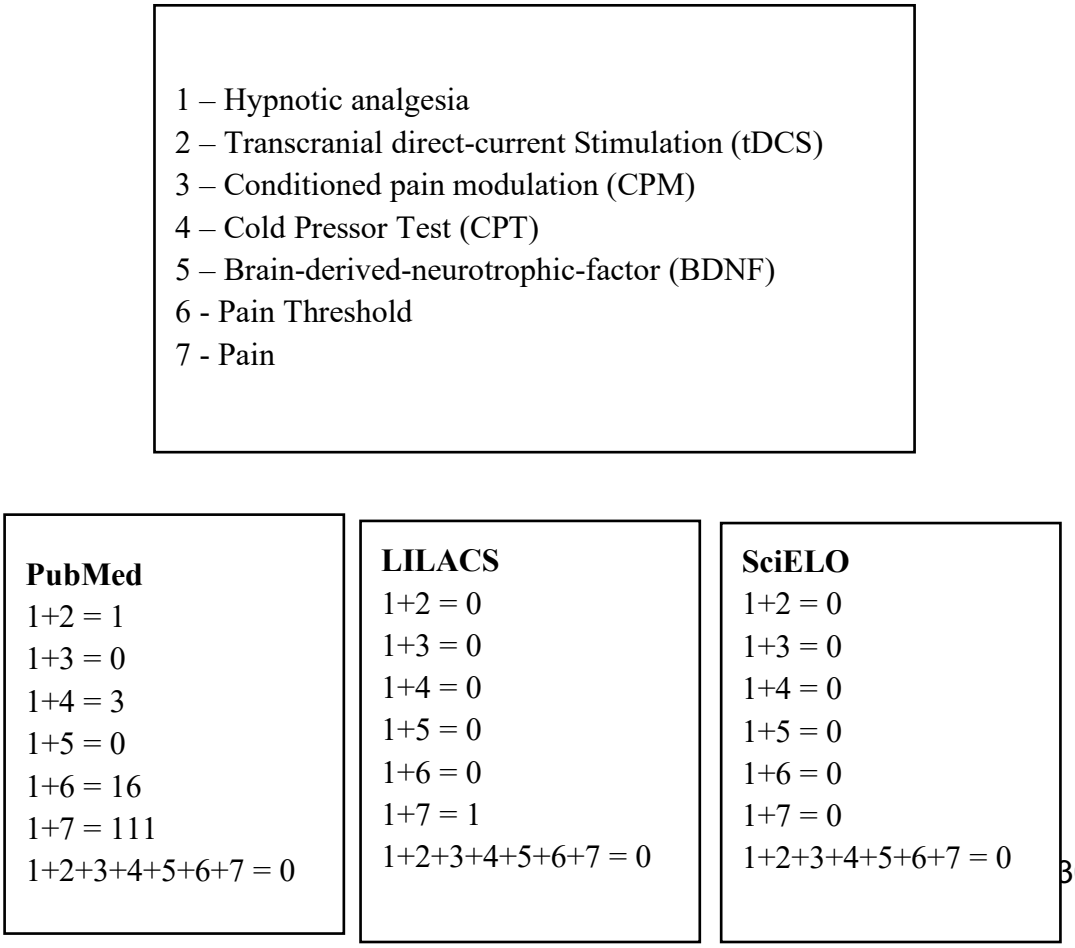
Tabla 1: Estrategia de búsqueda de referencias bibliográficas.

Palavras-chave	PubMed	EMBASE	Lilacs	SciELO
Transcranial direct current stimulation	4142	7232	37	32
Hypnosis	14612	18201	224	114
Hypnotic analgesia	4889	1926	46	7
Suggestion for hypnotic analgesia	82	76	0	0
Nociceptive pain	17572	24561	253	158
Pain perception	18743	24472	739	581
Pain threshold	22793	32345	273	145
Pain measurement	90778	34941	1.539	462
Descending pain modulatory system	180	254	0	0
Diffuse Noxious Inhibitory Control	179	384	0	0
Conditional Pain Modulation	31	34	0	0
Brain-derived neurotrophic factor	21044	36247	93	77

DNIC: *diffuse noxious inhibitory system*; CPM: *Conditional Pain Modulation*; *3*; tDCS: *Transcranial direct current stimulation*; BDNF: *Brain-derived neurotrophic factor*.

Para la elección de los artículos, se utilizaron los marcadores OR para el agrupamiento de las palabras clave relacionadas a los procesos de dolor, actividad cortical y neuromodulación. Se uso el marcador AND para relacionar los tres grupos de interés. Los artículos fueron rastreados por medio de los títulos y resúmenes y después de la exclusión de las duplicaciones y lectura de los resúmenes para confirmación de la relación con temas, 26 artículos fueron seleccionados para la lectura completa. Además, a lo largo de 3 años, se hizo un seguimiento de los artículos que explican el mecanismo de funcionamiento de los temas, por lo que estos artículos de referencia también se incluyeron. La figura 1 presenta la estrategia de búsqueda en cada base de datos.

Figura 1: Estrategia de la búsqueda sistematizada



2.2. El concepto del dolor y sus aspectos históricos

El miedo, el dolor y la ansiedad han sido consideradas emociones humanas fundamentales. Fueron estudiados desde enfoques filosóficos y biológicos, hasta que el sigo XX el dolor y la ansiedad surgieron como tema central en la vida moderna.

El hombre primitivo interpretaba el dolor como consecuencia de fluidos mágicos o de espíritus demoníacos en el interior del cuerpo. La historia muestra que la humanidad ha recorrido múltiples caminos con el intento de desaparecer el dolor. Oraciones, sacrificios a los dioses, uso de amuletos y talismanes eran inventadas para controlar el dolor, aunque, por el contrario, el dolor es una señal de alarma y de preservación[29].

El dolor fue el motivo más importante y decisivo en el desarrollo del arte de curar que comenzó con Aristóteles que interpretó el dolor como una experiencia típicamente desagradable, y sentida por el corazón como pasión del alma, esta idea del corazón perduró por mucho tiempo. Sin embargo, otros filósofos defendían al cerebro como los centros de las sensaciones. Por ejemplo, Descartes fue el primero en evidenciar la existencia de nervios capaces de recibir las informaciones sensoriales en la periferia y llevarlas hasta el cerebro [30].

En la edad media, se reconoció al dolor como una sensación de alerta y protección del organismo contra el daño físico, es aquí donde se estableció que el dolor era el resultado de un daño físico y su intensidad era proporcional al grado de lesión tisular, llamada de teoría de la especificidad .Otra teoría que predomino en la edad media fue la de somación, en la cual el dolor era el resultado de la suma de estímulos excesivos provenientes de receptores nociceptivas [31].Sim embargo, en el en el siglo XX, Melzack y Wall [32] revalorizaron las teorías de la especificidad y la suma y concluyeron que la primera estaba sólidamente basada en evidencias

fisiológicas, a partir de estudios en el sistema nervioso central en donde las evidencias científicas sugirieron que el dolor percibido es determinada por muchas variables fisiológicas y psicológicas. El dolor no sería exclusivamente causado por la actividad neural en vías nociceptivas, sino que resultaría de la interacción de las actividades de varias regiones del sistema nervioso, cada cual con su función especializada [32]. Esta teoría tiene en cuenta el papel de la especialización fisiológica, de la somación central, de la modulación de la señal nociceptiva y de la influencia de los factores psicológicos [32]. A partir de la mitad del siglo XX, con estudios sobre plasticidad y del sistema nervioso central, se hizo aún más importante entender los procesos perceptivos, afectivos y cognitivos del dolor en donde los descubrimientos de mayor interés explicaban que la corteza cerebral y otras estructuras centrales podrían ejercer controles inhibitorios o excitatorios sobre la transmisión del impulso nociceptivo [29].

En la década del 60, Melzack Wall y Melzack Casey desarrollaron un modelo para explicar cómo el daño tisular concomitantemente activaba los componentes afectivos, sensorial y motivacional del dolor. La naturaleza y la intensidad del dolor pasaron entonces a ser consideradas consecuencias de los mecanismos sensoriales, afectivos y cognitivos derivados del daño tisular[33]. La insuficiencia de este tipo de modelos mencionados anteriormente contribuyó a un creciente reconocimiento de que los factores psicosociales, como el estrés emocional, podrían afectar la notificación de los síntomas, los trastornos médicos y la respuesta al tratamiento[34]. George Engel (1977) fue uno de los primeros en pedir la necesidad de un nuevo enfoque de la filosofía reduccionista biomédica tradicional que dominó el campo de la medicina desde el Renacimiento. Esto condujo posteriormente al crecimiento del campo de la

medicina conductual y la psicología de la salud [35]. Una importante consecuencia, a su vez, fue el desarrollo y la evolución del modelo biopsicosocial. Este modelo ha sido especialmente influyente en el área del dolor crónico.

El modelo biopsicosocial se centra tanto en la enfermedad como en el enfermo, y el enfermo se considera una interacción compleja de factores biológicos, psicológicos y sociales. Gatchel y Turk & Monarch, argumentaron que la enfermedad se define como un evento biológico objetivo que involucra la alteración de estructuras corporales específicas o sistemas de órganos causados por cambios anatómicos, patológicos o fisiológicos. En contraste, el enfermo se refiere a una experiencia subjetiva o auto atribución de que una enfermedad está presente. Por lo tanto, enfermedad se refiere a cómo una persona enferma y los miembros de su familia viven con y responden a síntomas de discapacidad[31].

La distinción entre enfermo y enfermedad es análoga a la distinción que se puede hacer entre la nocicepción y el dolor. La nocicepción implica la estimulación de los nervios que transmiten información sobre el daño potencial de los tejidos al cerebro. En contraste, el dolor es la percepción subjetiva que resulta de la transducción, la transmisión y la modulación de la información sensorial. Esta entrada puede filtrarse a través de la composición genética de un individuo, la historia de aprendizaje previa, el estado psicológico actual y las influencias socioculturales. Para que el dolor se registre, el organismo debe estar consciente [31]. Hasta donde sabemos, los pacientes completamente anestesiados no perciben dolor; sin embargo, la nocicepción puede detectarse después de una incisión quirúrgica, incluso en ausencia de cualquier informe subjetivo. Waddell (1987) ha enfatizado que el dolor no puede evaluarse de manera exhaustiva sin una comprensión del individuo que está expuesto a la nocicepción. Para

comprender completamente la percepción de una persona y su respuesta al dolor y la enfermedad, las interrelaciones entre los cambios biológicos, el estado psicológico y el contexto sociocultural deben ser considerados. Cualquier modelo que se centre solo en una de estas dimensiones será incompleto e inadecuado.

En la actualidad y con el avance de a ciencia sabemos que el cerebro no es solo un receptor pasivo de información nociceptiva; al contrario, las neuronas de la médula espinal y el cerebro procesan y modulan activamente esa información. Además, no hay un "centro de dolor" en el cerebro. Pero si, existen múltiples redes de dolor integradas que trabajan juntas para contribuir a la experiencia global del dolor.

El dolor presenta cuatro componentes principales: sensorio-discriminativo, afectivo-motivacional evaluativo-cognitivo y social [36] El dolor puede ser clasificado como agudo y crónico. El dolor agudo tiene función de protección y adaptación, mientras que el dolor crónico no tiene función adaptativa. El dolor crónico se define considerando los aspectos cronológicos y fisiopatogénicos. En cuanto al tiempo de corte varía de 3 a 6 meses a partir del período esperado para resolución del proceso y en cuanto a la fisiopatogenia este implica un conjunto de modificaciones disfuncionales en las vías y en el procesamiento del sistema somatosensorial, que constituye un proceso de plasticidad mal adaptativa.[37]

2.2.1 Procesamiento fisiológico del dolor

En el procesamiento normal del dolor, su percepción implica dos principales grupos de vías neuronales: ascendente y descendente. Los nervios periféricos transmiten señales sensoriales nociceptivas para la médula espinal a través de la vía nociceptiva ascendente. Estas señales se emiten cuando los receptores situados en los nervios periféricos se activan mediante

el estímulo de la temperatura, la presión o el impacto. Las vías descendientes de modulación del dolor envían señales tanto facilitadoras, cuando inhibidores del cerebro para la médula espinal y para la periferia, aumentando o disminuyendo las señales nociceptivas que llegan al cerebro [38]

El dolor tiene mucho en común con otras modalidades sensoriales[39]. En primer lugar, hay receptores específicos para el dolor. Estas son las terminaciones nerviosas, presentes en los tejidos corporales, que solo responden a estímulos dañinos o potencialmente dañinos. Segundo, los mensajes iniciados por estos estímulos nocivos son transmitidos por nervios específicos identificados a la médula espinal. La terminación nerviosa sensible en el tejido y el nervio unido a él, forman una unidad llamada nociceptor aferente primario que capta la información y por el proceso de transducción cuando el estimulo es suficiente desencadena un potencial de membrana que producirá la conducción a lo largo del nervio poniéndose en contacto con las neuronas de transmisión del dolor de segundo orden en la médula espinal. Las células de segundo orden transmiten el mensaje a través de vías bien definidas hacia los centros superiores, incluida la formación reticular del tronco cerebral, el tálamo, la corteza somatosensorial y el sistema límbico. Se piensa que los procesos que subyacen a la percepción del dolor afectan principalmente al tálamo y la corteza [40].

2.2.2. Nocicepción

La nocicepción es el proceso sensorial que forma el componente sensorio-discriminatorio del dolor, siendo el primer paso en el procesamiento de la conversión de un estímulo nocivo (térmico, químico o eléctrico) detectado por terminaciones periféricas

(mecano-termo receptores y receptores polimodales) y transmitido bajo la forma de impulso nervioso hasta el encéfalo [41,42]. Los nociceptores son terminaciones nerviosas libres cuyos cuerpos celulares se localizan en los ganglios trigeminales (cara) y los ganglios espinales (cuerpo). Se activan cuando el estímulo alcanza un umbral nocivo y su respuesta es progresiva de acuerdo con la intensidad del estímulo [43]. Los nervios periféricos están constituidos por los siguientes grupos de fibras: las fibras A-α y A-β y las fibras A-δ y C, que conducen las señales nociceptivas [40].

Las fibras A-α y A-β (mielinizadas gruesas), caracterizadas por la conducción rápida de estímulos con bajo umbral, como el tacto y otros estímulos no nocivos [43]. El procesamiento sináptico de estos estímulos ocurre en neuronas de láminas espinales más profundas, especialmente la lámina III (figura 2) . Aunque no están involucradas en la percepción nociceptiva fisiológica del dolor, en condiciones anormales, estas fibras pueden ser reclutadas y pasan a transmitir señales percibidas como dolor [42]. Mientras que la **Las fibras A-δ** (mielinizadas de pequeño calibre) presentan conductividad lenta, están involucradas con la primera sensación de dolor (fuerte y localizada) y transmiten señales nociceptivas principalmente para neuronas en la lámina I y II (Figura 2A) [44]. Se dividen en dos tipos: las de umbral alto, las cuales responden sólo a la estimulación mecánica intensa y están las que responden al calor, tanto para las temperaturas nocivas, como para las temperaturas inocuas [43] y por ultimo **Las fibras C** (a-mielínicas de pequeño calibre) conducen la señal lentamente y transmiten las señales nociceptivas principalmente para neuronas en la lámina I y II del cuerpo posterior de la médula (Figura 2B)[44]. La mayoría de ellas están involucradas en la percepción del "segundo" dolor con características difusas, mal localizadas que conducen estímulos

captados por receptores polimodales y mecano-receptores, capaces de responder a estímulos nocivos mecánicos, térmicos y químicos [43]. las fibras sensoriales están situadas en los ganglios de la raíz dorsal (GRD) y los ganglios trigeminales. Son fibras que transmiten informaciones sobre estímulos nocivos e inocuos al cuerno dorsal y al tronco encefálico, lugar en que la información nociceptiva es recibida, procesada y modulada por el sistema descendente [45].

En la figura 2 se identifica y compara los diferentes axones aferentes primarios

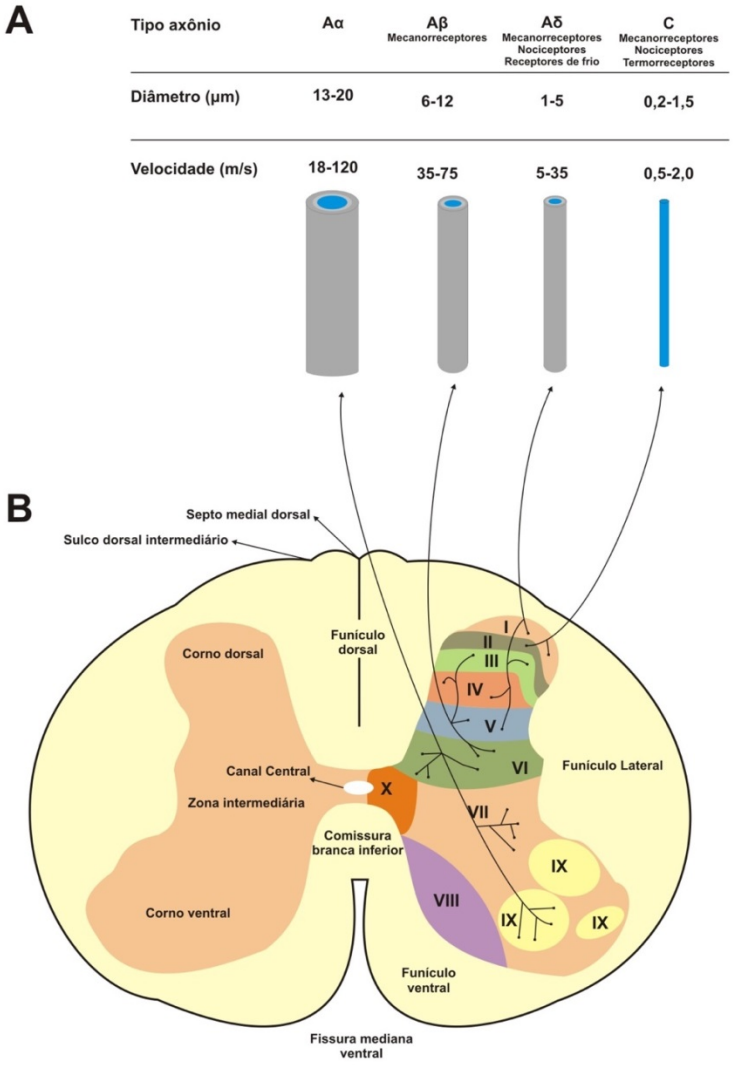


Figura 2|modificada (Julius 2001; Yaksh 2007) Caracterización de los axones aferentes primarios. En relación con: A) características y velocidad de respuesta e B) aferencias en corno dorsal.

Al existir una lesión, una cascada de eventos ocurre en las vías del dolor, cuyo estímulo es percibido por los nociceptores, produciendo una secuencia de eventos. La primera etapa

llamada la transducción, ocurre en los terminales periféricos de las neuronas aferentes primarias a partir de la información captada por los nociceptores, esta información se convierte en actividad eléctrica – llamada potencial de acción (PA), siendo así capaz de ser transmitida [43,46]. Este PA es conducido a las neuronas de primer orden situadas en el ganglio de la raíz dorsal (DRG), a las neuronas de segundo orden localizadas en el cuerno dorsal de la médula espinal (DHSC), a las neuronas de tercer orden localizadas en el tálamo y a las de cuarto orden localizadas en el mismo, córtex, por el proceso de transmisión [46].

A partir de la transmisión, ocurre el proceso por el cual la actividad neural puede ser alterada a lo largo de la vía de transmisión del dolor, llamado de modulación, que ocurre principalmente dentro del DHSC. Este nivel de procesamiento implica una multiplicidad de sistemas de neurotransmisores. La activación de sistemas de modulación del dolor generalmente resulta en menor actividad en la vía del dolor a partir de un estímulo nocivo. Los ejemplos de activación de este proceso incluyen analgesia inducida por el estrés. Sin embargo, en algunas circunstancias, la modulación también puede resultar en una mejora de la señalización del dolor (Figura) [46].

Tanto las fibras A-δ como las C al proyectarse hacia el cuerno posterior de la médula espinal, cruzan la línea media y ascienden a través del sistema antero lateral. En el corno posterior de la médula espinal, los axones de esas neuronas decrecen hacia el otro lado y transmiten el estímulo por los siguientes tractos: el espinotalámico (STT) y el espinorreticular, que van hacia el tronco cerebral y tálamo que luego se dirigen hacia la corteza somatosensorial (SI), secundario (SII), ínsula y para la corteza cingulada anterior (ACC), llevando a la percepción del dolor [46]. La percepción es, por lo tanto, la etapa final del proceso de

señalización del dolor por el cual la actividad neural en la vía de transmisión somatosensorial resulta en la sensación subjetiva de dolor. Así, las neuronas talámicas distribuyen la información nociceptiva para dos lugares: el SI, región en que el aspecto sensorio-discriminatorio del dolor es efectivamente procesado y para el SII, que parece tener un importante papel en el reconocimiento, aprendizaje y memoria de eventos dolorosos. La ínsula está involucrada en las reacciones autonómicas al estímulo doloroso y aspectos afectivos de la memoria y aprendizajes relacionados al dolor y por fin, el ACC está relacionado a la característica del displacer del dolor, pudiendo auxiliar en la integración del afecto general, cognición y selección de la respuesta frente a la respuesta de estímulos que provoquen dolor [45]. Por último, se presume que este proceso resulta de la activación simultánea de los córtex somatosensorial y límbico primario y secundario (Figura 3) [46] , lo que explica la cuestión subjetiva y emocional relacionada con la percepción del dolor.

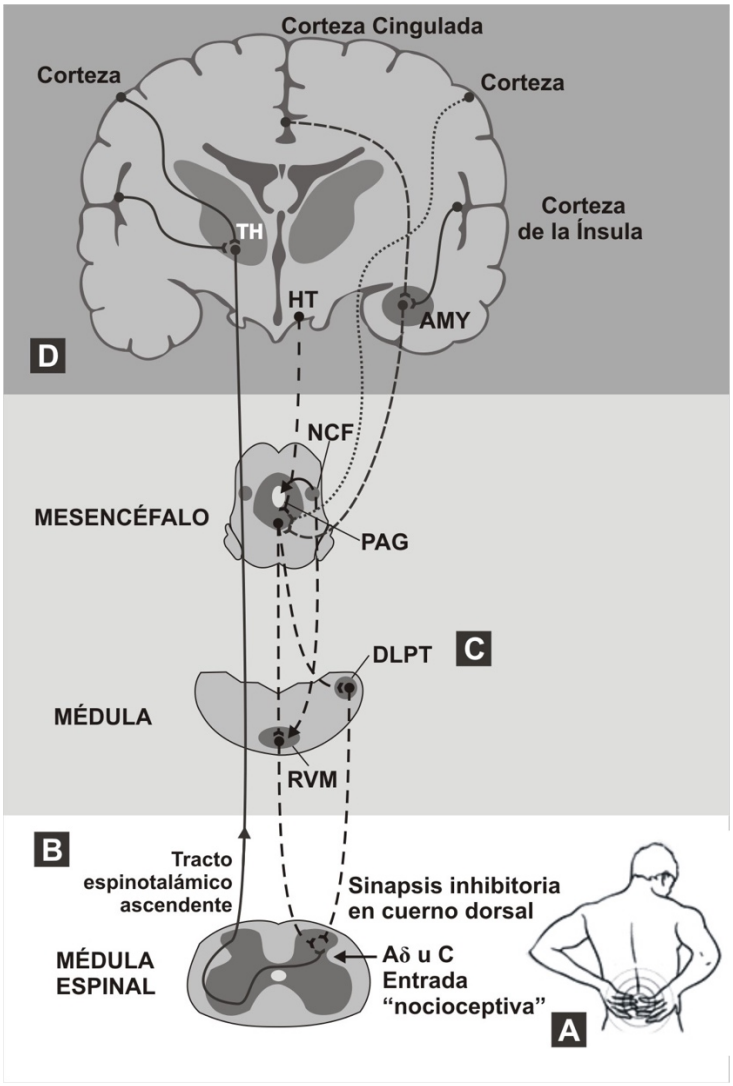


Figura 3|modificado de (Raja, Hoot, and Dougherty 2011).

Mecanismos de señalización del dolor. A) Transducción, transmisión y sensibilización periférica; B) transmisión sináptica, modulación y sensibilización central; C) Modulación descendientes D) Percepción, componentes sensoriales y afectivos del dolor. TH, tálamo; HT, hipotálamo; AMY, amígdala; NCF, núcleo cuneiformes; PAG, materia gris periacueductal; DLPT, tegmentum pontino dorsolateral; RVM, médula ventromedial rostral.

El estímulo nociceptivo llega al cuerno posterior de la médula, tanto por los impulsos conducidos por las fibras Aδ como las C. En el procesamiento del dolor a nivel central el glutamato es el principal neurotransmisor excitatorio. Actúa sobre todo en los receptores α-Amino-3-hydroxy-5-metil-4-isoxazolepropionic acid (AMPA) y el ácido N-metil-D-aspártico (NMDA). Mientras que la sustancia P (SP) actúa sobre el receptor NK1 y el péptido relacionado con el gen de la calcitonina (PRGC). Después de la activación del nociceptor, estos neurotransmisores se liberan en la sinapsis de la neurona de segundo orden en la médula espinal y periféricamente en el lugar de la lesión, lo que provoca los signos de eritema, edema y dolor [43].

Después de la estimulación de neuronas de segundo orden a partir de informaciones nociceptivas provenientes de las fibras A-δ y C, dos tipos de neuronas ejercen un papel fundamental en los mecanismos inhibitorios de supresión del dolor. Los llamados Wide Dynamic Range (WDR), que responden a estímulos dolorosos (A-δ y C) y no dolorosos (A-α y A-β) y las neuronas nociceptores específicos (NS)[47]. Las neuronas NS se activan preponderantemente por estímulos dolorosos provenientes de las fibras A-δ y C [48]. Además de recibir estímulos excitatorios de los nociceptores, las neuronas del DHSC son influenciadas por sinapsis inhibitorias y por células microgliales, que tiene por lo menos dos caminos [49]. En el primero, ocurre la liberación de glutamato a partir de una descarga de corta duración o de baja frecuencia que llega al terminal presináptico. En el segundo, el glutamato y el SP se liberan simultáneamente, en respuesta a un estímulo prolongado o de alta frecuencia que llega hasta la terminación pre-sináptica nociceptora [50,51].

La percepción del dolor en su procesamiento usual involucra dos grupos de vías neuronales principales: la vía aferente y la eferente. La vía nociceptiva ascendente involucra los nervios periféricos, que transmiten señales inductores de dolor (nociceptivo) a la médula espinal y luego al tálamo y posteriormente a la corteza y allí son procesados por la vía nociceptiva ascendente, que se compone de dos componentes cualitativos: un componente sensorio-discriminatorio lateral y un componente afectivo-motivacional medial [43]. La vía sensorio-discriminatoria lateral comprende el tracto neoespinotalámico que se proyecta hasta la corteza somatosensorial, lo que permite la localización del dolor en la corteza somatosensorial primaria. Esta vía mide el primer dolor, que cursa con sensación fuerte y bien localizada [50]. Las vías afectivas-motivacionales mediáticas son un conjunto de vías que comprenden las proyecciones para la formación reticular, el mesencéfalo, el tálamo, el hipotálamo y el sistema límbico [51], los cuales influencian las respuestas emocionales y viscerales al dolor, así como a la modulación descendente de dolor. Este conjunto de vías está compuesto por el trato espinomesencefálico, tracto espinoreticular, trato paleospinotalámico y fibras espino-hipotalámicas y vía espinolímbica. Al final de este proceso, la vía ascendente del dolor emite señales que activan los centros del tronco encefálico que envía las señales a las fibras modulatorias descendientes de la médula espinal [52][53].

2.2.3 Sistema modulatorio descendente del dolor

El Sistema modulatorio descendente del dolor es la vía que controla la respuesta al dolor, lo que garantiza que la intensidad de ella sea compatible con el estímulo ocurrido en condiciones fisiológicas. Las vías modulatorias descendientes envían señales de facilitación e inhibición del encéfalo para la médula espinal y siguen, hacia la periferia, aumentando o inhibiendo las señales nociceptivas ascendentes [53], lo que puede ejercer un control bidireccional sobre la nocicepción.

Las vías descendientes del dolor se originan de distintas áreas encefálicas, siendo la lámina II o sustancia gelatinosa de Roland uno de los principales sitios de modulación, existen dos estructuras claves del mesencéfalo en la modulación del dolor endógeno que son: la sustancia gris periaquedutal (PAG) que recibe entradas de centros cerebrales superiores y es capaz de activar un fuerte efecto analgésico[54] y el núcleo magno de rafe [54,55]. Además de estas dos estructuras, el núcleo magno del rafe en la médula rostral ventromedial (RVM) ejerce influencias tanto inhibitorias, como facilitadoras [54,56]. A su vez, el córtex pre-frontal, cingulado anterior (ACC) y la amígdala, con proyecciones para la PAG, coordinan las influencias inhibitorias y facilitadoras de la RVM en el procesamiento nociceptivo espinal [54,57,58]. Esta vía de modulación descendente del dolor es llamada de vía espinobulboespinal [55].

La PAG es la primera región del tronco encefálico que ha sido explícitamente demostrada para activar el sistema inhibidor de dolor endógeno [54]. El PAG influencia la modulación descendente del dolor a través de sus conexiones bidireccionales con la médula ventromedial rostral (RVM) y modula las entradas nociceptivas y la percepción del dolor por

medio de sus interacciones con proyecciones ascendentes y descendentes de innumerables regiones [54].

Las vías descendentes inhibitorias involucradas en este proceso son las serotoninérgicas, GABAérgica, adenosinérgica, opioidérgica y noradrenérgica[59,60]. Las proyecciones noradrenérgicas descendientes para el DHSC surgen de los grupos celulares adrenérgicos A5, A6 (locus coeruleus) y A7, y en la cuales estas regiones se comunican con RVM y PAG [54]. Así, estas proyecciones noradrenérgicas forman un componente importante de la modulación del dolor descendente para inhibir la transmisión nociceptiva tanto pre-sináptica y post-sináptica [54,61].

Todas estas vías modulan la entrada sensorial de fibras ascendentes y de las neuronas de proyección del DHSC y activan reflejos protectores que influencian en la percepción del dolor [55] . En muchos casos las vías neurales fisiológicas, tanto ascendentes como descendentes del dolor, pueden llegar a ser disfuncionales y llevar a estados mal adaptativos, perdiendo así la función protectora original [62] y evolucionando hacia el dolor crónico (DC). En resumen, el dolor puede convertirse en una enfermedad cuando ocurre en ausencia de lesión del tejido o cuando persiste después de la cicatrización adecuada de los tejidos dañados [46], perdiendo así su utilidad como un sistema de alerta [43].

2.3 Evaluación de la función del sistema modulatorio descendente del dolor

El sistema modulatorio descendente del dolor puede ser evaluado por medio de la modulación condicionada del dolor (CPM), que evalúa la respuesta de las vías endógenas descendentes inhibitorias del dolor. En el CPM ocurre una inhibición difusa y selectiva de las

neuronas de rango dinámico amplio (WDR) en el cuerpo dorsal de la médula espinal por medio de la aplicación de un estímulo condicionante estándar, aplicado en áreas remotas a un estímulo test. Este estímulo condicionante provoca activación de las fibras nociceptivas A-δ y C y induce una reducción en la percepción de la sensación del dolor al estímulo test [63,64].

El protocolo de evaluación del CPM se hace utilizando un estímulo condicionante estándar, aplicado en un área remota del cuerpo y un estímulo nociceptivo test, aplicado en un área contralateral[65]. Se pueden utilizar una variedad de estímulos, como el estímulo térmico al frío, el estímulo eléctrico y el estímulo isquémico [65,66]. Para el estímulo test, se pueden utilizar las respuestas electrofisiológicas, los umbrales de dolor a la presión y el estímulo térmico evaluados por medio de la prueba cuantitativa sensorial (*Quantitative sensory testing* - QST) [67–69], equipo validado en Brasil, en 2011 [70] Todos los estímulos deben cumplir las normas de seguridad para evitar lesiones [65].

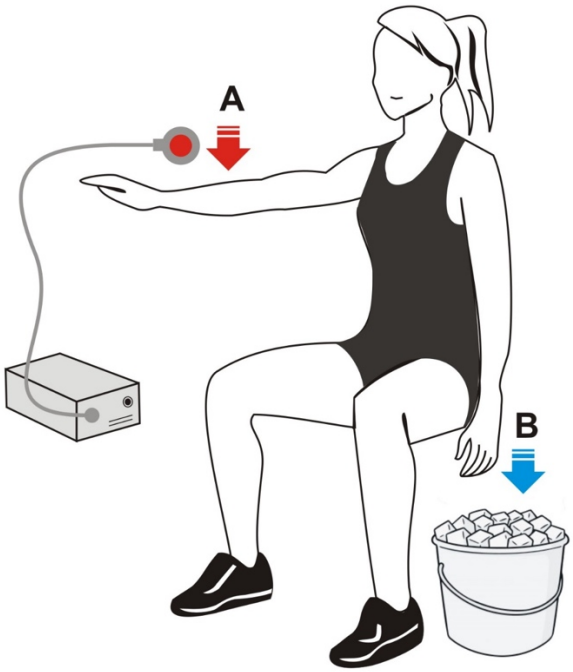


Figura 4| Evaluación de la función del sistema descendente del dolor con QST. A) Ejemplo de estímulo teste - QST. B) Ejemplo de estímulo condicionante – estímulo térmico al frio.

Para la evaluación del CPM es necesario medir la intensidad del dolor percibida por el estímulo, por medio de la escala numérica de dolor, en dos momentos: 1) el estímulo nociceptivo aplicado aisladamente hasta alcanzar una puntuación de 6 en la Escala Numérica de Dolor (NPS 0/10) y 2) durante la aplicación del estímulo condicionante, aplicado en el área remota. En este caso, se aplica el estímulo térmico, eléctrico o de presión con la intensidad para provocar dolor 6, aplicado concomitantemente al estímulo de contra-irritación a distancia. Se calcula entonces el valor de delta (Δ), restando la medida 2, definida por un estímulo moderado en la NPS obtenido en la medida 1. Cuando el valor de la medida 2 menos la medida 1 es

negativo, este resultado indica que el sistema modulatorio descendente del dolor es efectivo; sin embargo, cuando este valor es cero significa ausencia de función del sistema modulatorio descendente endógeno y cuando es positivo, indica efecto de somación [71].

Los resultados de la CPM-task con valor cero o positivo han sido asociados a varias condiciones de dolor crónico y en el caso de somación, en que hay una respuesta aumentada del dolor durante la prueba, existe un perjuicio en el funcionamiento de ese sistema, característico de pacientes con dolor crónico[72,73] . Estos resultados pueden relacionarse con factores como trastornos del sueño y el uso de medicación sedante, comunes en pacientes con dolor crónico, o la depresión [72,74].

2.4 Umbral de dolor al calor y al frío

La prueba cuantitativa sensorial (QST) permite investigar alteraciones somatosensoriales a través de un estímulo térmico que ayuda evaluar el umbral de dolor al calor y tolerancia térmica al dolor. A menudo se aplica en el brazo o la pierna[75]. El QST analiza la percepción en respuesta a un estímulo externo de intensidad controlada. En realidad, el umbral de dolor es detectado después de la aplicación del estímulo doloroso en la piel de modo creciente y decreciente. El QST es una prueba sensorial para evaluar la función de fibras finas, afectadas frecuentemente en los cuadros de dolor neuropático [76]. Se utiliza en investigaciones clínicas para predicción de resultados terapéuticos en el tratamiento del dolor. Además, proporciona información útil sobre el lugar del daño neural y sobre las anormalidades somatosensoriales[77].

Otra prueba que se utilizará para medir el dolor es el Cold Pressor Task (CPT), una

prueba de "presión al frío", utilizada como una medida experimental de dolor, documentada por Wolf y Hardy hace más de medio siglo [78]. Su aplicación es la inmersión de la mano del individuo en un recipiente con agua fría, con temperatura constante y usualmente entre 0 y 1 ° C, durante 60-120 segundos [78]. La duración que la mano permanecerá en contacto con el agua fría es controlada por medio de un cronómetro, pudiendo ser igual al nivel de tolerancia del individuo. Cabe destacar la importancia de atenerse al tiempo máximo de permanencia con la mano sumergida, ya que temperaturas muy bajas pueden provocar quemaduras en la piel [79].

En la literatura existen distintos protocolos para el uso de diferentes temperaturas, que varían entre niños, adultos, ancianos y también de acuerdo con la propuesta de cada estudio. El buen posicionamiento del individuo, la utilización de un soporte en el miembro que será probado, así como la temperatura ambiente agradable, son factores que disminuyen posibles errores durante la ejecución de la técnica [78,80].

A través del CPT es posible verificar una multiplicidad de medidas relacionadas al dolor, incluyendo umbral de dolor, tolerancia, clasificaciones de intensidad de dolor, además de respuestas autonómicas / cardiovasculares. Durante el CPT, están involucradas diferentes aferencias, como: canales específicos al frío, fibras Aδ cutáneas, nociceptores vasculares, cutáneos y nociceptores C perivasculares [80].

2.5 Marcador biológico de plasticidad asociado al dolor

Las diversas moléculas se han relacionado con el procesamiento del dolor, entre ellas el Factor Neurotrófico derivado del Cerebro (BDNF)[81]. El BDNF es una neurotrofina que está ampliamente distribuida en el sistema nervioso central y es reconocida como un importante marcador de plasticidad neuronal relacionada a receptores NMDA en vías nociceptivas ascendente y descendentes [82], asociado a la modulación y mediación del dolor y del humor. Su efecto neuroplástico surge con el objetivo de realizar reparación neuronal [83] y está asociado al fortalecimiento de las sinapsis excitatorias (sistema glutamatérgico) y debilitamiento de las inhibitorias (sistema gabaérgico) [4]. El BDNF se encuentra presente en el contexto de los síndromes de sensibilización central, definidos como "una amplificación de la señalización neural dentro del SNC, provocando hipersensibilidad al dolor" [84]. Como es el caso de las FM. BNDF también aumenta la amplitud y frecuencia de la corriente post-sináptica excitatoria espontánea (sEPSC) y posee la capacidad de alterar los caminos de dolor en todo el SN [85].

Un estudio reciente de nuestro grupo [86], evaluó la excitabilidad de la corteza motor y los niveles de BDNF en dolor crónico musculo esquelético de acuerdo con la patología estructural; en ese contexto la FM. Los resultados demostraron que el BDNF se correlacionó inversamente con la inhibición intracortical y con cambios en la escala numérica de dolor durante el CPM-test, sugiriendo una mayor desinhibición en la corteza motor y en el sistema inhibitorio descendente del dolor en FM y síndrome de dolor miofascial que en paciente con osteoartritis y sanos. Además, en pacientes con FM los niveles séricos de BDNF se encuentran aumentados (a la inversa correlación con los umbrales de dolor por presión [81].

2.6 Estimulación transcraneal de corriente continua (ETCC)

En el dolor crónico a menudo ocurre una pobre respuesta a la terapia convencional, sobre todo en las condiciones de los dolores musculoesqueléticos, neuropáticos y de origen central; por lo que el uso de recursos complementarios a la terapia clásica se hace necesario. En los últimos 10 años se han producido evidencias de que las técnicas de neuromodulación pueden modular redes neuronales corticales y subcorticales. En este contexto, se incluye la estimulación transcraneal de corriente continua (ETCC), la cual se aplica al tratamiento de enfermedades, disfunciones o lesiones que comprometen vías neurales, con presencia o no de lesión estructural[87]. En esta técnica se utiliza una corriente eléctrica continua y de baja intensidad (0,5-2 mA) que se coloca directamente en el cuero cabelludo en el área cerebral de interés, por medio de electrodos de esponja con suero fisiológico o electrodos de goma con gel conductor [88,89]. El ETCC ha sido utilizado para la modulación de la excitabilidad cortical, que ocurre en áreas próximas a los electrodos[90]. Entre los efectos del ETCC está la capacidad de modular el potencial de membrana neuronal, influenciando su excitabilidad, por medio de la polaridad específica de los electrodos [91]. Cuando el ánodo se coloca sobre el área a ser modulada, la estimulación se llama anódica, en contrapartida, cuando el cátodo es colocado, el estímulo es catódico [91]. El estímulo anódico aumenta la excitabilidad, mientras que el estímulo catódico causa hiperpolarización, reduciendo la excitabilidad de la membrana neuronal [90,92,93].

El posicionamiento del electrodo es importante para la determinación del flujo de corriente ya que lleva a la eficacia del tratamiento [88,94], pues los efectos del ETCC son dependientes de la posición y tamaño de los electrodos, así como de la duración e intensidad

del estímulo eléctrico [95]. El efecto tiende a ser acumulativo y inducido por sesiones repetidas .Está vinculado a la neuroplasticidad de la transmisión sináptica, tales como la potenciación de larga duración (LTP), con aumento del proceso de facilitación o depresión de larga duración (LTD) con disminución en el potencial de transmisión sináptica[90]. El LTP y LTD expresan el envolvimiento de los sistemas glutamatérgico y gabérgico, respectivamente.

Los experimentos con ETCC que comenzaron en finales de la década de 1980 hasta hoy, se enfocaron en aclarar los mecanismos de acción de esta técnica[96,97], establecer criterios de seguridad y pautas confiables [87,98], así como definir con más claridad qué condiciones clínicas responden mejor a esta técnica [99,100] y qué resultados pueden esperarse [101].

Las áreas corticales involucradas en el procesamiento del dolor, posibles de modulación por la ETCC, son la corteza motora primario (M1), la corteza prefrontal dorsolateral (DLPFC) y la corteza somatosensorial (S1). Algunos estudios de imagen indican que S1 es responsable del componente sensorial discriminatorio del dolor [102,103]. Independientemente de la localización del dolor, se sabe que la estimulación del DLPFC afecta la cognición, atención, anticipación, así como los aspectos emocionales del dolor [97,100].

El DLPFC generalmente se asocia con el mantenimiento y la regulación de la modulación de arriba hacia abajo, la conducción de respuestas de comportamiento apropiadas y con los procesos de el dolor. En particular, no es la única región activada, de lo contrario puede ser una área clave de redes implicadas en el procesamiento nociceptivo y la modulación del dolor. Específicamente, muestra activación en respuesta a estímulos nociceptivos en sujetos sanos, o muestra activación diferencial entre pacientes con dolor crónico y sujetos control. Se ha demostrado que está involucrado no solo en la supresión del dolor, en línea con su papel en

el control cognitivo y emocional, sino también en la detección del dolor[104].

En apoyo a lo anterior, un estudio informó que la actividad de la DLPFC se relacionó negativamente con el desagrado del dolor (la medida en que el dolor molesto al sujeto). Adicionalmente estudios informaron que el DLPFC está involucrada en la modulación del dolor con placebo. Sin embargo, el rol de la DLPFC en la detección del dolor se apoya en la observación de que la DLPFC mostró actividad binaria (todo o nada) en respuesta al dolor en una muestra de sujetos sanos, independientemente de los estímulos o las intensidades de dolor informadas. Varias líneas de evidencia apoyan un papel para la DLPFC en la supresión del dolor y el mantenimiento de la inhibición del dolor. Por ejemplo, los sujetos que reciben instrucciones para suprimir el dolor muestran un aumento de la activación de la DLPFC bilateral, pero en particular de la izquierda, durante la estimulación prolongada del dolor agudo. La activación bilateral de la DLPFC se asoció con una reducción del desagrado del dolor térmico[105]. Brascher et al informaron que el dolor incontrolable dio como resultado un aumento de la activación de áreas relacionadas con el dolor como el tálamo y la ínsula, pero que el DLPFC bilateral tuvo una mayor fuerza de conectividad negativa durante dolor controlable del tálamo y de la ínsula anterior derecha. En otras palabras, la DLPFC suprimió la actividad de la ínsula y el tálamo y redujo la sensibilización al dolor asociada con el dolor incontrolable[105]. Finalmente, la conectividad entre la DLPFC izquierda y la derecha se ha relacionado con la sensibilidad individual al dolor, de modo que una conectividad interhemisférica más fuerte se asoció con una mayor tolerancia al dolor[105].

En un estudio se vio que la estimulación anodal sobre el DLPFC izquierdo puede reducir el grado percibido de valencia emocional para cuadros emocionales negativos [106] y para

imágenes de expresiones de rabia [107]. Y que la estimulación anódica sobre el DLPFC aumenta las reacciones a estímulos emocionales positivos y la identificación de expresiones emocionales positivas [108].

Con esta perspectiva están otras investigaciones recientes, que se observó que la estimulación anodal en el DLPFC izquierdo puede aumentar significativamente el umbral de dolor a estímulos de calor en pacientes con fibromialgia, así como su tolerancia, debido a su efecto sobre el procesamiento sensorio discriminatorio del dolor. Los efectos analgésicos de la estimulación del DLPFC también pueden atribuirse a una inhibición neuromodulatoria de la actividad del tálamo, según Silva et al (2017).

Una opción bastante prometedora para optimizar los efectos de la ETCC es su uso combinado con otras intervenciones, que promuevan la activación de los mismos circuitos neurales. Por eso, se cree, y los estudios actuales demuestran que la estimulación, tanto en M1 como en DLPFC, combinada a las más variadas intervenciones (no farmacológicas y farmacológicas), potencie la respuesta analgésica promovida por la ETCC.

Los ensayos clínicos aleatorizados han evidenciado que, cuando se asocian a la electro acupuntura, la ETCC tiene su efecto analgésico potencializado [82,109]. De hecho, su asociación a otra técnica de estimulación periférica (TENS) para el manejo del dolor se mostró superiormente eficaz, incluso de modo agudo, en pacientes con dolor crónico refractario cuando comparadas de forma aislada [110]. Una revisión en diferentes poblaciones clínicas mostro que la asociación de la ETCC a realidad virtual fue benéfica en la rehabilitación de individuos en el manejo del dolor crónico [111]. Tal observación nos lleva a hipotética que la asociación de la ETCC podría también ser promisoria cuando asociamos con hipnosis analgésica, ya que la

realidad virtual no deja de ser una imaginación guiada, igualmente que la hipnosis.

En un estudio de ETCC con Fibromialgia, Valle et al. (2009) usaron ETCC a 2mA, durante 20 minutos, en el M1 y DLPFC durante 10 días, en mujeres con fibromialgia, observaron un importante efecto analgésico y mejora de la calidad de vida en ambos lugares de estimulación. [112]. En otro estudio con Fibromialgia, Fregni et al. (2006) realizaron pruebas para determinar si la estimulación activa del córtex motor primario (M1) o de la corteza prefrontal dorsolateral (DLPFC) con ETCC está asociada a la reducción del dolor y otros síntomas de fibromialgia en comparación con la estimulación simulad, la estimulación usando ETCC en el área de el DLPFC se eligió porque está fuertemente asociada a los efectos emocionales y trastorno de depresión mayor [113]. Se sabe que la estimulación del DLPFC afecta a la cognición, atención, anticipación, así como los aspectos emocionales del dolor durante el procesamiento del dolor [114–116]

Con los estudios mencionados los cuales soportan la noción de que un protocolo combinado de ETCC y otra intervención, sea cognitiva, conductual, sugestiva, atencional o motora, promueven la optimización de la respuesta terapéutica a corto y largo plazo[9,117] Nuestros estudio esta encaminado a combinar la ETCC con la hipnosis analgésica.

2.7 Hipnosis

La hipnosis es una interacción social en la que una persona (el sujeto) responde a las sugerencias dadas por otra persona (el hipnotizador) para producir experiencias creativas que involucran cambios en la percepción, memoria y control voluntario. En el caso clásico, estas

respuestas se asocian a un grado de convicción sobre la experiencia [118]. El tratamiento hipnótico estándar generalmente comienza con una inducción, proporcionando sugerencias para los pacientes, a fin de concentrarse en un solo estímulo (por ejemplo, un punto en la pared, sensaciones asociadas a la respiración) para experimentar cambios iniciales en las percepciones del individuo (por ejemplo, promoviendo la relajación [119]. La inducción hipnótica aumenta la apertura o la voluntad del sujeto para responder a las siguientes sugerencias [120]. La inducción hipnótica es seguida por las sugerencias, las cuales abordan los cambios en el problema presentado. Para los individuos con dolor, por ejemplo, éstos pueden incluir sugerencias para el confort o la capacidad de mantenerse funcional en presencia de dolor. Las sugerencias se suministran después de la inducción hipnótica pudiendo ser de varios tipos, conforme la tabla abajo [121].

Sugestões hipnóticas para o manejo da dor crônica

Sugestão direta direcionada para o conforto

Você está percebendo onde no seu corpo sente o maior conforto e permitindo que essa sensação de conforto se espalhe...

Sugestão indireta para os benefícios do tratamento

Me pergunto como você encontrará o maior benefício na sessão de hoje... talvez você vivencie uma sensação de relaxamento e uma maior habilidade de ignorar sensações desconfortáveis... mas eu sei que tu irá experienciar cada vez mais e mais conforto e controle...

Utilização de metáforas

Você pode experienciar quaisquer sensações desagradáveis como uma imagem ou objeto, como fogo, ou uma corda com um nó bem apertado... isso mesmo... e agora perceba como esse objeto muda... ficando mais e mais confortável

Sugestões pós-hipnóticas para manutenção dos ganhos

E o conforto, as percepções úteis, e outros benefícios que alcançaste por conta própria na sessão de hojevão durar até depois da sessão... automaticamente... pelo tempo que forem úteis pra ti... por minutos, por horas, dias, anos e décadas...

2.7.1 Analgesia Hipnótica

La investigación intrínseca ha permitido una mejor comprensión de los mecanismos neurales multidimensionales que subyacen a los procesos y respuestas hipnóticas: la analgesia hipnótica [122] . Una de las aplicaciones médicas más antiguas de la hipnosis se refiere al control del dolor, cuya eficacia, ha encontrado recientemente una confirmación indiscutible a nivel de la medicina basada en la evidencia en los metanálisis publicados en los estudios controlados aleatorios, tanto en casos agudos como crónicos. La analgesia hipnótica representa un paradigma importante de cómo la investigación neurofisiológica y neuropsicológica ha contribuido de manera decisiva a una mejor comprensión de los mecanismos de control del dolor multidimensional. Dado que el dolor tiene una estructura multidimensional que involucra aspectos sensoriales-discriminativos, motivacionales-afectivos y evaluativos – atencionales [123], es probable que la analgesia hipnótica implique múltiples mecanismos de modulación del dolor [124].

Los estudios recientes sobre esta técnica respaldan los sistemas de control durante las

sugerencias hipnóticas en diferentes niveles y sitios dentro del sistema nervioso. A nivel periférico, existe una evidencia controvertida de que la hipnosis puede modular la entrada nociceptiva al regular negativamente la estimulación de las fibras A delta y C, mientras que puede reducir significativamente la excitación simpática [124] relevante para inducir y mantener algunos estados de dolor crónico. A nivel espinal, es probable que la hipnosis active los sistemas inhibitorios descendentes, al reducir el reflejo nociceptivo R-III, paralelo a la reducción del dolor auto-informada [125,126]. A nivel cortical supraespinal, los estudios de neuroimagen y electrofisiológicos han demostrado que las sugerencias hipnóticas de analgesia pueden modular directa y selectivamente las dimensiones sensoriales y afectivas de la percepción del dolor, confirmando así, al menos en parte, la teoría de la neodisociación de Hilgard y Hilgard (1994). Además, los sujetos altamente hipnotizables poseen capacidades de filtrado de atención más fuertes que los sujetos con bajo nivel de hipnotización. Esta mayor flexibilidad cognitiva podría resultar en un mejor enfoque y desvío de la atención del estímulo nociceptivo, así como una mejor desviación de los estímulos irrelevantes en el medio ambiente. Los procesos de control cognitivo se asocian con un "sistema de atención supervisora", que involucran a las cortezas límbicas, temporales y frontales[127]. Esta red compleja podría representar la "neuroestructura" de la modulación hipnótica del dolor [124]. Cabe destacar que las estructuras involucradas en la percepción del dolor son las mismas que las involucradas en su modulación cognitiva y hipnótica, aunque la dinámica funcional de estos patrones complejos aún no se ha aclarado. La figura 5 muestra esquemáticamente los mecanismos de la analgesia hipnótica[124].

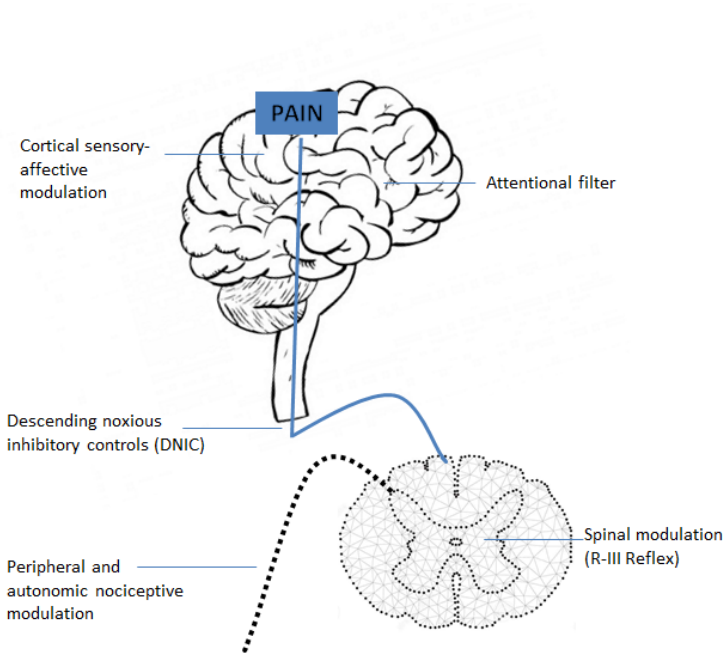


Figura 5: Autoría mecanismos putativos de la analgesia hipnótica.

2.7.2 Mecanismos corticales y subcorticales

Un número creciente de estudios han examinado los efectos de la analgesia hipnótica sobre áreas del cerebro, así como sobre los procesos neurofisiológicos subyacentes a la experiencia de dolor. De acuerdo con estudios de neuroimagen, la analgesia hipnótica disminuye la actividad en áreas supra-espinales identificadas como componentes de la matriz del dolor, incluyendo el tálamo, córtices sensoriales, ínsula, córtex anterior cingulado (CAC) y área frontal [128]. Además, la analgesia hipnótica disocia los componentes sensoriales y afectivos de la experiencia dolorosa de acuerdo con el contenido de las sugerencias [24,129] y modula la actividad / conectividad de la matriz dolorosa [130]. La analgesia hipnótica redujo

la incomodidad de la percepción del dolor térmico y modula la actividad neural en el CAC de acuerdo con los cambios en la percepción del dolor térmico evaluados por la tomografía por emisión de positrones (PET) [131].

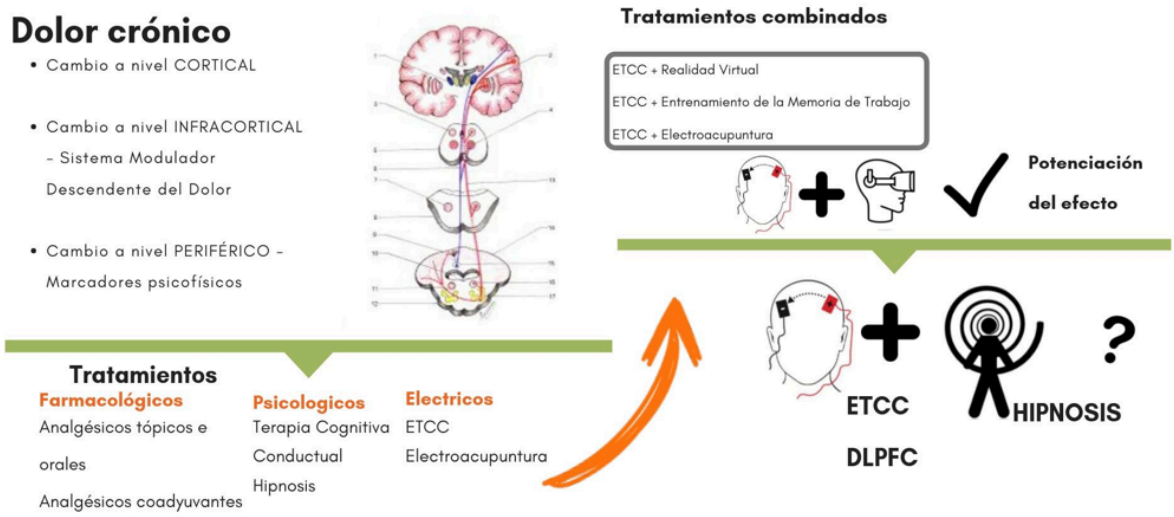
De acuerdo con un meta-análisis, que incluyó estudios clínicos y de laboratorio, el tamaño del efecto (ES) de la analgesia hipnótica fue moderado (ES = 0,71) [132]. Las sugerencias hipnóticas pueden aumentar la actividad en la corteza cingular anterior, que se identifica como una "parte importante de la red ejecutiva, pues está involucrada en la atención selectiva, el aprendizaje y la resolución de conflictos" [133]. Su efecto en el tálamo también está asociado a una "activación en el trayecto motor de los ganglios de la base para áreas motoras más altas" [133]. Además, los estudios mostraron que durante la prueba de presión fría (CPT), la analgesia hipnótica aumentó la tolerancia del dolor en aproximadamente el 62% y redujo la percepción del dolor y el reflejo nociceptivo para el 65% del área refleja basal [134].

Las alteraciones corticales debido al uso de técnicas neuromodulatorias pueden ser reflejadas en la actividad de las ondas oscilatorias cerebrales, medidas por EEG. Como ejemplo, tanto estudios con dolor agudo y crónico han demostrado cambios de aumento rápido de ondas beta (13-35 Hz) frecuencia de onda asociada al procesamiento de información como aumento de ondas alfa (8-12 HZ) y theta (7,5) -14Hz), ondas asociadas a la relajación [135]. Es interesante notar que la hipnosis y la meditación se han mostrado eficaces para aumentar la actividad de ondas lentas, especialmente theta. Con base en estos resultados, es razonable suponer que el aumento de la actividad de ondas más lentas (es decir, theta y alfa) y disminución

de actividad de las ondas más rápidas (por ejemplo, beta) están asociados a reducciones en la intensidad del dolor.

El potencial analgésico tanto de la hipnosis como de la ETCC ya ha sido evidenciado, sin embargo, todavía quedan lagunas en cuanto a la implicación de mecanismos involucrados en la percepción nociceptiva, así como la contribución de ambas técnicas en la producción de un potencial efecto sinérgico en las vías inhibitorias del dolor. En este contexto, es primordial conceptualizar, así como evaluar la posible combinación entre diferentes técnicas neuromodulatorias involucradas en el manejo del dolor crónico. Los datos actuales en la literatura justifican la necesidad de estudios destinados a explorar las posibles conexiones entre técnicas no farmacológicas dirigidas al manejo del dolor y parámetros corticales en la percepción nociceptiva [90,93]. Se cree que una alterada percepción de un estímulo nociceptivo consista en una barrera terapéutica en el manejo de pacientes refractarios, lo que, invariablemente, implica un peor pronóstico, una vez que la percepción juega un papel fundamental en la modulación de la respuesta frente al dolor.

Marco Teorico



3. Justificativa

Aunque el conocimiento entre las técnicas de hipnosis e ETCC han avanzado, es necesario estudios adicionales para comprender de qué manera estas técnicas actúan en el procesamiento del dolor y sus respuestas terapéuticas. Como se puede observar a partir del referencial teórico presentado y considerando la relación de las técnicas neuromodulatorias y su respuesta en la percepción del dolor, este estudio se justifica por su objetivo principal: evaluar el efecto de la combinación de la hipnosis analgésica con la estimulación transcraniana de corriente continua en la percepción del dolor.

Además, las dos técnicas neuromodulatorias combinadas podrían tener efectos potencializadores en la percepción del dolor, lo que aún no ha sido estudiado hasta el momento. Por lo tanto, es fundamental comprender cómo la hipnosis analgésica y la ETCC se relacionan entre sí en los procesos de dolor. Entonces es plausible argumentar que estas dos técnicas de neuromodulación externa pueden ser adecuadas para afectar los cambios específicos, en la percepción del dolor. Se debe considerar que la respuesta y el efecto de los métodos de neuromodulación farmacológica o no farmacológica son mediados por procesos de neuroplasticidad, lo que justifica la realización del estudio propuesto.

Por lo tanto, considerando que los síndromes dolorosos cursan con alteraciones perceptivas frente a un estímulo nociceptivo, se hace primordial la obtención de datos adicionales que permitan la comprensión de cómo la analgesia hipnótica y el ETCC puedan acceder, así como modular la percepción del dolor, que interactúan con vías neurobiológicas complejas

implicadas en el procesamiento del dolor. Se sabe que lo pacientes que tienen dolor crónica presentan diferencias en los sistemas corticales, infracorticales, así como a nivel periférico y que los tratamientos actúales actúan de manera parcial, por lo tanto, sabiendo que la ETCC y la hipnosis presentan efectos promisorios para el alivio del dolor insoladamente, nuestro estudio refuerza la necesidad de combinar las técnicas, para comparar sus efectos y sus combinaciones.

4. OBJETIVOS

5.1 Objetivo primario

El objetivo de este estudio es evaluar los diferencias en los efectos de la hipnosis analgésica y ETCC, así como comprender el efecto de su combinación en el processamiento del dolor por medio de las medidas psicofísicas y del sistema modulatorio descendente del dolor

.

5.2 Objetivos secundarios

Determinar si la hipnosis analgésica y ETCC tienen un efecto diferente sobre la percepción del dolor y sobre el sistema modulatorio descendiente del dolor (DPMS)

Comprender el efecto de la combinación de ETCC y hipnosis analgésica en la percepción del dolor y en el DPMS.

Determinar se el efecto de la analgesia hipnótica y ETCCen las medidas psicofísicas (HPT, HPTo, CPT *y CPM*, CPM) está asociado com los niveles de BDNF.

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5. ARTÍCULOS

7.1 ARTICULO 1

Comparison of hypnotic suggestion and transcranial direct-current stimulation effects on pain perception and the descending pain modulating system: a crossover randomized clinical trial

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Comparison of hypnotic suggestion and transcranial direct-current stimulation effects on pain perception and the descending pain modulating system: a crossover randomized clinical trial

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Abstract

Objectives: This paper aims to determine if hypnotic analgesia suggestion and transcranial direct-current stimulation (tDCS) have a differential effect on pain perception. We hypothesized that transcranial direct-current stimulation would be more effective than hypnotic analgesia suggestion at changing the descending pain modulating system, whereas the hypnotic suggestion would have a greater effect in quantitative sensory testing.

Design: This is a randomized, double blind and crossover trial.

Settings: All stages of this clinical trial were performed at the Laboratory of Pain and Neuromodulation of the Hospital de Clínicas de Porto Alegre.

Subjects: Were included 24 healthy females aged from 18 to 45 years old, with a high susceptibility to hypnosis, according to the Waterloo-Stanford Group Scale of Hypnotic Susceptibility, Form C (15).

Methods: The subjects received a random and crossover transcranial direct-current stimulation over the dorsolateral prefrontal cortex (2mA for 20min) and hypnotic analgesia (20min).

Results: Only hypnotic suggestion produced changes that are statistically significant from pre- to post-intervention in the following outcomes measures: heat pain threshold, heat pain tolerance, cold pressure test and serum brain-derivate-neurotrophic-factor. The analysis showed a significant main effect for treatment ($F=4.32$; $P=0.04$) when we compared the delta-(Δ) of conditioned pain modulation task between the transcranial direct-current stimulation and hypnotic suggestion groups. Also, the change in the brain-derivate-neurotrophic-factor was positively correlated with the conditioned pain modulation task.

Conclusions: The results confirm a differential effect between hypnotic suggestion and transcranial direct-current stimulation on the pain measures. They suggest that the impact of the interventions has differential neural mechanisms, since the hypnotic suggestion improved pain perception, whereas the transcranial direct-current stimulation increased inhibition of the descending pain modulating system.

Registration information clinicaltrials.gov: NCT03744897.

Perspective: These findings highlight the effect of hypnotic suggestion on contra-regulating mechanisms involved in pain perception, while the transcranial direct-current stimulation increased inhibition of the descending pain modulating system. They could help clinicians comprehend the mechanisms involved in hypnotic analgesia and transcranial direct-current stimulation and thus may contribute to pain and disability management.

Keywords: Hypnotic analgesia, Transcranial direct-current stimulation, Pain threshold, Conditioned pain modulation, Brain-derivate-neurotrophic-factor, Pain.

1. Introduction

The pain and emotion circuits are reciprocally interconnected, providing pro- and anti-nociceptive pain modulation [1]. Although sensory information is primarily transmitted by the ascending pathway, providing the sensory components of the pain experience, top-down neuromodulator techniques can affect both ascending and descending pain processing pathways. These approaches include noninvasive brain stimulation (NIBS) methods, which can alter aberrant activities within the pain processing circuit (e.g., transcranial direct-current stimulation [tDCS]), and psychological pain interventions, which improve the cognitive and emotional components of pain (e.g., meditation and hypnotic suggestions).

Previous studies have shown the efficacy of tDCS for the treatment of various chronic pain conditions (i.e., fibromyalgia, phantom pain, and trigeminal neuralgia) [2,3]. The primary target to apply the tDCS has been the primary motor cortex (M1) [4]. The stimulation of M1 enhances the strength of the descending pain modulating system (DPMS) in both, healthy subjects [5,6] and patients with chronic pain [4,7]. In addition, the dorsolateral prefrontal cortex (DLPFC) is a target for improving pain sensations and the emotional aspects linked to pain [2,3,6]. According to recent studies of fibromyalgia, anodal tDCS over the left DLPFC reduced pain sensations and fatigue [6], improved cognitive performance [8] and the performance of tasks related to attentional networks [6]. In addition, prefrontal tDCS might alter the function of emotion-related information processing circuits [9]. Specifically, anodal tDCS applied to the left DLPFC, with the cathode over the right DLPFC has been shown to either enhance neural activity and/or reduce neural activity in the right DLPFC[10].

The DLPFC is also involved in pain modulation through multiple psychological processes. As such, it is activated in experimental pain studies. For example, individuals that received instructions to suppress pain increased activation of bilateral, particularly in the left – DLPFC [11]. Besides, the bilateral tDCS, with anodal stimulation over DLPFC increased the connectivity strength in both regions, thalamus and right anterior insula [12]. While a single session of tDCS over the left DLPFC significantly increased the heat pain threshold in fibromyalgia [6]. Further to the factors related to the target to apply the stimulation, other factors can influence the tDCS effect, such as the type and duration of stimulation (anodal or cathodal), schedule and number of repetitive stimulations. In addition, according to previous studies, its effect is likely state-dependent neuroplasticity, since the brain-derived-neurotrophic-factor (BDNF) [8,13] predicted the impact of tDCS on short-term memory in patients with fibromyalgia [8] and the disability due to pain after hallux valgus surgery [14].

Regarding to hypnotic analgesia, the literature reports different responses to pain perception in highly susceptible subjects. Its effects include a decreased H-reflex amplitude [11]and decreased subjective pain perception. Also,–it reduces the neuronal activity of the primary somatosensory cortex (SI) [3]. According to a meta-analysis, which included both laboratory and clinical studies, the effect size (ES) of hypnotic analgesia was moderate (ES = 0.71) [15]. The-hypnotic suggestions can increase activity on anterior cingulate cortex, which is identified as an “important part of the executive network, as it is involved in selective attention, learning and conflict resolution” [12]. Its effect on the thalamus is also associated with an “activation of a critical node in the motor path from basal ganglia to higher motor areas” [12]. In addition, studies have shown that during the cold-pressor test (CPT), hypnotic analgesia

increased pain tolerance by a mean of approximately 62% and reduced both pain perception and the nociceptive reflex to 65% of the baseline reflex area [13]. However, diffuse noxious inhibitory control (DNIC) provoked by the CPT [13] was less effective during hypnosis than without hypnosis. A study suggested that the inhibition of DNIC does not involve spinal motoneuron excitability [14]. In short, these results, together with those of earlier studies [16,17], suggest that the inhibition of DNIC also involves supraspinal structures. During the last decade, the human DNIC counterpart has been identified and is referred to as conditioned pain modulation (CPM) [18].

As the application of tDCS can modulate thalamocortical synapses in a top-down manner, and the hypnotic suggestion can improve pain perception new insights are needed to compare how each one these two techniques changes the pain perception and the descending pain-modulating function. Thus, this study investigated whether the tDCS would be more effective than a hypnotic suggestion for improving the inhibitory functions of the DPMS, as assessed by reported changes on the Numerical Pain Scale (NPS ranging from 0–10) during the CPM test. And so, this study tested the following hypotheses: (i) hypnotic suggestion would have a superior effect on pain perception in response to the Quantitative Sensory Testing (QST) compared with anodal tDCS applied to the left DLPFC and cathodic tDCS applied to the right DLPFC, as assessed by changes in the heat pain threshold (Δ-HPT), heat pain tolerance (Δ-HPTo) and the cold pressure test (Δ-CPT). (ii) Anodic tDCS applied to the left DLPFC and cathodic tDCS applied to the right DLPFC would be superior to hypnotic suggestion at altering the DPMS function, as assessed by the delta (Δ)-value of the change on the NPS (scale of 0-10) during a CPM-test. In addition, we evaluated the influence of Δ-BDNF on the effects of tDCS

and hypnotic suggestion and performed exploratory analyses of the relationships between state anxiety and the Δ-value of the NPS (0-10) during the CPT and between Δ-BDNF and the Δ-value of the NPS (0-10) during the CPM test, according to treatment mode.

2. Material and method

2.1 Design overview, setting, and participants

All subjects provided written informed consent for their participation in this randomized double-blind crossover clinical trial, with a 1:1 allocation ratio. The protocol was approved by the Institutional Review Board (IRB no 63863816000005327 and 16-0635) and conducted according to the Declaration of Helsinki. Recruitment was undertaken in the time from July 2017 to November 2018. To assess clinical and psychological characteristics, we used a standardized questionnaire and administered scales validated to the Brazilian population. Additionally, we collected behavioral measurements (i.e., pain assessments). De-identified data relating to intervention and primary outcomes will be made available on request to Caumo W. (wcaumo@hcpa.edu.br) with no time restriction. The timeline of study is presented in Figure 1.

---Insert Figure 1---

2.2 Subjects

The volunteers were recruited from the general population by advertisements posted in the universities, on the internet and in public places in the city of Porto Alegre, Brazil. Subjects were considered eligible to participate if they were healthy women with more than 11 years of

education, ranging between 18 to 45 years old. Individuals were excluded if they presented hearing impairment or formal contraindication to transcranial direct-current stimulation (tDCS), according to current guidelines.

During the first contact, the researcher performed the Waterloo-Stanford Group Scale of Hypnotic Susceptibility Form C [19] and the subjects completed a structured questionnaire that assessed the following variables: current acute or chronic pain conditions, use of analgesics in the past week, rheumatologic disease, clinically significant or unstable medical or psychiatric disorder, history of alcohol or substance abuse in the past six 6 months, neuropsychiatric comorbidity, and use of psychotropic drugs. They were excluded if answered any of these questions positively. Subjects with a score greater than or equal to 8/12 on the Waterloo-Stanford Group C Hypnotic Susceptibility Scale (WSGC) were included in the later phases of the investigation, while subjects with Beck Depression Inventory [20] scores higher than 12 were excluded [21], as were those with positive screening higher seven for minor psychiatric disorders (somatic symptoms, depressive moods, depressive thoughts and decreased energy) on the World Health Organization (WHO) Self-Reporting Questionnaire (SRQ-20).

2.3 Experimental protocol

This was a double-blind randomized crossover trial. On admission to the study, participants were randomized to initially receive either tDCS or hypnotic analgesia sessions. For the tDCS condition, the anode electrode was positioned over the left dorsolateral prefrontal cortex (DLPFC) and a cathode electrode on the right (DLPFC). A constant current of 2mA was applied for 20min, with initial and ending ramps of 30s long stimulation. The hypnotic

analgesia session consisted of a 10min long standard induction. The protocol began with a set of suggestions to subjects to focus their attention on a single stimulus and they were encouraged to control their breathing, guiding subjects to progressive relaxation. After that, suggestions were given for comfort, in which the patient had to imagine being in a quiet and peaceful place. On the 10 final minutes of the induction, the hypnotic suggestions for analgesia were given targeting the decrease of the subject's pain and controls over her own sensations. As suggestion for hypnotic analgesia the patient was told that he no longer would feel pain. His mind would be able to control the sensations of his own body, preventing pain. This suggestion was based on Jensen's approach for hypnotic analgesia [22]. After the first intervention, to stimulate pain and determine the CPM, we used the difference between the pain score on NPS (0-10) QST during cold water immersion (QST+CPM) and the temperature of the point at which subjects felt 6/10 pain on the NPS scale (during the initial time period). To determine heat thermal thresholds (HTT), heat pain threshold (HPTh) and heat pain tolerance (HPTo) during the CPM task the QST was performed. The participants remained seated, and a thermode was positioned on the forearm of the dominant side of the body. The temperature started at 30°C, and the thermode was heated at a rate of 1.0°C/s to a maximum of 51°C, when the temperature began to drop. Besides that, the CPT was conducted to determine on subject's' response due to the physical cold stimulus per se and the reaction due to the cold pain. During the test, the participant was asked to immerse the dominant hand in ice-saturated water for a maximum of 2min. The participant returned for a second experimental session to receive the alternative intervention. The order of the experimental sessions was counterbalanced and separated by at least seven days to avoid carryover effects of the initial stimulation protocol.

2.4 Randomization

Randomized numbers in a 1:1 ratio were generated to allocate each participant to either the HSA or tDCS group. The randomization table was generated by appropriate software. Envelopes were prepared for randomization process and sealed with the subject's #24 sequence number on the outside of the envelope. The allocation was concealed so no investigator was aware of treatment allocations and therefore had control over the randomized order of patients.

2.5 Blinding

To control possible biases, the following strategies were established. Participants were instructed on all aspects related to the interventions during the evaluations. Two independent evaluators who were not aware of the treatment were trained to do the assessments. The brown envelopes were prepared before starting the study, sealed, initialed and numbered sequentially. The envelope contained the allocation interventions and was opened only after the participant had given her informed consent to participate in the study. The subject's name and number were immediately sent to those responsible for controlling the randomization process. The blinding was gauged at the end of each evaluation.

2.6 Interventions

2.6.1 Transcranial direct-current stimulation

TDCS was applied using Brain Monitoring and Stimulation Technologies (NE, Neuroelectrics Barcelona Sl, model Starstim). Cathodal and anodal electrodes covering an area

of 25 cm² each were surrounded by a water-soaked sponge. Electrodes were placed at spatial positions F3-(Anodal) DLPFC - L and F4-(Cathodal) DLPFC - R, according to the international 10-20 system for electroencephalogram electrode placement [23] that are commonly considered surface locations above the mid-dorsolateral prefrontal cortex [24]. Stimulation was delivered at an intensity of 2 mA for 20min, including a 30s ramp-up to 2 mA at the start and a 30s ramp-down to 0 mA at the end. During stimulation, participants were asked to relax while the upper limb was supported in a comfortable position.

2.6.2 Hypnotic analgesia suggestion protocol

The techniques of hypnosis developed for this study were based on the classical approach developed by the American clinician and Ph.D. Mark P Jensen. The hypnotic induction protocol was standardized to be equally applied to all subjects. The standard hypnotic protocol begins with an induction that is associated with breathing and relaxation, where subjects receive suggestions to focus their attention on a single stimulus. Then, direct suggestions are given for comfort and pain management [25]. The duration of experimental manipulation (induction + suggestions) is 20 min.

The protocol of suggestion followed these standardized steps, read by the researcher:

"And from now on, you will no longer feel any more pain ... you will not feel any kind of pain after waking up. Your mind will be able to control the sensations of your whole body ... Your mind controls the sensations of your body ... after you wake up, you will no longer feel any kind of pain ... From now on... and after you wake up you will no longer feel any kind of

pain ... I will count from one to ten and you will feel no pain anymore... your mind will control all the sensations of your body and after waking up you will not feel pain."

1: "*...you're feeling even more relaxed and comfortable...*"

2: "*...you will get even more relaxed and feeling good...*"

3: "*...feeling better and better, you will not feel pain after you're awake...*"

4: "*...even more relaxed...after waking up, a heat stimulus will be placed on your forearm...and your hand in the ice...and you will not feel any kind of pain...*"

5: "*...will be relaxed and painless...*"

6: "*...your mind will control all the sensations of your body...will control it and you will no longer feel pain after you wake...*"

7: "*...you relax even more...feeling very well...you relax and have good sensations...*"

8: "*...very deep, you will not be able to feel any kind of pain in the periphery of your body when you are awake...*"

9: "*...you will not feel pain...your brain controls all the sensations of the periphery of your body...you will not feel any kind of pain...*"

10: "*...your brain now controls all your sensations of your body...and you will not feel pain after you wake up...feel even more relaxed and comfortable...when you wake up you will no longer feel any kind of pain.*"

"*Now you are very relaxed and feeling comfortable, but very soon you will wake up. I will count from one to ten and to each number you will wake up even more...I will count from one to ten, and you will wake up, feeling good...and will no longer feel any kind of pain...I will count from one to ten and you will wake up, and you will not be able to feel any kind of pain in your*

body...one...you are gradually coming back and feeling your own body...two...you are gradually coming back...and you can remember that you will no longer feel any kind of pain...three...you can slowly feel your body and the energy increasing...four...you are waking up and feeling your body on the chair...five...halfway through...after waking up you will no longer feel any kind of pain...six...feeling good and slowly waking up...seven...slowly feeling the environment and the movements of your body...eight...you are almost awake...when I get to ten you will wake up feeling good and your brain will not allow you to feel any kind of pain...nine...you are waking up and being aware of your surroundings...waking up more and more...ten...now you wake up feeling good...open your eyes."

2.7. Instruments and assessments

The tools used to evaluate psychological state and hypnotic suggestion were validated to the Brazilian population. Two psychologists were trained to perform the psychological tests and scale of susceptibility. The Waterloo-Stanford Group Scale of Hypnotic Susceptibility, Form C [26] was used to assess hypnotic susceptibility, and depressive symptoms of patients were assessed by the depression inventory from Beck (BDI II) [20]. The Pain Catastrophizing Scale (PCS) measures a patient's catastrophizing, defined as "an exaggerated negative 'mental set' brought to bear during actual or anticipated pain experience" [27]. Central Sensitization Inventory (BP-CSI) is an instrument used to identify patients with central sensitization syndrome (CSS) and central sensitization (CS) symptoms. The self-report questionnaire (SRQ-20) was used to measure minor psychiatric disorders, somatic symptoms, depressive mood, depressive thoughts, and decreased energy, and the Pittsburgh quality of sleep index to assess

sleep quality, as higher scores indicate worse sleep quality [28]. We used the refined version of the State-Trait Anxiety Inventory (STAI) [29] using the Rasch model, which derived STAI-Form X scales from shorter state traits without threshold disorders and for differential element performance (DIF) problems. Scores on the state and trait evaluations vary from 13 to 52 and from 12 to 36, respectively. A standardized questionnaire was used to evaluate demographic data and medical comorbidities.

2.8 Outcomes

The primary outcomes were the NPS (0-10) during the conditioned modulated pain (CPM task) as assessed by the Δ-CPM (i.e., post- minus pre-intervention) and change on NPS (0-10) during the cold pressure test (Δ-CPT). The secondary outcomes were Δ-HPT and Δ-HPTo assessed by quantitative sensory testing (QST).

2.9 Outcomes assessment

In this study, we evaluated pain as a response to a nociceptive stimulus using quantitative sensory testing (QST), including the conditioned pain modulation task (CPM task) and the cold pressure test (CPT).

a) To perform QST, we have used a computerized version of the thermostat (Heat Pain Stimulator 1.1.10, Brazil) [30] to determine heat thermal thresholds (HTT), heat pain threshold (HPTh) and heat pain tolerance (HPTo) during the CPM task. The participants remained seated, and a thermode (30 x 30mm) was positioned on the forearm of the dominant side of the body. The temperature started at 30°C, and the thermode was heated at a rate of 1.0°C/s to a maximum

of 51°C, when the temperature began to drop. For the HTTh, the participants were asked to press a button when they "felt the first heat sensation" and were asked to press a button when they "felt the first heat pain". The heat thermal threshold and heat pain threshold were determined by the average of three evaluations with a 40s interval between them. Also, we assessed the HPTo.

b) To measure the CPM test, we evaluated the pain intensity in two tonics HPT test stimuli separated by a CPM test. We used the HPT as conditioning pain stimulus to elicit a prolonged pain sensation to trigger CPM. The CPM test consisted of immersion of the non-dominant hand in cold water at a temperature of 0°C to 1°C for one minute. A thermostat was used to control the temperature variation and to maintain the water temperature. The QST procedure was introduced after 30 seconds of cold-water immersion. To determine the CPM, we used the difference between the pain score on NPS (0-10) QST during cold water immersion (QST+CPM) and the temperature of the point at which subjects felt 6/10 pain on the NPS scale (during the initial time period).

***c*)** CPT was conducted to determine on subjects' response due to the physical cold stimulus per se and the reaction due to the cold pain [31]. During the test, the participant was asked to immerse the dominant hand in ice-saturated water for a maximum of 2min [32,33]. The temperature of the ice water was measured, and across all tests, it ranged from 9°C to 10°C. Perceived pain intensity was rated continuously on a 0–10 electronic visual analogue scale (VAS) with the non-dominant hand and stored electronically for 2min for subsequent analysis of peak pain intensity. If pain was intolerable before 2min, the subject could withdraw, in which case pain intensity was considered maximal until the end of the 2min period [31].

***d*)** To test serum levels of BDNF, blood samples were collected at baseline at pre-intervention and post-intervention. Using a ChemiKine BDNF Sandwich ELISA kit, CYT306 (Chemicon/Millipore, Billerica, MA, USA), serum BDNF was determined by the Enzyme-linked Immunoabsorbent Assay (ELISA). The lower detection limit of the kit is 7.8 pg/ml for BDNF.

2.10 Sample size

The sample size was estimated using the G*Power software, based on a previous study with a similar methodology (Effect of hypnotic suggestion on fibromyalgia pain: Comparison between HSA and relaxation, Antoni Castel). The calculus indicated that a sample size of 12 individuals would be necessary to detect a 3-point difference in the numerical scale of pain (average SD 0.59) (NPS) in pain levels to nociceptive stimuli, with a power of 0.95 and an α of 0.05. To ensure the power of the study, 15% was added in case of possible losses, totaling 24 subjects (12 per group). This also provided a power to detect a meaningful effect size (determination coefficient (f^2)=0.2) to detect differences between the two groups in the other outcomes.

The randomization table will be generated by computer program (Randomlogue). Random codes were placed in brown envelopes sealed. The sequence number is shown on the outside of the envelope.

2.11 Statistical analysis

Descriptive statistics were used to summarize the main socio-demographic features of the sample. T-tests for independent samples were used to compare continuously between groups. To compare the change within a group, Wilcoxon Signed Ranks tests were used. To test for normality, we used the Shapiro-Wilk test. After verifying the corresponding assumptions, a mixed ANCOVA model was used to analyze the main effect of interventions. Factors were the intervention (tDCS and hypnotic suggestion) and the order of the treatments. The order of interventions was included in the model to assess a possible carryover effect produced by the two sequences of treatment to which all subjects were randomly assigned. The outcomes were evaluated using the mean variation for delta ((Δ)-values, post-intervention minus pre-intervention) of the following measures: score on the NPS (0-10) during the CPT, change on NPS (0-10) during the CPM task, HPT and HPTo. The covariate included in all models was the change on serum BDNF (Δ-value, post-intervention minus pre-intervention). We performed all analyses by two-tailed tests, and they were corrected for multiple comparisons using the Bonferroni test. Within groups, the standardized mean difference (SMD) was computed in terms of the ratio between the mean change and the pool of baseline standard deviation (SD). The SMD was interpreted as follows: small, 0.20 to 0.4; moderate, 0.50 to 0.70; and large, 0.80 or higher, with respective confidence interval (CI) . We accepted a type I error of 5%. To perform the analyses, we used the software SPSS version 22.0 (SPSS, Chicago, IL, United States).

3. Results

3.1. Demographic and characteristics of the subjects

A total of 90 subjects were recruited to participate in this study. After applying the Waterloo-Stanford Group C (WSGC) Scale of Hypnotic Susceptibility, using a cutoff point (8/12) for susceptibility to hypnosis, 27 subjects were selected for the hypnosis experiment. These 27 subjects underwent screening for the presence of minor psychiatric disorders, as determined by the Self-Reporting Questionnaire (SRQ-20) and the Beck Depression Inventory-II (BDI-II). Three subjects were excluded because we identified the presence of minor psychiatric disorders or scores on the BDI-II that were higher than the cutoff point of 12. The final sample included 24 subjects who were randomized to receive either tDCS or hypnotic suggestion for analgesia. For each group, 12 participants were randomized and assigned in a crossover manner to participate in the two sequences of treatment. For all outcomes, 24 subjects were analyzed by the arm, as shown in the study flowchart.

---Insert Figure 2---

3.2. Primary and secondary outcomes: univariate analyses

The socio-demographic characteristics of the subjects according to the sequence allocation were comparable and are shown in Table 1. Twelve subjects were allocated to trial I, which received tDCS first, and twelve subjects were allocated to trial II, which received

hypnotic suggestions for analgesia first. All subjects completed the protocol to which they had been randomized.

---Insert Table 1---

The within and between groups comparisons of psychophysical measures (HPT, HPTo, CPT, and Δ-value of NPS during the CPM task) and serum levels of BDNF, according to the intervention, are presented in Table 2. Comparisons revealed that only hypnotic suggestions for analgesia produced significant changes between the pre and post-intervention measures for HPT, HPTo, CPT and serum BDNF levels.

---Insert table 2---

3.3. Primary Outcomes: multivariate analyses

3.3.1. Intervention Effect During the CPT

A mixed analysis of covariance (ANCOVA) model revealed a significant main effect of the intervention on the Δ-value of NPS during the CPT ($F = 15.98$; $P < 0.00$). An order effect was not observed ($F = 0.38$; $P=0.54$). The covariate included in the model was the change in serum BDNF level (Δ-value, post-intervention minus pre-intervention). The results of this analysis are presented in Table 3. In the tDCS group, the mean (SD) of the Δ-value of NPS during the CPT (mean post-intervention minus pre-intervention) without the adjustment for the Δ-BDNF value was -0.50 (1.19), while the mean (SD) with adjustment for the Δ-BDNF value was -0.29 (1.23). The pain perception due to the physical cold stimulus increased within the

tDCS group by 42%. The effect size of this increment was 0.22 [(mean difference: 0.50/1.19 (SD)]. In contrast, in the hypnotic suggestion group, the mean (SD) of the Δ-value of NPS during the CPT without adjustment for the Δ-BDNF value was -2.10 (1.80), while the mean (SD) with adjustment for the Δ-BDNF value was -2.29 (1.74). Pain perception decreased within the hypnotic suggestion group by 8.30%. The effect size of this decrement was 0.10 [(mean difference: 0.19/1.80 (SD)].

---Insert table 3---

The mean in the NPS (0–10) during the CPT is presented in Figure 3. A mixed ANCOVA model revealed a significant main effect of interventions on the Δ-value of NPS (0–10) during the CPT ($F = 15.98$; $P < 0.00$). An order effect was not observed ($F = 0.38$; $P=0.54$), neither influences in the change of serum BDNF.

---Insert Figure 3---

3.3.2. Intervention Effect on the CPM test

A mixed ANCOVA model revealed a significant main effect for treatment ($F=4.32$; $P=0.04$) when we compared the Δ-values of NPS during the CPM test (mean post-intervention minus mean pre-intervention) between the tDCS and hypnotic suggestion groups. The result of the analysis adjusting for the influences of Δ-BDNF revealed that changes in this neurotrophic

factor increased the inhibitory function of the DPMS in the tDCS group, whereas an opposing effect was observed in the hypnotic suggestion analgesia group. In the tDCS group, the mean (SD) of the Δ-value of NPS during the CPM test without adjusting for the Δ-BDNF value was -0.29 (1.75), while the mean (SD) with adjustment for the Δ-BDNF value was -0.45 (1.56). In the tDCS group, BDNF increased the inhibitory function of the DPMS by 39.20%. The effect size of this increment on inhibitory function within the group, as assessed by SDM, was 0.42 (mean difference: 0.74/1.75 (SD)). In contrast, in the hypnotic suggestion group, the mean (SD) of the Δ-value of NPS during the CPM test without adjustment for the Δ-BDNF value was 0.42 (1.30), while the mean (SD) with adjustment for the Δ-BDNF value was 1.11 (3.34). In the hypnotic suggestion group, BDNF decreased the inhibitory function of the DPMS by 165%. The effect size of this decrement on inhibitory function within the group, as assessed by SDM, was 0.53 [(mean difference 0.69/1.30 (SD)]. When interpreting these results, it is important to recognize that a higher Δ-value of NPS during the CPM task indicates the reduced inhibitory function of the DPMS. These findings show that the effect of BDNF on the DPMS is likely to be related to the effects observed for the interventions (tDCS or hypnotic suggestion) on the Δ-BDNF, as tDCS increased the BDNF level while hypnotic suggestion reduced the BDNF level (Table 2). When we analyzed the interaction between the Δ-BDNF value and the intervention group, we did not find an interaction between Δ-BDNF and intervention (F = 0.07; P=0.78) (Table 3). Based on these results, the changes in the neuroplasticity mechanisms induced by the type of intervention can explain their effects on the inhibitory function of the DPMS, which increased with tDCS and decreased with hypnotic suggestion.

----------Insert Figure 4----------

3.4. Intervention Effect on the Secondary Outcome: HPT and HPTo

The mixed ANCOVA analyses of the main effects of the intervention on the Δ-value of the HPT and HPTo are presented in Table 4. The mixed ANCOVA revealed a main effect of group on the Δ-HPTo (F=5.10; P=0.02). Hypnotic suggestion induced a more substantial impact during the CPT than tDCS. For the Δ-HPT, a we did not observe a significant difference between hypnotic suggestion and tDCS (F=3.14; P=0.08), nor was an order effect observed for either outcome.

---Table 4---

3.4.1 Relationships between State Anxiety vs. Baseline during the Δ-value of NPS during the CPT and between Δ-BDNF and Δ-value of NPS during the CPM test

The scatter plots of the raw data for state anxiety and Δ-value of NPS during CPT, according intervention, are shown in Fig.5 A and B, respectively. In the tDCS group, state anxiety and the Δ-value of NPS during the CPT showed a negative non-parametric correlation, demonstrating that patients with higher levels of state anxiety that received tDCS treatments showed a lower Δ-value of NPS during the CPT and indicating a larger effect of tDCS in these patients. The Spearman's correlation coefficient between state anxiety and the Δ-value of NPS during the CPT for the tDCS group was 0.43, with a 95% confidence interval (95% CI) of -0.71 to -0.03 (P=0.03). The Spearman's correlation coefficient between state anxiety and the Δ-value of NPS during the CPT for the hypnotic suggestion group was 0.05, with a 95% CI of -0.33 to 0.44 (P=0.8).

---Figure 5---

The scatter plots of the raw data for Δ-BDNF and Δ-value of NPS the CPM test, according intervention, are shown in Fig. 6 A and B, respectively. For the hypnotic suggestion group Δ-BDNF and Δ-value of NPS during the CPM test showed a positive non-parametric correlation, suggesting that patients that received hypnotic suggestions showed a lower Δ-value of NPS during the CPM test. It is important to recognize that higher Δ-values of NPS during the CPM test indicate the reduced potency of the DPMS. The Spearman's correlation coefficient between Δ-BDNF and Δ-value of NPS during the CPM test was 0.42, with a 95%CI of 0.02 to 0.70 (P=0.03). The Spearman's correlation coefficient between Δ-BDNF and Δ-value of NPS during the CPM test in the tDCS group was 0.22, with a 95%CI of -0.20 to 0.57 (P=0.26).

---Figure 6---

4. Discussion

These findings indicate that the effect of hypnotic suggestion on pain perception involves cortical pain processing, whereas tDCS induced either a downregulation of the pain-facilitating pathways or an upregulation of the inhibitory function of the DPMS. In short, these results provide new scientific insights concerning the effects of hypnotic suggestion and tDCS on pain processing, while simultaneously raising important questions regarding the extent to

which each intervention can alter pain perception. In addition, an exploratory analysis showed two distinct effects of these two interventions, and increased levels of state anxiety at baseline were correlated with a more substantial tDCS effect on the Δ-value of NPS during the CPT. For hypnotic suggestion, a higher change in the Δ-BDNF value associated with the magnitude of the Δ-value of NPS during the CPM test.

The effects of hypnotic suggestion on CPT and HPTo responses revealed that it was able to decrease pain perception. These results are in line with those reported by randomized controlled studies of clinical populations, which reported that hypnotic suggestion could improve pain conditions and analgesia [25,34]. These results also indicate that hypnotic suggestion might be an effective procedure for alleviating pain perception in experimental models [35,36]. Due to the complex mechanisms of pain, it is important to investigate the multiple methods through which hypnotic suggestion can influence pain perception, as evaluated by psychophysical pain measures. According to previous studies, the psychophysical measures that are suitable for activating pain pathways, can be measured by valid tests, and are reproducible have a strong probability of being correlated with pain perception. In addition, our results allow the discrimination of effects mediated primarily by cortical mechanisms (i.e., pain threshold and pain tolerance) from those mediated by infra-cortical mechanisms, such as the inhibitory functions of the DPMS, based in the paradigm of the CPM test [37].

In the present study, an exploratory analysis revealed that subjects with increased levels of state anxiety at baseline showed increased responses to anodal tDCS applied to the left DLPFC during the CPT. This result can be explained by the upregulation of reactions to positive emotional stimuli. In accordance with these results, a previous study reported that the anodal

stimulation of this region improved the identification of positive emotional expressions [38]. However, other studies found that anodal stimulation of this region may reduce the perceived degree of emotional valence for negative emotional pictures [39] and expressions of anger in images [40]. In addition, the right DLPFC may be involved in the upregulation of negative emotional outcomes. High-frequency (i.e., excitatory) treatment with repetitive transcranial magnetic stimulation (rTMS) applied to the right DLPFC resulted in impaired attention disengagement from a threat (angry faces) [40]. However, these results remain inconclusive, and further studies are necessary to clarify how the stimulation of both hemispheres affects pain tolerance according to the levels of state anxiety immediately prior to the application of tDCS.

The novelty of these results reveals that hypnotic analgesia induces a dissociative effect, which activates supra-spinal neural networks that reduce pain perception for both heat pain threshold and heat pain tolerance. Conversely, this effect is likely decoupled from the inhibition of the DPMS. When we analyzed the impact of the interventions on Δ-value of NPS during the CPM test within groups (hypnotic suggestion and tDCS) (Table 2), we found small effect sizes for both procedures (hypnotic suggestions ES=0.22 and tDCS ES=0.1). However, these effects changed when we adjusted the impact of the interventions by the Δ-BDNF values. The adjusted analysis revealed that hypnotic suggestion improved the inhibitory function of the DPMS. One hypothesis is that hypnotic suggestion reduced the experienced pain level; consequently, the DPMS was less activated by heterotopic painful stimuli. An alternative explanation is that both hypnotic suggestion and painful heterotopic stimuli compete for the same descending inhibitory pathways. In addition, according to the literature, the H-reflex amplitude decreases significantly during hypnosis in highly susceptible subjects, whereas the painful stimulus of the CPM test

does not affect this monosynaptic reflex excitability [11]. It is possible that the subjects may have experienced less pain during hypnotic suggestion and resulting in the observed decrement in the Δ-BDNF value. However, we do not have a clear explanation for how hypnotic suggestion affects BDNF secretion or whether the observed effects of hypnotic suggestion on the DPMS are dependent on changes in this neurotrophic factor.

The application of tDCS increased the function of the descending pain inhibitory system when we adjusted for changes in the BDNF levels. This result indicates that the bi-encephalic approach used to apply the tDCS altered neuroplasticity processes and improved the DPMS. Although the mechanisms underlying this effect are not entirely understood, it is plausible that tDCS can activate structures within the brainstem that are involved in the inhibitory function of the DPMS. This result demonstrates that the effects of tDCS on the descending inhibitory pathway may be linked to increased cortical excitability. This hypothesis is supported by evidence from left DLPFC studies, which indicate that the analgesic mechanisms of tDCS involve the activation of top-down downstream circuits to the anterior insula, the hypothalamus, the periaqueductal gray region, the nucleus accumbens and the rostroventral medulla [41]. Thus, cortical hyperexcitability and increased spinal inhibition could explain the observed changes in BDNF levels. Although these results help improve the understanding of the relationship between tDCS applied to the left DLPFC and serum BDNF and their effects on DPMS function, it is necessary to be judicious when interpreting these results. Because these results were observed in healthy subjects, in an experimental model after, one session of tDCS these findings may not be applicable to other situations. Thus, further studies are necessary.

In the current study, the bi-encephalic tDCS montage was not able to increase the pain threshold for the CPT. Our results are similar to those reported by another study using healthy subjects, where a significant effect on thermal thresholds was not found for tDCS applied to the left DLPFC [42]. However, studies with similar characteristics found considerable increases in the HPT when anodal tDCS was applied to both the M1 [43,44] and the right DLPFC [43]. Likewise, either low- or high-frequency rTMS applied to the right or left DLPFC has been reported to reduce cold- or heat-induced pain [45,46]. Three studies in healthy subjects found that rTMS applied to the right or left DLPFC reduced the sensitivity to thermal pain stimuli [45,47].

Although these results are intriguing, the effect sizes for both interventions were small in this experimental model using healthy subjects. Thus, further clinical studies are required, especially as the clinical administration of these procedures generally involves repeated sessions. In addition, although there are limitations on the translation of an experimental paradigm to the clinical setting, these experiments allow us to characterize the etiological components of pain (e.g., the nature, localization, intensity, frequency and duration of the trigger necessary to evoke pain). Thus, these results add to the body of research regarding the use of hypnotic analgesia in combination with psychophysical pain measures, which are widely used to evaluate the effects of interventions on pain processing. Furthermore, these studies permit the measurement of a dynamic series of multiple neurophysiological mechanisms that modulate pain perception.

There are several concerns related to the design and data interpretation of this study. First, we included only females because the literature has shown that the pain response is

increased in females compared to males. The differences between sex on pain perception have been attributed to physiological and psychological variables, including mechanisms of endogenous inhibition, the capability to endure pain, genetic factors, pain expectation and personality traits [48]. In addition, females are more prone to respond to negative emotional stimuli (i.e., stress, fear, and anxiety). Thus, sex could be a confounding factor. Second, BDNF levels indicate neuronal activity [49], and BDNF is able to cross the blood-brain barrier (BBB); therefore, the peripheral blood level of BDNF is a reliably good indicator of BDNF levels in the brain [50]. Thus, we assumed that increased serum BDNF levels indicated a diffuse increase in cortical excitability associated with anodal stimulation [51]. Third, although it is a crossover design with a small sample, this design can help prevent the overestimation of the benefits of the intervention being tested [52]. A potential advantage of this design is that it allows the subject to be the control [53]. Fourth, we did not observe a "carryover" effect, which means that the effects found for each phase of the experiment do not reflect the impacts of any residual effects of therapy provided during previous phases of the experiment [54]. Fifth, the findings may only apply to subjects with high levels of hypnotic susceptibility. Thus, further research should explore the beneficial aspects of hypnotic suggestion for chronic pain. In addition, these results provide new insights for psychologists, psychotherapists and hypnosis practitioners and suggest that hypnosis may represent an effective treatment for chronic pain, especially when coupled with its cost-effectiveness and minimal side effects. Sixth, although we did not formally measure the potential impact of awareness of the allocation group on the outcomes, a sham intervention that is meaningful for hypnosis is not feasible. Despite these limitations, our findings were evaluated using psychophysical parameters, which are less prone to assessment

bias than self-reported measures. Finally, we showed a dissociation between the effects of hypnotic suggestion and DPMS function. These findings provide additional insights into the integration of cortical and distant neural circuits in pain processing. While these results are essential to the understanding of the possible neurobiological mechanisms of hypnotic suggestion on the DPMS compared with tDCS, they do not support therapeutic decision-making in clinical settings.

In conclusion, these results confirm a differential effect between hypnotic suggestion and tDCS on pain measures. They suggest that the impacts of these interventions can be explained by differential effects on contra-regulating mechanisms involved in pain perception, as hypnotic suggestion improved pain tolerance, whereas tDCS increased inhibition in the DPMS. Furthermore, they highlight that Δ-BDNF value influenced the effect of these interventions differently with regards to the inhibitory function of the DPMS; hypnotic suggestion paradoxically decreased the inhibitory function of the DPMS, whereas tDCS increased the inhibitory function of the DPMS. Overall, these findings increase our understanding of the differential effects of these interventions on pain processing, and further studies should be performed that examine their combined effects.

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6. Author Contributions Statement

GB: Participated in the sequence alignment and drafted the manuscript. Conceived the study, participated in its design and coordination.

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Legends of Figures

Figure 1. Timeline of procedure of study.

Figure 2. Flowchart of study.

Figure 3. The change in Numerical Pain Scale (NPS 0–10) during cold pressure test with water at zero to 10ºC degree, assessed by the Δ-value (score post intervention minus pre-intervention) in the two experimental groups. The error bars indicate standard error of the mean. Asterisk indicates difference between two intervention groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni test for post hoc multiple comparisons.

Figure 4. The change in NPS (0–10) during CPM-test, assessed by the Δ-value (score post intervention minus pre-intervention) in the two experimental groups. The error bars indicate standard error of the mean. Asterisk indicates difference between two intervention groups. All comparisons were performed by a mixed analysis of variance model, followed by the Bonferroni test for post hoc multiple comparisons. Numerical Pain Scale (NPS 0–10).

Fig 5 A and B. Scatter plots of State-anxiety with Δ-value of the NPS (0-10) during CPT (i.e., after intervention minus baseline level) according to tDCS (5A) and hypnotic suggestion (5B).

Fig 6 A and B. Scatter plots of Δ-BDNF (post intervention minus pre-intervention) with Δ-Change on NPS (0-10) during CPM-test (i.e., after intervention minus baseline level) according to tDCS (6A) and hypnotic suggestion (6B)

Table 1 – Demographic and clinical characteristic of the sample. Data are presented as mean and standard deviation (SD) according to group in trial I (n=24).

	tDCS (n=12)	**Hypnotic suggestion (n=12)**	**P-value***
Demographic			
Age(years)	26.00 (7.66)	27.00 (10.66)	0.85
Level of education (years)	14.09 (2.76)	14.92 (2.76)	0.47
Psychological and sleep quality measures			
Waterloo-Stanford Group Scale of Hypnotic Form C (WSGC)	8.73 (1.00)	8.61 (1.04)	1.00
Self-Reporting Questionnaire (SRQ-20)	3.27 (1.34)	3.15 (2.30)	0.88
Beck Depression Inventory – BDI – II	6.08 (4.68)	4.95 (3.35)	0.34
Pain Catastrophizing Scale - PCS	10.66 (9.93)	10.91 (11.29)	0.93
Central Sensitization Inventory – BP–CSI	23.04(10.34)	23.54(9.74)	0.86
State-Trait Anxiety Inventory – STAI			
State-Anxiety (STAI)	21.54(6.82)	22.37 (5.60)	0.64
Trait-Anxiety (STAI)	18.91 (3.62)	19.75 (4.54)	0.48
Pittsburgh Sleep Quality Index – PSQI	4.75(1.79)	5.33 (2.18)	0.32

State-Trait Anxiety Involutory (STAI). * Compared using t-Test for independent samples.

Table 2. Psychophysical tests (HPT, HPTO, CPT, CPM-task and BDNF) according to intervention group. Dara are presented as mean and standard deviation (SD) and delta [Δ-value of means (post-intervention minus pre-intervention)] (n =24).

	Mean (SD) before intervention	**Mean (SD) after intervention**	**Δ-value**	*P-value £: between group*	*P-value ¥: within group*	***Effect size***
Heat pain threshold (HPT) ºC						
tDCS (n=12)	38.50 (2.12)	39.69 (1.81)	1.10 (1.62)	0.11	0.05	0.51
Hypnotic suggestion (n=12)	38.41 (1.47)	41.11 (3.47)	2.67 (1.62)		0.00	1.81
Heat pain tolerance (HPTO) ºC						
tDCS (n=12)	44.74 (2.44)	44.98 (1.78)	0.23 (1.76)	0.00	0.70	0.09
Hypnotic suggestion (n=12)	44.32 (1.96)	46.07 (2.84)	1.74 (2.22)		0.00	0.89
Score on NPS(0-10) during the (CPT)						
tDCS (n=12)	6.850 (2.26)	6.34 (2.67)	-.50 (1.19)	0.00	0.47	0.22
Hypnotic suggestion (n=12)	7.367 (2.06)	5.26 (2.78)	-2.10 (1.80)		0.00	1.01
Change on NPS(0-10) during the (CPM-test)						
tDCS (n=12)	-0.79 (2.84)	-1.08 (2.70)	-0.29 (1.75)	0.83	0.73	0.10
Hypnotic suggestion (n=12)	-1.83 (1.85)	-1.42 (2.74)	0.42 (1.30)		0.76	0.22
Brain-derived neurotrophic factor (BDNF)						
tDCS (n=12)	39.81 (19.17)	58.96 (35.82)	13.20 (35.72)	0.00	0.25	0.68
Hypnotic suggestion (n=12)	40.18 (22.04)	29.68 (16.63)	-14.49 (34.63)		0.01	0.66

Celsius degree (ºC); Numerical Pain Scale (NPS0-10),

£ comparison between group by Wilcoxon-Mann Whitney.

¥ comparison within group by Wilcoxon Signed Ranks tests.

The effect size within group as assessed by the standardized mean difference (SMD) was computed in

terms of the ratio between the mean change and the baseline standard deviation (SD).

Table 3. Mixed ANCOVA model to assess the treatment effect between groups on Δ-value of the primary outcomes measures [Δ values of the NPS (0-10) during CPT and the change on NPS (0-10) during the CPM-test] (n = 24).

	β	**SEM**	**df**	**t**	**P-value**	**CI 95%**
Dependent variable: Δ-Score on NPS (0-10) during Cold Pressure Test						
Intercept	-2.41	.415	32.06	-5.81	.00	(-3.26 to -1.57)
Order of intervention	.28	.464	43.79	.61	.54	(-.65 to 1.22)
Intervention tDCS	1.92	.480	42.70	3.99	**.00**	(.95 to 2.89)
Hypnotic suggestion	$0^{reference}$					
Δ-BDNF (post intervention minus pre-intervention	-.009	.006	40.84	-1.36	.17	(-.02 to 0.004)
Dependent variable : Δ-Change on NPS (0-10) during CPM-test						
Intercept	1.14	.523	43.086	2.18	.03	(.09 to 2.19)
Order of intervention	-.11	.736	41.06	-.15	.88	(-1.60 to 1.37)
Intervention tDCS	-1.50	.715	40.01	-2.09	**.04**	(-2.94 to -0.05)
Hypnotic suggestion	$0^{reference}$					
Δ-BDNF (post intervention minus pre-intervention)	.04	.0126	24.33	3.20	**.00**	(.014 to 0.07)
Interaction between Δ-BDNF vs. intervention						
Δ-BDNF *tDCS	-.03	.0192	41.92	-1.61	.11	(-.07 to 0.007)
Δ-BDNF * Hypnotic suggestion	$0^{reference}$					

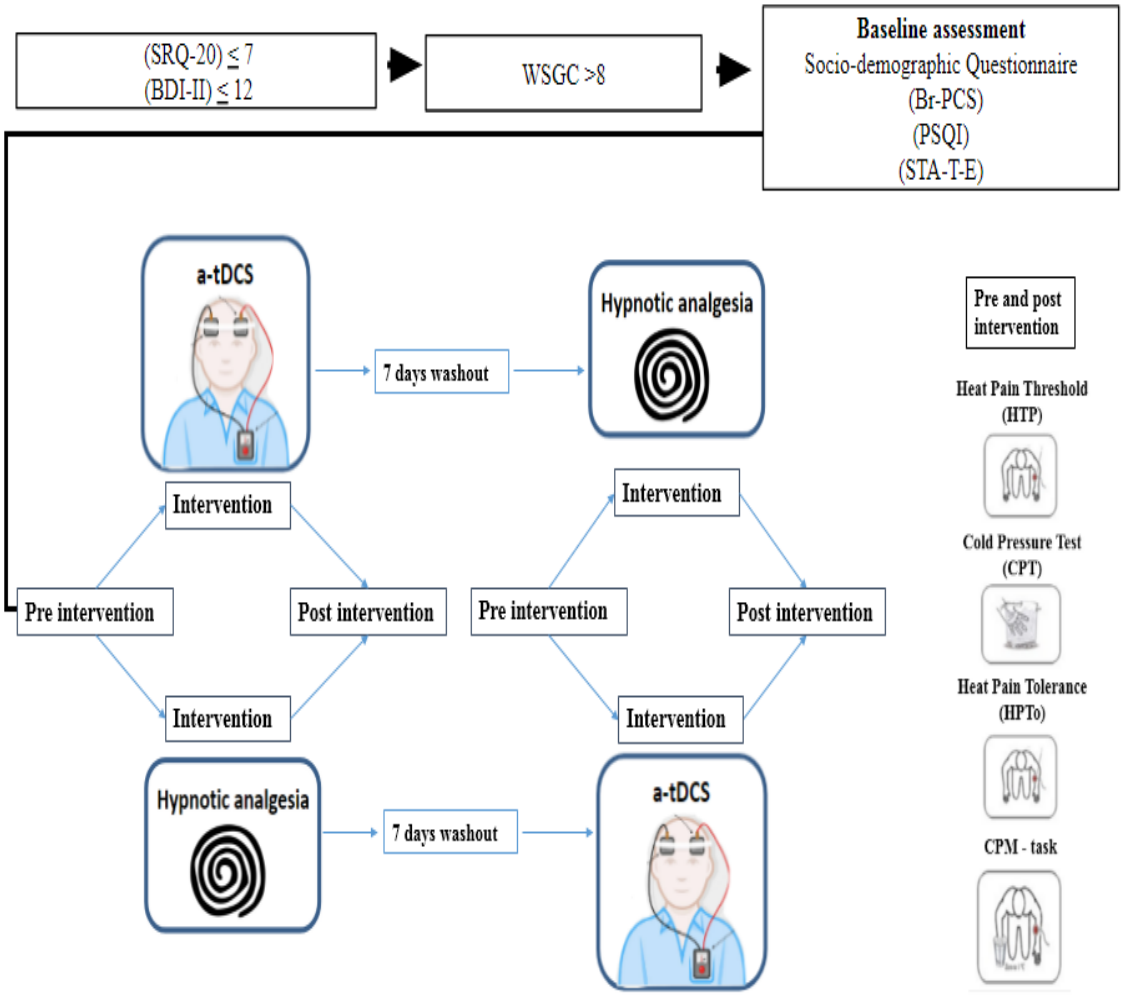
Brain derived neurotrophic factor (BDNF); β (beta value shows the estimated effect of the intervention (factor) and covariates); Standard error for mean (SEM); degrees of freedom (df).

Table 4. Mixed ANCOVA model to assess the treatment effect between groups on Δ-value of the secondary outcomes: HPT and HPTo (n = 24).

	β	**SEM**	**df**	**t**	**P-value**	**CI 95%**
Dependent variable : Δ-Heat pain threshold						
Intercept	2.901	.801	33.19	3.62	**0.00**	(1.27 to 4.53)
Order of intervention	-.30	.870	43.99	-.35	0.72	(-2.06 to 1.48)
Intervention tDCS	-1.60	.906	43.76	-1.77	**0.08**	(-3.45 to 0.21)
Hypnotic suggestion	0[reference]					
Δ-BDNF (post intervention minus pre-intervention	.006	.012	42.63	.20	0.83	(-0.03 to 0.03)
Dependent variable : Δ-Heat Pain Tolerance (HPTo)						
Intercept	1.91	.590	34.48	3.24	**.00**	(0.71 to 3.11)
Order of intervention	-.42	.617	43.37	-.67	.50	(-1.66 to 0.83)
Intervention tDCS	-1.45	.639	43.68	-2.25	**.02**	(-2.73-0.16)
Hypnotic suggestion	0[reference]					
Δ-BDNF (post intervention minus pre-intervention	-.005	.008	43.98	-.53	.59	(-.02 to -.01)

Brain derived neurotrophic factor (BDNF); β (beta value show the estimated effect of the intervention (factor) and covariates); Standard error for mean (SEM); degrees of freedom (df).

Figure 1



Self-Reporting Questionnaire ***(SRQ-20)*** *;Beck Depression Inventory* ***(BDI-II)*** *;Waterloo-Stanford Group Scale of Hypnotic Susceptibility* ***(WSGC);****Brazilian Portuguese Pain Catastrophizing Scale* ***(Br-PCS);****Pittsburg Sleep Quality Index* ***(PSQI)****;State-Trait Anxiety Inventory* ***(STA-T-E)****.*

Figure 2

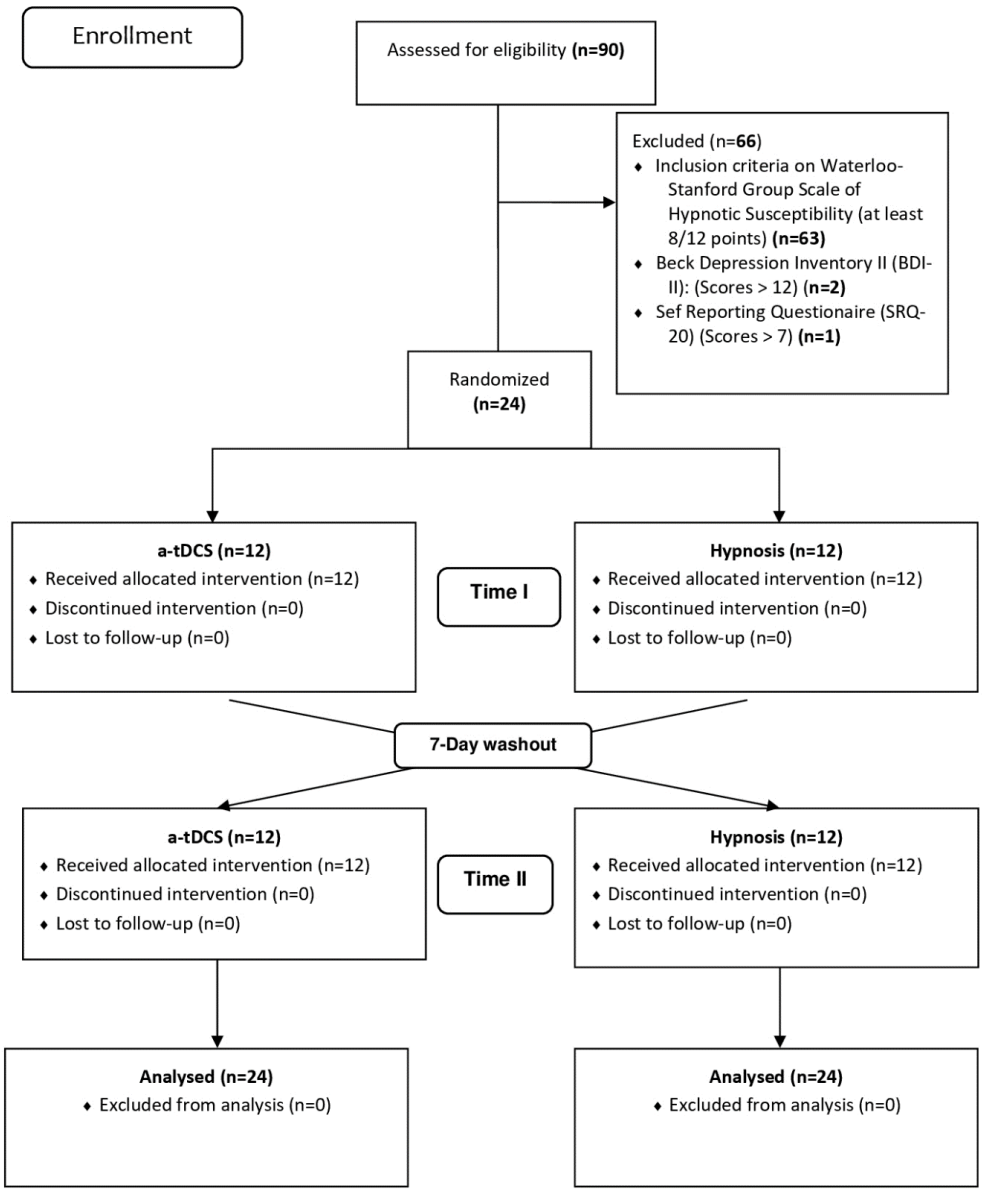

Enrollment
Assessed for eligibility (n=90)
Excluded (n=66)
Inclusion criteria on Waterloo-Stanford Group Scale of Hypnotic Susceptibility (at least 8/12 points) (n=63)
Beck Depression Inventory II (BDI-II): (Scores > 12) (n=2)
Sef Reporting Questionaire (SRQ-20) (Scores > 7) (n=1)
Randomized (n=24)
a-tDCS (n=12)
Received allocated intervention (n=12)
Discontinued intervention (n=0)
Lost to follow-up (n=0)
Time I
Hypnosis (n=12)
Received allocated intervention (n=12)
Discontinued intervention (n=0)
Lost to follow-up (n=0)
7-Day washout
a-tDCS (n=12)
Received allocated intervention (n=12)
Discontinued intervention (n=0)
Lost to follow-up (n=0)
Time II
Hypnosis (n=12)
Received allocated intervention (n=12)
Discontinued intervention (n=0)
Lost to follow-up (n=0)
Analysed (n=24)
Excluded from analysis (n=0)
Analysed (n=24)
Excluded from analysis (n=0)

Figure 3

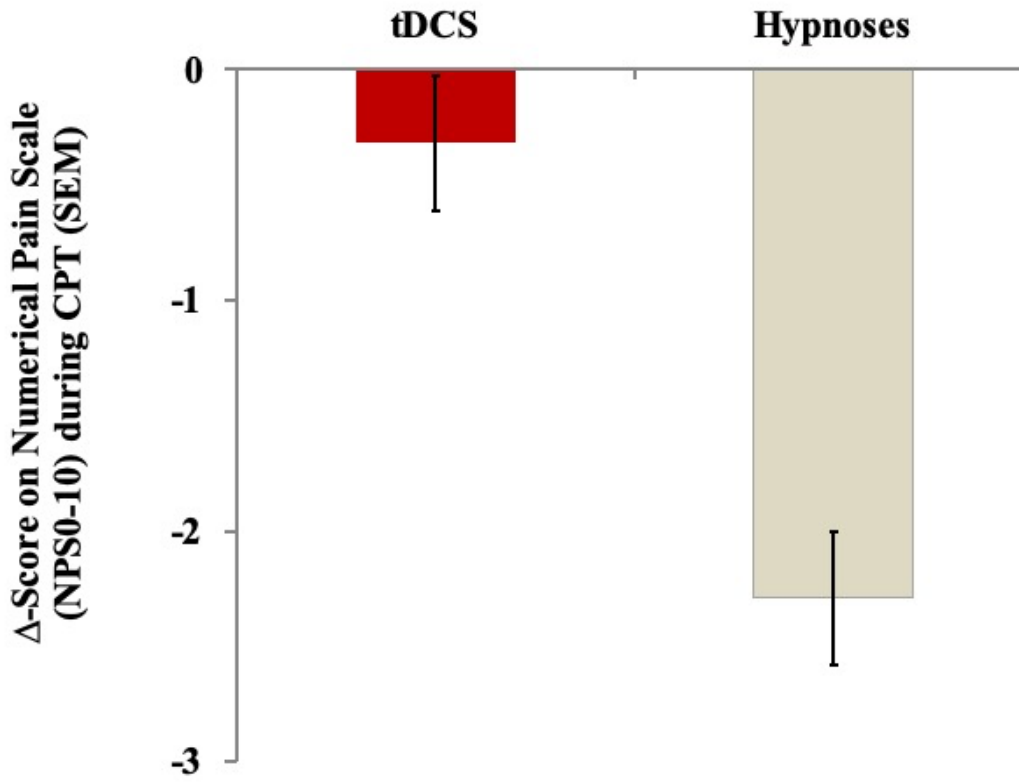

tDCS
Hypnoses
0
-1
-2
-3
Δ-Score on Numerical Pain Scale (NPS0-10) during CPT (SEM)

Figure 4

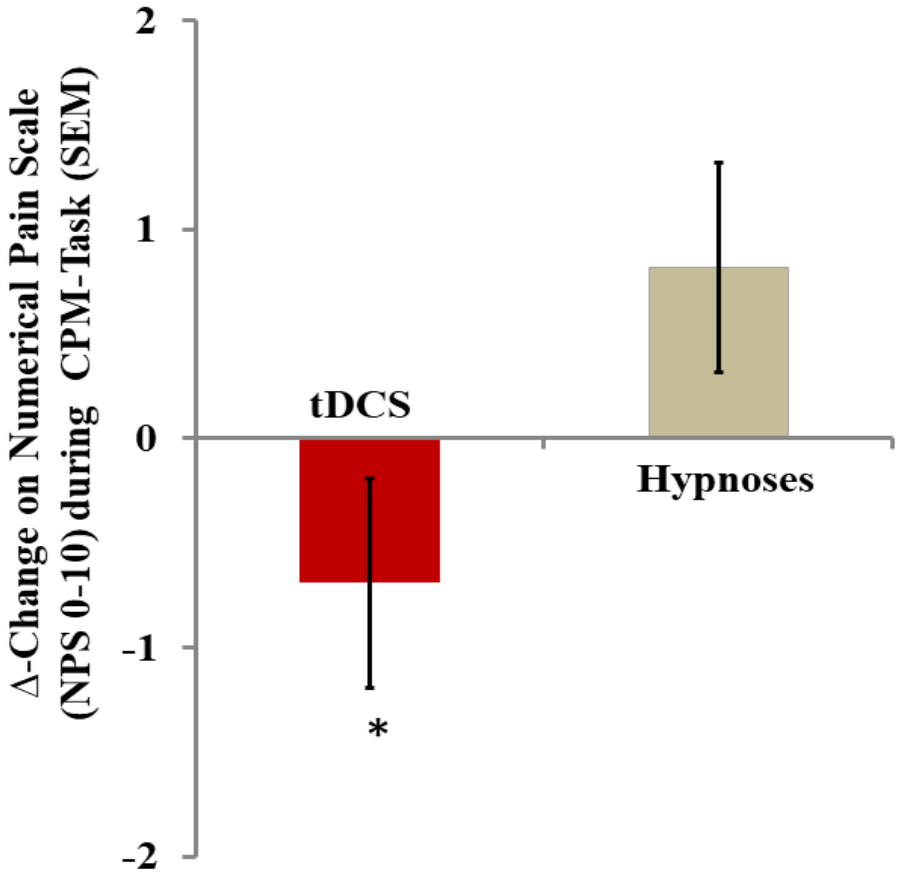

2
1
0
-1
-2
Δ-Change on Numerical Pain Scale (NPS 0-10) during CPM-Task (SEM)
tDCS
Hypnoses
*

Figure 5

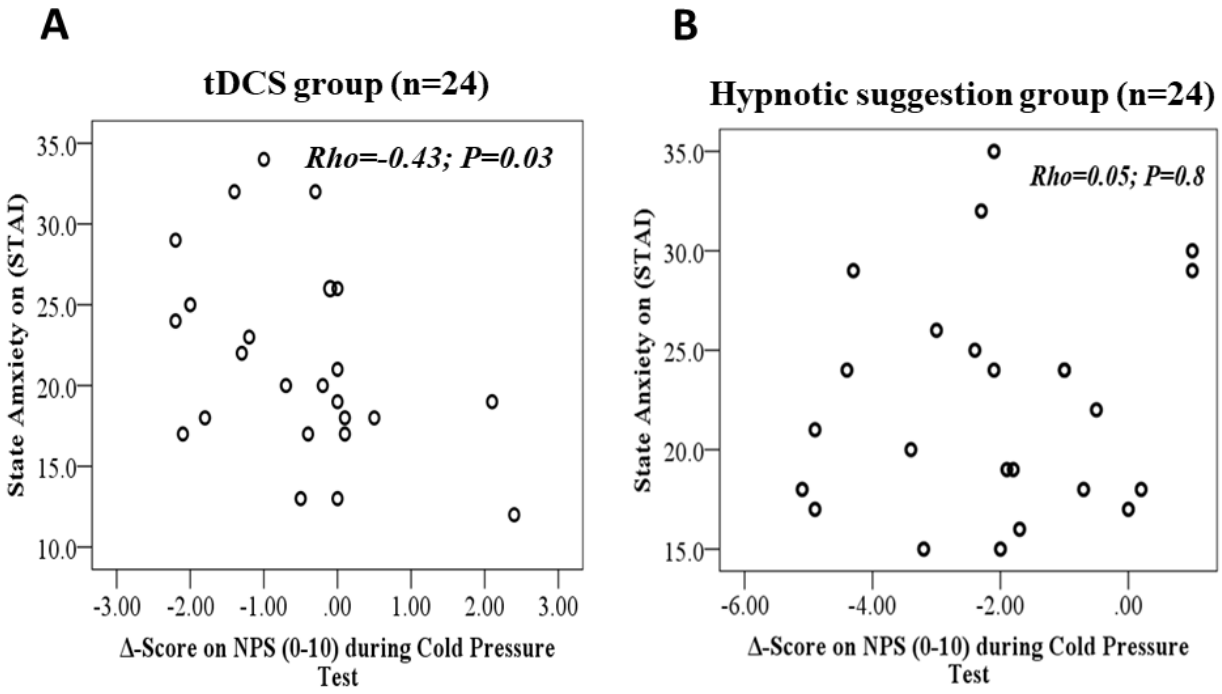
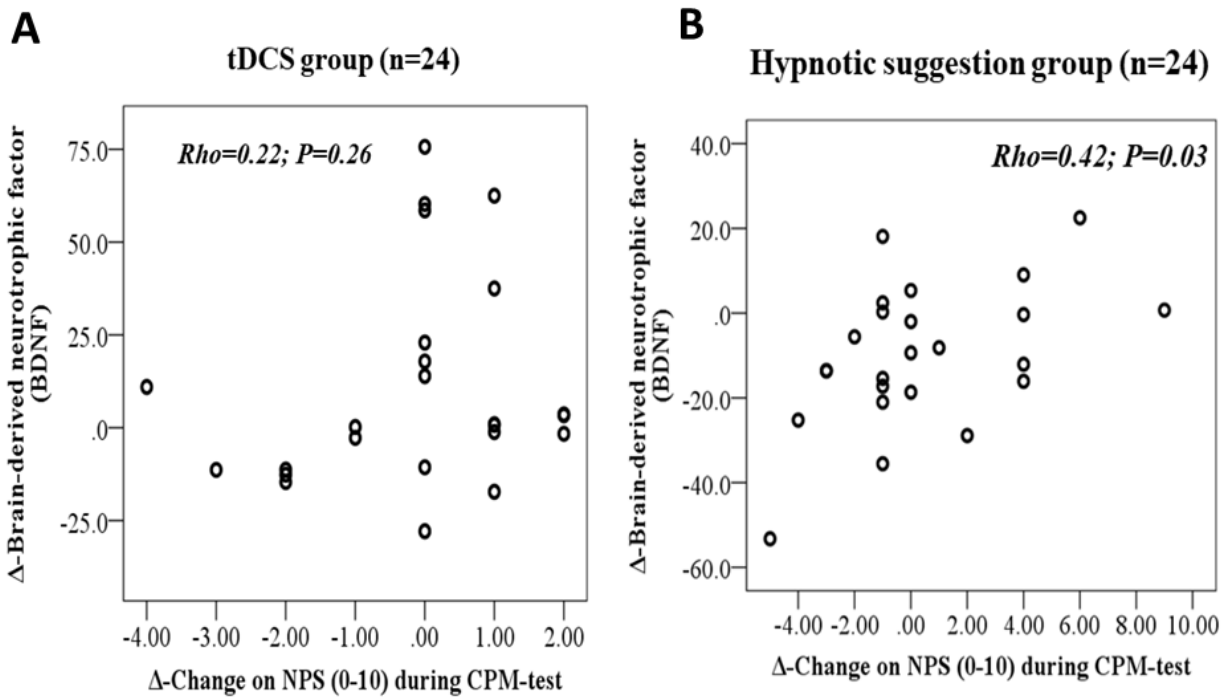

A
tDCS group (n=24)
Rho=-0.43; P=0.03
State Amxiety on (STAI)
35.0
30.0
25.0
20.0
15.0
10.0
-3.00
-2.00
-1.00
.00
1.00
2.00
3.00
Δ-Score on NPS (0-10) during Cold Pressure Test
B
Hypnotic suggestion group (n=24)
Rho=0.05; P=0.8
State Anxiety on (STAI)
35.0
30.0
25.0
20.0
15.0
-6.00
-4.00
-2.00
.00
Δ-Score on NPS (0-10) during Cold Pressure Test

Figure 6


A
tDCS group (n=24)
Rho=0.22; P=0.26
Δ-Brain-derived neurotrophic factor (BDNF)
75.0
50.0
25.0
.0
-25.0
-4.00 -3.00 -2.00 -1.00 .00 1.00 2.00
Δ-Change on NPS (0-10) during CPM-test
B
Hypnotic suggestion group (n=24)
Rho=0.42; P=0.03
Δ-Brain-derived neurotrophic factor (BDNF)
40.0
20.0
.0
-20.0
-40.0
-60.0
-4.00 -2.00 .00 2.00 4.00 6.00 8.00 10.00
Δ-Change on NPS (0-10) during CPM-test

Appendix

Hypnotic Induction

HYPNOTIC INDUCTION

Please make yourself comfortable. Close your eyes and let yourself relax. Take a few slow deep breaths, and notice that as you exhale, you can feel yourself becoming more and more relaxed. You can continue to relax, as I speak to you...and each time you exhale, you can feel yourself becoming more and more relaxed...more and more relaxed. But no matter how relaxed you become, you will hear my voice, and you will be able to respond to my suggestions. If you become at all uncomfortable, you can readjust your body and make yourself comfortable again, and that won't get in the way of your experience of hypnosis. If you need to speak to me, you will be able to do so easily, without disrupting your hypnotic experience.

Right now, you might want to relax even more, and as you relax, you may feel a slight tingly feeling and your fingers...or in your toes...and if you do, it can comfort you, because you will know that is a feeling of relaxation that some people have as they begin to experience hypnosis. Let your body relax. Just begin to feel a spreading sense of calm...and peace...letting go all of your cares and concerns... let them drift away, like clouds in the wind...dissipating...breaking up...just relaxing more and more...feeling more and more at peace, more calm.... more comfortable and secure...nothing to bother you... nothing to disturb...more and more deeply relaxed, as your enter I a pleasant comfortable state of hypnosis...becoming so deeply involved in hypnosis that you can have all the experiences you want to have...deep enough to experience whatever you want to experience...but only the experiences you want...just your own experiences.

And you can focus your attention on your toes...your right toe...and your left toe. Let your right toe relax...relax completely...and your left toe...letting your toes relax...more and more...more and more relaxed. And let the relaxation spread from your toes into your feet, and let your feet relax. Let them

become more and more relaxed...as you can feel so calm and at ease. And now pay attention to your ankles and to your calves... I wonder if you can begin to let go...let go and relax as you feel perhaps a comfortable sense of warmth in your ankles or in your calves...or perhaps it is a cool and easy feeling...in your right leg or in your left leg. Just let your legs relax...more and more relaxed...more and more completely relaxed.

And the relaxation can spread into your thighs...your thighs can relax more and more...just letting go. And you can let your pelvis relax. Just let it go loose and limp... loose and limp...relaxing more and more. Relax your stomach. Let your stomach become completely relaxed. Notice how it feels, can you let it feel completely relaxed...can you notice this now or a bit later? And let the relaxation spread upward into your chest. Let all the nerves and muscles in your chest relax completely...relaxed...loose and limp...feel the peace spreading as you feel so at ease...so secure...your body and mind so relaxed and at peace. And now your back can relax, and your shoulders. Let yourself feel the relaxation in your back and your shoulders...more and more relaxed...loose and limp...completely relaxed.

Let the relaxation spread through your arms, down into your hands and your fingers. Relax more and more...focus on the feelings in your arms and hands. Do your fingers feel heavier than light, or more light than heavier? Focuses in your right upper arm...right lower arm...your right hand... and fingers...relaxing completely, so relaxed...completely relaxed. And now your left arm...relaxing completely, so relaxed...completely relaxed. I wonder if you can go even deeper now. Deeper and deeper...just as you wish...just as comfortable and as deep as you would like to go.

You might like to imagine being somewhere peaceful and relaxing. I like to imagine lying on a quiet beach on a warm sunny day, with a beautiful blue sky and just a few billowy clouds floating by...I can imagine a feeling of a soft gentle breeze...smelling the salt sea air... but you can imagine being anywhere you like. It might be someplace you've been...or someplace you'd like to be. Or just a place in your imagination. It doesn't matter...all that matters is your comfort...your peace. Wherever it is, it is so

peaceful and calm...someplace where you can just be you...where you can feel completely at ease and content. And you can imagine yourself actually being there...seeing, in your mind's eye, the things that you would see if you were actually there now...feeling the things you would feel...hearing the sounds that you would hear...smelling the smells.

And while you are in your perfect place, I am going to count from one to ten. And with each counted you can drift more and more deeply into hypnosis...more and more...able to experience whatever you want to experience. One...drift, drift and deeper...two...more and more centered, and balanced...three...four...deeper and deeper...five ...six...seven...even deeper than before...so deep that you can experience whatever you wish to experience...eight...nine...ten...very deep now...very deep...completely at one with yourself...completely engrossed."

From Kirsch, I., Lynn, S.J., & Rhue, J.W. (1993). Introducion to clinical hypnosis. In J.W.Rhue, S.J. Lynn, & Kirsch (Eds), *Handbook of clinical hypnosis* (pp.12-14).Washington, D. C.: American Psychological Association.

"And from now on, you will no longer feel any more pain ... you will not feel any kind of pain after waking up. Your mind will be able to control the sensations of your whole body ... Your mind controls the sensations of your body ... after you wake up, you will no longer feel any kind of pain ... From now on... and after you wake up you will no longer feel any kind of pain ... I will count from one to ten and you will feel no pain anymore... your mind will control all the sensations of your body and after waking up you will not feel pain."

1: "*...you're feeling even more relaxed and comfortable...*"

2: "*...you will get even more relaxed and feeling good...*"

3: "*...feeling better and better, you will not feel pain after you're awake...*"

4: "*...even more relaxed...after waking up, a heat stimulus will be placed on your forearm...and your hand in the ice...and you will not feel any kind of pain...*"

5: "*...will be relaxed and painless...*"

6: "*...your mind will control all the sensations of your body...will control it and you will no longer feel pain after you wake...*"

7: "*...you relax even more...feeling very well...you relax and have good sensations...*"

8: "*...very deep, you will not be able to feel any kind of pain in the periphery of your body when you are awake...*"

9: "*...you will not feel pain...your brain controls all the sensations of the periphery of your body...you will not feel any kind of pain...*"

10: "*...your brain now controls all your sensations of your body...and you will not feel pain after you wake up...feel even more relaxed and comfortable...when you wake up you will no longer feel any kind of pain.*"

"*Now you are very relaxed and feeling comfortable, but very soon you will wake up. I will count from one to ten and to each number you will wake up even more...I will count from one to ten, and you will wake up, feeling good...and will no longer feel any kind of pain...I will count from one to ten and you will wake up, and you will not be able to feel any kind of pain in your body...one...you are gradually coming back and feeling your own body...two...you are gradually coming back...and you can remember that you will no longer feel any kind of pain...three...you can slowly feel your body and the energy increasing...four...you are waking up and feeling your body on the chair...five...halfway through...after waking up you will no longer feel any kind of pain...six...feeling good and slowly waking up...seven...slowly feeling the environment and the*

movements of your body...eight...you are almost awake...when I get to ten you will wake up feeling good and your brain will not allow you to feel any kind of pain...nine...you are waking up and being aware of your surroundings...waking up more and more...ten...now you wake up feeling good...open your eyes."

Based on the hypnotic analgesia approach from Jensen, M. P. (2011). *Hypnosis for chronic pain management: Therapist guide*. Oxford University Press.

7.2 ARTICULO 2

Combined transcranial direct current stimulation with hypnotic suggestion in acute experimental pain: A Proof of Concept cross-over sham-controlled randomized trial

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Combined transcranial direct current stimulation with hypnotic suggestion in acute experimental pain: A Proof of Concept cross-over sham-controlled randomized trial

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ABSTRACT

We evaluated the transcranial direct-current stimulation (tDCS) effect over the left dorsolateral prefrontal cortex (DLPFC) or hypnotic suggestion (HS) on pain perception and the function of the descending pain modulatory system (DPMS). Their effects were assessed alone or combined [a-tDCS; HS; a-tDCS/HS and sham-tDCS/HS) on the following outcomes: the function on DPMS (primary outcome). Heat pain threshold (HPT), heat pain tolerance (Δ-HPTo) and cold pressure test (Δ-CPT). We also examined whether their effects are related to neuroplasticity state evaluated by serum brain-derived-neurotropic factor (BDNF). Forty-eight females received active (a)-tDCS (2mA, 20min) or HS in a randomized crossover sequence. The a-tDCS/HS intervention increased the inhibitory function of the DPMS markedly compared to HS or s-tDCS/HS. All other interventions increased the HPTo compared to a-tDCS. The a-tDCS/HS intervention increased the CPT substantially compared to all other interventions. Also, higher baseline levels of BDNF were associated with a larger change in CPT and HPTo. These findings indicate that a-tDCS upregulates the inhibition on DPMS, while the HS improves HPTo and the a-tDCS/HS showed substantial effect upon the CPT. They indicate that these inventions modulate the pain processing distinctly and the combined effect did not improve the efficiency of inhibition in the DPMS.

Keywords: tDCS, hypnotic analgesia, Conditioned Pain Modulation, pain perception.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT03744897.

1. Introduction

Although we have witnessed a leap forward in comprehension in pain pathophysiology, a gap persists between pain research and pain management in clinical settings. Chronic pain management is challenging, and drug treatments provide insufficient relief in many patients.[1] The limited efficacy of pharmacological therapy corroborates statistical data that 3% to 4% of adult Americans are receiving long-acting opioid treatment.[2] Although the pharmacological treatment has advanced, the response is heterogeneous among patients with the same diagnosis by several factors, such as the severity of disease and genetic, emotional, hormonal, and other factors related to the dysfunction of descending pain modulating system.[3] Thus, transcranial direct current stimulation (a-tDCS) may represent a promising tool for modulating the membrane potential of neurons in the cerebral cortex and could change the dysfunctional plasticity within pain circuits; it may also affect the nuclei in the thalamus and sub-thalamic regions.[4] Cumulative evidence has demonstrated that a-tDCS montage applied over the primary motor cortex (M1) reduces pain levels,[5] whereas its effect over dorsolateral prefrontal cortex (DLPFC) with anodal at left and cathodal at right DLPFC can significantly improve pain perception.[6] With this montage, in a recent study in patients with fibromyalgia who received the home-based (HB) a-tDCS treatment during twelve weeks for a total of 60 sessions, we observed significant improvement on the cardinal symptoms of fibromyalgia: pain level, psychological symptoms, sleep quality, and disability due to pain.[7] Another early study found that anodal over the left DLPFC may reduce the perceived degree of emotional valence for negative emotional pictures[8] and images of anger expressions.[9] Furthermore, anodal stimulation over DLPFC upregulates reactions to positive emotional stimuli and the identification of

positive emotions.[10]

Aligned with these findings, other studies showed that the a-tDCS can modulate pain systems by altering cortical excitability,[11] and in patients with chronic pain, it enhancing the strength of the pain modulatory descending system (MDS).[12] A recent study found that anodal stimulation over left DLPFC and cathodal over right DLPFC improved the inhibitory function of the descending pain modulating system, while hypnotic suggestion changes pain perception. These results are intriguing and give us new insight into the mechanism involved in hypnotic suggestion analgesia, which likely changes the cortical pain processing, whereas a-tDCS induced either downregulation of the pain-facilitating pathways or upregulation of the inhibitory function of the DPMS.[13]

The influence of hypnotic analgesia to decrease the activity in supraspinal areas identified as components of the pain matrix, including the thalamus, sensory cortices, insula, anterior cingulate cortex (CCA), and the frontal area, has been demonstrated in previous studies.[14] Also, earlier studies found that hypnotic analgesia dissociates sensory and affective components of the pain experience according to the suggestion[15] and modulates the activity/connectivity of the pain matrix.[16] In the same way, hypnotic analgesia reduced the unpleasantness of thermal pain perception and modulates the neural activity in the CCA in accordance with the changes in thermal pain perception as assessed by positron emission tomography (PET).[16] Another study found that hypnotic anesthesia effects in the somatosensory brain processing may be related to top-down somatosensory inhibition.[17]

Based on the hypothesis that the hypnotic suggestion effects on pain perception involve mainly cortical pain processing, whereas a-tDCS likely induces either downregulation of the

pain-facilitating pathways or upregulation of the inhibitory function of the DPMS, we conduct this factorial study to comprehend more about the target of the effects of these two interventions on pain perception and explore if these two interventions can produce additive effects of pain perception. We considered that the complexity of chronic could introduce many confounders to understand the isolated and combined effect of these two interventions. Therefore, we conduct this study in healthy subjects to examine this paradigm in an experimental condition in which we can characterize the relationship between etiological components of pain (e.g., nature, localization, intensity, frequency, and duration of the trigger necessary to evoke pain). We tested the hypothesis that the combined therapy (a-tDCS/hypnotic suggestion) would be more efficacious than sham interventions (s-tDCS/hypnotic suggestion) or single interventions (a-tDCS or hypnotic suggestion). The effect of these interventions was evaluated within and between intervention groups on the following outcomes: descending pain modulating system as measured by the change on numerical pain scales (NPS0-10) during the conditioned pain modulating task (CPM-task) by the delta (Δ)-value from post-intervention to pre-intervention (primary outcome). Also, we evaluated the effectiveness of interventions in the delta-value from post-intervention to pre-intervention in the secondary outcomes: heat pain threshold (HPT), heat pain tolerance (Δ-HPTo), and cold pressure test (Δ-CPT). Furthermore, we examined whether the effect of a-tDCS and hypnotic suggestion on psychophysical measures (CPM-task, HPT, HPTo, and CPT) could be associated with the serum level of the BDNF.

2. Results

2.1 Demographic and characteristics of the subjects

A total of 140 subjects were screened to participate. After applying the Waterloo-Stanford Group C (WSGC) Scale of Hypnotic Susceptibility, using a cutoff point (8/12) for susceptibility to hypnosis, 50 subjects were selected for the hypnosis experiment. Two subjects were excluded because we identified the presence of minor psychiatric disorders. The final sample comprised 48 subjects were randomized to receive four intervention: a-tDCS, HS, a-tDCS/HS and s-tDCS/HS. For each group, 12 participants were randomized and assigned in a crossover manner to participate in the two sequences of treatment. For all outcomes, 48 subjects were analyzed. The socio-demographic characteristics of the subjects according to the sequence allocation were comparable and are shown in Table 1. All subjects completed the protocol to which they had been randomized.

--- Insert table 1---

2.2 Primary Outcome

2.2.1 Intervention Effect on the NPS (0-10) during the CPM test

The mean of interventions group before and after the intervention and the psychophysical measures assessed by the Δ-value of means (post-intervention minus pre-intervention) in the NPS during the CPM-test (primary outcome) are presented in Table 2. Their means were compared within the group by Wilcoxon Signed Ranks tests and the effect-size estimated by standardized mean difference (SMD) computed in terms of the ratio between the mean change by the baseline standard deviation (SD). The Δ-value means of the change on

NPS (0-10) during CPM-test among groups were compared by Kruskal-Wallis followed by Bonferroni correction to check for differences between groups.

---Insert table 2---

hange on NPS (0-10) during CP

2.2.2 Change NPS (0-10) during the CPM test – multivariate analysis

A generalized linear model revealed a significant main effect for interventions group (Wald $\chi2 = 8.786$, Df = 3, P <0.032) when we compared the Δ-values of NPS during the CPM test (mean post-intervention minus mean pre-intervention) among interventions groups. This result is presented in Table 3. It showed that all three groups were statistically different than s-tDCS. The covariates were age and BDNF. The analysis showed that the increase in age is positively correlated with the score on NPS (0-10) during CPM-test. That is, the increase in age is associated with lower efficiency of DPMS. One could realize that higher values in the change of NPS (0-10) during CPM-test indicates lower efficiency of descending pain inhibitory system. The interaction analysis showed that the effect of interventions was independent either age or serum BDNF.

---Insert table 3---

The mean Δ-value (SD) of NPS during the CPM-test in the a-tDCS compared to HS was -0.54 (0.41) vs. -0.01 (0.41), respectively. The a-tDCS compared to HS improved the efficiency of DPMS more than filthy four -fold. The mean Δ-value (SD) of NPS during the

CPM-test in the a-tDCS compared to the a-tDCS/HS was -0.54 (0.41) and -0.25(0.43), respectively. The combined intervention reduced the efficiency of DPMS by 53.70%. Whereas, the mean Δ-value (SD) of NPS during the CPM-test in the HS compared to the s-tDCS/HS was -0.01 (0.41) and 0.19 (0.43), respectively. This combined intervention decreases the efficiency of DPMS almost 20 times. Whereas the group of a-tDCS/HS compared to s-tDCS/HS was -0.25(0.43) and -0.19 (0.43), respectively. The s-tDCS/HS reduced the efficiency of DPMS by 24%. The comparisons of means, according to the intervention group are presented in Figure 3.

---Insert figure 2---

2.3 Secondary outcomes

2.3.1 HPT, HPTo, and CPT – univariate analysis

The mean of interventions group before and after the intervention and the psychophysical measures assessed by the Δ-value (means post-intervention minus pre-intervention) in the HPT, HPTo, and CPT are presented in Table 4. Wilcoxon test was used to compare the mean change within the group, and the effect size estimated by standardized mean difference (SMD) computed in terms of the ratio between the mean change by the baseline standard deviation (SD). The Δ-value means on the HPT, HPTo, and CPT among groups were compared by Kruskal-Wallis followed by Bonferroni correction to check for differences between groups.

----Insert table 4---

2.3.2 Secondary outcomes: HPT, HPTo, and CPT – Multivariate analysis

Generalized linear model analyses of the main effects of the intervention on the Δ-value of the HPT, HPT, and CPT are presented in **Table 5**. GLM revealed a main effect of the interventions on the Δ-HPTo (Wald $\chi2$ =8.936, Df = 3, P <0.030) and the Δ-CPT (Wald $\chi2$ = 10.233, Df = 3, P <0.017). For the Δ-HPT, we did not observe a significant difference between the interventions (Wald $\chi2$ =6.299, Df = 3, P <0.098). The covariates were age and BDNF. The analysis showed that higher levels of BDNF in the baseline is positively correlated with a larger change in the Δ-value of CPT and in the HPTo. Age was not correlated with the change in these psychophysical measures.

---Insert table 5---

The Δ-value (SD) of HPTo in the a-tDCS compared to the a-tDCS/HS was 0.12 (0.41) and 1.45 (0.42) respectively. The combined intervention increased the HPTo by 120.8%. Whereas, the Δ-value (SD) HPTo in the a-tDCS compared to s-tDCS/HS was 0.12 (0.41) and 1.40 (0.42), respectively. The combined intervention increases HPTo almost 16 times. Whereas in the HS compared to the s-tDCS/HS, the mean (SD) was 1.73 (0.41) and 1.40 (0.42), respectively. The s-tDCS/HS reduced the HPTo by 19.07%. The Δ-value mean (SD) of HPTo in the HS compared to the a-tDCS/HS was 1.73 (0.41) and 1.45 (0.42), respectively. The a-tDCS/HS reduced the HPTo by 16.18%.

The Δ-value (SD) of CPT in the a-tDCS group compared to the a-tDCS/HS was 4.63 (4.41) and 24.20 (4.62), respectively. The combined intervention increased the CPT almost six times. Whereas, the Δ-value (SD) CPT in the a-tDCS compared to s-tDCS/HS was 4.63 (4.41)

and 9.55 (4.64), respectively. The combined intervention increased the CPT by 206.23%. The Δ-value mean (SD) CPT in the HS compared to the a-tDCS/HS was 15.48 (4.44) and 24.20 (4.62), respectively. The a-tDCS/HS increased the CPT by 156.33%.

3. Discussion

The study tested hypotheses concerning differential effects of a-tDCS, HS, and the combination of both interventions (a-tDCS/HS or s-tDCS/HS) in DPMS efficiency. They corroborate the assumption that a-tDCS induces downregulation of the pain-facilitation pathways or upregulation of the inhibitory function of the DPMS. While the HS modulated the pain tolerance, a-tDCS improved the efficiency of DPMS more than fourfold compared to s-tDCS/HS, an effect with statistical significance and a large ES. The HS, a-tCDS/HS, and s-tDCS/HS improved pain perception via pain HPTo and CPT. Furthermore, a-tDCS/HS produced a distinct impact on the CPT compared to the other three interventions. Also, baseline BDNF was positively correlated with the changes in HPTo and CPT, while age was associated positively with the variations of Δ-value on NPS (0-10) during CPM-test.

The a-tDCS effect on the function of DPMS aligns with previous studies with acute experimental pain,[13] as well as with the treatment of chronic pain in osteoarthritis.[18] Earlier studies in acute experimental pain have found that HS improves pain perception compared to a-tDCS[13] and also in chronic pain conditions.[19] Here, the combined intervention a-tDCS/HS reduced the efficiency of DPMS by 53.70%. Although this result contrasts with our initial hypothesis that the a-tDCS combined with the HS would have an additive effect to improve the DPMS efficiency, it is incompatible with floor or ceiling effects or with “inverted U-shaped

dose-effect curve," since to justify an inverted U-shaped effect, it should increase up to a maximum and then decrease. Hence, this result suggests that their effects are dissociated and by distinct mechanisms.

The a-tDCS impact on the DPMS indicates that it increased the inhibitory function in the cortical spinal pain pathways. Although its underpinning mechanisms are not fully understood, it is plausible that tDCS activates structures within the brain stem involved in the descending pain inhibitory system. This hypothesis is supported by previous studies that applied the a-tDCS over the primary motor cortex (M1).[20,21] Likewise, a similar effect was found when applying a-tDCS over DLPFC.[13] These findings indicate that the analgesic mechanisms of tDCS over DLPFC may involve the activation of descending circuits. This effect finds support from the anatomical and neurophysiological perspective, since the DLPFC is a critical structure for attention functions[22] and modulates the inhibition of neuronal coupling along the ascending midbrain-thalamic-cingulate pathway through descending fibers from the prefrontal cortex.[23] Also, DLPFC can activate the descending nociceptive inhibitory control system (DNIC) and is involved in pain control, pain expectation, and placebo effect.[24]

Concerning the effect of HS on DPMS, it is conceivable that it shifted participants' attention and may have produced altered brain processing related to pain. Areas such as anterior cingulate and insular cortex have been shown to change their activity during an HS.[25] Furthermore, healthy individuals engaged in high demanding attentional tasks showed differential activation in the anterior cingulate and insula.[26] Thus, the strategy of focus-then-orient attention toward a sub-nociceptive image has been associated with response inhibition through an event-related potential experiment.[27] Also, the hypothesis of a "distraction effect"

is coherent with a previous trial that indicated that emotion and attention influence pain through different modulatory mechanisms at spinal and supraspinal levels.[28] This study found that the emotional valence modulated pain ratings and distraction by viewing neutral pictures reduced pain.[29] Thus, it is plausible that the HS modulates pain perception at the supraspinal level, and it supports the idea that the descending pain inhibitory system may be related to the effects of emotions on pain processing.

In addition, this study supports the finding that HS may modulate the pain reaction to distinct thermal stimuli, such as HPTo and CPT, differently. These results revealed no enhancement for HPTo for a-tDCS/HS compared to the interventions in which techniques were applied alone. However, an additive effect was observed for the a-tDCS/HS in the CPT, which increased more than two-fold compared to s-tDCS/HS (206.23%), 156.33% compared to HS, and almost six-fold to a-tDCS. Earlier investigations found that a-tDCS is better than s-tDCS on acute pain perception in different conditions such as in experimental pain in healthy subjects,[30] postoperative acute pain,[20] and various symptoms related to chronic pain syndromes.[18] A critical factor that may contribute to a difference in the a-tDCS effect on pain perception is the site of stimulation, which in these studies was applied over M1,[31] while in the current study, it was over the left DLPFC.

Concerning the impact of HS on the CPT, the results are consistent with previous studies[32] that showed that those who rate higher on measures of suggestibility during experimental pain paradigms (e.g., cold pressor tasks, painful heat stimuli) tend to demonstrate more significant responses to analgesia suggestions.[33] Cold and heat pain sensations have been linked to different psychological and biological mechanisms.[34] According to meta-analysis,

different thermal stimuli (e.g., heat or cold) both activate the anterior cingulate cortex (ACC) and insula.[35] However, cold noxious stimuli activated right subgenual ACC and the amygdala,[35] while the noxious heat stimulus was likely to activate the left ACC and the right thalamus.[35] Thus, the more substantial effect related to a-tDCS/HS upon the CPT may be linked to a more unpleasant sensation compared to the HPTo.[36]

This hypothesis is supported by the previous study that showed that the a-tDCS applied over the DLPFC reduced amygdala threat reactivity, and this prevents the acquisition of fear conditioning. This amygdala reactivity activates attentional control networks involved in the detection of behaviorally relevant stimuli, such as attentional control[37] and action selection.[38] Also, this effect on the CPT is in line with the noxious cold meta-analysis, which also found that negative affect related to the cold noxious stimuli has more probability of being processed in areas such as the amygdala, insula, and ACC.[39] Hence, the a-tDCS/HS combines two techniques that modulate the activation in the ACC, which is involved in the modulation of pain unpleasantness.[40] Also, the effect of tDCS over DLPFC may modulate the neuronal firing frequencies in the ACC, which is correlated with stimulus intensity.[41]

Additionally, the more substantial effect of a-tDCS/HS may be linked to effects on the inhibitory control, as found in studies with fibromyalgia patients who received a-tDCS over DLPFC coupled with a Go/No-go task to modulate attentional networks.[6] The a-tDCS combined with the cognitive task to induce inhibition increased HPT and HPTo compared to s-tDCS.[6] Another study in healthy subjects showed that a-tDCS over DLPFC combined with a brief cognitive intervention increased HPT and HPTo.[42] Accordingly, it has been suggested that tDCS is state-dependent[43] and can modulate prefrontal circuitry and also induce a priming

effect. Thus, a-tDCS/HS may work together to enhance capacity to tolerate and downregulate the emotional component of the pain experience. Thus, HS can maintain these gains and create a potential synergistic effect. In this study, we did not observe the impact of a-tDCS on HPT. Our result aligns with another study with healthy subjects who received a-tDCS over the left DLPFC, which did not produce an effect with significant impact on the HPT.[44]

The study has some methodological limitations that should be addressed. First, we included females in our sample since sex differences in response to pain have been found.[45] Gender may be a confounding factor, since females are more prone to activating brain areas involved in pain processing upon negative emotional responses such as stress, fear, and anxiety, including the CPM-test response. We are conscious that the exclusion of men reduces external validity. However, we aimed to comprehend neurophysiological mechanisms. Thus, a homogeneous sample can minimize the risk of bias. Second, the absence of a group of subjects with a low hypnotizable propensity limits the scope of generalization of our results. Third, the effects of hypnosis after dehypnotization have been observed in earlier studies.[46] However, it is difficult to quantify a potential residual effect and, if it exists, to identify how much of it has a real impact on the outcomes. From a pragmatic view, we would need counterbalancing with the effect of inter-individual variability if we had compared different individuals on physiological measures, as in the case of psychophysical tests. This is mainly because in this case, the starting point of these measures are individualized, and the reactivity to the same stimulus can vary from one individual to another. Taking this into account, we conducted a crossover study to consider this effect, if it exists, as an inherent characteristic of this intervention type. Even though we cannot exclude some carryover effect, we believe that it is

unlikely to change the conclusions because the aim is to explore the combined intervention (e.g., tDCS/HSA) and in both arms of combined interventions, the device was used, which can offer active or sham stimulation, according to randomization. Regarding the t-DCS, previous studies have shown that one session induces some aftereffect that can persist for one hour if we applied for only a course. Thereby, we assumed that the seven-day washout period is sufficient to prevent cumulative effects.[47] Fourth, the design of this study helps control inter-individual differences related to the impact of stimulation since computational models have suggested that head anatomy may alter the dispersion of the electric field. Hence, a uniform dose of tDCS for all individuals may produce results with a variable level of efficacy.[48] In short, we used this study design so each one could be in control.[48] To support this assumption, according to previous studies, we found that the tDCS effect is likely related to the BDNF levels, which suggests that is a neuroplasticity state dependent response.[5] Fifth, we did not observe a carryover effect, which means that the results for each phase of the experiment do not reflect the impacts of any residual effects of therapy provided during previous periods of the trial.[49] Sixth, although we did not formally measure the potential impact of awareness of the allocation group on the outcomes, a sham intervention that is meaningful for hypnosis is not feasible. Despite these limitations, our findings were evaluated using psychophysical parameters, which are less prone to assessment bias than self-reported measures. Finally, heat and cold pain have specific physiological mechanisms and can be interpreted in a very particular way,[34] which may limit deducing the same effects to other somatosensorial modalities, such as mechanical pain.

In summary, these results indicate that a single-session intervention of a-tDCS over left DLPFC in healthy subjects upregulates the inhibition on DPMS, while HS improves

HPTo and a-tDCS/HS showed substantial effect upon the CPT. They indicate that these inventions modulate pain processing distinctly and the combined effect did not improve the efficiency of inhibition in the DPMS.

4. Material and method

4.1 Design overview, setting, and participants

We conducted a randomized blinded crossover sham-controlled clinical study. The sections were reported according to CONSORT guidelines (von Elm 2008), and the study is registered in ClinicalTrials.gov under the number NCT03744897. This study was previously approved by the Research Ethics Committee (CEP) at the Hospital de Clínicas de Porto Alegre (HCPA) (Plataforma Brasil CAAE: 63863816000005327 and CEP nº: 16-0635) according to international ethical standards based on the Declaration of Helsinki. All participants were given written informed consent. To assess clinical characteristics, we used a standardized questionnaire and scales validated to the Brazilian population to evaluate psychological characteristics. Additionally, we collected behavioral measurements associated with pain assessments. Figure 1 represents the timeline of this study.

---Insert figure 1a----

---Insert figure 1b---

4.2 Subjects

The volunteers were healthy women ranging between 18 to 45 years old with more than 11 years of studies. They were recruited from the general population by advertisement postings in the universities, on the internet, and personal divulgation and invitation in public places in the Porto Alegre area.

We selected 48 subjects who completed the Waterloo-Stanford Group Scale of Hypnotic Susceptibility, Form C.[50] The subjects with a score greater than or equal to 8/12 on the scale were included in the later phases of the investigation. The participants also answered a structured demographic questionnaire assessing the following variables: current acute or chronic pain conditions, use of analgesics in the past week, rheumatologic disease, clinically significant or unstable medical or psychiatric disorder, history of alcohol or substance abuse in the past 6 months, neuropsychiatric comorbidity, and use of psychotropic drugs. They were excluded if presenting any of these variables or if hearing impairment or formal contraindication to transcranial direct-current stimulation (a-tDCS), according to current guidelines. Subjects with scores higher than 12 on Beck Depression Inventory[51] were also excluded, as were those with positive screening (>7) for minor psychiatric disorders (somatic symptoms, depressive moods, depressive thoughts, and decreased energy) on the World Health Organization (OMS) Self-Reporting Questionnaire (SRQ-20).

4.3 Experimental protocol

This was a participant blind randomized crossover trial. On admission, participants were randomized into four groups: (1) a-tDCS, (2) hypnotic suggestion (HS), (3) a-tDCS/HS and (4) s-tDCS/HS. For the a-tDCS condition, the anode electrode was positioned over the left dorsolateral prefrontal cortex (DLPFC) and the cathode electrode to the right DLPFC. A constant electric current of 2mA was applied for 20 min, with initial and ending ramps of 30s stimulation. In the s-tDCS, current was initially ramped to 2 mA over 30s and then immediately ramped down to 0 mA. The hypnosis session consisted of a 20 min long standard induction, beginning with a set of suggestions to participants to focus their attention on a single stimulus and associated with breathing and guiding a progressive relaxation. After that, suggestions of well-being related thoughts and sensations were given, in which the patient had to imagine being in a peaceful place. Through the 8 final minutes of the induction, suggestions were used to reduce the pain of the participants and increase control over their own sensations. After the first intervention, participants returned for a second experimental session to receive an alternative intervention. Group 1 (a-tDCS) went on to receive the group 2 (hypnotic suggestion) intervention and vice versa. The same was applied between groups 3 (a-tDCS/HS) and 4 (s-tDCS/HS). The order of the experimental sessions was counterbalanced and separated by at least 7 days (mean 25.0 (SD 14.0) days) to avoid carryover effects of the initial stimulation protocol.

4.4 Interventions

4.4.1 Transcranial direct current stimulation

tDCS was applied using Starstim 8 equipment (Neuroelectrics, Barcelona, Spain). Cathodal and anodal electrodes featured an area of 25 cm² each placed inside a round sponge soaked in saline solution. Electrodes were placed at spatial positions F3-(Anodal) and F4-(Cathodal) according to the international 10-20-system for electroencephalogram electrode placement,[52] commonly considered surface locations above (mid-) left and right DLPFC, respectively.[53] Stimulation was delivered at an intensity of 2 mA for 20 min, including a 30s ramp up to 2 mA at the start and a 30s ramp down to 0 mA at the end. In the sham group, current was initially ramped to 2 mA over 30 s and then immediately ramped down to 0 mA. In the final 30 s, the current ramped up to 2mA and back to 0 mA. During stimulation, participants were asked to relax their bodies while sitting and supported in a comfortable position.

4.4.2 Hypnotic analgesia suggestion protocol based on the Classical Approach

The techniques of hypnosis developed for this study are founded on the classical approach developed by the American clinician and Ph.D. Mark P Jensen. The hypnotic induction protocol was standardized to be equally applied to all subjects. The standard hypnotic protocol begins with an induction to the subjects to focus their attention on a single stimulus and associate this with breathing and relaxation. After that, suggestions are given for comfort and pain management. [54] The duration of experimental manipulation (induction + suggestions) is 20 min.

4.4.3 a-tDCS/Hypnotic Suggestion and s-tDCS/Hypnotic suggestion

In group 3 (a-tDCS/HS), both techniques were performed concomitantly. When the a-tDCS stimulator was turned on, the therapist began the hypnosis induction as previously described. This session was conducted throughout the 20 min of the tDCS, ending with the final stimulation ramp, with the amperage reduced to zero. Group 4 (s-tDCS/HS) also had a protocol of 20 min duration. The hypnosis induction started with the initial 30s current ramp up to 2 mA, and then the current was immediately ramped down to 0 mA. In the final 30 s, the current was ramped up to 2mA and back to 0 mA.

4.5 Instruments and assessments

The tools used to evaluate the psychological state and hypnotic suggestion were validated in the Brazilian population and conducted by two psychologists, trained to perform the psychological tests. We used the refined version of the State-Trait Anxiety Inventory (STAI). [55] The scores in the state- and trait- score ranges from 13 to 52, and 12 to 36, respectively. A standardized questionnaire was applied to assess demographic data and medical comorbidities.

4.6 Outcomes

The primary outcome was the change on numerical pain scales by the delta (Δ)-value (from post-intervention to pre-intervention) in the NPS (0-10) during the conditioned pain modulation test (CPM-test). Secondary outcomes assessed the pain perception by delta (Δ)-value (from post-intervention to pre-intervention) in the HPT, HPTo and Cold Pressor Test

(CPT).

4.6.1 Outcomes assessment

In this study, we evaluated pain as a response to a nociceptive stimulus using the Quantitative Sensory Testing (QST), including the Conditioned Pain Modulation task (CPM-task) the Cold Pressor Test (CPT).

a) In order to perform the QST, we have used a computerized version of the thermostat (Heat Pain Stimulator 1.1.10, Brazil). [56]The HPT was used to determine the heat thermal thresholds (HTT), heat pain threshold (HPTh) and heat pain tolerance (HPTo) during the CPM task. The participants remained seated, and a thermode (30x30 mm) was positioned on the forearm of the dominant side of the body. The temperature started at 30°C, and the thermode was heated at a rate of 1.0°C/sec to a maximum of 51°C when the temperature began to drop. For the HTTh, the participants were asked to press a button when they "felt the first heat sensation" and were asked to press a button when they "felt the first heat pain". The heat thermal threshold and heat pain threshold was determined by the average of three evaluations with a 40s interval between them. Subsequently, "when the pain had reached its maximum" for the HPTo (this was done only once because of the possible sensitization effect).

b) To measure the CPM-task we evaluated the pain intensity in two tonics HPT test stimuli separated by a CPM-task. We used the HPT as conditioning pain stimulus to elicit a prolonged pain sensation to trigger CPM. The CPM-task consisted of immersion of non-dominant hand in cold water at a temperature of 0oC to 1oC for one minute. A thermostat was used to control the temperature variation and to maintain the water temperature from 0oC to 1oC. The QST

procedure was introduced after 30 seconds of cold-water immersion. To determine the CPM we have used the difference between the pain score on NPS (0-10) QST during cold water immersion (QST+CPM) and the temperature of the point at which subjects felt 6/10 pain on the NPS scale (during the initial time period).This procedure is a standardized method to assess the DPMS. [57]

c) Cold pressor pain test (CPT) was induced by the submergence of the hand in cold water. During the test, the participant was asked to immerse the dominant hand in ice-saturated water for a maximum of 2 min (41–44). The temperature of the ice water was measured and across all tests, it ranged from 0-1 °C. If pain was intolerable before 120 seconds, the subject could pull out the hand, in this case pain intensity was maximal until the end of the 120 seconds. [58] The CPT is suggested to be a method that mimics the effects of chronic conditions effectively because of its unpleasantness, and it has excellent reliability and validity.[58]

d) BDNF. The laboratory outcome measured was the serum level of BDNF. We collected the blood samples before starting the assessment and after finishing the intervention. We centrifugate the blood samples for 10 min, at 4500 rpm in 4°C and stored in -80°C for the hormone assay. The serum BDNF was determined using Enzyme-Linked Immunosorbent Assay (ELISA), using monoclonal specific antibodies for BDNF (R&D Systems, Minneapolis, United States #DY248, BDNF lowest detection limit =11.7pg/mL).

4.7 Sample size

The sample size was estimated by the G*Power software, based on previous study with similar methodology (**Beltran 2019**). The calculus indicated that would be necessary a sample

size of 12 individuals to detect a 3-point difference in the numerical scale of pain [average SD 0.59 (NPS) in pain levels to nociceptive stimuli, with a power of 0.95 and an α of 0.05. To ensure the power of the study, 15% was added in case of possible losses, totaling 48 subjects (12 per group). For an error type I of 5% and error type.

4.8 Randomization

The randomization to allocate each participant was generated by a computer program (Randomlogue) in a ratio of 1:1:1:1. They were allocated to receive the following interventions in incomplete crossover manner: a-tDCS/HS, s-tDCS/HS, HS or a-tDCS. Those received a-tDCS/HS in the first trial received s-tDCS/HS in the second trial or vice-versa, whereas who received the hypnotic suggestion in the first trial received a-tDCS in the second trial or vice-versa. Random codes were placed in brown envelopes sealed with the subject's sequence number # 48 on the outside of the envelope. The allocation concealment was reached on account of no investigator involved in the assessments was aware of treatment allocations.

4.9 Blinding

To control possible biases, the following strategies were established: Participants were instructed on all aspects related to the interventions during the evaluations. Two independent evaluators who were not aware of the treatment received were trained to do the assessments. The brown envelopes were prepared before starting the study, sealed, initialed and numbered sequentially. Inside the envelope were the interventions allocation, and it was open only after the participant had given her informed consent to participate in the study. The subject`s name

and number were immediately sent to those responsible for controlling the randomization process. The blinding was gauged at the end of each evaluation.

4.10 Statistical analysis

Descriptive statistics were used to summarize the main socio-demographic features of the sample. To test for normality, we used the Shapiro-Wilk test. After verifying the corresponding assumptions, to compare changes in mans within group was used the Mann-Whitney U test. To compare the mean of Δ-value between groups in univariate analysis was used the Kruskal-Wallis followed by Bonferroni correction to check for differences between groups. A generalized lineal model (GLM) was used to analyze the main effect of the interventions group (a-tDCS, HS, a-tDCS/HS and s-tDCS/HS) in the mean delta(Δ)-values (average at interventions end minus baseline means) of the following outcomes measures: score on the HPT, HPTo, CPT and the change on the NPS (0-10) during the CPM-test. In the GLM the factors was the interventions group and the dependent variables the outcomes. It recognized that psychophysiological measures show individual reactivity to a stimulus of the same intensity. For example, one individual may be highly reactive into painful stimuli, whereas another shows limited changes receiving the same stimulus. Especially with clinical studies, it may be necessary to not only look at the difference but to acknowledge the starting situations (i.e., adjust for the baseline value). Thus, to control for the inter-individual variability changes in these psychophysical, we compared the effect of treatment on the Δ-values from the baseline to their levels at treatment end. [59] The covariate included in all models was the serum baseline BDNF since the BDNF has been related to the effect of a-tDCS in previous studies [13] and age

has been associated with decreases in BDNF signaling, which may cause changes for excitatory and inhibitory synaptic in prefrontal cortex[60] Within groups, the standardized mean difference (SMD) was computed in terms of the ratio between the mean change and the pool of baseline standard deviation (SD). The SMD was interpreted as follows: small, 0.20 to 0.40; moderate, 0.50 to 0.70; and large, 0.80 or higher. To perform the analyses, we used the software SPSS version 22.0 (SPSS, Chicago, IL, United States).

5. Author Contributions Statement

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6. Data Avaliability

The data that support the findings of this study are available from the corresponding autor, WC, upon reasonable request.

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Figure 1 a.

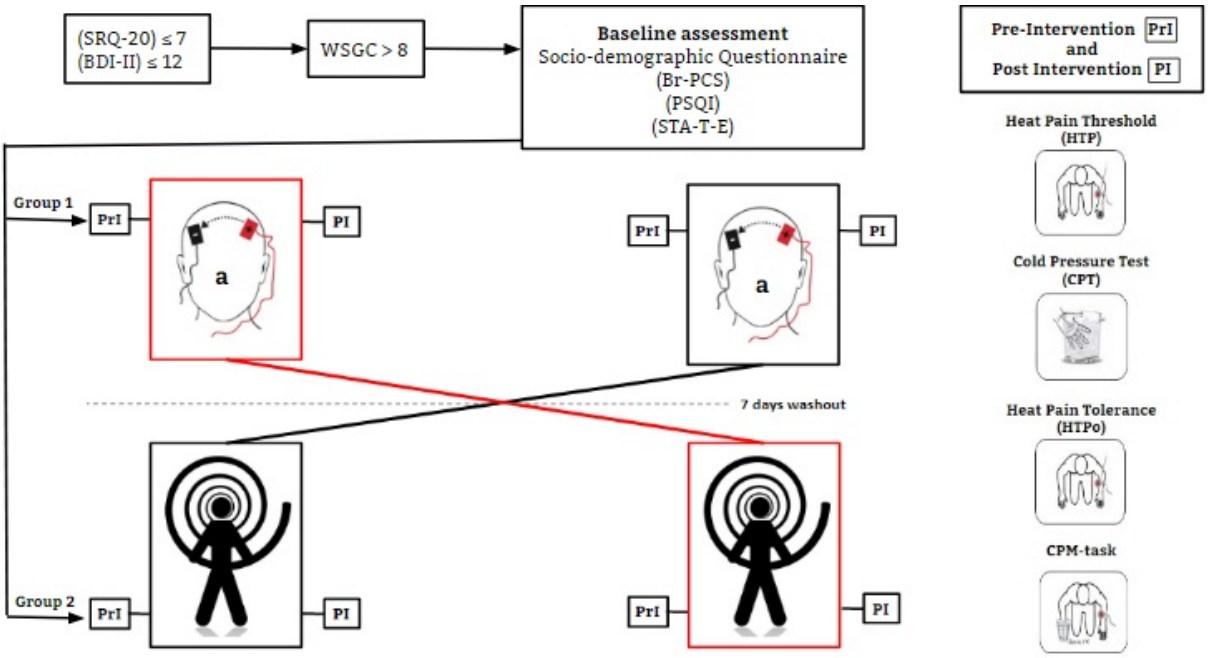

Figure 1 b.

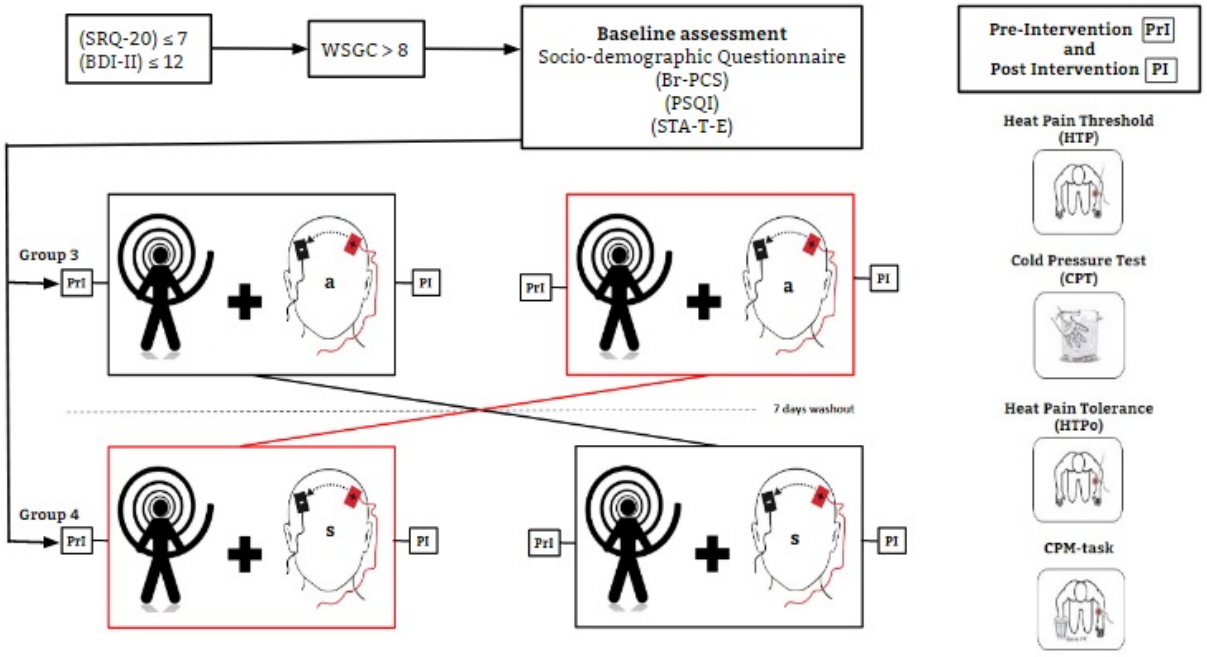

Figure 2. The change in NPS (0–10) during CPM-test, assessed by the Δ-value (score post-intervention minus pre-intervention) in the four experimental groups. The error bars indicate standard error of the mean. The asterisk indicates all interventions were significantly different ($P < 0.05$). All comparisons were performed by a GLM, followed by the Bonferroni test for post hoc multiple comparisons. Numerical Pain Scale (NPS 0–10).

Table 1 – Demographic and clinical characteristic of the sample. Data are presented as mean and standard deviation (SD) according to group in trial I (n=48).

	a-tDCS	**Hypnotic suggestion**	**a-tDCS/ Hypnotic suggestion**	**a-tDCS/ Hypnotic suggestion**	***P-value*** *
Demographic					
Age(years)	26.21 (7.71)	26.21 (7.78)	24.55 (6.75)	24.55 (6.75)	0.756
Education Level (years)	15.67 (4.15)	15.25 (3.138)	15.27 (3.298)	15.27 (3.29)	0.972
Psychological and sleep quality measures					
Waterloo-Stanford Group Scale of Hypnotic Form C (WSGC)	8.58 (0.776)	8.58 (0.776)	8.95 (1.090)	8.95(1.09)	0.316
Self-Reporting Questionnaire (SRQ-20)	2.83 (2.565)	2.71 (3.407)	2.73 (3.326)	2.91 (2.617)	0.995
Beck Depression Inventory – BDI – II	8.00 (7.913)	6.04 (8.137)	7.41 (9.555)	5.36 (6.94)	0.680
Pain Catastrophizing Scale - PCS	23.50(13.46)	23.29(11.67)	27.68(14.31)	25.27 (14.12)	0.667
Central Sensitization Inventory - BP–CSI	10.517(2.14)	13.917(2.841)	11.835(2.52)	11.185 (2.38)	0.893

State-Anxiety (STAI)	7.354 (1.501	6.307 (1.287	4.943 (1.054)	6.597 (1.40)	0.654
Trait-Anxiety (STAI)	4.194 (7.35)	5.162 (6.307)	4.433 (4.943)	4.380 (6.59)	0.913
Pittsburgh Sleep Quality Index – PSQI	2.085 (0.42)	1.911 (0.390)	3.161 (0.674)	2.319 (0.49)	0.804

Table 2. The primary outcome as measured by the change on NPS (0-10) during CPM-test according to the intervention group. Data are presented as mean and standard deviation (SD) and delta [Δ-value of means (post-intervention minus pre-intervention)] (n=48).

	Mean (SD) pre-intervention	Mean (SD) post-intervention	Δ-value	P-value £	SDM £	P-value ¥	SDM ¥
Primary outcomes							
Change on NPS (0-10) during CPM-test							
a-tDCS (1)	-0.97 (2.51)	-1.33 (2.69)	-0.54 (0.41) [2.3.4]	0.030	1.70	0.994	0.14
Hypnotic suggestion (2)	-1.91 (1.72)	-2.04 (2.80)	-0.01 (0.41) [1,3,4]		0.46		0.07
a-tDCS/Hypnotic suggestion (3)	-1.58 (1.85)	-1.59 (2.55)	-0.25(0.43) [1,2.4]		0.44		0.01
s-tDCS/Hypnotic suggestion (4)	-1.89 (2.21)	-1.86 (2.37)	0.19 (0.43)		0[a]		0.01

Numerical Pain Scale (NPS0-10). The differences are indicated via superscript numbers, which correspond to the respective groups labeled 1 to 4.
Symbol (£) indicates comparisons between groups. We compared the change in the means between groups using the Δ-value by Kruskal-Wallis followed by Bonferroni correction to check for differences

between groups. The effect size between groups was assessed by the standardized mean difference (SMD) was computed in terms of the ratio between each one three groups compared to the s-tDCS/Hypnotic suggestion group by the standard deviation (SD) of the Δ-value
Symbol (¥) indicates comparisons within groups. Mean of groups compared within by Wilcoxon Signed Ranks tests. The effect size within the group was assessed by the standardized mean difference (SMD) computed in terms of the ratio between the mean change and the baseline standard deviation (SD).

Table 3. GLM to assess the treatment effect among groups on Δ-value of the change on NPS (0-10) during the CPM-test] (n=48).

Dependent Variable	B	SEM	CI 95%	Wald χ2	Df	P-value
Primary outcome						
Δ-Change on NPS (0-10) during CPM-test						
Intervention						
a-tDCS	-4.77	2.20	(-9.10 to – 0.44)	4.66	1	0.037
Hypnotic suggestion	-4.65	2.21	(-8.98 to -0.32)	4.43	1	0.023
a-tDCS/Hypnotic suggestion	-5.58	2.36	(-10.22 to -0.93)	5.54	1	0.015
s-tDCS/Hypnotic suggestion	0[a(referece)]					
Intercept	1.07	1.72	(-2.30 to 4.45)	0.38	1	0.004
Age	0.06	0.06	(-0.19 to 0.70)	0.84	1	0.011
BDNF baseline	0.01	0.00	(-0.00 to 0.03)	1.49	1	0.219
Intervention				8.78	3	0.032
Intervention * Age				7.31	3	0.062

Table 4. Secondary outcomes. Psychophysical tests (HPT, HPTo, CPT) according to the intervention group. Data are presented as mean and standard deviation (SD) and delta [Δ-value of means (post-intervention minus pre-intervention)] (n=48).

	Mean (SD) pre-intervention	**Mean (SD) post-intervention**	**Δ-value**	***P-value £***	***SD M £***	***P-value ¥***	***SDM ¥***
Heat pain threshold (HPT) ºC							
a-tDCS (1)	34.30 (0.66)	35.34(0.96)	1.28 (0.44)	0.175	0.65	0.034	-0.03
Hypnotic suggestion (2)	34.18 (0.67)	36.79 (0.67)	2.78 (0.45)		2.53		2.10
a-tDCS/Hypnotic suggestion (3)	33.93 (0.66)	35.63(0.73)	1.69 (0.46)		0.21		1.66
s-tDCS/Hypnotic suggestion (4)	34.12 (0.77)	36.37(0.69)	1.59 (0.47)		0[a]		0.14
Heat pain tolerance (HPTo) ºC							
a-tDCS (1)	45.16 (2.54)	45.16 (2.22)	0.12 (0.41) [2,3,4]	0.003	3.04	0.011	0.0
Hypnotic suggestion (2)	44.71 (2.23)	46.10 (2.66)	1.73 (0.41) [1]		0.78		0.62
a-tDCS/Hypnotic suggestion (3)	44.44 (2.49)	46.00 (2.53)	1.45 (0.42) [1]		0.11		0.62
s-tDCS/Hypnotic suggestion (4)	45.59 (2.28)	47.08 (2.61)	1.40 (0.42) [1]		0[a]		0.65

Cold Pressor Test (CPT) in seconds							
a-tDCS (1)	60.38 (39.74)	64.92 (39.83)	4.63 (4.41) [3]	0.007	1.10	0.002	0.11
Hypnotic suggestion (2)	56.54 (38,93)	69.04 (39.45)	15.48 (4.44) [3]		1.27		0.39
a-tDCS/hypnotic suggestion (3)	53.91 (33.73)	81.50 (35.85)	24.20 (4.62) [1,2,4]		3.15		0.81
s-tDCS/ hypnotic suggestion (4)	72.50 (2.12)	86.32 (2.61)	9.55 (4.64) [3]		0[a]		6.51

Symbol (£) indicates comparisons between groups. We compared the change in the means between groups using the Δ-value by Kruskal-Wallis followed by Bonferroni correction to check for differences between groups.

The differences are indicated via superscript numbers, which correspond to the respective groups labeled 1 to 4.

Symbol (£) Indicates the effect size between groups assessed by the standardized mean difference (SMD) computed in terms of the ratio between each one three groups compared to the s-tDCS/HS group by the standard deviation (SD) of the Δ-value.

Symbol (¥) indicates comparisons within groups. Mean of groups compared within by Wilcoxon Signed Ranks tests. The effect size within the group was assessed by the standardized mean difference (SMD) computed in terms of the ratio between the mean change and the baseline standard deviation (SD).

Table 5. GLM to assess the treatment effect among groups on [Δ-value of means (post-intervention minus pre-intervention)] on HPT, HPTo, and CPT (n=48).

	B	Std. Error	CI 95%	Wald $\chi 2$	Df	P-value
Δ-value-Heat pain threshold (HPT) ºC						
(Intercept)	2.234	1.0553	(0.16 to 4.30)	4.481	1	.034
Age (ys)	-.041	.0322	(-0.10 to 0.02)	1.611	1	.204
Brain-derived neurotrophic factor (ng/mL)	.009	.0103	(-0.01 to 0.03)	.771	1	.380
a-tDCS (1)	-.313	.6452	(-1.58 to 0.95)	.235	1	.628
Hypnotic suggestion (2)	1.187	.6533	(-0.09 to 2.46)	3.300	1	.069
a-tDCS/Hypnotic suggestion (3)	.101	.6579	(-1.18 to 1.39)	.023	1	.878
s-tDCS/ Hypnotic suggestion (4)	0[a(reference)]					
Δ-value-Heat pain tolerance (HPTo) ºC						
(Intercept)	1.130	.9807	(-0.79 to 3.05)	1.328	1	.249
Age (ys)	-.040	.0307	(-0.10 to 0.02)	1.678	1	.195
Brain-derived neurotrophic factor (ng/mL)	.029	.0093	(0.01 to 0.05)	9.769	1	.002
a-tDCS (1)	-1.275	.5926	(-2.43 to -0.11)	4.628	1	.031

Hypnotic suggestion (2)	.336	.5958	(-0.83 to 1.50)	.317	1	.573
a-tDCS/Hypnotic suggestion (3)	.054	.5961	(-1.11 to 1.22)	.008	1	.927
s-tDCS/Hypnotic suggestion (4)	0[a(reference)]					
Δ-value-Cold Pressor Test (CPT)						
(Intercept)	12.170	10.4816	(-8.37 to 32.71)	1.348	1	.246
Age (ys)	-.489	.3195	(-1.11 to 0.13)	2.339	1	.126
Brain-derived neurotrophic factor (ng/mL)	.224	.1022	(0.023 to 0.43)	4.795	1	.029
a-tDCS (1)	-4.918	6.4082	(-17.47 to 7.64)	.589	1	.443
Hypnotic suggestion (2)	5.937	6.4887	(-6.78 to 18.65)	.837	1	.360
a-tDCS/Hypnotic suggestion (3)	14.656	6.5347	(1.84 to 27.46)	5.030	1	.025
s-tDCS/Hypnotic suggestion (4)	0[a(reference)]					

Confidence interval (CI)

8. CONSIDERACIONES FINALES

Los resultados obtenidos con esta tesis de doctorado permiten las siguientes consideraciones:

Los resultados confirman un efecto diferencial entre la sugerencia hipnótica y la estimulación de corriente directa transcraneal en las medidas del dolor. Sugieren que el impacto de las intervenciones tiene mecanismos neurales diferenciales, ya que la sugerencia hipnótica mejoró la percepción del dolor y la estimulación por corriente continua transcraneal aumentó la inhibición del sistema de modulación del dolor descendente.El efecto combinado no mejoró la eficiencia de la inhibición en la DPMS. Y por ultimo el a-tDCS / HS mostró un efecto adictivo sustancial sobre el CPT. Sin embargo, se necesita más investigación para probar el impacto a largo plazo de estos dos enfoques.

9. PERSPECTIVAS FUTURAS

Sesta perspectiva futura, diagnósticosherramientas deneuromodulación

10. Anexos

10.1 TCLE

Nº do CAAE_63863816.0.0000.5327

Título do Projeto: **EFEITO HIPNOSE COMBINADA A ESTIMULAÇÃO TRANSCRANIANA DE CORRENTE CONTÍNUA NA ATIVIDADE CORTICAL E NA PERCEPÇÃO DA DOR EM SUJEITOS SAUDÁVEIS**

Você está sendo convidada a participar de uma pesquisa cujo objetivo é avaliar se a hipnose associada à estimulação elétrica de baixa voltagem na cabeça pode influenciar na percepção de dor. Esta pesquisa está sendo realizada pelo Laboratório de Dor & Neuromodulação do Hospital de Clínicas de Porto Alegre (HCPA).

A hipnose é uma técnica que a pessoa responde a informações oferecidas por um instrutor sobre um determinado assunto. Após isto, a pessoa experimentará relaxamento.

A estimulação transcraniana de corrente continua é uma técnica em que se aplica uma corrente elétrica fraca contínua por 20 minutos, na qual iremos utilizar eletrodos (dispositivos circulares metálicos) que serão colocadas na sua cabeça e pelos quais passarão a corrente elétrica que não causa dor. Esta técnica já é aplicada como tratamento da dor e outras condições.

Se você aceitar participar da pesquisa você terá que comparecer ao Centro de Pesquisa Clínica (CPC) do HCPA para os seguintes perdimentos:

1. Responder algumas perguntas (questionários) sobre dor, a capacidade de ser hipnotizado e perguntas gerais sobre seu estado de ansiedade e de ânimo, sentimentos que você

tem em relação à sua rotina.

2. Coleta de 10mL de sangue para avaliação de algumas proteínas relacionadas a sensação de dor.

3. Avaliação da dor: Será colado no seu antebraço um instrumento (aparelho) que aquece a temperaturas seguras (não queima a pele, em torno de 35ºC). Enquanto este aparelho aquece, você deverá responder algumas perguntas sobre a sua sensibilidade a essas temperaturas, atribuindo uma nota. Durante um dos momentos de aquecimento do aparelho você colocará sua mão em um recipiente com água gelada por até um minuto e atribuirá uma nota à sensibilidade ao calor do braço com o aparelho. Ao mesmo tempo em que esses testes estejam acontecendo, você colocará na cabeça uma touca com eletrodos para avaliar a sua atividade cerebral. Essa touca emitirá corrente elétrica e você não perceberá essa corrente.

4. Sessão de hipnose: durante a sessão de estimulação elétrica, será realizada uma sessão de hipnose, na qual o pesquisador falará frases que induzem o estado de relaxamento. Esta etapa durará em torno de 20 minutos.

5. Novamente será realizada outra avaliação da sensação de dor, conforme explicado na etapa 3.

6. Por último, a etapa 2 será realizada uma outra vez.

Todos esses procedimentos terão duração de aproximadamente 3 horas.

Após uma semana, você repetirá pela última vez as etapas de 1 a 6.

Os possíveis riscos ou desconfortos decorrentes da participação na pesquisa são, dor e hematoma (mancha roxa) no local da retirada da amostra de sangue, sensação de calor e frio durante os testes. Na avaliação da sua atividade cerebral, você terá o desconforto de colocar

uma touca com eletrodos que podem causar sensações de coceira no seu couro cabeludo acionado pela estimulação elétrica. Não são esperados riscos ou desconfortos relacionados à hipnose, contudo, você poderá experienciar um processo de relaxamento profundo e perder a noção de tempo após o processo, por exemplo: após à técnica, ter a percepção de ter permanecido sob este estado de relaxamento profundo durante três horas, enquanto, de fato, permaneceu apenas 20 minutos. Caso você tenha algum desconforto maior durante a aplicação dos procedimentos, poderá desistir de participar a qualquer momento.

Os possíveis benefícios decorrentes da participação na pesquisa não são diretamente relacionados a você. Caso tenha interesse, receberá os resultados das aplicações realizadas. A pesquisa contribuirá para uma melhor compreensão dos estudos com dor.

Sua participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir de participar e retirar seu consentimento, não haverá nenhum prejuízo ao atendimento que você recebe ou possa vir a receber na instituição.

Não está previsto nenhum tipo de pagamento pela sua participação na pesquisa e você não terá nenhum custo com respeito aos procedimentos envolvidos, porém, poderá ser ressarcido por despesas decorrentes de sua participação, cujos custos serão absorvidos pelo orçamento da pesquisa.

Caso ocorra alguma intercorrência ou dano, resultante de sua participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Caso você tenha dúvidas, poderá entrar em contato com o pesquisador responsável Prof. Dr. Wolnei Caumo, pelo telefone (33596377), com o pesquisador Gerardo Beltran, pelo telefone (33596377) ou com o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (HCPA), pelo telefone (51) 33597640, ou no 2º andar do HCPA, sala 2227, de segunda à sexta, das 8h às 17h.

Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

_____

Nome do participante da pesquisa

_____

Assinatura

_____

Nome do pesquisador que aplicou o Termo

_____

Assinatura

Local e Data: _____

10.2 INDUÇÃO HIPNÓTICA

INDUÇÃO HIPNÓTICA

Por favor coloque-se numa posição confortável. Feche os seus olhos e permita-se relaxar. Faça algumas respirações lentas e profundas e repare que à medida que expira, você pode sentir que fica mais e mais relaxado. Você pode continuar a relaxar, enquanto eu falo consigo... e de cada vez que expirar, pode sentir que fica mais e mais e mais relaxado... mais e mais relaxado. Mas independentemente do grau de relaxamento que atingir, vai continuar a ouvir a minha voz, e poderá responder às minhas sugestões. Se ficar desconfortável, poderá reajustar o seu corpo para ficar confortável outra vez e isso não interferirá na sua experiência de hipnose. Se necessitar falar, poderá fazê-lo facilmente, sem perturbar a sua experiência hipnótica.

Agora, poderá querer relaxar mesmo mais, e à medida que relaxa, poderá sentir uma ligeira impressão nos seus dedos... ou nos seus dedos do pé... e se sentir isso, isso dar-lhe-á conforto, porque saberá que essa é uma sensação de relaxamento que algumas pessoas têm quando começam a experimentar a hipnose. Deixe o seu corpo relaxar. Comece apenas a sentir uma sensação de calma espalhando-se ...de paz... deixando para trás todos as suas preocupações... deixe-as vaguear para longe, como nuvens dissipando-se ao vento..... desfazendo-se... relaxando apenas mais e mais... sentindo-se mais e mais em paz, mais calmo.... Mais confortável e seguro... nada que o incomode... nada que o perturbe... mais e mais profundamente relaxado... como se entrasse num confortável estado de hipnose, ficando tão profundamente envolvido na hipnose que poderá ter todas as experiências que quiser ter... tão profundamente que poderá experimentar todas as experiências que desejar... apenas as suas experiências

E agora pode concentrar a sua atenção nos seus dedos do pé... nos seus dedos do pé direito... e nos seus dedos do pé esquerdo. Deixe os seus dedos do pé direito relaxar... relaxar completamente... e os seus dedos do pé esquerdo... deixe os seus dedos do pé relaxar... mais e mais... mais e mais relaxados. E deixe o relaxamento espalhar-se dos seus dedos para os seus pés, e deixe os seus pés relaxar... deixe-os tornarem-se mais e mais relaxados... à medida que

se sente tão calmo e à vontade.... E agora preste atenção aos seus tornozelos e às barrigas das pernas... será que poderá começar a deixar-se ir... deixar-se ir e relaxar à medida que sente talvez uma confortável sensação de calor nos seu tornozelos ou nas suas barrigas das pernas... ou talvez seja uma sensação de frescura e à vontade... na sua perna direita ou na sua perna esquerda. Deixe apenas as suas pernas relaxar... mais e mais relaxadas... mais e mais completamente relaxadas.

E o relaxamento pode espalhar-se até às suas ancas... as suas ancas podem relaxar mais e mais... apenas deixando que isso aconteça. E pode deixar o seu pélvis relaxar. Apenas deixe que ele fique solto e suave, solto e suave...soltos e suave...relaxando mais e mais. Relaxe o seu estômago, deixe o seu estômago tornar-se completamente relaxado. Repare como ele se sente, pode permitir que ele se torne completamente relaxado... poderá reparar nisto agora ou um pouco mais tarde? E deixe a sensação de relaxamento espalhar-se para cima para o seu peito. Deixe que todos os nervos e músculos do seu peito relaxem completamente... relaxados...soltos e suaves... sinta a paz espalhando-se à medida que se sente tão à vontade... tão seguro... o seu corpo e a sua mente tão relaxados e em paz. Agora as suas costas podem relaxar, e os seus ombros. Permita-se sentir o relaxamento nas suas costas e nos seus ombros...mais e mais relaxado... solto e suave... completamente relaxado.

Deixe que a sensação de relaxamento se espalhe pelos seus braços para baixo até às suas mãos e dedos. Relaxe mais e mais... concentre-se nas sensações dos seus braços e mãos. Os seus dedos estão mais pesados do que leves, ou mais leves do que pesados? Concentre-se na parte superior do seu braço direito...parte inferior do seu braço direito..... sua mão direita... e

dedos... relaxando completamente, tão relaxados... completamente relaxados. E agora o seu braço esquerdo... relaxando completamente, tão relaxado... completamente relaxado. Será que você pode mergulhar ainda mais profundamente agora? Mais profundo e mais profundo... apenas como você deseja... apenas tão confortável e tão profundamente como você gostaria de ir

Você poderá gostar de imaginar estar em algum lugar calmo e relaxar. Eu gosto de imaginar que estou numa praia sossegada num dia de sol morno, com um céu azul bonito e apenas algumas nuvens que flutuam no céu... eu posso imaginar uma sensação de uma brisa delicada e suave... um cheiro no ar de mar salgado... mas você pode imaginar que está em qualquer lugar você goste. Pôde ser nalgum local onde já foi... ou aonde gostava de ir. ou apenas um lugar na sua imaginação. Não importa... a única coisa que importa é o seu conforto... a sua paz. Onde quer que esse local seja... é tão calmo e cheio de paz... um local onde você pode apenas ser você ...onde você pode sentir-se completamente à vontade e satisfeito. E pode imaginar-se realmente lá... vendo com os olhos da sua mente, as coisas que veria se realmente estivesse lá agora... sentindo as coisas que sentiria... escutando os sons que ouviria... cheirando os cheiros...

E enquanto está no seu lugar perfeito, eu vou contar de um até dez. E em cada numero você vai mergulhar mais profundamente na hipnose... mais e mais... sendo capaz de experimentar o que quer que queira experimentar. **Um**... mergulhando, mergulhando mais e mais profundo... **dois** mais e mais centrado, e equilibrado... **três**... **quatro**... mais e mais profundo e... **cinco... seis... sete**... mais profundo do que antes... tão profundo que pode experimentar o que quer que deseje experimentar... **oito... nove... dez.**..... muito profundo... agora muito profundo...completamente em união consigo mesmo...completamente absorvido

In Kirsch, I., Lynn, S.J., & Rhue, J.W. (1993). Introducion to clinical hypnosis. In J.W.Rhue, S.J. Lynn, & Kirsch (Eds.), *Handbook of clinical hypnosis* (pp.12-14).Washington, D. C.: American Psychological Association.

10.3 CONSORT

2010 – Cheklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page ART 1	Reported on page ART 2
		Title and abstract		
	1a	Identification as a randomised trial in the title	86	145
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	88	147
		Introduction		
Background and objectives	2a	Scientific background and explanation of rationale	90	148
	2b	Specific objectives or hypotheses	92	150
		Methods		
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	93	151
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA	NA
Participants	4a	Eligibility criteria for participants	94	152
	4b	Settings and locations where the data were collected	88	151
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	97	154
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	100	155
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA	NA
Sample size	7a	How sample size was determined	102	158
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA	NA
Randomisation:				

Sequence generation	8a	Method used to generate the random allocation sequence	103	158
	8b	Type of randomization; details of any restriction (such as blocking and block size)	96	158
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	96;103	159
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	96	159
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	96	159
	11b	If relevant, description of the similarity of interventions	96	159
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	103	159
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	103	159
		Results		
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	104;105	160
	13b	For each group, losses and exclusions after randomisation, together with reasons	104;105	160
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA	NA
	14b	Why the trial ended or was stopped	NA	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	105	161
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	105	160
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated	105	161

		effect size and its precision (such as 95% confidence interval)		
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	107	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA	NA
		Discussion		
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	114	169
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	110	165
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	110	165
Other information				
Registration	23	Registration number and name of trial registry	87	146
Protocol	24	Where the full trial protocol can be accessed, if available	89	147
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	87	146

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.