SINTOMATOLOGÍA NEGATIVA DE LA ESQUIZOFRENIA EN UN MODELO GENÉTICO DE RATAS: VALIDACIÓN CONDUCTUAL Y FARMACOLÓGICA EN LAS CEPAS DE RATAS ROMAN

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DISERTACIÓN DOCTORAL
PARA OPTAR AL GRADO DE
DOCTOR EN NEUROCIENCIAS

PRESENTADA POR
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TUTORIZADO Y DIRIGIDO POR EL
DR. ALBERTO FERNÁNDEZ TERUEL

- “El paso más importante que puede dar alguien.
No es el primero, ¿verdad?
Es el próximo. Siempre el próximo paso, Dalinar”-
Brandon Sanderson, Archivo de las Tormentas III: Juramentada

La investigación de la presente Disertación Doctoral ha contado con el apoyo de la financiación siguiente:

Ministerio de Economía, Industria y Competitividad de España -PID2020-114697GB-I00 (ref. AEI/10.13039/501100011033) i – PSI2017-82257-P.
Agencia de Gestión de Ayudas Universitarias y de Investigación de la Generalitat de Catalunya – 2017SGR-1586.
Institución Catalana de Investigación y Estudios Avanzados - ICREA-Academia- 2013

Agradecimientos

¡Vamos allá! Primero de todo, dejarme explicaros un símil que usó una persona para definirme hace poco. Me describió como una foca bostezando, a la que le cae el pescado para comer sin ella buscarlo. Que quiere decir esto, pues bien, que soy una persona con mucha suerte sin quererlo. Es totalmente cierto, soy esa foca. Debido a ello, este es tranquilamente el apartado más difícil de escribir de toda esta Disertación Doctoral. No porque no tenga nada que agradecer, todo lo contrario, porque al tener mucha, pero mucha, muchísima suerte, me atora pensar en todo lo que debo agradecer. Tantas cosas y tanta gente a la que le debo tanto y sin la cual nada de esto sería posible, ni tendría sentido, que no sé por dónde empezar. Espero no dejarme a nadie…

Primeramente, me gustaría agradecerte, Alberto, la oportunidad que me has brindado de poder formar parte del grupo de investigación y por la oportunidad laboral. Durante estos años, he podido formarme como investigador y ha sido, sin duda alguna, gracias a tus directrices, consejos y ejemplo. También quiero agradecerte el buen ambiente de trabajo que has fomentado todos estos años entre el grupo, gracias a ello realizar mi trabajo y esta tesis ha sido una alegría y no tanto un sufrimiento. Agradecerte Adolf tus ideas y tu punto de vista global y clínico que han sido de gran ayuda durante las reuniones durante estos años. Quisiera agradecerte Susana, la oportunidad de formar parte de tu grupo de investigación en Copenhague, aunque breve. me sentí como en casa desde el minuto uno. Gracias.

También agradecer al Institut de Neurociències de la UAB por sus servicios. A Mar por ayudarnos con todo el proceso de la inmunohistoquímica, y a Meri por toda la ayuda con las fotos en microscopía. Hicisteis que el trabajo quedara perfecto. Agradeceros Amalia, Tina y Pilar por formar parte de la comisión de seguimiento cada año y guiarme, cuestionarme y hacerme pensar; sin duda la tesis está como está gracias a vosotras. Gracias.

Quisiera aprovechar para agradecer a todas aquellas personas que desafortunadamente no conozca demasiado bien pero que sin duda habéis hecho mi vida en la universidad más fácil y cómoda: al personal administrativo, al personal de limpieza, al personal de mantenimiento, al personal del comité de empresa, al personal de seguridad, al personal del estabulario y al personal de la cafetería. Gracias.

Agradeceros también a todos los que habéis formado o formáis parte de la *Unitat de Psicologia Mèdica.* A los profesores, compañeros y amigos que habéis estado cada día en el pasillo, comedor o laboratorio. A Anna, Ignasi, Carles y Cristóbal por vuestra ayuda y compañía durante las largas horas de conducta e investigación. Desde el momento que llegué como estudiante de máster, hasta ahora, me habéis ayudado en todo lo que he necesitado. Ha sido un privilegio poder aprender de los seniors. Agradeceros Francesco, Pilar, Valeria y Pau por vuestro tiempo ayudándome con los experimentos durante vuestras estancias o durante vuestras prácticas de máster. Espero que os haya podido enseñar algo, sin duda yo he aprendido de vosotros. Gracias.

A Anastasia, Juan, Bernat, Paula, Aida, Adam y Alex por compartir que la ciencia y la investigación también son los viernes de cerveza y charlas. Por entender que se puede compaginar ocio y tesis. Especials ànims a vosaltres, Adam i Alex! Agrair-vos el suport moral mutu mentre avançàvem en aquesta aventura que es fer una tesis; ja s'acaba. Gràcies.

Agrair-te especialment a tu, Toni, cada minut que hem treballat junts. Em costa imaginar un millor ambient de treball, unes millors reflexions i una millor forma de entendre i relacionar-me amb les persones que les que he pogut aprendre de tu. Entendre i preguntar-me "Quina part de responsabilitat tinc en tot això que està passant? Que puc fer-ne?" m'ha servit per relativitzar les coses i no angoixar-me mes del compte. No tinc cap dubte que he pogut fer aquesta tesis, i la meva feina, perquè he tingut la sort de tenir-te com a company i amic. Moltes gràcies.

Al amics que conservo de la carrera. Agrair-vos Anna, Raquel, Efren i Gin l'amistat que vam plantar i que encara cuidem, que no mori mai. A la Gemma, xoxi moltes gràcies per treure'm de casa i compartir *pisito* amb mi. M'encanta que entris per la porta al crit de HIHIII!, m'alegra l'ànima. Espero escoltar-ho una mica més. Tots sou part molt important de la meva vida. Moltes gràcies.

Al amics de tota la vida, als amics de Granollers. A en Carles, l'Arnau, la Silvia, el Guille, la Mar, el Lax, l'Enric, el Mere, l'Alberto, la Laura, en Xexi, el Luna, en Piñe, la Clàudia, la Silvia i en Pol agrair-vos la sort que hem tingut en aquesta vida de trobar-nos tant ràpidament i tenir-nos com amics des de ven petits. Agrair-te especialment Enric, la *pedazo* de portada que has fet per aquesta tesis, sento tots els canvis de títols que t'he fet fer. Gent, considero una victòria de tots tenir un grup d'amics i amigues com el nostre on hi te lloc tothom i tot. Moltes gràcies.

Roser, agrair-te el temps que portem junts. Perquè el millor durant aquests anys de doctorat no ha estat el coneixement obtingut o les aptituds adquirides ni el possible títol, ha estat sens dubte coneixe't. Parlar, discutir, aprendre de tu, i estimar-te es un regal que espero seguir agraint-te. Moltes gràcies per tenir-nos poc exigents i indulgents l'un a l'altre, perquè *t'estimo molt i també tinc por*.

Por último, a aquellas personas que han sido, son y serán mi familia. Las personas que me animaron a tomarme en serio la competición "absurda" de la casa; creo que voy ganando. A mi hermano Pedro, por ser mi primer amigo, por quererme y estar siempre disponible para hablar de cualquier cosa, escucharte siempre es sinónimo de aprender. Decirte también que, aunque estos últimos años no lo hayas tenido tan fácil, he podido aprender el significado real de resiliencia, esfuerzo y perseverancia solo con verte. Casi siento todos los deseos de cumpleaños que he gastado en tu contra. Finalmente, a la Mama y al Papa, agradeceros la vida que nos habéis dado. Os amo, porque me amasteis primero y así, siempre es más fácil. Agradeceros el ejemplo y la educación que nos habéis dado, con ello he podido llegar hasta aquí. Espero estar haciéndolo bien. Estoy orgulloso de vosotros. Muchas gracias, os quiero mucho a los tres.

…Espero seguir siendo aquella foca muchos años más. ¡Gracias! Gràcies!

Resumen

La presente Disertación Doctoral concentra una serie de estudios que añaden nuevas evidencias conductuales, farmacológicas y neuroanatómicas (de actividad celular cerebral) en apoyo de la validez de un modelo animal de rasgos relevantes para la esquizofrenia, la cepa de ratas "*Roman* de alta evitación" (RHA; comparadas con las "*Roman* de baja evitación", o RLA). A lo largo de las últimas décadas nuestro grupo de investigación se ha centrado en aspectos del modelo relacionados con la sintomatología positiva y cognitiva de la esquizofrenia, sin profundizar en aspectos de la sintomatología negativa. Los estudios que aquí se presentan van destinados a investigar por primera vez aspectos relacionados con la sintomatología negativa de la esquizofrenia, como la asociabilidad o falta de interés por la conducta social.

El aspecto conductual sintomatológico común entre los distintos estudios aquí presentados es la asociabilidad. En la esquizofrenia, la asociabilidad o retraimiento social, es uno de los síntomas negativos más importantes, y puede entenderse como una reducción en la motivación para crear nuevas relaciones con los demás. Por lo tanto, modelos animales capaces de modular diferentes aspectos del desinterés por la interacción social pueden ser valiosos para ampliar el conocimiento sobre las características neurobiológicas subyacentes a este aspecto de la sintomatología negativa de la esquizofrenia.

En los primeros estudios (Estudios 1-3) hemos observado diferencias entre las cepas de ratas *Roman* (RHA vs. RLA) en relación con la conducta social. Hemos observado que la cepa de ratas RHA presenta una menor preferencia social que la cepa de ratas RLA (Estudio 1). Además, los resultados revelan una reducción en preferencia social de los animales adultos en comparación con los animales jóvenes, así como que las hembras adultas de la cepa RHA muestran una mayor preferencia social que los machos de la misma cepa (Estudio 3).

Del mismo modo, hemos estudiado los efectos del tratamiento ambiental de estimulación neonatal (NH, del inglés *"neonatal handling"*) sobre la conducta social y sobre la expresión de c-Fos (como medida de activación celular) en varias áreas cerebrales en ambas cepas (Estudio 2). El tratamiento de NH ha sido capaz de aumentar la interacción social en ambas cepas con un efecto más marcado en las ratas RHA, así como de aumentar de forma específica la expresión de c-Fos en zonas de la corteza prefrontal y subregiones de la amígdala de las ratas RHA (Estudio 2).

Por último, y continuando con la validación del modelo, en los estudios siguientes (Estudio 4-5) se abordó el objetivo general de la Disertación Doctoral desde una perspectiva farmacológica. Así, la administración de un antagonista del receptor glutamatérgico NMDA, el MK801, produce un mayor déficit de conducta social y una mayor hiperactividad (ambos efectos considerados como modelos de aspectos

relacionados con la esquizofrenia) en las ratas RHA que en las ratas RLA. Así mismo, la administración de varios antipsicóticos atípicos atenúa los efectos del MK801 sobre la preferencia social y la actividad locomotora en mayor medida en la cepa RHA que en la cepa RLA, siendo el antipsicótico aripiprazol el que presenta mayor actividad "terapéutica" (Estudio 4). De modo similar, la administración concomitante de MK801 y oxitocina, un neuropéptido con propiedades antipsicóticas naturales, produjo una atenuación de los efectos negativos del MK801 sobre la conducta social y la hiperactividad. Dicho efecto resultó aparentemente más marcado en la cepa RHA que en la cepa RLA (Estudio 5).

Los datos que se muestran en esta Disertación Doctoral añaden validez aparente, de constructo y predictiva al modelo animal propuesto, las ratas RHA, como un recurso útil para la investigación y mejor comprensión de aspectos neurobiológicos y conductuales relevantes para la esquizofrenia.

Resum

La present Dissertació Doctoral concentra una sèrie d'estudis que afegeixen noves evidències conductuals, farmacològiques i neuroanatòmiques (d'activitat cel·lular) en suport de la validesa d'un model animal de trets rellevants per a l'esquizofrènia, la soca de rates "Romanes de alta evitació" (RHA; comparades amb les "Romanes de baixa evitació", o RLA). Al llarg de les darreres dècades, el nostre grup d'investigació s'ha centrat en aspectes del model relacionats amb la simptomatologia positiva i cognitiva de l'esquizofrènia, sense aprofundir en aspectes de la simptomatologia negativa. La recerca que aquí es presenta va destinada a investigar per primera vegada aspectes relacionats amb la simptomatologia negativa de la malaltia, com l'*associalitat* o manca d'interès per la conducta social.

L'aspecte conductual simptomatològic comú entre els diferents estudis aquí presentats és *l'associalitat,* o retraïment social, un dels símptomes negatius més importants de l'esquizofrènia. Per tant, models animals capaços de modular diferents aspectes del desinterès per la interacció social poden ser valuosos per ampliar el coneixement sobre les característiques neurobiològiques subjacents a aquest aspecte de la simptomatologia negativa de l'esquizofrènia.

En els primers estudis (Estudis 1-3), hem observat diferències entre les soques de rates Romanes (RHA vs. RLA) en relació amb la conducta social. Hem observat que la soca de rates RHA presenta una menor preferència social que la soca de rates RLA (Estudi 1), i que les femelles adultes de la soca RHA mostren una major preferència social que els mascles de la mateixa soca. A més a més, els resultats revelen una reducció en la preferència social dels animals adults en comparació amb els animals joves (Estudi 3).

D'altre banda, hem estudiat els efectes del tractament ambiental d'estimulació neonatal (NH, de l'anglès "*neonatal handling"*) sobre la conducta social i sobre l'expressió de c-Fos (com a mesura d'activitat cel·lular) en diferents àrees cerebrals en ambdues soques (Estudi 1-2). El tractament NH ha estat capaç d'augmentar la interacció social en ambdues soques amb un efecte més marcat en la soca RHA, així com d'augmentar de forma específica l'expressió de c-Fos en zones de l'escorça prefrontal i subregions de l'amígdala de les rates RHA (Estudi 2).

Per últim, i continuant amb la validació del model, en els estudis següents (Estudi 4-5) es va abordar l'objectiu general de la Dissertació Doctoral des d'una perspectiva farmacològica. Així, l'administració d'un antagonista del receptor glutamatèrgic NMDA, el MK801, vam veure que produeix un major dèficit de la conducta social i una major hiperactivitat en rates RHA que en les rates RLA (ambdós efectes considerats com a models d'aspectes relacionats amb l'esquizofrènia). D'altra banda, vam observar que l'administració de varis antipsicòtics atenua els efectes del MK801 sobre la preferència social i l'activitat locomotora, en major mesura en la soca RHA que en la soca RLA, essent

l'antipsicòtic aripiprazol el que presenta major activitat "terapèutica" (Estudi 4). De forma similar, l'administració concomitant de MK801 i oxitocina, un neuropèptid amb propietats antipsicòtiques naturals, va produir una atenuació dels efectes negatius del MK801 sobre la conducta social i la hiperactivitat. Aquest efecte va resultar aparentment més marcat en la soca RHA que en la soca RLA (Estudi 5).

Les dades que es mostren en aquesta Dissertació Doctoral afegeixen validesa aparent, de constructe i predictiva al model animal proposat, les rates RHA, com un recurs útil per la investigació i millor comprensió d'aspectes neurobiològics i conductuals rellevants per l'esquizofrènia.

Abstract

This Doctoral Dissertation focuses on a series of studies that add new behavioural, pharmacological, and neuroanatomical evidence (of cell activity) in support of the validity of an animal model of schizophrenia-relevant traits, the “Roman High Avoidance” strain of rats (RHA; compared to “Roman Low Avoidance”, or RLA). Along the last decades, our research group has focused on aspects of the model related to the positive and cognitive symptomatology of schizophrenia, without delving into aspects of negative symptomatology. The studies presented here intended to investigate, for the first time, aspects related to the negative symptomatology of schizophrenia, such as asociality or lack of interest in social behaviour.

The common symptomatologic behavioural aspect among the different studies presented here is asociality. In schizophrenia, asociality or social withdrawal is one of the most important negative symptoms and it can be understood as a reduction in the motivation to create new relationships with others. Therefore, animal models recapitulating different aspects of lack of motivation for social interaction may be valuable to expand our knowledge about the neurobiological characteristics underlying this aspect of the negative symptomatology of schizophrenia.

In the first studies (Studies 1-3) we observed differences between the Roman rat strains (RHA vs. RLA) in relation to social behaviour. We have observed that the RHA rat strain exhibits lower social preference than the RLA rat strain (Study 1). In addition, the results reveal a reduction in social preference of adult animals compared to young animals, as well as that adult females of the RHA strain show a greater social preference than males of the same strain (Study 3).

Furthermore, we have studied the effects of neonatal handling (NH; a neonatal environmental stimulation treatment) on social behaviour and c-Fos expression (as a measure of neuronal activation) in various brain areas in both rat strains (Study 1-2). NH treatment was able to increase the social preference of both strains with a more marked effect in RHA rats, and the treatment specifically increased the expression of c-Fos in areas of the prefrontal cortex and subregions of the amygdala of RHA rats (Study 2).

Finally, in the following studies (Study 4-5) the general objective of the Doctoral Dissertation was addressed from a pharmacological perspective. Thus, the administration of a glutamatergic NMDA receptor antagonist, MK801, produces greater deficits in social behaviour and greater hyperactivity (both effects are considered to model aspects related to schizophrenia) in RHA rats than RLA rats. Likewise, the administration of various atypical antipsychotics attenuates the effects of MK801 on social preference and locomotor activity to a greater extent in the RHA strain than in RLA rats, with the antipsychotic aripiprazole showing the greatest "therapeutic" activity (Study 4). Similarly, concomitant administration of MK801 and oxytocin, a neuropeptide with natural

antipsychotic properties, resulted in attenuation of the negative effects of MK801 on social behaviour and hyperactivity. This effect was apparently more marked in the RHA strain than in RLA rats (Study 5).

The data reported in this Doctoral Dissertation add face, construct, and predictive validity to the proposed animal model, the RHA rats, as a useful tool for research and for a better understanding of neurobiological and behavioural aspects relevant to schizophrenia.

Tabla de Contenido

Abreviaciones

$\alpha_{1\text{-}2}$: Subtipos de receptores adrenérgicos

5-CSRTT: Tarea de tiempo de reacción serial de cinco opciones

5-HT: Serotonina

5-$HT_{1\text{-}7,1A,2A,2C}$: Subtipos del receptor de la serotonina

AC-1: Anticuerpo primario

AC-2: Anticuerpo secundario

ACC: Corteza cingulada anterior

ACTH: Hormona adrenocorticotropa

ADN: Ácido desoxirribonucleico

AMPA: Ácido α-amino-3-hidroxi-5-metil-4-isoxazolpropiónico

AMY: Amígdala

ANOVA: Análisis de la varianza

APO-SUS: Susceptible a apomorfina

APO-UNSUS: No susceptible a apomorfina

ARI: Aripiprazol

ASR: Reflejo de respuesta de sobresalto acústico

BDNF: Factor neurotrófico derivado del cerebro

BLA: Amígdala basolateral

CF: Corteza frontal

Cg1: Corteza cingulada, área 1

CLZ: Clozapina

CNS: Sistema nervioso central

CNV: Variación en el número de copias

CRT: Terapia de remediación cognitiva

CTX: Contexto

$D_{1\text{-}4}$: Subtipos de receptores de dopamina

DA: Dopamina

DISC1: Gen Alterado en esquizofrenia 1

DOI: 2,5-dimetoxi-4-iodoamfetamina

DPBS: Solución salina de fosfato de Duelbecco

DSM: Manual diagnóstico y estadístico de los trastornos mentales

ErbB4: Proteína tirosina quinasa receptora

FDA: Administración de alimentos y medicamentos

FGA: Primera generación de antipsicóticos

GABA: Ácido gamma-aminobutírico

Grm2: Gen para el receptor mGlu2

H1: Subtipo de receptor histaminérgico

HAL: Haloperidol

HC: Caja nido

HPA: Hipofisiario-Pituitario-Adrenal

HPC: Hipocampo

IL: Infralímibco

LI: Inhibición latente

LSD: Dietilamida de ácido lisérgico

m1: Subtipo de receptor colinérgico muscarínico

MAM: Metilazoximetanol

MATRICS: Investigación de medidas y tratamientos para mejorar la cognición en la esquizofrenia

MePD: Amígdala medial posterodorsal

MePV: Amígdala medial posteroventral

$mGlu_{1\text{-}8}$: Subtipos del receptor de glutamato

mPFC: Corteza prefrontal medial

MK801: Dizocilpina

MRI: Imagen de resonancia magnética

mRNA: Ácido ribonucleico mensajero

MWM: Laberinto acuático de Morris

NaCl: Cloruro sódico

NDS: Suero de burro normal

NGF: Factor de crecimiento nervioso

NK: Asesina natural

NaOH: Hidróxido de sodio

NH: Estimulación neonatal

NIMH: Instituto Nacional de Salud Mental

NIH-HS: Instituto nacional de salud - Cepa de ratas genéticamente heterogéneas

NMDA: Ácido N-*metil*-D-aspartato

NMDAR: Receptor N-*metil*-D-aspartato

NOE: Exploración del objeto novedoso

NRG1: Gen Neuregulina 1

OLA: Olanzapina

OXT: Oxitocina

PBS: Solución tampón de fosfato

PCP: Fenciclidina

PFC: Corteza prefrontal

PND: Día post-natal

Poly I:C: Ácido poliinosínico-policitidílico

PPI: Inhibición prepulso de la respuesta de sobresalto

PrL: Prelímbico

PV: Parvalbúmina

RHA: *Roman* de alta evitación

RIS: Risperidona

RLA: *Roman* de baja evitación

SEM: Error estándar

SERT: Transportador de serotonina

SGA: Antipsicótico de segunda generación

SI: Interacción social

SNARE: Receptores de proteínas de fijación soluble del factor sensible a la N-etilmaleimida

SNP: Polimorfismo de un solo nucleótido

SPSS: Paquete estadístico para las ciencias sociales

Tran: Transportador

TWAA: Evitación activa en dos direcciones

VEH: Vehículo

ZPR: Ziprasidona

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Tablas

01 /
Introducción

1.1 Historia de la Esquizofrenia

Según el Instituto Nacional de la Salud Mental (*NIMH* por sus siglas en inglés*, National Institute of Mental Health*) la esquizofrenia es definida como una enfermedad mental crónica, severa e incapacitante que interfiere en como una persona piensa, siente y se comporta, y que afecta al 1% de la población mundial (NIMH, 2022). Las personas con esquizofrenia suelen ser diagnosticadas en un rango de edad comprendido entre los 16 y los 30 años después del primer episodio de psicosis (NIMH, 2022; Owen et al., 2016). No solo la enfermedad es un desafío económico para los gobiernos y los sistemas de salud pública, sino que para los pacientes también supone una disminución de la esperanza de vida en 10-20 años, así como unos niveles de desempleo de un 80-90% (Hjorthøj et al., 2017; Owen et al., 2016)

A mediados del siglo XIX, el psiquiatra alemán Emil Kraepelin (1856-1926) propuso una integración de cuadros clínicos bajo el nombre de "*dementia praecox*" basándose en estudios longitudinales que resultaban en un deterioro cognitivo y conductual (Jablensky, 2010; Volk, 2015). En abril del 1908, el psiquiatra suizo Eugen Bleuler acuño por vez primera el término "esquizofrenia" procedente del griego clásico "*schizein* " (dividir, escindir, romper) y "*phrē*n*"* (mente, entendimiento, razón) (Moskowitz & Heim, 2011). Aun así, la terminología de Bleuler no hace referencia a múltiples personalidades o personalidad dividida sino a una fragmentación del pensamiento (Jablensky, 2010). Desde entonces la definición y concepto de esquizofrenia ha ido cambiando, basándose en la observación y la clasificación de los síntomas sin ver modificado su vocablo.

1.2 Síntomas de la Esquizofrenia

La contribución de Bleuler no solo se limitó a una nomenclatura nueva de la enfermedad, sino que introdujo una distinción fundamental entre síntomas básicos (obligatorios) y síntomas accesorios (suplementarios) (Jablensky, 2010). Los accesorios hacen referencia a lo que actualmente conocemos como síntomas "positivos", a saber: delirios y alucinaciones. Mientras que los básicos hacían referencia a incoherencia del habla y del pensamiento, abolición, incongruencia afectiva y autismo (Jablensky, 2010). En la actualidad, los clínicos han trabajado y redefinido el concepto y el criterio de diagnóstico mediante la elaboración del Manual Diagnóstico y Estadístico de los Trastornos Mentales o DSM-5 (por sus siglas en inglés, *Diagnostic and Statistical Manual of Mental Disorders 5th Edition*) escrito por la Asociación Estadounidense de Psiquiatría el año 2013. Como se puede observar en la Tabla 1, el DSM-5 muestra los requisitos para el diagnóstico de esquizofrenia (American Psychiatric Association, 2013; Marder & Cannon, 2019; McCutcheon et al., 2020b). Estos requisitos consisten en dos síntomas de los cinco del criterio "A", de los cuales uno debe ser delirios, alucinaciones o habla desorganizada. Además, estos síntomas deben tener como mínimo una duración de 6 meses, incluidos un mes de síntomas de la fase activa (American Psychiatric Association, 2013; Marder & Cannon, 2019).

Tabla 1: Criterio de diagnóstico para la esquizofrenia, adaptado (American Psychiatric Association, 2013)

A. Dos (o más) de los siguientes ítems, cada uno presente por una duración de tiempo de un mes (o menos si se trata con éxito). Al menos uno de ellos debe ser el (1), (2), o el (3): 1. Delirios 2. Alucinaciones 3. Habla desorganizada (p.ej. incoherencia) 4. Comportamiento desorganizado o catatónico 5. Síntomas negativos (*i. e.* disminución de la expresión emocional o disminución de la expresividad)
B. Durante una parte clínicamente significativa del tiempo transcurrido desde el inicio de la perturbación, el nivel de funcionamiento en una o más áreas principales (por ejemplo: trabajo, relaciones personales o autocuidado) está marcadamente por debajo del nivel alcanzado antes de la perturbación.
C. Los signos continuos de la alteración persisten durante un periodo de al menos 6 meses, que debe incluir al menos 1 mes de síntomas (o menos si se trata con éxito) que cumplan el Criterio A (p. ej. síntomas de fase activa) y que puedan incluir periodos del prodrómico o síntomas residuales. Durante el periodo prodrómico o residual, los signos de la perturbación pueden manifestarse únicamente con síntomas negativos o con dos o más síntomas de la lista del Criterio A presentados en una forma atenuada (p. ej. creencias extrañas, experiencias perceptivas inusuales).
D. Se ha descartado el trastorno esquizoafectivo y el trastorno depresivo o bipolar con características psicóticas porque no se han producido: (1) episodios depresivos o maníacos simultáneamente con los síntomas de la fase activa, o (2) cualquier episodio del estado de ánimo ocurrido durante los síntomas de la fase activa que han estado presentes en una minoría de la duración total de los periodos activos y residuales de la enfermedad.
E. La alteración no es atribuible a los efectos fisiológicos de una sustancia u otra afección médica.
F. Si hay antecedentes de trastorno del espectro autista o un trastorno de comunicación en el inicio de la infancia, el diagnóstico adicional de esquizofrenia se realiza solo con delirios o las alucinaciones prominentes, además de los otros síntomas requeridos de la esquizofrenia, también presentes durante al menos 1 mes (o menos si se trata con éxito).

Aparte del criterio de diagnóstico descrito en el DSM-5, hoy día la clasificación más aceptada divide los síntomas de la esquizofrenia en tres categorías principales: síntomas positivos, síntomas negativos y síntomas cognitivos (Fernández-Teruel et al., 2021; Khan et al., 2015; McCutcheon et al., 2020b; Owen et al., 2016; Sawa & Snyder, 2002).

1.2.1 Síntomas Positivos

Los síntomas positivos son conductas y pensamientos que no están presentes normalmente. Se pueden entender como una "pérdida de contacto con la realidad" y se manifiestan cómo: delirios, alucinaciones y discurso y conducta desorganizado o errática.

1.2.2 Síntomas Cognitivos

Teniendo un origen independiente de la psicosis o de los síntomas negativos, los déficits cognitivos son una característica principal de la esquizofrenia presentes en el 98,1% de los pacientes (Wu et al., 2021). Los déficits cognitivos incluyen déficits en memoria y aprendizaje, déficits de memoria de trabajo, atención, resolución de problemas, velocidad de procesamiento y cognición social. Como consecuencia de la importancia de los síntomas cognitivos, en el año 2003, se estableció la iniciativa "MATRICS" (por sus siglas en inglés, *Measurement and Treatment Research to Improve Cognition in Sczhizophrenia*), que fue esponsorizada por el NIMH con colaboración con la Administración de Alimentos y Medicamentos (*FDA* por sus siglas en inglés, *Food and Drug Administration*) del gobierno de los Estados Unidos (Millan et al., 2012). La iniciativa tenía como objetivo crear un consenso sobre la naturaleza del deterioro cognitivo en la esquizofrenia, la evaluación cognitiva y la confección de un marco para el reconocimiento de los tratamientos que abordan específicamente los déficits cognitivos asociados con la esquizofrenia independientemente de una mejora en la psicosis (Kuo et al., 2020; Millan et al., 2012).

1.2.3 Síntomas Negativos

Son aquellos relacionados con el síndrome amotivacional. Formado por dos dimensiones que incluyen cinco dominios, los síntomas negativos son: (1) la dimensión de la expresión reducida que incluye la alogia (pobreza en el habla) y el aplanamiento afectivo; (2) la dimensión de la motivación reducida (también llamada la dimensión apática) que incluye la asocialidad (o aislamiento social), la abolición (la incapacidad para iniciar y persistir en actividades dirigidas a un objetivo) y la anhedonia (la incapacidad de sentir placer)(Wu et al., 2021). Recientemente, MATRICS está centrando su atención en la sintomatología negativa. Es evidente que el impacto, tanto de los síntomas negativos como cognitivos, es sustancial para los pacientes. Aunque los síntomas positivos estén controlados, eso no quiere decir que se traduzca en una mejora funcional para los pacientes. Con una prevalencia del 60%, la completa recuperación funcional y social ocurre en menos del 15% de los individuos con esquizofrenia, donde la sintomatología negativa juega un rol fundamental (Mitra et al., 2016; Remington et al., 2016; Wu et al., 2021).

1.3 Factores de Riesgo

Pese a que el origen de la esquizofrenia aún es incierto y desconocido, está demostrado que la interacción de tres factores puede ser la causante de la enfermedad. Las tres causas son: los factores genéticos, los factores ambientales y factores del neurodesarrollo (Marder & Cannon, 2019; McCutcheon et al., 2020b).

Figura 1: Esquema de los factores de riesgo asociados en la aparición de la esquizofrenia. Factores genéticos, factores ambientales y factores del neurodesarrollo.

1.3.1 Factores genéticos

La esquizofrenia es altamente poligenética con una heredabilidad estimada en un 80% usando metaanálisis de estudios con gemelos (Zamanpoor, 2020). Estudios han identificado varias regiones cromosómicas o locis que sugieren que los polimorfismos de un solo nucleótido o SNP (por sus siglas en inglés, *Single-Nucleotide Polymorphism,*) con un rango de frecuencias poblacionales contribuyen al riesgo. Así mismo, también se han identificado 11 variaciones en el número de copias o CNV (por sus siglas en inglés, C*opy Number Variants*) que individualmente confieren un riesgo relativamente alto de padecer esquizofrenia (Owen et al., 2016; Stilo & Murray, 2019; Zamanpoor, 2020).

Además, los factores genéticos parecen ser altamente pleiotrópicos (*i. e.* un gen o alelo puede afectar múltiples rasgos fenotípicos aparentemente no relacionados) y no se relacionan con las definiciones existentes de enfermedad (Owen et al., 2016). Un estudio mostró también que existe un intercambio significativo de variantes de riesgo comunes entre la esquizofrenia y otros trastornos como el trastorno bipolar, el trastorno depresivo mayor y el trastorno del espectro autista (Lee et al., 2013).

1.3.2 Factores ambientales

Aparte de los factores genéticos, los factores ambientales juegan un papel muy importantes en el desarrollo de la esquizofrenia (Marder & Cannon, 2019; McCutcheon et al., 2020b; Owen et al., 2016; Zamanpoor, 2020). Estos factores ambientales se pueden dividir dependiendo de si su intervención ocurre durante el período prenatal o durante el período postnatal.

Muchos son los factores que pueden afectar al desarrollo normal del feto y que pueden dar lugar a padecer esquizofrenia. Las infecciones durante el embarazo de virus u otros agentes infecciosos (como el virus de la influenza, el virus del herpes o el toxoplasma) o durante el tiempo de la concepción están asociados con un riesgo

posterior de sufrir desordenes psicóticos. Mediante procesos psicológicos e inmunológicos que incluyen desencadenar respuestas de citoquinas proinflamatorias, o por la liberación de hormonas del estrés, se puede producir hipoxia, hipertermia o desnutrición provocando un aumento del factor de riesgo (Stilo & Murray, 2019; Zamanpoor, 2020). Así mismo, complicaciones obstétricas como el nacimiento prematuro, un bajo peso al nacer o deficiencia nutricional prenatal son causas bien documentadas como factores de riesgo de esquizofrenia (Zamanpoor, 2020). Otros factores de riesgo son la edad avanzada de los progenitores, que mediante procesos epigenéticos en errores de impresión del ácido desoxirribonucleico (ADN) y cambios de metilación en varios genes expresados en el cerebro contribuyen a padecer la enfermedad. También se ha observado que el momento de la concepción es un factor de riesgo a tener en cuenta, siendo finales de invierno o primavera períodos con mayor factor de riesgo (Khan et al., 2015; Stilo & Murray, 2019; Zamanpoor, 2020).

Por otro lado, el periodo desde la concepción hasta el final de la adolescencia también es una ventana donde determinados factores ambientales pueden provocar el desarrollo del trastorno. Factores socioeconómicos, adversidades durante la niñez o traumas, nacer o criarse en zonas urbanas e inmigración (de primera o de segunda generación) se han asociado con un mayor riesgo de padecer esquizofrenia. El consumo de cannabis durante la adolescencia, lesiones en la cabeza, epilepsia, enfermedades autoinmunes e infecciones severas también se han asociado con un incremento del factor de riesgo (McCutcheon et al., 2020b; Stilo & Murray, 2019).

Finalmente, como resume la Figura 2 se entiende la esquizofrenia desde la interacción y suma de factores ambientales y genéticos que ocurren durante el desarrollo embrionario y durante los estadios posnatales y de la adolescencia tardía.

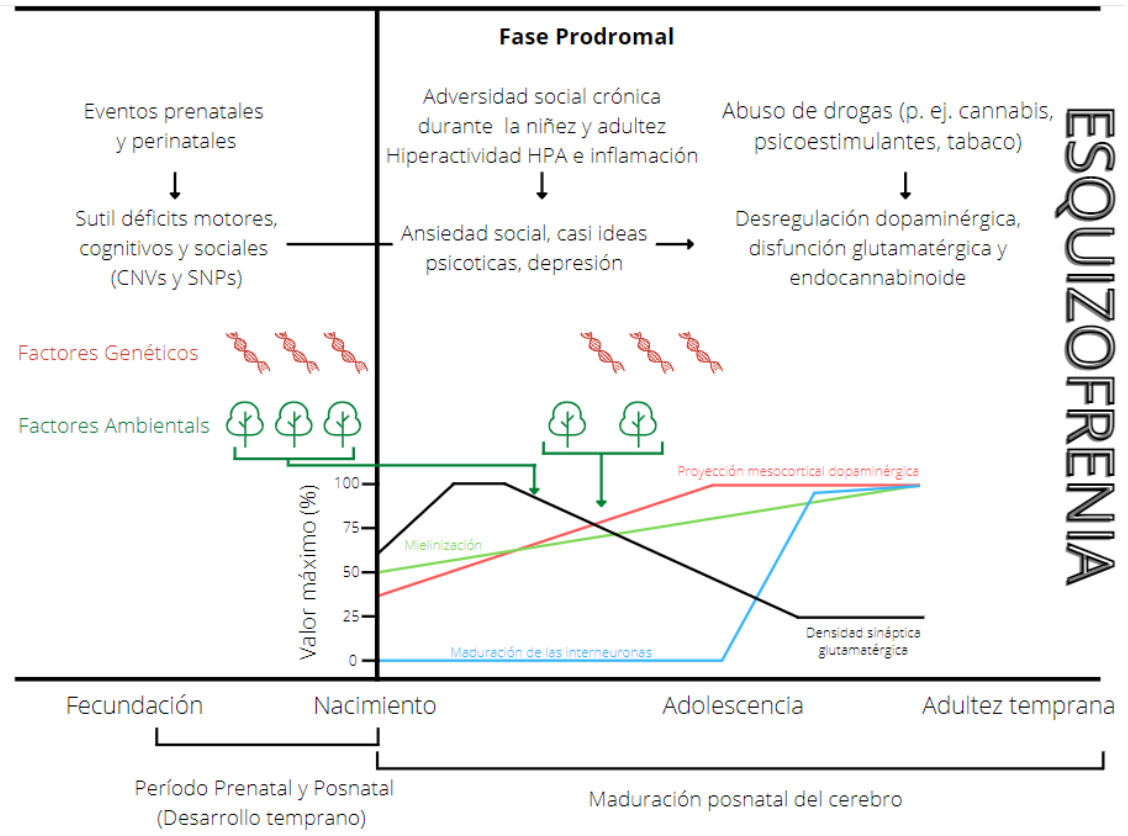


Figura 2: Interacción entre factores ambientales y genéticos en el desarrollo de la esquizofrenia. (Adaptado de Owen et al. 2020 y Stilo et al. 2019). Varios factores de riesgo genéticos y ambientales pueden afectar los procesos de desarrollo neurológicos a largo plazo y contribuir en la aparición de la esquizofrenia. El trastorno se presenta típicamente cuando el primer episodio de psicosis ocurre en la adolescencia tardía o en la adultez temprana, pero con frecuencia está precedido por una fase prodrómica; en algunos casos, los deterioros premórbidos en la cognición o el funcionamiento social, o ambos, se observan muchos años antes del primer episodio de psicosis. Se propuso que las alteraciones causadas por los genes de susceptibilidad y los efectos negativos ambientales durante el desarrollo temprano y adolescencia alteran la maduración cerebral posnatal. Es probable que estos factores perjudiquen algunos de los procesos cruciales en el desarrollo temprano, incluida la proliferación de células progenitoras, la migración neuronal y la ramificación y crecimiento dendrítico. Independientemente de tales riesgos y factores nocivos iniciales, los factores intrínsecos asociados con la enfermedad también podrían afectar directamente la maduración del cerebro posnatal. La acumulación de estas agresiones nocivas da como resultado una alteración general de la maduración cerebral posnatal, incluida la maduración de las interneuronas y las proyecciones dopaminérgicas, la eliminación de las sinapsis glutamatérgicas y la mielinización. La maduración de las interneuronas se representa como un aumento en la respuesta de las interneuronas a los agonistas de la dopamina D2 en la corteza prefrontal, mientras que la proyección dopaminérgica mesocortical se basa en los niveles de tirosina hidroxilasa. La densidad de sinapsis glutamatérgica y de mielinización están representados como niveles relativos.

1.4 Etiología

La etiología de la esquizofrenia se explica mejor mediante modelos poligenéticos multifactoriales que implican factores de riesgo genéticos modificados por el entorno (Zamanpoor, 2020) provocando un deterioro de circuitos neuronales en distintas áreas cerebrales (Elert, 2014; Millan et al., 2016). Cuatro hipótesis principales son las que intentan explicar los mecanismos neuronales subyacentes de los síntomas de esquizofrenia: (1) la hipótesis dopaminérgica, (2) la hipótesis glutamatérgica, (3) la hipótesis serotoninérgica y (4) la hipótesis GABAérgica.

1.4.1 Hipótesis dopaminérgica

La dopamina (DA) es un neurotransmisor que se produce en la sustancia negra y en las regiones tegmentales ventrales del cerebro cuyas proyecciones se dividen en la vía nigroestriada, la vía mesolímbica y la vía mesocortical (Brisch et al., 2014; Stępnicki et al., 2018). La hipótesis dopaminérgica surge principalmente de tres observaciones clínicas: la primera, explica que la hiperactivación del receptor D2 en la vía mesolímbica producido por la administración de anfetamina o cocaína (agonistas dopaminérgicos) produce, en pacientes sanos, efectos paranoides psicóticos similares a los observados en esquizofrenia (Stahl, 2018). La segunda observación se centra en el hecho que los antipsicóticos típicos (como el haloperidol o la clorpromazina) que bloquean los receptores D_2 de DA en el núcleo estriado reducen los síntomas positivos (Brisch et al., 2014; Seeman, 2021; Stahl, 2018). La tercera observación se refiere a estudios *post mortem* y estudios de tomografía por emisión de positrones, que muestran un mayor nivel de receptores D_2 en el estriado en cerebros de pacientes con esquizofrenia (Reynolds, 2022). Sin embargo, este aumento también podría entenderse como una adaptación a los fármacos antipsicóticos en vez de una anormalidad patológica de la enfermedad (Harrison, 2000; Marder & Cannon, 2019; McCutcheon et al., 2020a, 2020b; Quednow et al., 2010; Reynolds, 2022). Hay también una acumulación de evidencias que apuntan a una alteración en el almacenamiento presináptico, el transporte vesicular, la liberación, la recaptación y los mecanismos metabólicos en la vía mesolímbica de la dopamina (Quednow et al., 2010). Así mismo, la hipótesis dopaminérgica, aparte de proponer esta hiperactividad DA en las áreas mesolímbicas, también propone una hipoactividad DA en la corteza prefrontal que explicaría parte de los síntomas negativos de los pacientes esquizofrénicos (Brisch et al., 2014; Szczypiński & Gola, 2018).

Como puede observarse en la Figura 3, toda la etiología de la esquizofrenia no puede explicarse únicamente teniendo en cuenta un solo neurotransmisor. En la década de los 80, se descubrieron los antipsicóticos de segunda generación (*SGA*, por sus siglas en inglés, *Second-Generation Antipsychotics*) que actúan en otros receptores, aparte del receptor de dopamina D2.

1.4.2 Hipótesis glutamatérgica

El glutamato es el principal neurotransmisor excitatorio del cerebro, cuya función es crucial durante las etapas de la niñez temprana del neurodesarrollo (McCutcheon et al., 2020b). El glutamato actúa tanto en receptores ionotrópicos (receptores de N-metil-D-aspartato o NMDA, receptores de kainato y receptores del ácido α-amino-3-hidroxi-5-metil-4-isoxazolpropiónico o AMPA) como en receptores metabotrópicos (mGlu1-8). La hipótesis glutamatérgica se centra en el hecho que un exceso de glutamato liberado por regiones corticales hacia regiones subcorticales provoca un aumento de dopamina en el sistema mesolímbico, como puede observarse en la **Figura 3** (McCutcheon et al., 2020a, 2020b; Stahl, 2018; Stępnicki et al., 2018; Uno & Coyle, 2019). Se basa también en la evidencia de que fármacos antagonistas glutamatérgicos como la fenciclidina (PCP), la ketamina o la dizocilpina (MK801) producen síntomas positivos, negativos y cognitivos similares a los observados en esquizofrenia, en contraposición de los agonistas dopaminérgicos (anfetamina o apomorfina) que sólo replican la sintomatología positiva de la enfermedad (Brisch et al., 2014; McCutcheon et al., 2020a; Neill et al., 2014). Así mismo, también hay evidencias en estudios *post mortem* que muestran alteraciones estructurales en las neuronas glutamatérgicas en la arborización dendrítica, la densidad de las espinas y la expresión de sinaptofisina a través de regiones frontales y temporales (Harrison, 2000; McCutcheon et al., 2020a).

1.4.3 Hipótesis serotoninérgica

La serotonina es un neurotransmisor que media entre transmisiones excitatorias e inhibitorias mediante la modulación de la liberación de distintos neurotransmisores como el GABA, la dopamina, el glutamato, etcétera (López-Giménez & González-Maeso, 2018; Stępnicki et al., 2018). Los receptores de serotonina están compuestos por receptores metabotrópicos (5-$HT_{1,2,4-7}$) e ionotrópicos (5-HT_3)(Meltzer et al., 2003). La hipótesis serotoninérgica de la esquizofrenia surge de la observación de los efectos de los antagonistas de la serotonina como son la dietilamida de ácido lisérgico (LSD) o el 2,5-dimetoxi-4-iodoamfetamina (DOI), así como de los antipsicóticos atípicos que son ligandos de receptores dopaminérgicos y serotoninérgicos (como son la clozapina, la risperidona o la olanzapina) (Aghajanian & Marek, 2000; López-Giménez & González-Maeso, 2018; Meltzer et al., 2003). Se sugirió que la combinación de un alto nivel de antagonismo del receptor 5-HT_{2A} combinado con el antagonismo del receptor de dopamina D_2 podría ser la clave farmacológica para los antipsicóticos. Este hecho diferencia los antipsicóticos de primera generación, o típicos, respecto a los de segunda generación, o atípicos (Meltzer et al., 1989; Stępnicki et al., 2018). Como se observa en la **Figura 3**, la hiperactivación de los receptores serotoninérgicos en las neuronas glutamatérgicas de la corteza prefrontal provoca la sobreactivación de estas, activando la vía mesolímbica de la dopamina. Así mismo, los estudios *post mortem* también han mostrado un incremento de expresión de los receptores 5-HT_{1A} y un aumento de los receptores 5-HT_{2A} en la corteza frontal, así como alteraciones en el expresión del transporte de serotonina (Harrison, 2000; Quednow et al., 2010).

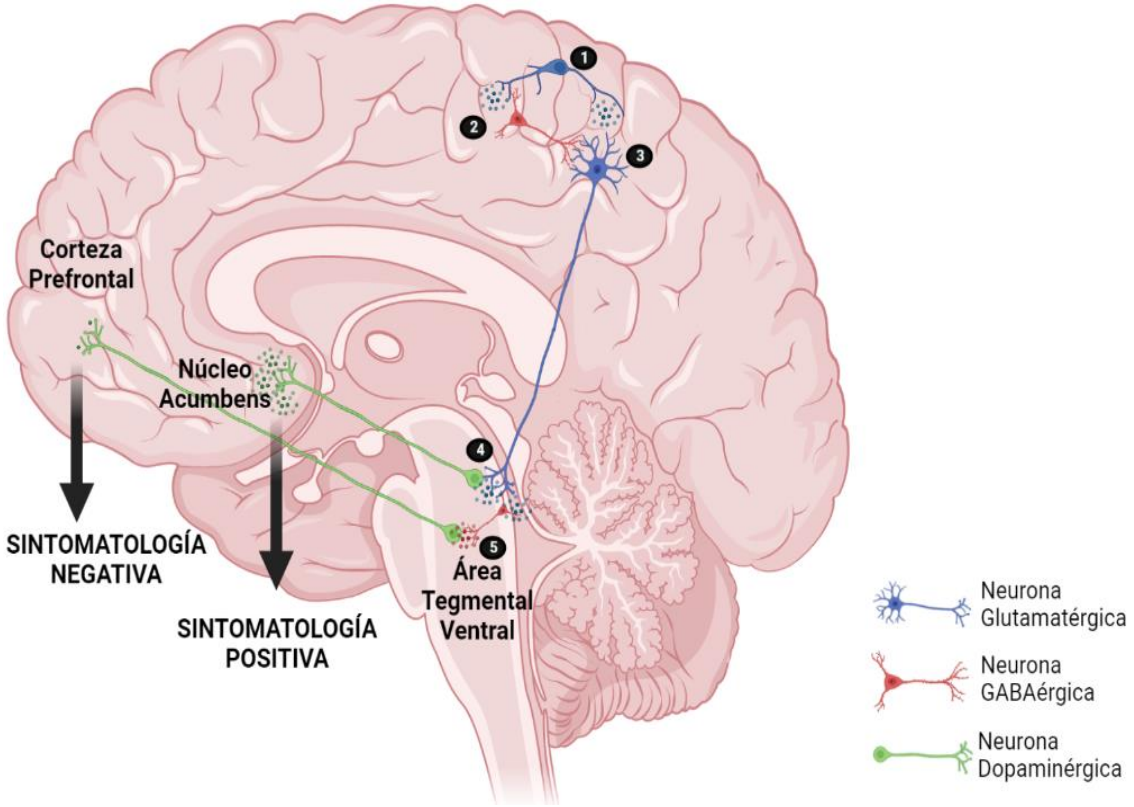


Figura 3: Esquema de la interacción entre las hipótesis etiológicas de la esquizofrenia. (Adaptado de Stahl et al., 2018 y Elert et al., 2014). **(1)** Cuando hay una activación de las neuronas glutamatérgicas corticales, estas liberan glutamato. Activando, por un lado, otras neuronas glutamatérgicas con proyección a áreas subcorticales y, por otro lado, a interneuronas GABAérgicas. **(2)** En la esquizofrenia, los receptores glutamatérgicos NMDA de las neuronas GABAérgicas son anormales evitando la unión del neurotransmisor. Esta hipoactivación de la interneurona provoca que no haya liberación de GABA evitando la inhibición de la neurona glutamatérgica. **(3)** La neurona glutamatérgica activada inerva hacia la región tegmental ventral activando, por un lado, **(4)** neuronas dopaminérgicas que proyectan en el núcleo accumbens (en el estriado ventral). Esto provoca una hiperactivación dopaminérgica, resultando en un exceso de dopamina que es responsable de los síntomas positivos (p. ej. alucinaciones y delirios). **(5)** Por otro lado, la neurona glutamatérgica activa interneuronas GABAérgicas que inhiben neuronas dopaminérgicas que, a su vez, proyectan hacia la corteza prefrontal. Esto provoca un déficit de dopamina en estas zonas resultando en la aparición de la sintomatología negativa (p. ej. ansiedad y asilamiento social).

1.4.4 Hipótesis GABAérgica

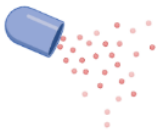
El ácido gamma-aminobutírico o GABA es el principal neurotransmisor inhibitorio del sistema nervioso central (*CNS,* por sus siglas en inglés, *Central Nervous System*), que juega un papel principal en las interneuronas GABAérgicas (Stępnicki et al., 2018). La perturbación de señalización de GABA, que se observa en pacientes con esquizofrenia, causa un desequilibro entre la excitación e inhibición(balance excitatorio/inhibitorio) de la corteza prefrontal, influyendo en mecanismos de percepción, memoria de trabajo y cognición, dando lugar a la sintomatología cognitiva (Blum & Mann, 2002; Pratt et al., 2008; Stępnicki et al., 2018). GABA se une tanto a receptores ionotrópicos (GABA-A) como metabotrópicos (GABA-B). La hipótesis GABAérgica se relaciona con la hipótesis glutamatérgica en cuanto que la disfunción de las interneuronas GABAérgicas de la corteza prefrontal aumenta la activación del área tegmental ventral, como se observa en

la Figura 3 (Stahl, 2018). También se ha observado una disminución de la síntesis de GABA en la subpoblación de neuronas GABAérgicas que contienen parvalbúmina (PV) y una alteración de estas en pacientes con esquizofrenia (Benes et al., 2007).

Cabe destacar el rol de las interneuronas GABAérgicas en las redes generadores de ritmos cerebrales y la sincronía de las oscilaciones neuronales, ya que estas son cruciales para procesos cognitivos (Stępnicki et al., 2018). Además, las alteraciones en la señalización de GABA pueden provocar cambios en la sincronía neuronal, cambios en las oscilaciones gamma que se han asociado a déficits en memoria de trabajo (Uhlhaas & Singer, 2010). Los estudios *post mortem* apoyan la teoría GABAérgica sobre un cambio en la transmisión de GABA en pacientes con esquizofrenia (Harrison, 2000; Stępnicki et al., 2018).

Tanto los descubrimientos hallados con estudios *post mortem,* como otras evidencias de los roles y funciones de los principales neurotransmisores implicados en las distintas hipótesis pueden encontrarse resumidas en la Figura 3 y la Tabla 2.

Tabla 2: Resumen de los principales neurotransmisores implicados en la esquizofrenia y los principales descubrimientos *post mortem* junto con otras evidencias relacionados con su expresión. Adaptado de Harrison (2000).

	Dopamina	**Glutamato**	**Serotonina**	**GABA**
Principales descubrimientos *post mortem* 	Aumento en la densidad de receptores D_2 Disminución de la inervación de DA en la corteza Aumento de la unión al receptor tipo D_4 Alteraciones en el receptor D_3	Marcadores presinápticos disminuidos Disminución de la expresión del receptor AMPA y kainato en el HPC Cambios menores en las subunidades del receptor NMDA en la CF Fibras de glutamato alteradas en la corteza cingulada	Disminución en la expresión del receptor 5-HT_{2A} en la CF Aumento en los receptores 5-HT_{1A} en la CF Mayor afinidad por el transporte de 5-HT Funciones tróficas y del desarrollo de la 5-HT	Disminución en la densidad de terminales GABAérgicos en la CF Aumento de la unión del receptor $GABA_A$ en áreas límbicas Expresión alterada de las subunidades del receptor $GABA_A$ en la CF Disminución en la expresión de la descarboxilasa del ácido glutámico en la CF Densidad alterada de las células GABAérgicas en el cingulado
Otras evidencias 	Los agentes liberadores de DA producen psicosis La mayoria de los antipsicóticos son antagonistas D_2 Aumento en la liberación de DA en el estriado *in vivo*	Los antagonistas del receptor NMDA producen psicosis similares a la esquizofrenia Funciones de los receptores NMDA en el desarrollo y la neurotoxicidad Los agonistas parciales del receptor NMDA tienen algunos beneficios terapéuticos	Los agonistas del 5-HT_2 (p. ej. LSD) son psicomiméticos Polimorfismos del receptor 5-HT_2 están asociados a la esquizofrenia y a la respuesta a la clozapina Los antipsicóticos atípicos tienen alta afinidad por varios receptores 5-HT	Rol del GABA en el estrés y la neurotoxicidad

$D_{2\text{-}4}$=Receptor de dopamina; DA=Dopamina; AMPA=α-amino-3-hidroxi-5metilo-4-isoxazolprpiónico; HPC=Hipocampo; CF=Corteza frontal; NMDA=N-metil-D-aspartato; 5-HT=Serotonina; LSD= Dietilamida de ácido lisérgico; GABA=Ácido gamma-aminobutírico.

1.5 Tratamientos farmacológicos

Debido al escaso conocimiento de las causas de la esquizofrenia, el primer tratamiento centrado en reducir los síntomas de la enfermedad son los antipsicóticos (Horacek et al., 2006; Remington et al., 2016; Stępnicki et al., 2018). El objetivo de los tratamientos es reducir el sufrimiento de los pacientes y mejorar las funciones cognitivas y sociales. No obstante, pese a que los síntomas positivos (alucinaciones y delirios) están bastante controlados con los tratamientos farmacológicos actuales, la efectividad de estos se ve reducida cuando hablamos de la sintomatología negativa y cognitiva (McCutcheon, et al., 2020b; Stępnicki et al., 2018).

1.5.1 Antipsicóticos típicos o antipsicóticos de primera generación

Los antipsicóticos típicos o, también llamados, antipsicóticos de primera generación (*FGA,* por sus siglas en inglés, *First-Generation Antipsychotics*) fueron descubiertos en la década de los 50 cuando el químico francés Paul Charpentier sintetizó por vez primera la clorpromazina cuando buscaba un nuevo antihistamínico (Stępnicki et al., 2018). La característica principal de los antipsicóticos típicos es que actúan bloqueando el receptor de DA D_2 en el cerebro, disminuyendo así los síntomas positivos de la esquizofrenia (McCutcheon, et al., 2020b; Meltzer et al., 1989; Wu et al., 2021; Zamanpoor, 2020). No obstante, no presentan una selectividad especifica por ninguna vía dopaminérgica en el SNC y, por tanto, pueden provocar efectos secundarios tales como: síntomas extrapiramidales, incremento de la prolactina (mediante la vía tuberoinfundibular), distonía, parkinsonismo o acatisia, que ocurren como consecuencia del bloqueo del receptor de D_2 en la vía nigroestriada. También, elevadas dosis de antipsicóticos típicos pueden inducir síntomas negativos y cognitivos como resultado de bloquear el receptor D_2 de la vía mesocortical (Brisch et al., 2014; Stępnicki et al., 2018; Volk & Lewis, 2015). El haloperidol, la clorpromazina y la flufenazina, son ejemplos de FGA.

1.5.2 Antipsicóticos atípicos o antipsicóticos de segunda generación

Casi cuarenta años después de la introducción de la clorpromazina, la FDA aprobó el uso de la clozapina (CLZ) como tratamiento para la esquizofrenia (Stępnicki et al., 2018; Zamanpoor, 2020). La CLZ es pues el primer antipsicótico atípico o antipsicótico de segunda generación (SGA). Generalmente, los SGA muestran mayor afinidad para el bloqueo del receptor 5-HT_{2A} que del receptor de dopamina D_2 en comparación con los FGA (Brisch et al., 2014; Khan et al., 2015; McCutcheon et al., 2020b). También, el antagonismo del receptor D_2 es menor en los SGA comparados con los FGA, provocando así menores efectos secundarios extrapiramidales. Estas características podrían estar relacionas con un mayor efecto terapéutico de los SGA sobre los síntomas negativos y cognitivos comparados con los FGA (Huang & Song, 2019; Mauri et al., 2014; Meltzer & Massey, 2011b; Miyamoto et al., 2004), aunque este extremo es aún controvertido. Los

principales antipsicóticos atípicos son: la clozapina, la olanzapina, la quetiapina, la risperidona, la paliperidona, la molindona y la ziprasidona.

Cabe destacar que actualmente el uso de los antipsicóticos atípicos no se reduce al tratamiento de la esquizofrenia, sino que también se utilizan para tratar otras trastornos como el trastorno bipolar, algunos trastornos de ansiedad, el trastorno obsesivo compulsivo, la agitación asociada a demencia y trastornos del espectro autista (Maher & Theodore, 2012). Aunque los SGA hayan supuesto una mejora en cuanto el tratamiento de la esquizofrenia y provoquen menos síntomas extrapiramidales que los FGA, también presentan limitaciones y efectos secundarios, como pueden ser: sedación, agranulocitosis, excesiva salivación, hipotensión, taquicardia o aumento de peso (Stępnicki et al., 2018).

Tanto la eficacia de unión con algunos receptores como el mecanismo de acción de los antipsicóticos atípicos principales pueden observarse en las Tablas 3-4.

1.5.3 Antipsicóticos de tercera generación

Un grupo nuevo de fármacos ha sido aprobado por la FDA en los últimos años, los antipsicóticos de tercera generación (Maher & Theodore, 2012). Comprendidos por el aripiprazol, el brexpiprazol y la cariprazina (entre otros), este grupo se ha separado de los SGA debido a su mecanismo de acción sobre los receptores de DA. A diferencia de los FGA y de los SGA, los antipsicóticos de tercera generación no son antagonistas del receptor para la dopamina $D_{2,}$, sino que son agonistas parciales (Mailman & Murthy, 2010; Stępnicki et al., 2018; Tuplin & Holahan, 2017). Pese a su afectación sobre otros receptores, se pueden entender como "estabilizadores de la dopamina" (Mailman & Murthy, 2010). En consecuencia, cuando hay una situación con un elevado nivel de DA extracelular (por ejemplo, en las áreas mesolímbicas implicadas en la sintomatología positiva), el agonismo parcial compite con la DA causando un antagonismo parcial, ofreciendo efectos clínicos positivos. Sin embargo, en situaciones donde hay una concentración baja de DA extracelular (por ejemplo, en circuitos dopaminérgicos involucrados en memoria de trabajo), ocupan receptores adicionales causando una activación parcial (Mailman & Murthy, 2010; Stip & Tourjman, 2010). Los antipsicóticos de tercera generación tampoco están exentos de provocar efectos secundarios, aunque inducen un menor riesgo de sufrir alteraciones metabólicas y endocrinas, síntomas extrapiramidales y disquinesia tardía (Stip & Tourjman, 2010).

Usando de ejemplo de estos antipsicóticos al aripiprazol, podemos observar su eficacia con la unión de distintos receptores, así como el efecto que provoca sobre ellos en las Tablas 3-4.

1.5.4 Oxitocina

Crecientes evidencias muestran las limitaciones de los SGA para constituir un tratamiento altamente efectivo para aliviar los síntomas negativos de la esquizofrenia, además de acarrear problemas relacionados con los efectos secundarios. Pese a que se han realizado varios enfoques farmacológicos e intervenciones de orientación psicosocial para mejorar la cognición social de los pacientes, la existencia de un tratamiento eficaz para los déficits sociales sigue, hoy en día, siendo un obstáculo clínico (Goh et al., 2021). A raíz de esta situación, se ha centrado el foco en otros tratamientos farmacológicos con menores efectos secundarios que los antipsicóticos.

Un ejemplo de ello es el neuropéptido, conservado entre especies, oxitocina (OXT). La OXT, que se sintetiza en el núcleo paraventricular y supraóptico del hipotálamo (Emiliano et al., 2006; Lopatina et al., 2012), ha sido propuesto como un antipsicótico natural alternativo tanto en algunos modelos animales como en pacientes humanos (Feifel et al., 2010; Goh et al., 2021; Kohli et al., 2019). Algunos estudios también han mostrado como la administración de OXT modula el comportamiento social y mejora los déficits sociales presentes en pacientes con esquizofrenia (Shilling & Feifel, 2016; Zimmermann et al., 2016). Además, se ha observado también que bajos niveles de OXT en el plasma sanguíneo pueden estar relacionados con varias afectaciones psiquiátricas, como los trastornos del espectro autista, la depresión y la esquizofrenia (Cochran et al., 2013).

Tabla 3: Eficacia de unión de los principales antipsicóticos a distintos receptores. Adaptado de Miyamoto et al., (2014) y Mauri et al., (2014).

	1º GENERACIÓN	2º GENERACIÓN				3º GENERACIÓN
RECEPTOR	**HAL**	**CLZ**	**RIS**	**OLA**	**ZPR**	**ARI**
D_1	+	+	+	++	+	-
D_2	++++	+	+++	++	+++	++++
D_3	+++	+	++	+	++	+++
D_4	+++	++	-	++	++	+++
5-HT_{1A}	-	-	-	-	+++	-
5-HT_{1D}	-	-	+	-	+++	-
5-HT_{2A}	+	+++	++++	+++	++++	+
5-HT_{2C}	-	++	++	++	++++	-
5-HT_6	-	++	-	++	+	-
5-HT_7	-	++	+++	-	++	-
α_1	+++	+++	+++	++	++	+++
α_2	-	+	++	+	-	-
H_1	-	+++	-	+++	-	-
m_1	-	++++	-	+++	-	-
DA tran		++		++		
NA tran		+		++	++	
5-HT tran					++	

-=Mínimo; +=Bajo; ++=Moderado; +++=Alto; ++++=Muy Alto; HAL=Haloperidol; CLZ=Clozapina; RIS=Risperidona; OLA=Olanzapina; ZPR=Ziprasidona; ARI=Aripiprazol; D1-4=Subtipo de receptores de dopamina; 5-HT1-7=Subtipos de receptores de serotonina; α1-2=Subtipos de receptores adrenérgicos; H1=Subtipo de receptor histaminérgico; m1=Subtipo de receptor colinérgico; DA=Dopamina; NA=Noradrenalina; 5-HT=Serotonina; Tran=Transportador.

Tabla 4: Mecanismos de acción propuestos para la acción de los antipsicóticos atípicos. Modificado de Mauri et al., (2014).

MODULACIÓN DOPAMINÉRGICA	
Bloqueo de D_2	El bloqueo del 65-75% conduce a la efectividad con seguridad preservada (menores efectos extrapiramidales e hiperprolactinemia).
Bloqueo de D_1	Se localiza en el PFC, mostrando efecto terapéutico sobre síntomas negativos y cognitivos. D1 modula la actividad de D2. El antagonismo solo de D1 no provoca efectos antipsicóticos.
Bloqueo de D_4	Disminuye la catalepsia e induce la liberación de DA en los ganglios basales y en el PFC. El antagonismo solo de D4 no tiene efecto antipsicótico.

Bloqueo de D_2/D_3	Antagonismo preferencial de los autorreceptores inhibitorios D2. Mayor liberación de DA en el estriado (menor riesgo de efectos extrapiramidales) y en el área neocortical (mejora de los síntomas negativos y cognitivos).
Bloqueo de D_3 en la corteza temporal	Conduce a estereoselectividad y es eficaz contra los síntomas positivos sin inducir síntomas extrapiramidales.
Rápida disociación de D_2	Una menor duración de la unión del fármaco a D2 es suficiente para un efecto antipsicótico pero insuficiente para inducir síntomas extrapiramidales e hiperprolactinemia (es el caso de la clozapina y la quetiapina).
Agonismo parcial D_2	En el caso del aripiprazol, un 30-40% del agonismo intrínseco del receptor D2 en relación con el alto bloqueo D2 provoca un efecto antipsicótico con bajo riesgo de síntomas extrapiramidales e hiperprolactinemia.
MODULACIÓN SEROTONINÉRGICA	
Bloqueo del 5-HT_{2A}	Los receptores 5-HT2A integran entradas corticales y subcorticales. Su antagonismo bloquea los efectos de los antagonistas NMDA e induce liberación dopaminérgica en el estriado y en la zona neocortical.
Agonismo 5-HT_{1A}	Induce la liberación de dopamina en el estriado y en el neocórtex (su acción es análoga al bloqueo de 5-HT2A), y también en estructuras límbicas.
Bloqueo del 5-HT_{2C}	Induce la liberación de dopamina en el neocórtex.
Modulación del 5-$HT_{1A,2A,2C}$	La modulación por sí misma no tiene efectos antipsicóticos.
INDUCCIÓN DE LA PLASTICIDAD	
La fosforilación de receptores, la potenciación de glutamato/glicina y la inducción de factores de crecimiento neural (como el NGF y el BDNF) provocan el refuerzo de la actividad del receptor NMDA y el desarrollo de nuevas sinapsis o de su remodelación.	

D1-4=Subtipos de receptores de dopamina; 5-HT1A,2A,2C=Subtipos de receptores de serotonina; PFC=Corteza prefrontal; DA=Dopamina; NMDA=N-metil-D-aspartato; 5-HT=Serotonina; NGF=Factor de crecimiento nervioso; BDNF=Factor neurotrófico derivado del cerebro.

1.5.5 Tratamientos No Farmacológicos

Teniendo en cuenta el fuerte impacto que puede tener el ambiente sobre el individuo y la interacción de este con la expresión génica, se han descrito y utilizado varios tratamientos no farmacológicos para tratar distintos trastornos psiquiátricos y alteraciones en la conducta.

En primer lugar, la terapia psicológica tiene una gran influencia en el sistema de valores de una persona, en sus sistema emocional y conductual, y en la estructura y función del cerebro (Barsaglini et al., 2014). Se ha demostrado que terapias psicológicas

cognitivo-conductuales como la terapia de remediación cognitiva (*CRT,* por sus siglas en inglés, *Cognitive Remediation Therapy*) tienen un impacto positivo sobre el cerebro de los pacientes con trastornos psiquiátricos. Estudios de neuroimagen funcional han encontrado evidencias de que la esquizofrenia está asociada con interacciones anormales entre regiones de una red que incluye la corteza prefrontal dorsolateral, la corteza cingulada anterior, y las regiones temporal lateral y medial (Barsaglini et al., 2014; Thomas et al., 2014). Se ha demostrado que la intervención no farmacológica de CRT provoca un incremento en la actividad frontal de las regiones implicadas con la atención y la memoria de trabajo. Los estudios sugieren que CRT en pacientes con esquizofrenia tiende a la normalización de los patrones de activación en áreas fronto-corticales, provocando una reducción en la hipofrontalidad presente antes del tratamiento (Barsaglini et al., 2014; Haut et al., 2010; Thomas et al., 2014).

Otro tipo de tratamiento no farmacológico, que se ha demostrado que tiene efectos positivos en varias condiciones pediátricas, es el masaje terapéutico en recién nacidos. Varios estudios sugieren que los posibles mecanismos subyacentes de la terapia de masaje incluyen una mayor activación del nervio vago y una disminución de las hormonas del estrés (Fernández-Teruel 2022; Field, 2016, 2019; Pepino & Mezzacappa, 2015). El incremento de la actividad del nervio vago, a su vez, conduce a una disminución del cortisol y, también, a un aumento y actividad de las células NK (por sus siglas en inglés, *Natural Killer*). Una función inmunológica mejorada conduce pues a una mejor salud. El aumento de la actividad parasimpática también provoca un aumento de 5-HT (químico natural contra el dolor) y una disminución de la sustancia P (causante de dolor) (Field, 2019; Pepino & Mezzacappa, 2015).

En relación con la terapia de masaje, en roedores se ha implementado el tratamiento de estimulación neonatal (*NH,* por sus siglas en inglés, *Neonatal Handling* (Raineki et al., 2014). Seymour Levine fue el primero en proponer y demostrar efectos a largo plazo provocados por el NH (Levine et al., 1956). Demostró que un período breve (menos de 15 minutos) de separación de las crías de su madre, dejando individualmente cada cría en una caja con una toallita de papel en su base, y aplicando un masaje con la mano desnuda por la parte dorsal del animal durante los PND1-21, producía una reducción permanente de la respuesta de estrés del eje Hipofisario-Pituitario-Adrenal (HPA) evaluada durante la adultez (Fernández-Teruel et al., 2002; Levine et al., 1956; Raineki et al., 2014; Todeschin et al., 2009). Además, entre otros muchos efectos psico- y neuro-biológicos, también se ha demostrado que el NH provoca mejoras en la ejecución de tareas cognitivas (memoria de trabajo, memoria espacial y memoria de referencia) y atencionales durante la adultez y previene la neurodegeneración hipocámpica relacionada con la edad (Cañete et al., 2015; Fernández-Teruel et al., 2022; Raineki et al., 2014; Río-Álamos et al., 2017,2019). Todo ello pone de manifiesto que cierto tipo de intervenciones ambientales muy tempranas pueden afectar aspectos/procesos

psicológicos y neurobiológicos que se han relacionado con la esquizofrenia, y de ahí el interés del tratamiento de NH en el presente trabajo.

Hay tres componentes fundamentales en el tratamiento de NH: (1) aislamiento/separación de la madre, ya que parece que hay un incremento de cuidados maternos hacia las crías cuando estas vuelvan a la caja-nido. (2) La exposición a un ambiente novedoso y el aislamiento de pocos minutos también tiene un papel fundamental en los efectos a largo plazo (Macrì et al., 2008; Raineki et al., 2014). (3) Otro componente es la estimulación táctil por parte de los investigadores y las investigadoras, que también es capaz de producir efectos positivos a largo plazo (Field, 2016).

El tratamiento de NH tendrá un papel relevante en algunos estudios de esta Tesis Doctoral.

1.6 Neuroanatomía

Las alteraciones moleculares y celulares de la esquizofrenia están muy alejadas de los síntomas conductuales y del curso del trastorno. Para poder superar esta brecha, la neuroimagen y la neurociencia de sistemas han demostrado ser una herramienta útil.

Mediante la neuroimagen estructural se ha observado que el volumen de los cerebros, medidos mediante imágenes de resonancia magnética (MRI), es anormal en pacientes con esquizofrenia (Kasai et al., 2002). Reducciones tanto de materia blanca como materia gris también se han observado en pacientes con esquizofrenia, comparado con pacientes sanos (Kasai et al., 2002; Khan et al., 2015). El volumen de los ventrículos también se ha visto aumentado, así como cambios en el grosor cortical (principalmente disminuido) (Goghari et al., 2010). Se ha observado una disminución de los volúmenes en varias regiones del cerebro como: la ínsula bilateral, la corteza cingulada anterior, el hipocampo (HPC), el tálamo y la amígdala (AMY). Esta disminución cortical se va generalizando provocando empeoramientos de las funciones cognitivas (Goghari et al., 2010; Khan et al., 2015).

El uso de la neuroimagen ha proveído de datos que muestran una alteración en la activación de las estructuras corticales y subcorticales en pacientes con esquizofrenia (Kasai et al., 2002). Los síntomas positivos se caracterizan por un procesamiento relevante anormal y la aparición de las alucinaciones. El procesamiento relevante depende de señales de neuronas dopaminérgicas del mesencéfalo que se proyectan hacia el cuerpo estriado ventral y la corteza prefrontal dorsolateral. Los estudios de neuroimagen molecular de captación de DA han revelado un aumento de DA en el estriado de pacientes con esquizofrenia y, esta se ha relacionado con la actividad de la corteza prefrontal (Khan et al., 2015; McCutcheon et al., 2020a, 2020b). También se han encontrado aumentos en la actividad del mesencéfalo tanto en pacientes con esquizofrenia como con alto riesgo de padecerla (Kasai et al., 2002).

Por lo que se refiere a la sintomatología negativa, también se han observado alteraciones. El estriado ventral está reducido en pacientes esquizofrénicos en comparación con sujetos sanos (Goghari et al., 2010). De igual forma, en cuanto a la regulación emocional mediada por la activación de la amígdala también se ha observado una reducción de su activación (Fallon et al., 2003). Relacionado con esto, las conexiones amígdala y corteza cerebral también están reducidas en pacientes con esquizofrenia. Las regiones del llamado "cerebro social" (en particular, la corteza prefrontal, la unión temporoparietal y la amígdala) se ha demostrado que son anormales en personas con esquizofrenia y podrían estar relacionados con déficits prominentes en la cognición social que ocurren en el trastorno (Goghari et al., 2010; Rasetti et al., 2009).

Finalmente, las alteraciones en las funciones cognitivas que muestran los pacientes con esquizofrenia se han relacionado con alteraciones a nivel de sistemas. Por ejemplo, los sustratos neurales de los procesos ejecutivos implicados en memoria de trabajo se han visto alterados cuantitativamente en el PFC dorsolateral, el córtex cingulado anterior parte rostral y el lóbulo parietal inferior (Khan et al., 2015; Uddin, 2014).

1.7 Modelos animales

Los modelos animales de trastornos psiquiátricos heterogéneos y complejos tienen un valor enorme para investigar las bases neurobiológicas en investigación preclínica o básica (Jones et al., 2011).

Para que un modelo animal sea útil para comprender en mayor profundidad un trastorno o enfermedad debe cumplir varios criterios de validez: (1) la validez aparente, según la cual deberá mostrar síntomas similares a los observados en la condición clínica; (2) la validez de constructo, según la cual deberá replicar los fundamentos neurobiológicos teóricos y patológicos que se correspondan con el trastorno y, finalmente, (3) la validez predictiva, según la cual el modelo deberá mostrar la respuesta farmacológica esperada, o la ausencia de ella, para los tratamientos conocidos así como posibles nuevas terapias aún por desarrollar (Jones et al., 2011; Winship et al., 2019). Algunos de los fenotipos que comúnmente se asocian con los modelos animales de esquizofrenia pueden observarse en la Tabla 5.

Los modelos animales existentes pueden clasificarse en cuatro categorías: modelos del neurodesarrollo, modelos farmacológicos, modelos de lesiones y modelos genéticos (Jones et al., 2011; Vales & Holubova, 2021; Wang et al., 2021; Winship et al., 2019).

Tabla 5: Fenotipos comunes asociados con modelos animales de esquizofrenia.
Adaptado de Winship et al., (2019).

CATEGORÍA	MEDIDA	DESCRIPCIÓN
CONDUCTUAL		
	Actividad locomotora (síntomas positivos)	Se calcula el movimiento en respuesta a un ambiente nuevo, al estrés o a los efectos farmacológicos (potenciadores de DA, antagonistas del receptor NMDA).
	Preferencia por la sacarosa (síntomas negativos)	Se mide la preferencia de agua azucarada sobre el agua no azucarada.
	Interacción social (síntomas negativos y cognitivos)	Se mide el tiempo transcurrido cerca o en contacto directo con animales no familiares o poco familiares.
	Inhibición latente (procesos atencionales, síntomas cognitivos)	Un procedimiento de condicionamiento clásico por el cual los estímulos previamente no reforzados son más lentos (menos eficientes) en generar una respuesta condicionada que los estímulos nuevos.
	Memoria (síntomas cognitivos)	Pruebas que miden aspectos de la memoria de trabajo, memoria declarativa, memoria de reconocimiento, (dominios visuales, espaciales y olfatorios) y condicionamiento.
	Razonamiento y resolución de problemas (síntomas cognitivos)	Pruebas de flexibilidad conductual en respuesta a entornos dinámicos que incluyen cambios de escenarios y aprendizaje nuevo.
	Inhibición prepulso (déficits sensoriomotores)	Normalmente se observa una reducción en la respuesta de sobresalto acústico si el estímulo de sobresalto

		está precedido en el tiempo por un pulso de baja intensidad.
	Comportamiento similar a la ansiedad	Patrones de exploración en entornos cómo el laberinto en cruz elevado, campo abierto o la caja claro-oscuro.
MORFOLOGÍA/FISIOLOGÍA		
	Ventrículos laterales agrandados	Mediciones del volumen de los ventrículos laterales utilizando técnicas de imagen *in vivo* (resonancia magnética) o *post mortem*.
	Morfología de las áreas corticales, estriada, talámica y límbica	Incluye medidas de tamaño/volumen de las áreas del cerebro, cantidad de neuronas, cambios dendríticos y proteínas sinápticas.
	Mielinización de las células gliales	Evaluación de la extensión de la mielinización, particularmente de los axones corticales. Cambios en la glía.
	Integridad de la matriz extracelular	Alteraciones en estructuras como redes perineurales y proteínas, incluida la reelina.
	Alteraciones relacionadas con neurotransmisores	Efectos sobre la DA, glutamato, 5-HT, GABA (especialmente las interneuronas que contienen parvalbúmina), glicina D-serina y esteroides.
	Patrones de actividad cerebral	La actividad neuronal, neuronas de DA, actividad y patrones de actividad oscilatoria se miden utilizando técnicas de electrofisiología *in vitro* e *in vivo*.

Pese a que las anteriores medidas se relacionan con la esquizofrenia, se debe tener en cuenta que ninguna es exclusiva para el trastorno. GABA=Ácido gamma-aminobutírico; 5-HT=Serotonina; NMDA=N-metil-D-aspartato.

1.7.1 Modelos del neurodesarrollo

Aunque los síntomas de la esquizofrenia aparecen típicamente en la adolescencia o la adultez temprana, hay una amplia línea de investigación que relaciona la esquizofrenia con factores ocurridos durante el período prenatal (ver apartado "*1.3.2 Factores ambientales*"). Los modelos del neurodesarrollo implican manipulaciones ambientales y/o administración de fármacos durante los períodos perinatales y/o postnatales. Un ejemplo de manipulación ambiental es el modelo animal por aislamiento (Jones et al., 2011; Oliveras et al., 2016; Winship et al., 2019). El aislamiento social postnatal, o durante el periodo juvenil, se manifiesta con cambios en la conducta cuando el animal ha alcanzado la adultez, provocando hiperactividad locomotora, mayor respuesta emocional a la novedad, déficits en el filtraje atencional, deterioro cognitivo y un aumento en la ansiedad y la agresión (Winship et al., 2019). La administración de la neurotoxina metilazoximetanol (MAM) en madres gestantes, también es un modelo del neurodesarrollo. MAM es un agente metilador del ADN que afecta específicamente la proliferación de neuroblastos o produciendo efectos teratogénicos en órganos periféricos. La administración de MAM se traduce en cambios neuroanatómicos, electrofisiológicos y modificaciones en la conducta (Wang et al., 2021). Otra estrategia consiste en inducir activación inmunitaria maternal mediante la inyección de agentes víricos como, por ejemplo, el ácido poliinosínico-policitidílico (Poly I:C). Esto provoca cambios en las interneuronas de PV y en sus redes perineurales (estructura de la matriz extracelular implicada en la plasticidad sináptica y en su estructura) en el PFC y el HPC, provocando déficits cognitivos (Jones et al., 2011; Wang et al., 2021; Winship et al., 2019).

Estos modelos permiten investigar los procesos que conducen al desarrollo de la esquizofrenia durante la fase prodrómica de la enfermedad y el desarrollo potencial de tratamientos profilácticos para prevenir la progresión a la psicosis.

1.7.2 Modelos farmacológicos

Los fármacos más comunes usados para mimetizar síntomas de la esquizofrenia son, o bien, potenciadores de la DA (p. ej. anfetamina, cocaína y apomorfina) o antagonistas no competitivos del receptor del glutamato NMDA (p. ej. PCP, ketamina o el MK801) (Jones et al., 2011).

La administración de anfetamina provoca hiperactividad (sintomatología positiva) pero no induce déficits de interacción social (sintomatología negativa), y solo es capaz de afectar tareas dependientes de la función cortical (sintomatología cognitiva) dejando el HPC prácticamente inalterado (Jones et al., 2011).

Por otro lado, siguiendo la hipótesis glutamatérgica de la esquizofrenia se han propuesto fármacos antagonistas no competitivos del receptor de glutamato NMDA como modelos capaces de provocar aspectos de la sintomatología negativa, sintomatología cognitiva y la sintomatología positiva de la esquizofrenia. Un antagonista del receptor del NMDA ampliamente usado es el MK801 (dizocilpina). Gran variedad de

estudios avalan el efecto del MK801 sobre la conducta social, provocando aislamiento social en roedores, una disminución del consumo de una solución acuosa placentera como la sacarosa (anhedonia), déficits en PPI, deterioros cognitivos e hiperactividad (Neill et al., 2014; Rung et al., 2005ª,2005b; Vardigan et al., 2010).

1.7.3 Modelos de lesiones

Mediante la lesión del HPC ventral en ratas en el PND7 con una inyección local de excitotoxinas (como es el ácido iboténico) se consigue un modelo animal de esquizofrenia por lesión (Winship et al., 2019). Este proceso causa anormalidades en la conducta que emergen durante la pubertad y que comprometen la integridad de la corteza prefrontal medial y del núcleo accumbens, ambas regiones densamente inervadas por estructuras subcorticales (Tseng et al., 2009). Una característica del procedimiento es que el tiempo de la lesión es crucial (antes del PND14), y que debe ser bilateral para provocar la totalidad de los cambios (Jones et al., 2011). La lesión del HPC ventral provoca que a medida que el animal va creciendo va mostrando conductas que simulan sintomatología de esquizofrenia, mostrando déficits de memoria espacial y aprendizaje (PND25), déficits en interacción social y un aumento de la agresividad (PND35), hiperactividad locomotora y un aumento de la sensibilidad para los agonistas de DA y los antagonistas de NMDA (PND 56) (Jones et al., 2011; Lipska et al., 2003)

1.7.4 Modelos genéticos

La creación de modelos de ratas modificadas genéticamente ha evolucionado recientemente. Los primeros modelos genéticos se basaban en la crianza selectiva y en la alteración de secuencias génicas de forma "espontánea" (Ellenbroek & Karl, 2016). Con la llegada de la ingeniería genética y el uso de las células madre embrionarias se han podido crear modelos basados en la eliminación (*knock-out* en inglés), en la reducción de la expresión de un solo gen (*knock-down* en inglés) y en la inserción o sustitución de secuencias de ADN específicas (*knock-in* en inglés) (Ellenbroek & Karl, 2016; Jones et al., 2011; Río et al., 2014).

En relación con los modelos animales creados por ingeniería genética encontramos varios ejemplos. El modelo *DISC1* (por sus siglas en inglés, *disrupted-in-schizophrenia 1*) se basa en la pérdida total o parcial del gen que expresa para una proteína sináptica expresada durante las etapas tempranas del desarrollo e involucrada en el desarrollo pre- y postnatal de las neuronas, provocando morfologías del cerebro consistentes con las observadas en la esquizofrenia (Winship et al., 2019). Otro modelo es el modelo de rata *knock-out* para el gen transportador de la serotonina (*SERT*¸ por sus siglas en inglés, *Serotonin Transporter*), que provoca un incremento de la ansiedad y síntomas parecidos a la depresión, un incremento de la actividad locomotora, déficits en conducta social y déficits en funciones cognitivas (Ellenbroek & Karl, 2016). El modelo de rata *knock-out*

para el gen de la neuregulina 1 (NRG1) es otro modelo válido de esquizofrenia capaz de producir reducción en la PPI, detrimento en memoria de trabajo, así como una reducción en la densidad de espinas dendríticas en neuronas piramidales del HPC (Wang et al., 2021; Winship et al., 2019).

En cuanto a los modelos de cruces selectivos encontramos modelos como la rata Brattleboro, que se basa en una mutación de un solo gen que provoca déficits en la liberación de vasopresina. Esta mutación provoca déficits en la inhibición prepulso, en la inhibición latente, en la habituación de la respuesta de sobresalto, hiperactividad locomotora y provoca así mismo cambios dopaminérgicos (Ellenbroek & Karl, 2016; Río et al., 2014). Otro modelo es la línea de ratas seleccionadas por Alta o Baja inhibición prepulso (PPI) de la respuesta acústica de sobresalto, un endofenotipo para estudiar los mecanismos neurobiológicos de la esquizofrenia (Río et al., 2014). Otro modelo parte de la selección por la susceptibilidad a la apomorfina, estableciendo así las ratas APO-SUS (susceptibles) y las APO-UNSUS (no susceptible)(Ellenbroek & Karl, 2016; Río et al., 2014). Esta selección provoca diferencias conductuales en pruebas de residente-intruso, en respuestas inducidas por novedad y en estereotipias inducidas por agonistas de DA, además de diferencias en el sistema endocrino e inmunológico (Ellenbroek et al., 1995; Ellenbroek & Karl, 2016).

Un modelo animal genético por cruce selectivo que usamos desde hace años en el grupo de investigación son las cepas *Roman* de alta (*RHA*¸ por sus siglas en inglés, *Roman High Avoidance*) y baja (*RLA*, por sus siglas en inglés, *Roman Low Avoidance*) evitación. Como constituyen el modelo central de la presente Disertación Doctoral, ampliaremos su descripción en el subapartado siguiente.

1.7.4.1 Ratas *Roman*

En la década de los 60 se establecieron en Roma las cepas de ratas *Roman* mediante la selección bidireccional y el cruce selectivo de ratas Wistar que mostraban altas (RHA) o bajas (RLA) frecuencias de evitación activa en dos sentidos en la caja de lanzadera (o "*shuttle box*") (Ellenbroek & Karl, 2016; Fernández-Teruel et al., 2021; Río et al., 2014). La colonia se fue desplazando y creando nuevas sublíneas de ambas cepas en ciudades (entre otras) como Birmingham (Reino Unido), Zúrich (Suiza), Cagliari (Italia) y Génova (Suiza), así como en Barcelona (España) en el año 1995 (Fernández-Teruel et al., 2021; Giorgi et al., 2019).

Comparada con la cepa RHA y otras cepas estándar de ratas de laboratorio (p. ej. Sprague Dawley, Wistar, entre otras), las ratas RLA presentan un perfil conductual de mayor ansiedad, miedo y una mayor respuesta hormonal al estrés (Díaz-Morán et al., 2012; Fernández-Teruel et al., 2021) cuando se encuentran en situaciones de amenazas incondicionadas o condicionadas, así como signos de frustración potenciados en tareas de devaluación de la recompensa (Fernández-Teruel et al., 2021; Giorgi et al., 2019; Papini et al., 2015).

En cambio, las ratas RHA presentan un amplio perfil conductual y neurobiológico relacionado con la adicción y la esquizofrenia. Se han observado perfiles conductuales relacionados con la sintomatología positiva, como puede ser un incremento de la actividad locomotora en respuesta a la novedad y una mayor sensibilidad a fármacos psicomiméticos/psicoestimulantes (Giorgi et al., 2007). Relacionado con la sintomatología negativa las RHA muestran una empeorada conducta maternal, una mayor conducta agresiva en ciertas situaciones y una menor conducta social "no agresiva" (Fernández-Teruel et al., 2021; Oliveras et al., 2022; Sampedro-Viana et al., 2021; presente Disertación Doctoral). El modelo también muestra alteraciones relacionadas con la sintomatología cognitiva, como déficits en memoria de trabajo y de flexibilidad conductual/cognitiva en el laberinto acuático de Morris (MWM) y el laberinto acuático de "Hebb-Williams", déficits en filtraje atencional (PPI), déficits en inhibición latente y otros déficits cognitivos generales (Fernández-Teruel et al., 2021; Giorgi et al., 2019).

A nivel neuroanatómico la cepa de ratas RHA muestra una disminución en volumen y actividad neuronal en áreas como el PFC, el HPC, la AMY (Fernández-Teruel et al., 2021; Meyza et al., 2009; Río-Álamos et al., 2019; Tapias-Espinosa et al., 2019). Presentan también volumen de los ventrículos laterales aumentado y una reducción de activación en neuronas GABAérgicas positivas de parvalbumina (PV) (Tapias-Espinosa et al., 2023). A nivel neuroquímico, las RHA muestran un incremento del tono funcional en la vía dopaminérgica mesolímbica, mayor densidad de receptores de DA $D_{1\text{-}3}$, menor densidad de receptores D_2, un aumento de niveles de 5-HT en la corteza, incremento de receptores 5-HT1A y 5-HT2A, así como incrementos de expresión de BDNF, NRG1 y Homer 1 (Elfving et al., 2019; Fernández-Teruel et al., 2021; Giorgi et al., 2019).

En la **Figura 4** se muestra un resumen de las alteraciones principales del modelo RHA de esquizofrenia y de su adaptación a la psico- y neuro-biología del trastorno a nivel molecular, celular y conductual.

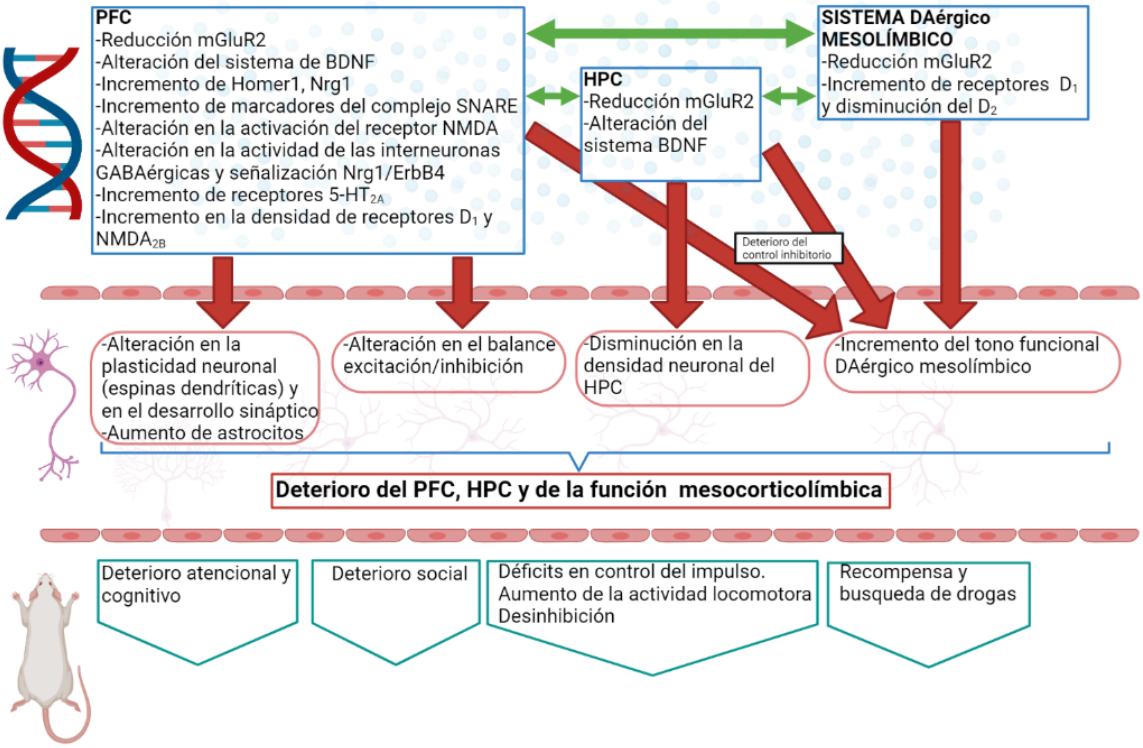


Figura 4: Esquema de las principales alteraciones moleculares, celulares y conductuales del modelo de rata RHA para el estudio de la esquizofrenia. Adaptado de Fernández-Teruel et al., (2021). Las flechas verdes indican "interacción" mientras que las flechas rojas muestran "alteración" o "deterioro". PFC=Corteza prefrontal; HPC=Hipocampo; DA=Dopamina; mGluR2=Receptor metabotrópico de glutamato 2; BDNF= Factor neurotrófico derivado del cerebro; Nrg1=Neuregulina 1; SNARE= Receptores de proteínas de fijación soluble del factor sensible a la N-etilmaleimida; NMDA= N-metil-D-aspartato; ErbB4= Proteína tirosina quinasa receptora; 5-HT= Serotonina.

02 /
Objetivos

El objetivo general de la presente disertación doctoral es profundizar en la validación del perfil conductual, neurobiológico y farmacológico de un modelo animal genético de rasgos relevantes de la esquizofrenia, las ratas RHA, centrando su foco en la asocialidad o falta de interés social. Pese a que el modelo se ha validado en varias ocasiones con respecto a los aspectos de sintomatología positiva y cognitiva del trastorno, existe hasta ahora una casi total carencia de información respecto a la sintomatología negativa.

A partir de este objetivo general se han planteado cinco estudios con objetivos específicos:

Estudio 1: *Decreased social interaction in the RHA rat model of schizophrenia-relevant features: Modulation by neonatal handling.*

- Establecer un procedimiento de interacción social útil y capaz de reproducir aspectos de asociabilidad o alteración en la motivación por la conducta social en las ratas RHA (vs. las RLA).
- Establecer si la cepa de ratas RHA muestran una disminución de interacción social en comparación la cepa RLA.
- Explorar si el tratamiento de estimulación neonatal (NH) puede mejorar el comportamiento social de las RHA en comparación con las RLA.

Estudio 2: *Neonatal handling treatment increases the c-Fos expression in some social brain areas.*

- Establecer diferencias en expresión de *c-Fos* en las cepas de ratas *Roman* después de la prueba de interacción social en distintas regiones del "cerebro social".
- Observar diferencias regionales de expresión de *c-Fos* tras una experiencia de interacción social debidas al tratamiento ambiental de estimulación neonatal (NH) y en función de la cepa de ratas.

Estudio 3: *Social preference in Roman rats: Age and sex variations relevance for modeling negative schizophrenia-like features.*

- Dilucidar si los déficits de interacción social presentados por la cepa de ratas *Roman* RHA están también presentes durante su adolescencia.
- Observar si hay diferencias entre ambos sexos de ambas cepas de ratas en cuanto a la conducta social en diferentes etapas del neurodesarrollo

Estudio 4: *Atypical antipsychotics attenuate MK801-induced social withdrawal in the RHA rats: a model of schizophrenia-relevant features.*

- Evaluar el efecto de la administración de un antagonista de los receptores glutamatérgicos NMDA, el MK801, en la conducta social y actividad locomotora de las cepas de ratas *Roman* RHA y RLA.

- Estudiar si distintos antipsicóticos atípicos (como son la clozapina, la ziprasidona y el aripiprazol) pueden atenuar o revertir las alteraciones conductuales producidas por el MK801 y si su efecto depende de la cepa de ratas *Roman*.

Estudio 5: *Effects of oxytocin on dizocilpine-induced impairment of social behavior and hyperactivity in the Roman rat strains*

- Evaluar los efectos de la oxitocina sobre los efectos perjudiciales de la administración de MK801 en la conducta social y actividad locomotora de las ratas *Roman* RHA y RLA.

03 /
Resultados

ESTUDIO 1

Decreased social interaction in the RHA rat model of schizophrenia-relevant features: Modulation by neonatal handling

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Artículo publicado

Behavioural Processes; IF(2021)=1.729

https://doi.org/10.1016/j.beproc.2021.104397

ABSTRACT

The Roman-Low (RLA) and High-Avoidance (RHA) rat strains have been bidirectionally selected and bred, respectively, for extremely poor vs. rapid acquisition of the two-way active avoidance task. Over 50 years of selective breeding have led to two strains displaying many differential specific phenotypes. While RLAs display anxious-related behaviours, RHA rats show impulsivity, and schizophrenia-like positive and cognitive symptoms or phenotypes. Neonatal handling (NH) is an environmental treatment with long-lasting anxiolytic-like and anti-stress effects. NH also reduces symptoms related to schizophrenia, such as pre-pulse inhibition (PPI) impairment and latent inhibition (LI) deficits, and improves spatial working memory and cognitive flexibility.

The present work was aimed at exploring whether RHAs also display negative schizophrenia-like symptoms (or phenotypes), such as lowered preference for social interaction (i.e. asociality), and whether NH would reduce these deficits. To this aim, we evaluated naïve inbred RHA and RLA rats in a social interaction (SI) test after either long- or short-term habituation to the testing set up (studies 1–2). In Study 3 we tested untreated and NH-treated RHA and RLA rats in novel object exploration (NOE) and SI tests. Compared with RHAs, RLA rats displayed increased anxiety-related behaviours in the NOE (i.e. higher behavioural inhibition, lesser exploration of the novel object) and SI (i.e. higher levels of self-grooming) tests which were dramatically reduced by NH treatment, thus supporting the long-lasting anxiolytic-like effect of NH. Remarkably, RHA rats showed decreased social preference in the SI test compared with RLAs, evidencing that RHAs would present a relative asociality, which is thought to model some negative symptomatology (i.e. social withdrawal) of schizophrenia. NH increased absolute levels of social behaviour in both strains, but with a more marked effect in RHA rats, especially in the first 5 min of the SI test. Thus, it is hypothesized that, apart from its effects on anxiety-related behaviours, NH might have long-lasting positive effects on behavioural and neurobiological processes that are impaired in schizophrenia.

Keywords: RHA and RLA rats, Schizophrenia, Social interaction, Asociality, Neonatal handling

1. INTRODUCTION

Schizophrenia is a chronic and disabling mental disorder that affects approximately 1% of the population worldwide. The multifactorial etiology, the heterogeneity of symptomatology and time course of the disease are major challenges for its study and the development of effective drug treatments (Millan et al., 2016). Schizophrenia's symptoms can be grouped into three clusters: (i) positive symptoms (understood as distortion or exacerbation of normal functions) include delusions, hallucinations, thought disorder and conceptual disorganization; (ii) negative symptoms (understood as deficits of normal function) include emotional blunting, anhedonia, social withdrawal, apathy, poverty of thought and speech, and (iii) cognitive symptoms, which refer to impaired working memory and other cognitive/executive functions, and attention dysfunctions (Millan et al., 2016; del Río et al., 2014).

Valid animal models are an essential tool for the research on the neurobiological bases of schizophrenia (and other psychopathologies) as well as to discover and evaluate new potentially effective drug treatments (Giorgi et al., 2019; Hayward et al., 2016; Jones et al., 2011; del Río et al., 2014). The Roman Rat lines were developed in Rome in the 1960's trough bidirectional selective breeding of Wistar rats for their rapid (Roman high-avoidance, RHA) or extremely poor acquisition (Roman low-avoidance, RLA) of the two-way active avoidance task (Bignami, 1965). Over 50 years of research have corroborated the existence of important phenotypic differences between RLA and RHA rats in anxiety/fearfulness traits, coping styles and stress sensitivity. Thus, RLA rats display increased levels of anxiety in both unconditioned and conditioned tests compared with their RHA counterparts (Giorgi et al., 2019). Additionally, compared to RHAs, RLA rats also exhibit elevated levels of hormonal (ACTH, corticosterone, and prolactin) responses to stress (Giorgi et al., 2019; Río-Álamos et al., 2015, 2017, 2019).

Importantly, the two Roman rat strains diverge in other phenotypic traits. Thus, compared with RLAs, RHA rats are characterized by (i) impulsive behaviour in the 5-choice serial reaction time test (5-CSRTT) and delay discounting task; (ii) novelty-, psychostimulant- and NMDA-antagonist-induced hyperactivity; (iii) vulnerability to psychostimulant-induced locomotor and mesolimbic dopaminergic sensitization; and (iv) a sensation-seeking behavioral profile and enhanced vulnerability to drug abuse (Giorgi et al., 2019). These phenotypes could be explained by (or are thought to be related to) alterations in the mesolimbic dopaminergic system, as well as in central 5-HT and glutamatergic function in the RHA strain (reviewed by Giorgi et al., 2019). On the basis of the above mentioned and other findings, RHA rats have been proposed as a potential animal model for schizophrenia-relevant features (reviewed by Giorgi et al., 2019). Impairments in pre-pulse inhibition (PPI) and latent inhibition (LI) are two characteristic symptoms of schizophrenia which evidence deficits in sensorimotor gating and attention. Compared with RLAs, Sprague-Dawley and genetically heterogeneous (outbred) rats, RHA

rats also display deficits in PPI of the acoustic startle response and show impairments of LI of both the fear-potentiated startle response and in the two-way active avoidance task (for review see Giorgi et al., 2019). Moreover, RHA rats also present other schizophrenia-like cognitive symptoms, such as deficits in working memory, in spatial reference learning/memory, and in cognitive flexibility in the Morris water maze (Giorgi et al., 2019). Collectively, the above phenotypic profiles make the RHA rat strain a model of schizophrenia–linked traïts having face validity for this disorder (or for its symptoms).

The schizophrenia-like phenotypes or symptoms of the RHA strain are accompanied by neural correlates which evidence the construct validity of the model. Thus, RHA rats display an enhanced mesolimbic dopaminergic functional tone and a lower density of dopamine D2 receptors in the nigrostriatal pathway, linked to increases in locomotor activity and disruptions in PPI. Importantly, recent findings showed that mGlu2 and 5HT2A receptors would act as a unique receptor complex (5HT2A/mGlu2) in the PFC, which is targeted by hallucinogenic and pro-psychotic drugs and is disrupted in untreated schizophrenia patients (González-Maeso et al., 2008). RHA rats display a dramatic reduction of mGlu2 receptors in the PFC, HPC and striatum, and increased 5HT2A receptor density in the PFC (Klein et al., 2014). Structural magnetic resonance imaging reveals that, compared with their RLA counterparts, RHA rats present reductions of medial PFC and HPC volume and dramatically enlarged lateral ventricles (Río-Álamos et al., 2019; Tapias-Espinosa et al., 2019). Moreover, RHA rats also display deficits of neural activity in PFC and HPC linked to PPI and various novelty tests (Meyza et al., 2009; Tapias-Espinosa et al., 2019). Altogether, neurochemical, neuroanatomical and neurofunctional studies reveal some characteristics of RHA rats that have also been found in schizophrenic patients (reviewed by Giorgi et al., 2019), thus conferring construct validity to the RHA model.

Neonatal handling (NH) is an environmental treatment administered to pups usually during the first 3 weeks of life. Research on NH has accumulated evidence that the treatment has long-lasting anxiolytic-like and anti-stress effects and, more specifically, it improves the ability to cope with stressful situations in rats, including the Roman rats (e.g. Fernández-Teruel et al., 2002, 1997; Levine, 1956; Raineki et al., 2014; Río-Álamos et al., 2019, 2017, 2015).

Besides its anxiolytic-like effects, NH affects attentional/cognitive functions that are altered in schizophrenia. Río-Álamos et al. (2019) found that RHA rats submitted to NH displayed improved PPI, showed better working memory and a more efficient cognitive flexibility in a reversal spatial learning task, which replicate and extend the findings of Escorihuela et al. (1995) and Aguilar et al. (2002; see reviews by Fernández-Teruel et al., 2002, 1997; Raineki et al., 2014). In addition, it is noteworthy that NH treatment has been found to reduce apomorphine-induced disruption of PPI in male Wistar rats (Pryce et al.,

2001) as well as to improve LI in rats of both sexes (Peters et al., 1991; Pryce et al., 2001; Shalev et al., 1998). There are also reports on positive enduring effects of NH on neurotrophic factor signalling and plastic neuronal processes in the HPC, which may have relevance given that neural/functional alterations in this region are thought to play a relevant role in schizophrenia (e.g. Fernández-Teruel et al., 2002, 1997; Giorgi et al., 2019; Katsouli et al., 2014; Río-Álamos et al., 2019; Tang and Zou, 2002; Tapias-Espinosa et al., 2019; Zou et al., 2001).

To sum up, NH of rats is able to long-lastingly improve attentional/ cognitive functions and some behavioural responses related to the positive symptom spectrum of schizophrenia. However, there is a paucity of studies devoted to assessing the enduring effects of NH on processes or behavioural responses related to the negative symptom spectrum of the disease, such as asociality (del Río et al., 2014). Therefore, the general aims of the present work were (i) to establish whether the RHA rats show less social interaction (SI) behaviour than their RLA counterparts, and (ii) to explore whether NH can improve social behaviour in RHA vs RLA rats, using a SI test that models asociality. One specific objective was to establish a procedure of SI including previous habituation to the experimental set up that could show sensitivity to strain differences and that could be of practical application to test the maximum number of rats per week. For this reason, we started by carrying out a procedure involving four habituation sessions before the SI test (study 1), similar to Gururajan et al's (2012) procedure, and then we carried out a second study (study 2) in which only one habituation session was performed before the SI test. Since study 2 showed strain differences similar to those found in study 1, for practical reasons the procedure used in the former was selected to carry out study 3.

2. MATERIALS AND METHODS

2.1 Animals

A total of 232 naïve male rats from the RHA and RLA strains were used. All rats came from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine (School of Medicine, Autonomous University of Barcelona, Spain), since 1996. The rats from each experimental group of the different studies came from 10 to 15 different litters and all were experimentally naïve. They were approximately 3–4 months old at the beginning of the experiments (except otherwise indicated) with an average weight of 409 ± 30 g (mean ± SD). Animals were housed in same-sexed pairs in standard macrolon cages (50 × 25 × 14 cm) and maintained under a 12:12 h light-dark cycle (lights on at 08:00 a. m), with controlled temperature (22 ± 2 ◦C) and humidity (50–70 %). They had food and water available ad libitum.
All testing was carried out between 09:00 and 14:00 h. All procedures were carried out in accordance with the Spanish Legislation (Royal Decree 53/2013, 1st February 2013) and the current regulation related to "Protection of Animals used for Scientific Purposes" established by the European Union (2010/63/UE, 22 September 2010).

2.2 Behavioural procedures: social interaction (SI) test, novel object exploration (NOE) test and neonatal handling (NH) treatment.

2.2.1 Habituation sessions and social interaction test

Social interaction (SI) set-up test was adapted from Gururajan et al. (2012). Two acrylic boxes (65 ×23 × 20 cm) were placed in front of each other at 12 cm, to prevent physical contact between the animals, in a red-lit room. Each box had two holes at the ends of 3 cm diameter. The hole facing to the other box was named as "social hole", while the opposite (distal) hole was named as "non-social hole" (see Drawing 1). All the procedure was recorded by a camera placed on the ceiling and connected to a TV outside the experimental room.

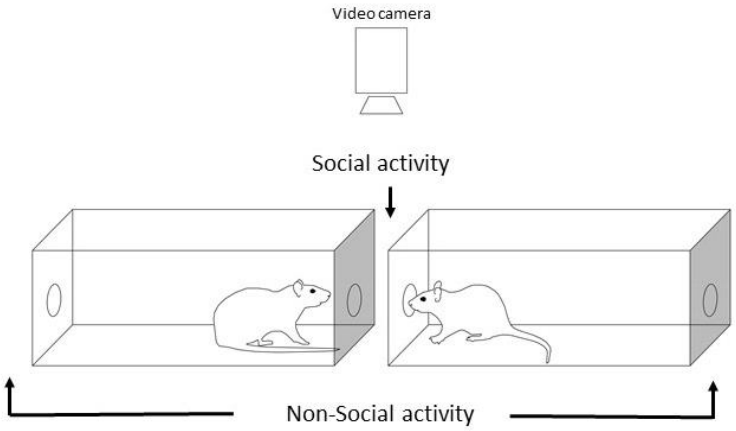
In Study 1 the animals underwent four 30-min habituation sessions in 4 consecutive days in order to acclimatize the rats to the testing room and set-up. The SI test was performed 24 h after the last habituation session (the 5th day). For the first three habituation sessions the animals were individually placed in a standard home-cage (containing clean sawdust) to acclimatize to the testing room. For the fourth habituation day, all the holes of the social interaction set-up were covered with tape and a barrier was placed between the two boxes to limit the exploration activity of the next box. A pair of non-familiar weight-matched animals of the same strain were placed into the testing boxes (one rat in each box) for the 4th 30-min habituation period. For SI assessment, in the 5th day, the holes were opened, and two weight-matched non-familiar rats of the same strain were placed (one rat in each box) into the set-up for a 15- min test. The SI

boxes were cleaned with a 70 % ethanol solution and dried with a paper towel between every pair of animals.

For Study 2, due to our objective of testing as many rats per week as possible, we tested a modification of the above habituation procedure. Thus, in this study animals were submitted to only one habituation session (30 min) to both the testing room and the social interaction set-up 24 h before the SI test. This habituation session was equivalent to the 4th day of the procedure used in Study 1. Similarly, the boxes were cleaned with a 70 % ethanol solution and dried with a paper towel between every pair of animals.

In the SI test, the time spent exploring (i.e. nose poking) the social hole (Social Time) and the non-social hole (Non-Social Time) were measured by two trained observers blind to strain and treatment conditions (reliability between their measurements, $r > 0.97$) and, from these measures, the percentage (%) time spent in the social hole (Social preference) was calculated according to the following formula:

%Social Preference = Social Time (Social Time + Non-Social Time) × 100



Drawing 1. Schematic drawing of the experimental SI set up. Modified from Deak et al., (2009) and Gururajan et al., (2012).

The time spent self-grooming (Grooming Time) and the total number of crossings (Crossings) were also recorded (each box was divided into three equal squares by lines painted on the floor to measure horizontal activity –crossings-).

2.2.2. Novel object exploration (NOE) test

A Novel Object Exploration (NOE) test was carried out to test anxiety-related behaviour (i.e. behavioural inhibition) in response to novelty and to compare with previous results (Río-Álamos et al., 2015). At PND 60, food was removed from the home cage (except 4 food pellets that were left in every cage), and each cage was pulled 20 cm

from the rack. One hour later, the novel object (graphite pencil Staedtler Noris, HB nº2) was vertically introduced in the cage through the grid until it touched the cage bedding. In order to differentiate both animals of the same home-cage, the animals were marked with a permanent-coloured mark on their tails one week before the NOE test. One well-trained observer measured the latency to the first exploration, defined as the first contact with the pencil for each rat (NOE Latency Time), and the total time of exploration, defined as the total time spent exploring the pencil by touching it with the forepaws and/or the nose (NOE Exploration Time). The measures were scored in a 3-min test by one observer standing approximately 50 cm away from the cage front (see Río-Álamos et al., 2015).

2.2.3. Neonatal handling (NH) treatment

Neonatal handling (NH) treatment was administered twice a day (at 9:30 h and 17:30 h) between postnatal days (PND) 1 21. At the beginning of each handling session, the mother was first removed from the litter. Then, the pups were placed individually in plastic cages (35 × 15 × 25 cm) lined with a paper towel, placed in a room with a temperature of 22 ± 2 ◦C, and were gently stroked for 3–4 s at 0, 4 and 8 min with bare hands. After these 8 min of individual separation from the mother, the pups were returned to their home cages with their mother and the rest of the litter. Non-handled rats ("C" groups), which were placed in the same colony room as NH rats, were left relatively undisturbed, except for checking for births and sexing the pups at PND 2–3 and for the regular home cage cleaning once a week. Every day some investigator came into the colony room to check for environmental conditions, and food and water were checked twice a week. After weaning (PND 21), rats were housed in same-sexed pairs of the same experimental group in standard macrolon cages (50 ×25 ×14 cm) and remained in the same colony room until the end of the experiments.

2.3 Studies and experiment design

2.3.1 Study 1: basal social interaction after four habituation sessions

In Study 1, the animals (RHA, n = 12; RLA, n = 12) were tested in the SI test in the 5th day, after 4 habituation sessions, as described above (section 2.2.1).

2.3.2 Study 2: basal social interaction after one habituation session

Study 2 followed an identical SI testing procedure, but only one habituation session (30 min, 24 h before SI testing; see section 2.3.1 above) was administered. Number of animals per group was RHA, n = 40, and RLA, n = 38.

2.3.3. Study 3: NOE and SI after neonatal handling treatment

A total number of 62 RHA (Control, n = 34; NH n = 28) and 68 RLA (Control, n = 34; NH n = 34) were evaluated in the NOE test at PND 60. Randomly selected rats from each of these groups (RHA, Control, n = 14; NH n = 12; and RLA, Control, n = 14; NH, n = 12) underwent SI testing 24 h after a single habituation session (as in Study 2). The remaining

rats (not tested for SI) were used in other experiments. See experimental overview in Figure 1.

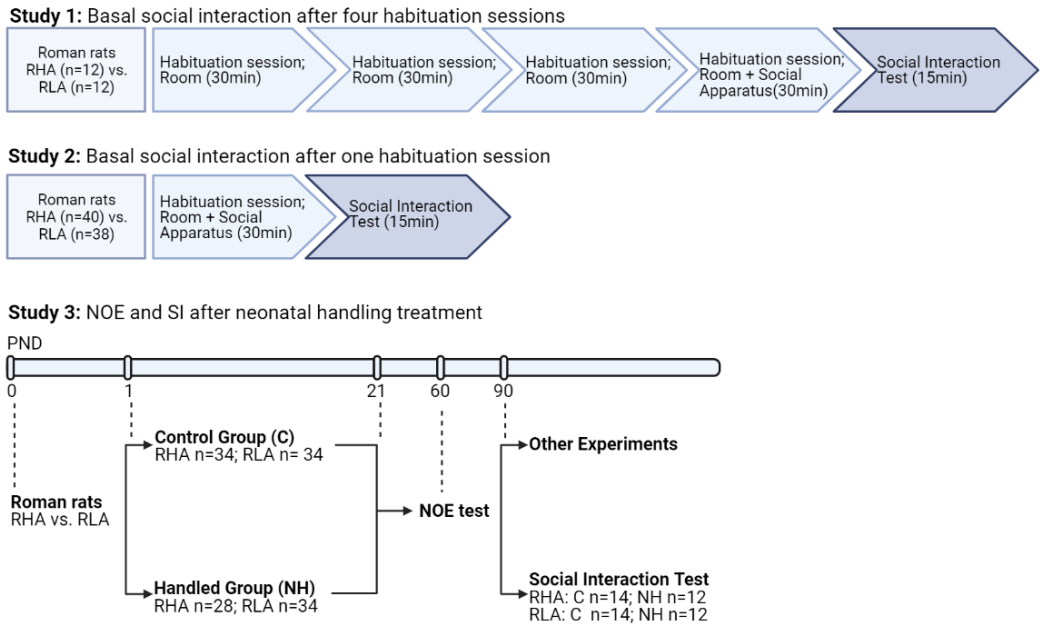


Figure 1. Overview of the experimental schedules (see "Materials and methods"). Study 1: Social interaction after four habituation sessions. RHA (n = 12) and RLA (n = 12) rats underwent three habituation sessions to the testing room (30 min each) plus one habituation session to the testing room + social apparatus (30 min). Finally, on the 5th day (24 h after the last habituation session) the animals underwent the SI test. Study 2: Social interaction after one habituation session. RHA (n = 40) and RLA (n = 38) underwent a single habituation session to the testing room + social apparatus (30 min) and, 24 h later, were evaluated in the SI test (15 min). Study 3: NOE and SI after neonatal handling treatment. A total of n = 62 RHA and n = 68 RLA rats were divided into two groups (Control group; Neonatal handling group, NH). The Control groups (RHA n = 34, RLA n = 34) were left relatively undisturbed during PND1 to 21 (but see "Material and methods" for details of the Control condition). The NH groups (RHA n = 28, RLA n = 34) received the neonatal handling treatment during PND1-21 (see "Materials and methods"). After weaning, at PND21, all animals were housed in pairs of the same sex, strain and condition. At PND60 all the animals underwent the Novel Object Exploration (NOE) test. At PND90, randomly-selected rats from each group of the NOE test were evaluated in the SI test (RHA, C n = 14 and NH n = 12; RLA, C n = 14 and NH n = 12).

2.4. Statistical analyses

Statistical analyses were performed using the "Statistical package for social science" (SPSS, version 17). The data from studies 1 and 2 were evaluated with one-way ANOVAs and those from Study 3 with two-way ANOVAs (2 "strain" x 2 "treatment" levels). If the two-way ANOVA revealed "Strain", "Treatment" or "Strain x Treatment" effects in Study 3, post hoc pair wise contrasts were performed with Duncan's multiple range tests to explore differences between groups, since we hypothesized that: (i) RHA rats would show less preference for social interaction than their RLA counterparts; (ii) NH groups would show higher levels of social behaviour and/or social preference, as well as higher levels of exploration of the novel object in the NOE test and lower levels of self-grooming

behaviour; and. (iii) NH effects on social preference would be more marked in those rats with relative social deficits. Significance level was set at $p \leq 0.05$.

3. RESULTS

3.1 SI under different habituation conditions in RHA and RLA rats (studies 1-2)

In Study 1, involving four habituation sessions, ANOVA of SI test data showed a "Strain" effect ($F(1, 22) = 10.5$, $p = 0.004$) on non-social time, as RHA rats spent more time exploring the non-social hole than RLAs (**Figure 2a**) while no differences were observed in social time (**Figure 2b**). RHA rats also exhibited less (%) social preference ($F(1,22) = 13.6$, $p = 0.001$; **Figure 2c**) and higher activity levels ($F(1, 22) = 84.9$, $p < 0.001$; **Figure 2d**) than RLA rats. RLA also exhibited more grooming time ($F(1,22) = 72.9$, $p < 0.001$; **Figure 2e**) than RHA rats. Similarly, in Study 2, involving only one habituation session, ANOVA again revealed that RHA rats spent more time exploring the non-social hole ($F(1, 76) = 7.0$, $p = 0.01$; **Figure 3a**), lesser (%) social preference ($F(1, 76) = 12.6$, $p = 0.001$; **Figure 3c**) and higher locomotor activity levels ($F(1, 76) = 87.7$, $p = 0.001$; **Figure 3d**) than their RLA counterparts. As in Study 1, no strain-dependent differences were found regarding social time (in Study 2, **Figure 2b**), and a strain difference was observed in time spent grooming (RLA > RHA; $F(1,76) = 25.6$, $p < 0.001$; **Figure 3e**).

3.2 Study 3: NOE test

In the NOE test, ANOVA showed "Strain" ($F(1,126) = 51.4$, $p < 0.001$; **Figure 4a**), "NH" ($F(1,126) = 51.0$, $p < 0.001$; **Figure 4a**) and "Strain x NH" ($F(1,126) = 46.4$, $p < 0.001$; **Figure 4a**) effects on "NOE latency". Post hoc Duncan's test revealed that NH reduced the latency to the first exploration of the novel object in RLA rats to the level of RHA rats (see Duncan's test in **Figure 4a**). ANOVA on "Time" spent exploring the novel object revealed "NH" ($F(1,126) = 211.5$, $p < 0.001$; **Figure 4b**) and "Strain x NH" ($F(1,126) = 106.5$, $p < 0.001$; **Figure 4b**) effects, as NH treatment globally increased scores of this variable in both rat strains, but this effect was especially marked in RLA rats (see post hoc Duncan's test in **Figure 4b**).

3.3. Study 3: SI test

In the SI test, ANOVA revealed a "NH" effect on total "Social time" ($F(1,48) = 28.5$, $p < 0.001$; **Figure 5b**) as well as on "Social time 0-5 min" ($F(1,48) = 11.2$, $p = 0.002$; **Figure 5e**; see also Duncan's tests in **Figure 5b and 5e**), which are explained by the global increase in these measures in both strains after NH. Regarding non-social time, ANOVA showed a "Strain" effect (RHA >RLA; $F(1,48) = 6.1$, $p = 0.018$; **Figure 5a**) which was not present during the first 5 min of the SI test (**Figure 5d**).

There was also a "strain" effect on (%)social preference (RLA > RHA; $F(1,48) = 8.4$, $p = 0.006$; **Figure 5c**) and, most interestingly, ANOVA revealed a "Strain x NH" effect on that measure during the first 5 min of SI testing ($F(1,48) = 5.4$, $p = 0.025$; **Figure 5f**), clearly indicating that NH increased (%) social preference in RHA (but not RLA) rats at the beginning of the SI test (see Duncan's test in **Figure 5f**).

Concerning locomotor activity (i.e. "crossings", Figure 6a), ANOVA showed a "Strain" effect ($F(1,48) = 74.7$, $p < 0.001$), as RHA rats displayed many more crossings than their RLA counterparts (Figure 6a). There were "Strain" ($F(1,48) = 12.5$, $p = 0.001$; Figure 6b) and NH ($F(1,48) = 12.6$, $p = 0.001$; Figure 6b) effects on grooming and, most importantly, a "Strain x NH" interaction ($F(1,48) = 8.1$, $p = 0.007$; Figure 6b) indicating that the NH-decreasing effect on that measure was very significant (only) in RLA rats (see Duncan's test in Figure 6b).

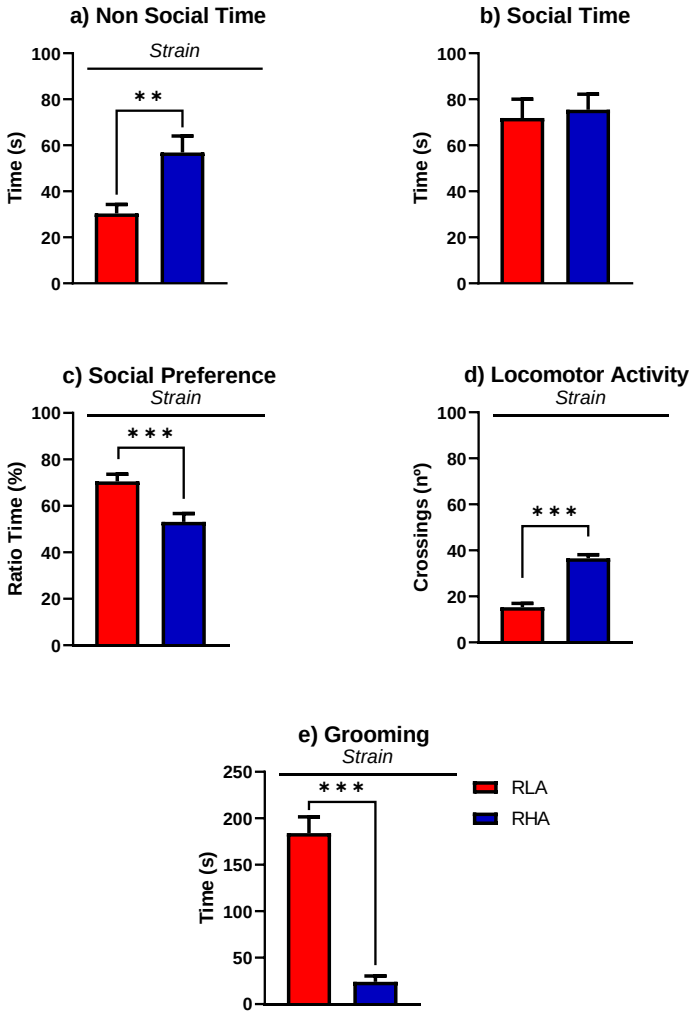


Figure 2. Social interaction differences between the Roman rat strains after a long habituation period (4 habituation days and 1 testing day). a) RHA rats spent more time exploring the non-social hole than their RLA counterparts. b) No strain-dependent difference was found in the total time spent exploring the social hole. c) RLA rats show higher % social preference than RHA rats (Ratio Time %=(social time/(social time + non-social time)) x 100). d) RHA rats present higher locomotor activity than RLA rats. e) RHA rats present less grooming time than RLA rats. N = 12/strain. Values are mean ± SEM. Strain effect (ANOVA). **p < 0.01, ***p<0.001.

4. DISCUSSION

This study was aimed to investigate the between-strain (RHA vs RLA) differences in SI, considered as a model of asociality, a negative symptom of schizophrenia, as well as the long-lasting modulation of SI by NH treatment. RHA and RLA rats were tested for SI either after a long (four 30-min sessions along 4 consecutive days; Study 1) or short (one 30-min session; Study 2) habituation to the SI testing set up. In Study 3, we treated RHA and RLA rats with NH during PND1-21, and at PND60 and PND90 control (non-handled) and handled RHA and RLA rats were tested in the NOE and SI tests, respectively. The main findings of the present study were the following: (1) for the first time it is shown that RHA rats display lowered social preference under both habituation conditions (studies 1–2); (2) RLA rats display increased levels of anxiety-related behaviours (i.e. behavioural inhibition) than RHA rats, as indicated by the NOE test and by the "grooming" measure from the SI test, and NH reduces these anxiety-related responses in both tests, an effect that is especially marked in RLA rats; (3) NH increases social interaction, i.e. "social time" and "social time 0- 5 min", in both strains (see the global ANOVA "NH" effects on both variables) and, (4) in the first 5 min of the SI test it is observed that NH produces a significant increase of social interaction preference (i.e. in % of preference for the social hole) in RHA rats, as indicated by the "strain x NH" interaction and Duncan's test (see Figure 5f).

For comparison, we have also included in Table 1 the results of a study using outbred rats from the heterogeneous NIH (National Institutes of Health) rat stock (HS rats; see characteristics of these rats in Hansen and Spuhler, 1984, and López-Aumatell et al., 2009). Although this study with HS rats was carried out 45 days after Study 1 and for this reason is not directly comparable with Study 1, the results shown in Table 1 are compatible with the idea that RHA rats are relatively "abnormal" in the present SI procedure, as they present a 53 % of social preference, which means that they do not prefer the social over the non-social hole (see Table 1). This is in contrast with the 70 % and 71 % preference for the social hole shown by RLA and HS rats, respectively (Table 1). This observation is consistent with several previous studies showing that RLA and HS rats are relatively similar in many behavioural (including schizophrenia-related) phenotypes in which RHA rats usually present differences with respect to both HS and RLAs (Díaz-Morán et al., 2013, 2012; Gómez et al., 2009; López-Aumatell et al., 2009; Martínez-Membrives et al., 2015; Oliveras et al., 2015; Tapias-Espinosa et al., 2018). However, a direct and simultaneous comparison among both Roman rat strains and outbred HS rats within a single SI experiment is warranted to definitively clarify this point.

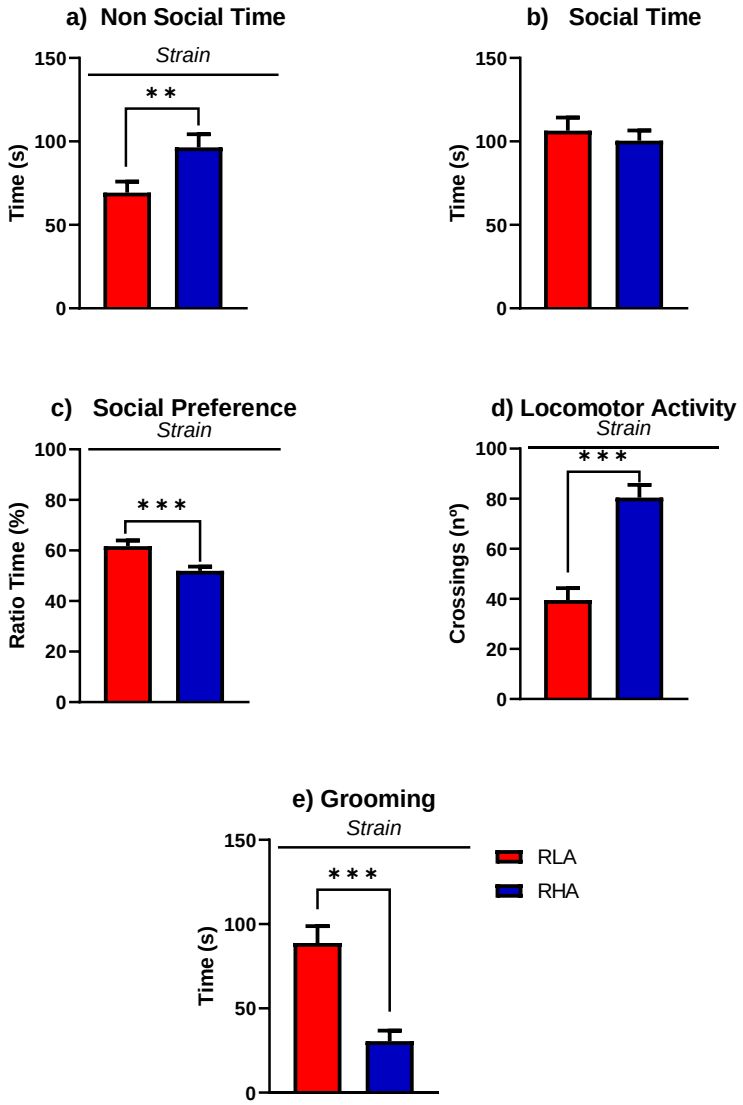


Figure 3. Differences in social interaction between the Roman rat strains after a short-term habituation period (1 habituation day and 1 testing day). a) RHA rats spent more time on the non-social hole than their RLA counterparts. b) There were no differences in the total time spent exploring the social hole. c) RLA rats show higher social preference than RHA rats (Ratio Time %= (social time/(social time + non-social time)) x 100). d) RHA rats present higher locomotor activity than RLA rats. e) RHA rats show less grooming time than RLA rats. RHA, n = 40; RLA, n = 38. Values are mean ± SEM. Strain effect (ANOVA). ** $p < 0.01$; ***$p < 0.001$.

In line with the hypothesis that the RHA strain presents a relative degree of asociality, control RHA rats from studies 1–3 exhibit a mean % social preference that is very close to 50 % (i.e. random exploration of both holes), whereas control RLA rats display in all cases a 60 % or higher score of social preference. This finding coheres with the face validity of the RHA model as an analogue of schizophrenia-relevant features, since it adds a negative symptom-like phenotype (i.e. decreased social preference) to the known profile of positive-like symptoms (e.g. novelty-induced hyperactivity, psychostimulant- and NMDA

antagonist-induced hyperactivity; reviewed by Giorgi et al., 2019), as well as attentional and cognitive deficits that characterize the RHA strain (e.g. Giorgi et al., 2019; Río-Álamos et al., 2019; Tapias-Espinosa et al., 2019; and unpublished data).

To the best of our knowledge, the present is the first study exploring NH long-lasting effects on social interaction from the perspective of studying phenotypes that may model some negative symptoms of schizophrenia. This is a relevant issue, since social interaction in rats can be studied from different theoretical perspectives (for example, as a measure of anxiety, or as a measure of schizophrenia-related asociality), and the specific SI testing procedure used should be appropriate for the underlying theoretical framework. In this regard, SI tests aimed at measuring social interaction to model "asociality" as a negative symptom of schizophrenia need to be devoid (as much as possible) of influences of novelty or anxiety components. For these reasons, habituation of the animals to the test conditions prior to SI testing (as done in the present studies) is necessary for the SI test to be able to measure the motivation (or preference) for social interaction while minimizing anxiety influences or interferences of novelty as much as possible.

Previous studies have tested NH effects on social interaction, but in relation to (or with the confound of) anxiety, which have involved the use of SI procedures and/or NH treatments that are markedly different from the ones used in the present study. In fact, for example, several of these works have used NH procedures involving handling of all rats pups from the same litter at the time, for 1 min every day and from PND 1–10, which contrasts with our NH procedure of 21 days, involving several minutes of individual (not group) separation from the mother every day and several seconds (approx. 25-30 s) of daily individual (not group) gentle stroking with bare hands (e.g. Denenberg and Grota, 1964; Padoin et al., 2001; Raineki et al., 2009; Todeschin et al., 2009; for review see Raineki et al., 2014). Hence, due to these important methodological differences, regarding both the NH treatment and SI testing procedures, our present findings cannot be compared with those studies.

Turning back to the treatment effects observed in Study 3, it is worth noting that NH increases social interaction in both strains, as indicated by the measure of "Social Time" during the whole SI test. Remarkably, our hypothesis that the effect of NH would be more marked in those rats with relative social deficits seems to be confirmed when we analyze the first 5 min of the SI test. Thus, in both the "social time 5 min" (ANOVA NH significant effect; **Figure 5e**) and "social preference 5 min" (ANOVA "strain x NH" effect; **Figure 5f**) NH-increasing effects have been observed specifically in RHA rats (see Duncan's tests in **Figure 5e and 5f**, respectively). Thus, these results would cohere with the notion of a global positive NH effect on social interaction. Moreover, these findings would also indicate a greater NH effect in RHA rats (particularly in the early phases of the SI test), which exhibit relatively decreased social interaction (or relative social withdrawal). It is worth noting that the effects of NH on social interaction are very specific, as the treatment was devoid of effects on non-social behaviour or activity (i.e., crossings) in any

rat strain. Thus, NH does not increase general activity or general exploration of the holes, but it specifically results in an increased "sociability" ("social time" and "% social preference"), particularly in RHA rats.

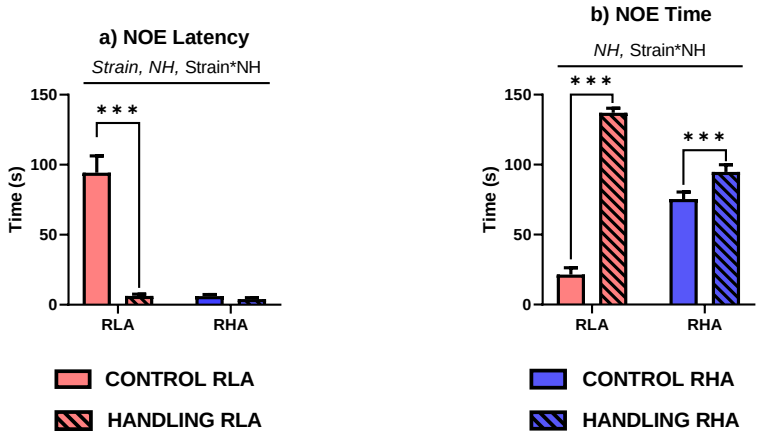


Figure 4. Differences in the novel object exploration (NOE) test between the Roman rat strains and effects of neonatal handling. Differences between strains and between handled ("Handling") and control animals in "NOE latency" (time elapsed until the first exploration of the novel object) and in total time spent exploring the novel object ("NOE time"). a) Control RLA rats take much more time than RHAs to explore the novel object for the first time ("Strain" effect). Handling dramatically reduces "NOE latency" in RLA rats (global "NH" effect, and "Strain x NH" effect). No differences between RHA-C and RHA-NH groups. b) Handling-treated rats spent more time exploring the novel object than Control rats of both strains (global "NH" effect), and the exploration-increasing (anxiolytic-like) effect of neonatal handling was much more marked in RLA rats than in their RHA counterparts ("strain x NH" effect). RLA-NH, n = 34; RLA-C, n = 34; RHA-NH, n = 28; RHA-C, n = 34. Values are mean ± SEM. Strain, NH (neonatal handling), Strain*NH (strain x neonatal handling) effects (ANOVA). ***p < 0.001 (Duncan's multiple range test).

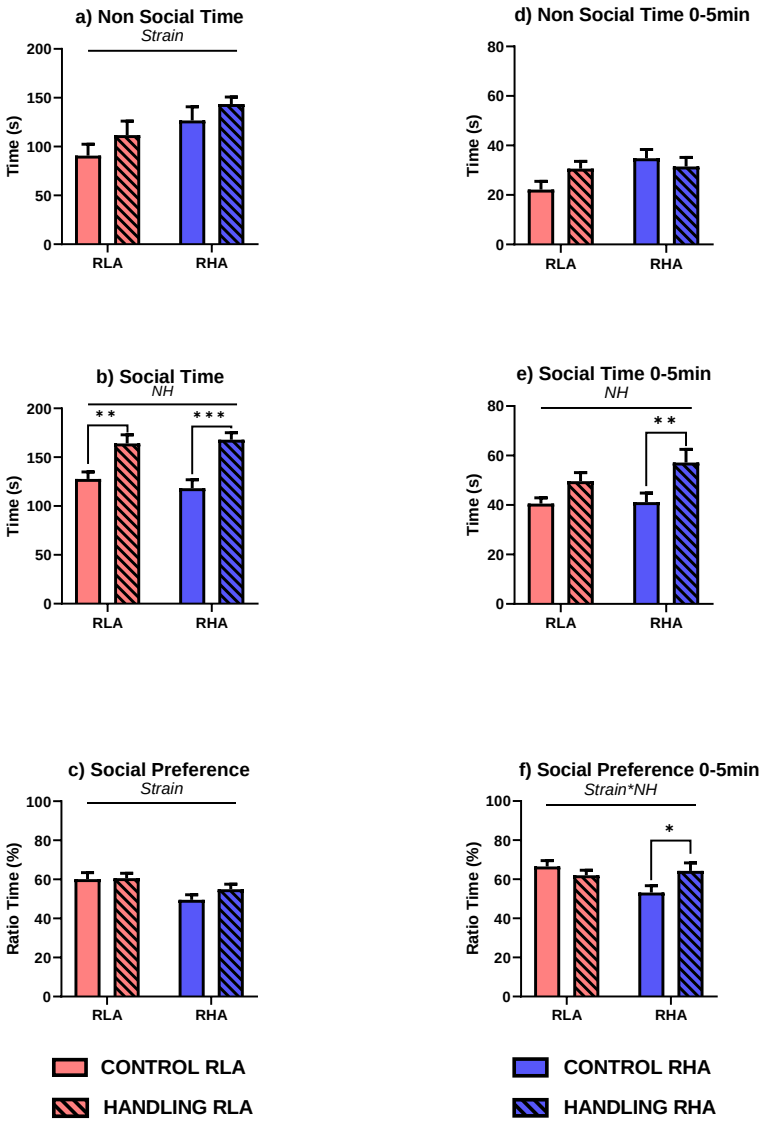


Figure 5. Social interaction results for the total 15-min test (a-c) and for the first 5 min of the test (d-f). a) Total non-social time results showed significant global differences between the strains ("Strain" effect), but no effect of Handling. b) Handled animals from both strains spent more time exploring the social hole than the Control animals ("NH" effect, and Duncan's test). c) RLA rats overall show a higher % preference for the social hole than RHA rats ("Strain" effect). d) There were no significant effects on non-social time during the first 5 min of the test. e) RHA handled animals spent more time on the social hole than RHA control animals during the first 5 min of testing ("NH" effect and Duncan's test). f) RHA handled animals show higher % social preference than RHA Control animals during the first 5 min of testing ("Strain x NH" effect, and Duncan's test). RLA C n = 14, RLA NH n = 12, RHA C n = 14, RHA NH n = 12. Values are mean ± SEM. Strain, NH (neonatal handling), Strain*NH (strain x neonatal handling) effect (ANOVA). *p < 0.05, **p < 0.01, ***p < 0.001 (Duncan's multiple range test).

Further in support of the independency of social (and non-social) interaction measures from locomotor activity it is worth mentioning that Pearson's correlation coefficients between activity and the different social and non-social interaction variables (including % social preference) were all non-significant, ranging from r=-0.09-0.16, r=- 0.09-0.42 and r=-0.01 0.06 (non-significant in all cases; studies 1–3, respectively) for RLA rats, and from

r=-0.34-0.14, r=-0.06-0.26 and r = 0.07-0.32 (all non-significant; studies 1–3, respectively) for RHA rats.

Some authors have discussed on whether the effects of NH are due to its positive effects or to adverse effects from the relatively unstimulated "control" condition (e.g. Peters et al., 1991; Pryce et al., 2001). These authors proposed that the NH groups would be the "normal" animals, given that 3-month-old NH-treated male rats showed latent inhibition (LI) under their conditions (which was the normal result they obtained in different studies), whereas "non-handled control" males (which, in these studies, were unstimulated, i.e. they were left completely undisturbed from birth until weaning; Peters et al., 1991) did not show LI. Conversely, the control rats in the present study are very similar to the "normal husbandry" controls used by those authors (Pryce et al., 2001), since our "control" and NH rats were placed in a colony room in which there were also other rats (but no other breeders) and there was no restriction of regular room entry (i.e. every day) to check for environmental conditions, and cage cleaning was done once per week, so that both "control" and NH rats were handled 2–3 times between PND1 and 21 (for cage cleaning, usually the mother was first placed in the new/clean cage, and then all the pups from the litter were again placed with the mother at the same time). Thus, control (and NH) litters received such a moderate stimulation (of weekly cage changes) 2–3 times during PND1-21, plus the gently manipulation involved in checking birth of litters and sexing the pups on PND2–3. Moreover, food and water control occurred twice per week in the colony room of control and NH rats. Thus, our present control rats were not as unstimulated as the "non-handled control" rats in the abovementioned studies (Peters et al., 1991), rather they received some moderate environmental and experimenter-applied stimulation and, in spite of this our NH treatment had still clear effects on both anxiety-related measures (i.e. novel object exploration and self-grooming) and social interaction.

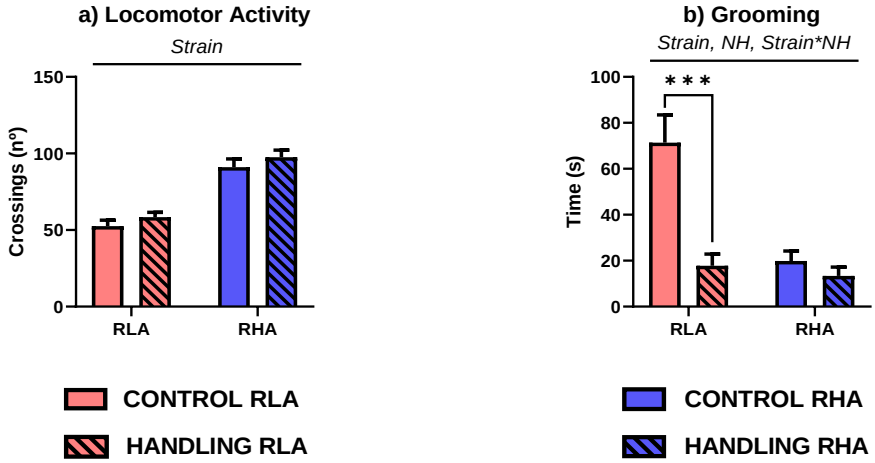


Figure 6. Differences in locomotor activity and self-grooming between the Roman rat strains and effects of neonatal handling during the SI test. a) RHA displayed overall more locomotor activity than RLA rats ("Strain" effect). b) RLA rats spent overall more time self-grooming than RHA rats ("Strain" effect). Handling treatment reduces grooming activity (global "NH" effect), and this effect is much more marked in RLA rats ("Strain x NH" effect). See "n"/group in **Figure 5**. Values are mean ± SEM. Strain, NH (neonatal handling), Strain*NH (strain x neonatal handling) effect (ANOVA). ***p < 0.001 (Duncan's multiple range test).

Table 1. Social and non-social behaviour in the SI test in the Roman strains and in the outbred HS rat stock.

	RHA (n = 12)	RLA (n = 12)	HS (n = 80)
Non-social time (s)	56.9 ± 7.2	30.4 ± 3.9	41.1 ± 4.7
Social time (s)	75.5 ± 6.8	71.9 ± 9.8	121.2 ± 12.4
% Social Preference	53.1 ± 3.6	70.5 ± 3.1	71.4 ± 2.7

RHA and RLA rats are from Study 1. HS rats (3-4 months old; naïve males from 30 different litters) are from a study performed 45 days after Study 1, and under the same experimental conditions as Study 1.

Neuropeptides such as oxytocin and vasopressin have been widely related to the regulation of many aspects of social behaviour, including aggression, social recognition, social motivation, and maternal nurturing (e.g., for reviews see Raineki et al., 2014, and Veenema, 2012). Thus, these hormones might be seen as good candidates to mediate/modulate the influences of differential early experiences on latter sociality/asociality traits, including the enduring effects of neonatal handling on adult social behaviour (Raineki et al., 2014; Veenema, 2012). However, the findings reported thus far with both neuropeptides in response to NH treatment are rather contradictory (see Veenema, 2012). For example, using a NH procedure consisting of handling all pups from each litter together, for 1 min and during PND 1–10, "deficits" of social behaviour,

as well as a decrease of oxytocin neurons in the paraventricular nucleus and amygdala, have been reported (Todeschin et al., 2009; for review see Raineki et al., 2014, and Veenema, 2012). These findings are hard to reconcile with our present behavioural results, as well as with recent findings from our group showing that NH reduces anxiety, the HPA axis endocrine responses to stress and amygdala volume in the more anxious/fearful RLA rats (Río-Álamos et al., 2019, 2017, and references therein). Studies of the oxytocinergic-vasopressinergic systems in the Roman strains and using our NH procedure are therefore warranted.

On the other hand, NH of rats results in (sex-dependent) long-lasting alterations in brain dopaminergic (DA) and serotoninergic (5-HT) systems (e.g. Fernández-Teruel et al., 2002; Panagiotaropoulos et al., 2004; Peters et al., 1991), both of which are thought to be involved in the etiopathogenesis of psychoses and schizophrenia. NH has also been reported to lead to enduring changes in glutamate AMPA and NMDA receptor density and subunit expression (and related neural plasticity processes) in limbic and cortical regions of rats (e.g. Stamatakis et al., 2009 and references therein). The Roman rat strains are known to differ in various aspects of the function of DA, 5-HT and glutamate systems (particularly NMDA and mGlu2 receptors; see Elfving et al., 2019; Klein et al., 2014; Wood et al., 2017; for review see Giorgi et al., 2019), which have been reported to be affected by NH (e.g. Fernández-Teruel et al., 2002; Katsouli et al., 2014; Stamatakis et al., 2009). Thus it seems conceivable that some of these systems, including neuropeptides such as oxytocin and vasopressin, might have a modulatory role in the effects of NH on social behaviour seen in the present study. Some of these possibilities are being addressed in current studies at our laboratory. On the other hand, although for practical reasons only male rats were used in the present work, future studies should address the gender-specific effects of NH on the social behaviour of Roman rats, since we and others have shown that neurobehavioral effects of NH depend on gender (e.g. Fernández-Teruel et al., 1991, 1992; Katsouli et al., 2014; Panagiotaropoulos et al., 2004; Peters et al., 1991; Pryce et al., 2001; Río-Álamos et al., 2015, 2019; Shalev et al., 1998; Stamatakis et al., 2009).

The present work is in line with all the evidence accumulated along more than four decades concerning the behavioural inhibition and anxiety-related traits of the Roman rats (for review see Giorgi et al., 2019). Accordingly, our results show that, compared with RHAs, RLA rats display increased anxiety-related behaviour, as suggested by the decreased exploration of the novel object (NOE test) and increased self-grooming behaviour in the SI test (Estanislau et al., 2019; Fernández-Teruel and Estanislau, 2016; Kalueff et al., 2016; Río-Álamos et al., 2015). NH dramatically reduces the above-mentioned anxiety-related behaviours. Moreover, this effect is much more marked in RLA than RHA rats, consistent with findings from previous studies (e.g. Río-Álamos et al., 2019, 2017, 2015; Steimer et al., 1998). It is important to clarify that NH effects over sociability seem to be independent from the NH reduction of anxiety. As said above, it is known that

RHA rats display lower levels of anxiety than their RLA counterparts, and this is further supported by the present NOE and grooming results. Therefore, if social behaviour in the present SI test procedure would be critically influenced by anxiety levels, we would expect that those rats with lower levels of anxiety would display higher social preference and spent more time socializing. Nevertheless, our results point just in the opposite direction: the "more anxious" RLA rats show a greater preference for social interaction than RHAs. In addition, while NH reduces anxiety more markedly in RLA rats, the effects of NH on social behaviour are more marked in RHA rats, which is particularly evident during the first 5 min of the SI test. Thus, it can be proposed that NH treatment has a genuine effect of increasing social activity, and anxiety does not appear to interfere in the present procedure of SI testing.

In conclusion, this work is the first to report that in a SI procedure including previous habituation to the testing set up the RHA rat strain displays relatively reduced social interaction preference (a model of schizophrenia's negative symptomatology, i.e. asociality) as compared to RLA rats. In addition, it is also reported for the first time that NH treatment long-lastingly increases social interaction preference in the Roman rats, and this effect is more pronounced in the RHA strain.

The present findings should be considered together with previous works that had also reported benefits of NH on other aspects of schizophrenia-related phenotypes. Among them, an example is the work of Río-Álamos et al. (2019) showing that NH improves PPI deficits and increases working memory and cognitive flexibility in RHA rats. In the same vein, Pryce et al. (2001) also reported that NH attenuated apomorphine-induced impairments in PPI and improved latent inhibition (Peters et al., 1991; Pryce et al., 2001; Shalev et al., 1998). The integration of all these results allow us to propose that some types of early-life stimulation, such as neonatal handling-like procedures, may be associated to long-lasting positive effects on psychological and neurobiological processes related to the schizophrenia spectrum (see Fernandez-Teruel et al., 2002; Río-Álamos et al., 2019, and references therein).

Credit authorship contribution statement

Daniel Sampedro-Viana: Investigation, Data analysis, Formal analysis, Supervision, Writing - original draft. **Antoni Cañete:** Investigation, Data analysis, Formal analysis, Supervision, Writing - original draft. **Francesco Sanna:** Investigation, Data analysis, Formal analysis, Writing - original draft. **Bernat Soley:** Resources, Data curation, Data analysis, Writing. **Osvaldo Giorgi:** Conceptualization, Writing - review & editing. **Maria G. Corda:** Conceptualization, Writing - review & editing. **Pilar Torrecilla:** Investigation, Data curation. **Ignasi Oliveras:** Investigation, Resources, Writing. **Carles Tapias-Espinosa:** Investigation, Resources, Writing. **Cristóbal Río-´Alamos:** Investigation, Resources, Writing. **Ana Sánchez-González:** Resources, Writing. **Adolf Tobeña:** Conceptualization, Writing - review & editing, Funding acquisition. **Alberto Fernández-Teruel:** Conceptualization, Methodology, Formal analysis, Data analysis, Writing - original draft, Supervision, Project administration, Funding acquisition.

Acknowledgements

Work supported by PSI2017-82257-P (MINECO), 2017SGR-1586 and ICREA-Academia 2013 (to AF-T). I.O is recipient of a “Juan de la Cierva” postdoctoral fellowship (FJC2018-038808-I). F.S. is the recipient of a PhD fellowship funded by the Italian Ministry of University and Research (MIUR). C.T-E is recipient of a PhD fellowship FPU15/06307.

Conflict of interest

The authors present no conflict of interest.

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ESTUDIO 2

Neonatal handling treatment increases the c-Fos expression in some Social Brain Areas in the RHA rat model

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Manuscrito pendiente de publicar

ABSTRACT

Neonatal handling (NH) procedure is an environmental manipulation that induces long-lasting changes in behavioural, neuroendocrine, and neuroanatomical processes. In previous studies we have reported that NH treatment increases social interaction preference in an animal model for studying some schizophrenia-relevant symptoms, the Roman High Avoidance (RHA) rats. In the present study was aimed at evaluating whether the increase of social behaviour/preference due to the NH treatment in the RHA rats is associated with differences in c-Fos expressions levels in some of the brain areas associated with the "social brain". To this aim, we evaluated the performance of adult male rats from both Roman strains (RHA vs. RLA), either untreated (control) or treated with NH (administered during the first 21 days of life) in a social interaction task. For the analyses of the immediate early gene expression (c-Fos) the animals were divided into three different experimental conditions: undisturbed home cage controls (HC) animals, exposed to the testing set-up context (CTX) animals, and social interaction (SI) tested animals. It was found that, compared with their RLA counterparts, NH treatment increases social behaviour in RHA rats and also specifically enhances c-Fos expression in the NH-treated RHA rats in some brain areas related to the social behaviour, i.e. the infralimbic (IL) and the medial amygdala posterodorsal (MePD) region.

Keywords: Schizophrenia; Neonatal handling; c-Fos expression; Social brain areas; Prefrontal Cortex; Amygdala; RHA and RLA rats

1. INTRODUCTION

Social behaviour, including social preference, is impaired in several neurodevelopmental disorders, such as schizophrenia (Albers, 2012; McCutcheon et al., 2020; Mitra et al., 2016). Social preference could be understood as the behavioural choices between social and non-social outcomes (Hackenberg et al., 2021).

The "social brain" is highly conserved across species, and refers to those brain structures that are engaged in social cognitive processes such as attention, memory, motivation and emotion (Fernández et al., 2018; Ko, 2017). These brain regions involve the prefrontal cortex (PFC) as a top-down modulatory system for social behavior, the amygdala (AMY) for encoding emotion processing, the hypothalamus for stress modulation and synthesis of neuropeptides (e.g., oxytocin, vasopressin), the hippocampus (HPC) for memory processing, the nucleus accumbens (NAc) for social motivation (related to incentive/reward seeking), the anterior cingulate cortex (ACC) for the detection and evaluation of social processes, and some somatosensory and temporal cortical areas that process sensory and motor inputs and outputs (Adolphs, 2010; Bickart et al., 2014; Fernández et al., 2018; Gangopadhyay et al., 2021; Porcelli et al., 2019; Sherwin et al., 2019).

The Roman rat strains have been selectively and bidirectionally bred for their very good (RHA) vs. extremely poor (RLA) ability to acquire the two-way active avoidance task, a "passive avoidance/active avoidance" conflict that involves anxiety (Fernández-Teruel et al., 2021; Giorgi et al., 2019). As a consequence of such a bidirectional selection, many other phenotypes/traits differ between both Roman rat strains. Thus, relative to RHAs, RLA rats show enhanced signs of anxiety/fear in many unconditioned and conditioned tasks/tests, and also more intense stress-induced HPA-axis and prolactin responses. Moreover, compared with RLAs and other rat strains, the phenotypic profile of RHAs suggests that this strain may be considered as a valid animal model for studying some schizophrenia-relevant symptoms or features (Fernández-Teruel et al., 2021). Among other schizophrenia-related phenotypes, RHA rats show enhanced impulsive behavior, deficits in latent inhibition, impaired prepulse inhibition of the startle response, worsened working memory, spatial reference learning/memory and cognitive flexibility, enhanced novelty-induced locomotor activity as well as increased locomotor and mesolimbic dopaminergic sensitization to dopaminergic psychostimulants (Fernández-Teruel et al., 2021; Giorgi et al., 2019). Regarding the negative symptom domain, we have recently shown that adult male RHAs display deficits in social interaction compared to their RLA counterparts and heterogeneous (outbred) stock rats (Oliveras et al., 2022; Sampedro-Viana et al., 2021). In addition, the RHA rats also present some neurobiological alterations in the mesolimbic dopaminergic system, along with some alterations of central serotoninergic and glutamatergic transmission, and in the function of the prefrontal cortex and hippocampus, that resemble the neurochemical and neuroanatomical traits found in patients with schizophrenia (e.g. Río-Álamos et al., 2019;

Tapias-Espinosa et al., 2019) (reviewed by Fernández-Teruel et al., 2021 and Giorgi et al., 2019).

As to the effects of neonatal handling (NH) treatment in RHA and RLA rats, we have reported that NH stimulation induces very long-lasting strain-dependent effects, such as improvements of attentional- and cognition-related processes, reductions of various anxiety- and stress-related responses/behaviors, and volume alterations of the AMY and HPC (e.g. Aguilar et al., 2002; Escorihuela et al., 1995; Fernández-Teruel et al., 1991, 1992, 1997, 2002; Río-Álamos et al., 2015, 2017, 2019; Steimer et al., 1998). Moreover, we observed that NH treatment increases social interaction preference, being the effect more marked in the RHAs than in their RLA counterparts (Sampedro-Viana et al., 2021).

In the present study, we aimed at elucidating whether the increase of social behaviour/preference due to the NH treatment in the RHA rats is associated with differences in c-Fos expression levels in some of the brain areas associated with the social brain. We evaluated c-Fos expression, an index of neuronal activity, to identify brain areas activated by the exposure to NH. Taking into account previous results of NH treatment in the Roman rats (e.g. Río-Álamos et al., 2017, 2019; Sampedro-Viana et al., 2021), we expected to find some strain-dependent c-Fos expression differences between treatment (NH vs untreated rats) and testing (i.e. social interaction or control) conditions, as well as differential strain- and/or treatment-dependent effects depending on the brain region examined (i.e. medial prefrontal cortex, anterior cingulate cortex, amygdala nuclei).

2. METHODS

2.1 Subjects

Animals used for the present study were naïve male rats from the inbred Roman high- (RHA, n=36) and low-avoidance (RLA, n=32) strains, from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine (School of Medicine, at the Autonomous University of Barcelona, Spain), since 1996.

Animals were housed in macrolon cages (standard size) in same-sexed pairs. They were maintained under a 12:12h light-dark cycle (lights on at 8:00h) with controlled temperature (22ºC ± 2ºC) and humidity (50-70%). Food and water were available *ad libitum*. All testing was performed in the morning between 8:00 and 14:00h. All the experimental procedures agreed with the Spanish legislation on "Protection of Animal Used for Experimental and Others Scientific Purposes" (RD 53/2013) and the European Communities Council Directive (2010/63/EU).

2.2 Social interaction test

The social interaction (SI) procedure was described by Sampedro-Viana et al., (2021). Two transparent acrylic boxes (65 x 23 x 20 cm) placed facing one to another at 12 cm, to prevent any physical contact between the animals in the box, in a red-lit room. A 3-cm diameter hole were at the ends of each box. The holes facing the other box was named "social hole", while the opposite was named "non-social hole". All the behavioural procedure was recorded by a camera connected to a TV monitor outside the experimental room. A trained observer, who was blind to strain and treatment, scored the behaviour.

Two phases made up the procedure: habituation day and testing day. During the habituation phase a 30-min habituation period to the set-up was carried out with all four holes covered with tape and a barrier between the two SI boxes. A pair of non-familiar weight-matched animals were placed into the boxes (one rat per SI box).

For the SI testing day all the holes were opened, the barrier was removed, and a two weight-matched non-familiar rats of the same strain and treatment were placed into the set-up for a 15-min SI test. Time spent exploring the social hole (Social time) and the non-social hole (Non-social time) were measured. The ratio (%) of social preference for the social hole was calculated according to the following formula: %Social Preference= (Social time/ (Social time + Non-social time)) x 100.

Number of crossings was also measured (dividing each box into three squares to measure horizontal activity). Each box was cleaned with a 70% ethanol solution and dried with a paper towel before testing every pair of animals.

2.3 Neonatal handling (NH) treatment

Neonatal handling (NH) treatment was administrated twice a day at 9:30 h and 17:30 h between PND 1 to 21. Before the handling session, the mother was removed from the home cage leaving the litters. Then, the pups were individually placed in plastic cages (35 x 15 x 25 cm) lined with a paper towel, placed in a temperature (22 ± 2 ºC) controlled room. Each pup was gently stroked for 3 – 4 s at 0, 4 and 8 min with bare hands by well-trained investigators. After the 8-min separation, the pups were returned to their home cage with the mother and the rest of the litter. Non-handled rats (control "C" group) were placed in the same colony room as NH rats, and were left relatively undisturbed, except for the habitual home cage cleaning once a week. After weaning (PND 21), rats were housed in same-sexed pairs of the same experimental group in standard macrolon cages and remained in the same colony room.

2.4 Immunohistochemistry of c-Fos

Tissue processing, c-Fos immunohistochemistry, and microscopy c-Fos immunostaining was performed with modifications as described in Tapias-Espinosa et al., (2019). Animals were euthanatized 120 min following the habituation phase (CTX) or the social interaction (SI) task or were taken directly from their home cages (HC) in a counterbalanced manner with the other two (CTX and SI) experimental groups. Brains were removed by trained investigators and immersed in paraformaldehyde 3,7-4,0% (Casa Alvarez FO090101) to fix the tissues. 24 h later, two consecutive washes with phosphate buffer (PB) 0.1 M were made and samples were submerged in saccharose (SIGMA 84100) solution 30% for 3-4 days. Before storing the samples in -80 ºC, the brains were frozen with 2-methylbutane (isopentane, SIGMA 320404) between -40º C to -60º C. Coronal 30-µm thick sections were cut in a cryostat (Leica CM3050S) and stored free floating in an antifreeze solution (PB 0.1 M; Ethylene glycol, SIGMA 102466; Glycerol, SIGMA G7757) at -20º C.

Once the slices were selected for the immunohistochemistry, they were washed four times for 5 min in DPBS buffer 0.01 M. Endogenous peroxidase activity was quenched with 2% hydrogen peroxide (SIGMA 216763) in 70% methanol (SIGMA 179337) in 28% DPBS, followed by several washes in DPBS-T (0.2% Triton, SIGMA X-100). Afterwards, endogenous protein was blocked incubating the slices with 5% normal donkey serum (NDS, Jackson 017-000-121) for 30 min. After that, incubation with primary antibody (Abcam 6167, anti-c-Fos antibody raised in sheep, 1:200) in 1% NDS in DPBS-T for 44h at 4º C was done. On the next day, slices were washed in DPBS-T, and were then incubated with the secondary antibody (Jackson 713-035-147, Peroxidase AffiniPure Donkey Anti-Sheep IgG, 1:200) for 2h. After washes in DPBS-T, DPBS and TB-HCl, the samples were incubated with 3,3'-diaminobenzidine tetrahydrochloride DAB (SIGMA D5637; + 99ml of TB + 33 microliters of H_2O_2) for 60 min. Afterwards, slices were washed in TB, dehydrated with ethanol, cleared with xylene, and cover-slipped with DPX (SIGMA 6522).

Microphotographs were captured using an Eclipse 80i Nikon microscope and Eclipse 90i Nikon, both attached to a Nikon DXM1200F70 digital camera at x10 magnifications. The regions of study were the Cg (Anterior Cingulate; bregma: 4.20 to 0.72 mm), PrL (Prelimbic; bregma: from 4.68 to 2.52 mm), IL (Infralimbic; bregma: 3.72 to 2.52 mm), BLA (Basolateral amygdala; bregma: -2.04 to -3.36 mm), MePD (Medial amygdaloid nucleus posterodrosal part; bregma: -2.76 to -3.60 mm) and MePV (Medial amygdaloid nucleus posteroventral part; bregma: -2.76 to -3.60 mm). The borders of each area were identified with the help of a rat brain atlas (Paxinos & Watson, 2013) (see Figure 1 for details of the regions of interest). The ImageJ software ("analyze particles" function) was employed to automatically identify and count the number of c-Fos immunostained nuclei in 25-30 (Cg and PrL) and 8-15 (IL, BLA, MePD and MePV) histological sections of the brain region/mm^2. Particle size and appropriate grey threshold were set for each region and maintained for all subjects.

2.5 Statistical analyses

All the analyses were performed employing the "Statistical Package for Social Sciences" (SPSS). Significance level was set at $p < 0.05$.

A 2 x 2 (2 "Strains" x 2 "Treatments") ANOVA followed by post hoc Duncan's multiple range tests were used to determine differences between both strains in the social interaction test related with the NH treatment.

A 2 x 2 x 3 (2 "Strains" x 2 "Treatment" x 3 "Conditions") ANOVA followed by post hoc Duncan's test were used to determine differences between the RHA and RLA rats in the different conditions (i.e., Home-Cage –HC-, Context –CTX-, social interaction –SI-) of the c-Fos study.

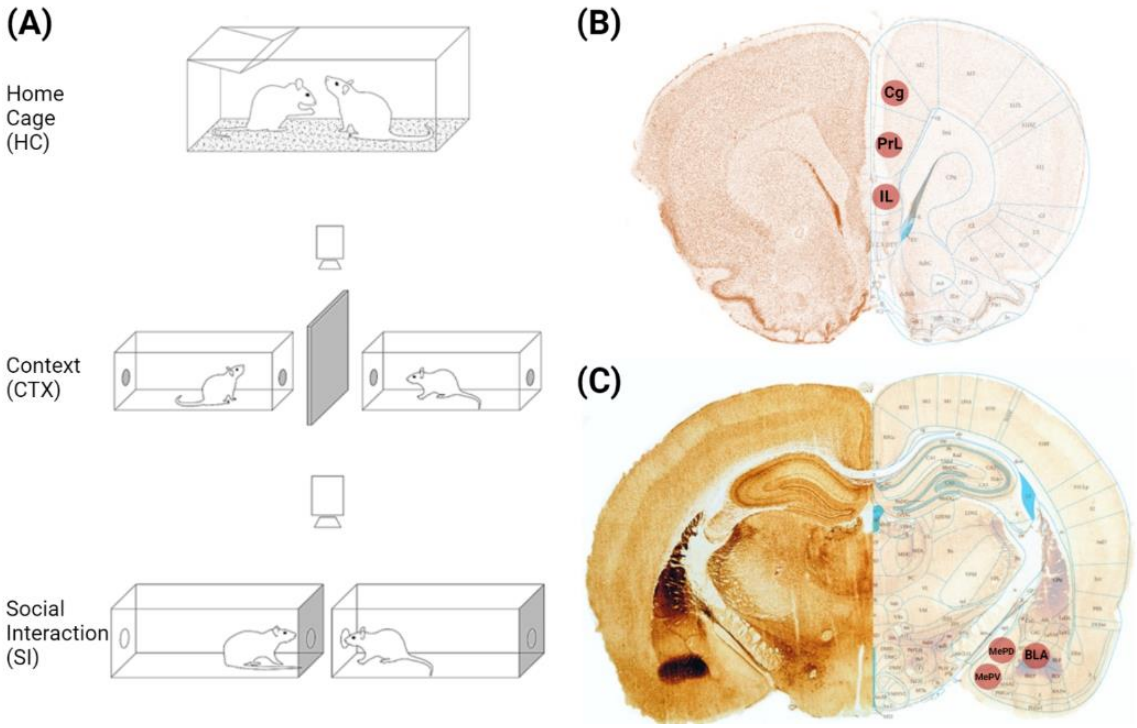

Figure 1: Representation of the experimental conditions and the brain areas: **(A)** randomly assigned rats to one of the three different conditions, home cage (HC), context (CTX) or social interaction (SI) for the assessment of c-Fos expression. HC animals stayed in their home cage throughout the testing procedure. CTX animals were exposed to the behavioural testing set-up, with both holes covered with tape and a barrier placed between the two SI boxes, for 30 min. SI animals were placed into the set-up 30 min with the same conditions as the CTX animals. 24 h after de habituation session, a pair of non-familiar (same strain and treatment) conspecifics was placed into the apparatus for a 15-min social interaction (SI) test. Rats were returned to their home cage after habituation or testing for 90 min prior to tissue collection. **(B)** Regions of interest for analysis of c-Fos related with the prefrontal regions (Bregma 2.52 mm) in a rat brain. **(C)** Regions of interest for analysis of c-Fos expression related with the amygdala region (Bregma -3.24 mm) in a rat brain. Cg = Cingulate cortex; PrL = Prelimbic area; IL = Infralimbic area; BLA = Basolateral amygdala; MePD = Medial amygdaloid nucleus posterodorsal part; MePV = Medial amygdaloid nucleus posteroventral part. The border of each area were identified with the help of a rat brain atlas (Paxinos & Watson, 2013).

3. RESULTS

The results of the present work are presented in Figure 2-4.

Behavioural analysis

Factorial ANOVA (2 "Strains" x 2 "Treatments") revealed a "Treatment" ($F(1,64)=19.656$; $p<0.001$) effect on "Social Time", indicating a significant difference between the groups with the environmental treatment and the control groups (Figure 2a, and Duncan's). As for the first five minutes of the behavioural test, ANOVA revealed "Strain" ($F(1,64)=4.072$; $p<0.05$) and "Treatment" ($F(1,64)=8.982$; $p<0.01$) effect, indicating that RHA rats spent more time in the social hole compared with RLA rats, and NH globally increased the time spent in the social hole (Figure 2b, and Duncan's)

Regarding the "Non-Social Time" variable, a "Strain" ($F(1,64)=6.680$; $p<0.05$) effect was observed, reflecting that the RHA rats spent more time in the non-social hole than their RLA counterparts (Figure 2c, and Duncan's). ANOVA also showed a "Strain" ($F(1,64)=6.933$; $p<0.05$) effect on "Non-Social Time 5 min", indicating that RHA spent more time in the non-social hole than their RLA counterparts already during the first 5 minutes of SI testing (Figure 2d, and Duncan's).

As for "Social Preference", there was a "Strain" ($F(1,64)=9.448$; $p<0.01$) effect, showing that the RHA rats presented a lower social preference than RLAs (Figure 2e, and Duncan's). During the first five minutes of the social interaction task, the ANOVA revealed a "Strain*Treatment" ($F(1,64)=4.440$; $p<0.05$) interaction, showing that the treatment affects differently depending on the strain, i.e. it tends to increase social preference in RHA rats whereas a trend to a decrease is observed in RLA rats (Figure 2f, and Duncan's).

ANOVA also revealed a "Strain" ($F(1,56)=100.899$; $p<0.001$) effect on "Locomotor Activity", as RHA rats presented global higher levels of activity than RLA rats (Figure 2g, and Duncan's). A "Strain" ($F(1,56)=43.333$; $p<0.001$) effect was also observed during the first five minutes for the "Locomotor Activity" variable, indicating that RHA rats presented a higher number of crossings compared to RLA rats (Figure 2h, and Duncan's).

Prefrontal cortex areas

Factorial ANOVA (2 "Strains" x 2 "Treatments" x 3 "Conditions") revealed a "Condition" ($F(2,48)= 42,282$; $p<0.001$) effect on c-Fos expression in the Cg in both strains, indicating that c-Fos levels are globally increased by both the CTX and SI conditions (Figure 3a-b, and Duncan's test).

Similarly, in the PrL area a "Condition" ($F(2,48)= 9,044$; $p<0.001$) effect was observed, indicating that both the CTX and SI conditions increased c-Fos expression in both rat strains (Figure 3c-d, and Duncan's test).

With respect to the levels of c-Fos in the IL, a "Condition" ($F(2,48)=12,957$; $p<0.001$) effect was observed (Figure 3e-f). However, further Duncan's post-hoc analysis revealed significant differences between the SI group and both the CTX and HC groups NH-treated RHA rats (Figure 3f).

Amygdala areas

The results of the three-way ANOVA with the c-Fos expression in the BLA as the dependent variable and with Strain (RHA and RLA), Treatment (control and neonatal handling), and condition (home cage, context and social interaction) as between-subjects factors showed significant effect of the "Strain" ($F(1,48)=5,337$; $p<0.05$) and "Condition" ($F(2,48)=21,123$; $p<0.001$) effects, indicating that the c-Fos expression in the BLA subregion differ depending the experimental situation and the rat strain. A "Strain x Condition" interaction was revealed, indicating that c-Fos levels of SI condition is higher in the RHA rats compared to their counterparts RLA rats (Figure 4a-b, and Duncan's). Student's *t*-test ($t(1,8)= 7.298$; $p<0.01$) was performed between the RLAs control-CTX and neonatal handling-CTX group (Figure 4a), showing that the NH-CTX group exhibited lower novelty-(CTX)induced c-Fos expression than the untreated control-CTX group in the BLA (Figure 4a).

Regarding the c-Fos expression in the MePD region, "Condition" ($F(2,48)=28,847$; $p<0.001$) and "Strain x Condition" ($F(2,48)=3,711$; $p<0.05$) effects were observed. This interaction indicates that the experimental condition (CTX, SI) affects differently depending on the strain, and reflects the fact that NH-RHA rats tested for SI present higher c-Fos expression levels than their CTX comparison group (Figure 4c-d, and Duncan's).

ANOVA also revealed a "Condition" ($F(2,48)=32,948$; $p<0.001$) effect on c-Fos expression in the MePV subregion, indicating a global increasing effect of both the CTX and SI conditions on both rat strains (Figure 4e-f, and Duncan's).

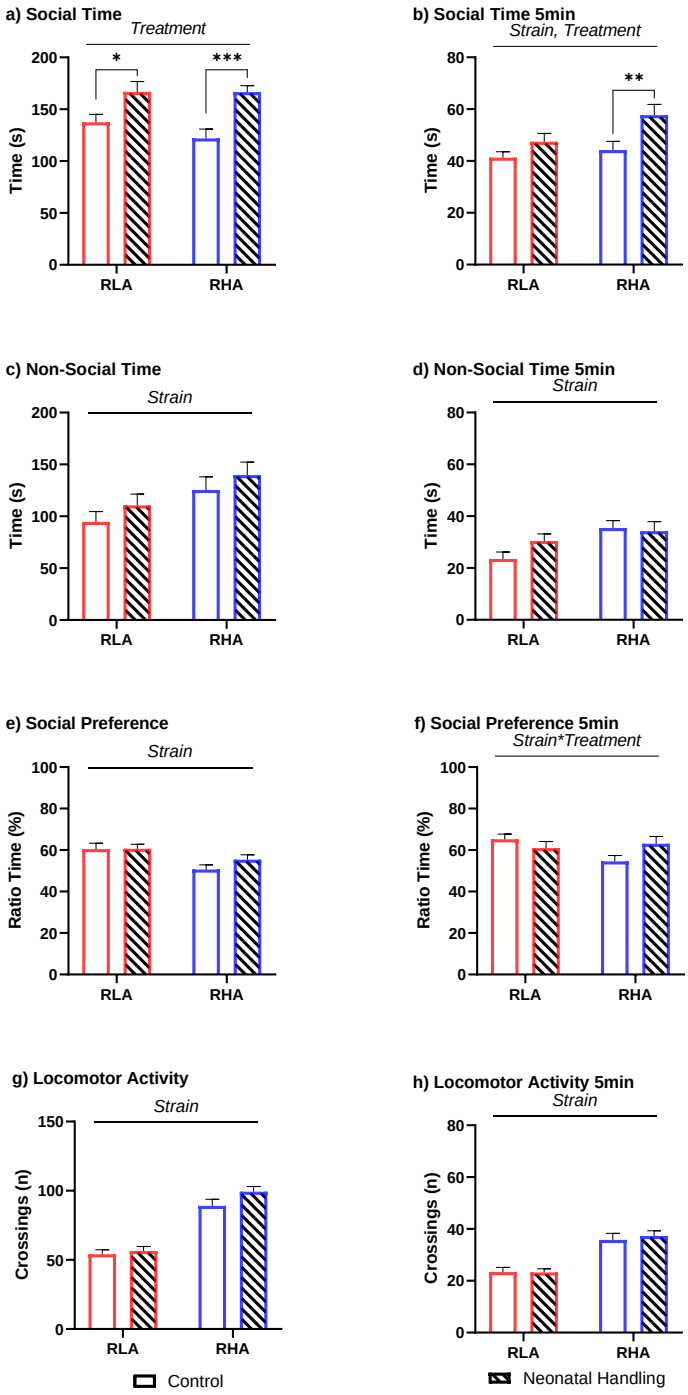

Figure 2: Principal variables in the social interaction task. a) Mean social time (±SEM) of RLA and RHA is shown for each treatment. **b)** Mean social time (±SEM) of the first five minutes of RLA and RHA is shown for each treatment. **c)** Mean non-social time (±SEM) of RLA and RHA is shown for each treatment. **d)** Mean non-social time (±SEM) of the first five minutes of RLA and RHA is shown for each treatment. **e)** Mean social preference (±SEM) for RLA and RHA is shown for each treatment. Mean social preference (±SEM) of the first five minutes of RLA and RHA is shown for each treatment. **f)** Mean number of crossings (±SEM) of RHA and RLA is shown for each treatment. **g)** Mean number of crossings (±SEM) of the first five minutes of RHA and RLA is shown for each treatment. Values are mean ± SEM. Control n=18/strain; Neonatal Handling n= 16/strain. "Strain" and "Treatment" effects (ANOVA). * $p < 0.05$; *** $p < 0.001$ (Duncan's multiple range test).

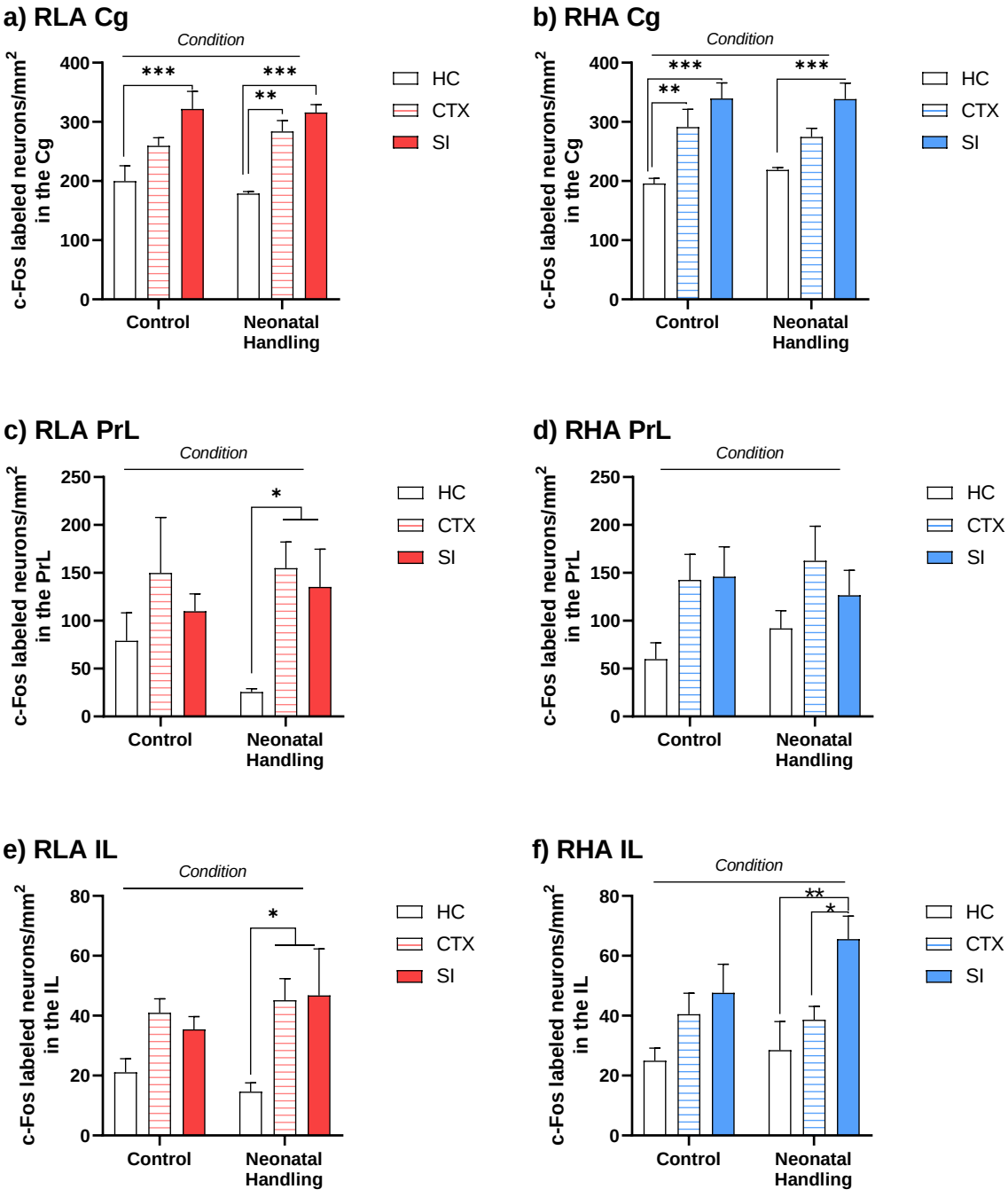

Figure 3: c-Fos expression in different prefrontal cortex areas in different conditions in the Roman rat strains. a) RLA and Cg. **b)** RHA and Cg. **c)** RLA and PrL. **d)** RHA and PrL. **e)** RLA and IL. **f)** RHA and IL. RLA=Roman low avoidance; RHA=Roman high avoidance; Cg=Cingulate cortex; PrL=Prelimbic area; IL=Infralimbic area. Values are mean ± SEM. RLA and RHA N=5/group. "Condition" effect (ANOVA). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Duncan's multiple range test).

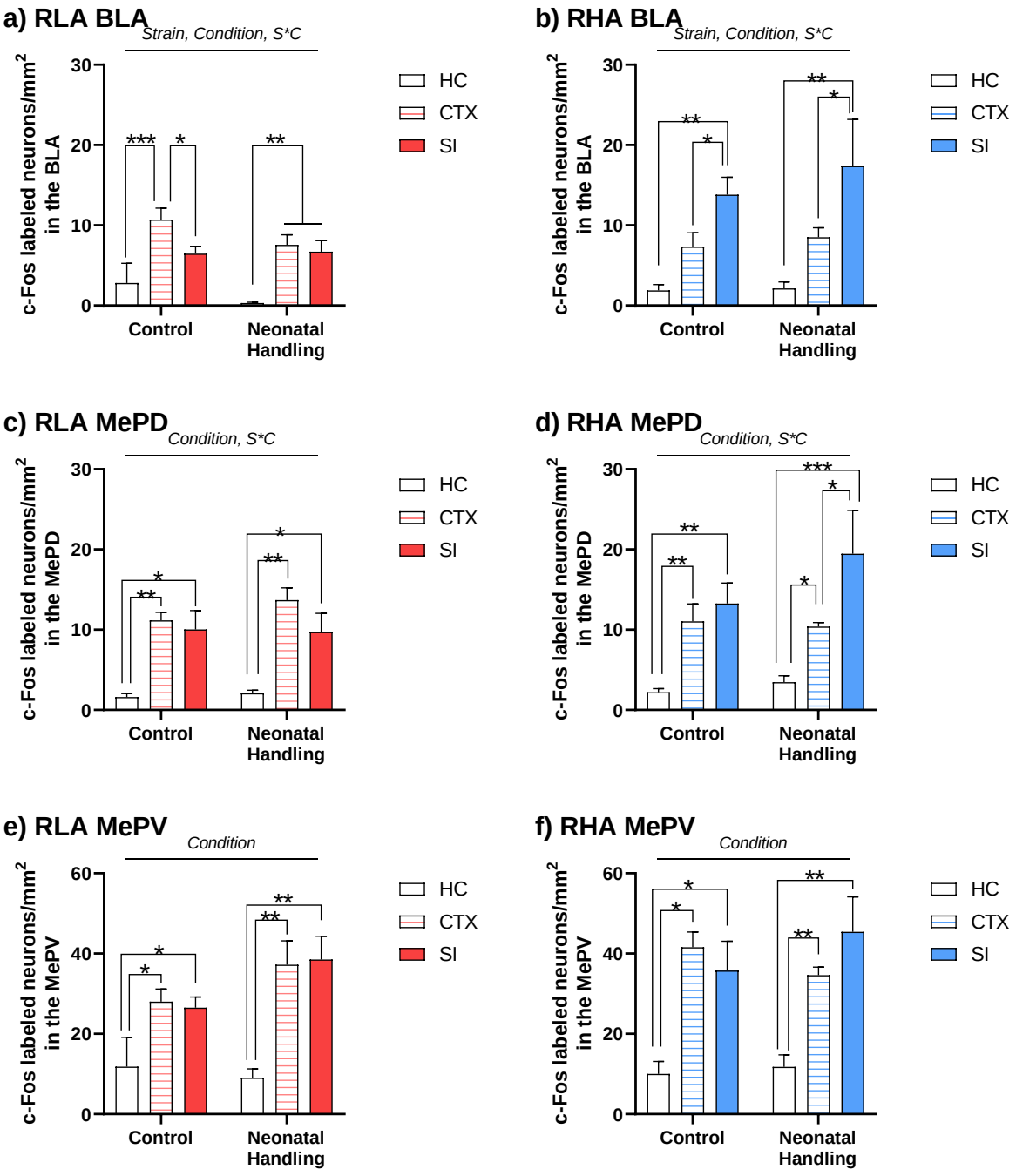

Figure 4: c-Fos expression in different amygdaloid nucleus in different conditions in the Roman rat strains. a) RLA and BLA. **b)** RHA and BLA. **c)** RLA and MePD. **d)** RHA and MePD. **e)** RLA and MePV. **f)** RHA and MePV. RLA=Roman low avoidance; RHA=Roman high avoidance; BLA=Basolateral amygdala; MePD=Medial amygdala posterodorsal; MePV=Medial amygdala posteroventral. Values are mean ± SEM. RLA and RHA N=5/group. "Strain", "Condition", and "Strain*Condition" (S*C) effects (ANOVA). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Duncan's multiple range test).

4. DISCUSSION

The present results show for the first time the effects of the NH treatment on social interaction and c-Fos expression in the Roman rat strains, in various brain regions related to social behaviour. We found that the NH treatment increased social interaction in RHA rats and enhanced c-Fos expression following SI in some of the "social brain" areas specifically in the RHA rats.

The present results of the SI test are consistent with our previous report (Sampedro-Viana et al., 2021), showing that NH leads to increases of social interaction, which are more pronounced in RHA rats.

Results of c-Fos expression (Figure 3 and Figure 4) generally show that CTX and SI conditions activate c-Fos expression in all regions (statistically significant "Condition" effects), thus indicating that exposure to the testing context (CTX condition) and/or to the SI test (SI condition) leads to neuronal activation in the regions investigated. The "Strain x Condition" effects found in BLA and MePD regions reflect the fact that both the CTX and -in particular- the SI condition lead to a higher c-Fos increase in RHA than in RLA rats. It is also worth noting that in several cases, i.e. Cg in both strains (Figure 3a,b), IL in NH-treated RHA rats (Figure 3f) and BLA and MePD in RHA rats (Figure 4b,d), c-Fos activation was apparently greater in the SI than in the CTX condition. This seems to indicate that while exposure to a novel context (CTX) significantly increases c-Fos activation by itself in most regions (see Cg in Figure 3a-b, PrL in Figure 3c, IL in Figure 3e, BLA in Figure 4a, MePD and MeAD in Figure 4c-f), the experience of social interaction (SI condition) in the same context is associated with further neuronal activation in some areas, such as the Cg (Figure 3a-b), the IL in NH-RHAs (Figure 3f), the BLA in RHAs (Figure 4b) and the MePD in NH-RHAs (Figure 4d). Habituation to the context (and thus, the CTX group) was included to discriminate the effect of novelty exposure (and thus, stress) from the actual increase due to social behaviour. It seems reasonable to infer that with a more extended habituation to the context this increase of c-Fos under the CTX condition would be lower, which perhaps would allow detection of greater differences between the SI and CTX groups.

With regard to the effects observed in the cingulate cortex (Cg), it is worth to comment that the SI condition increased c-Fos in all cases, whereas the CTX condition failed to significantly increase neuronal activation in two cases, the control-RLA and NH-RHA groups (Figure 2a-b). The Cg has been linked to the processing of remote contextual fear and potential threats, and consistent with this the Cg is interconnected with amygdala and the hippocampus (e.g. Fiddick, 2011; Frankland et al., 2004; McNaughton & Corr, 2004). Apart from its involvement in these aversive processes, the Cg has been implicated in modulation of (non-aggressive) social behaviour in humans, macaques, rats and mice (e.g. Guo et al., 2019; Mielnik et al., 2014; Rudebeck et al., 2007; see review by Rudebeck et al., 2008). The present c-Fos results in the Cg do not allow to draw any definitive

conclusion on specific strain, treatment and/or SI-condition effects related with Cg activation, although as seen in Figure 2 there appears to be a general (non-significant) trend to a higher response in the social (SI) condition than in the CTX condition. Again, as discussed above, the fact that CTX condition generally elicits very pronounced enhancements of c-Fos expression may be a factor preventing clearer effects of the SI condition.

It seems particularly outstanding that NH treatment increases neuronal activation specifically in some brain areas associated with the social (SI) experience only in RHA rats, as it is particularly clear in the IL and MePD regions of the NH-RHA group tested for SI (Figure 3f and Figure 4b). It is worth to mention here the evidence indicating opposing functional roles for PrL and IL in some behavioural responses (the "PrL-go/IL-stop" model) (Felix-Ortiz et al., 2016; Gourley & Taylor, 2016; Minami et al., 2017). For instance, PrL activity seems to be associated with increases of freezing (fear-related) behaviour, whereas IL stimulation decreases freezing (Minami et al., 2017; Vidal-Gonzalez et al., 2006). This notion on the opposing or complementary role of PrL and IL regions on fear-related responses might be applied to behaviors related with social interaction, as "approach/contact" and "leaving the partner" behaviors in a social interaction test have been related to activation of PrL or IL, respectively (Minami et al., 2017). Remarkably, in the present study the PrL was not responsive to social interaction, as the SI condition did not induce any increase of c-Fos activation over the level of the CTX condition in any strain, while a specific SI-induced c-Fos activation was observed in the IL of NH-treated RHA rats. Notably, RHA rats, which show lowered social preference than RLAs (see Figure 2), were the ones that showed a greater effect of NH treatment on social interaction behavior (NH-RHA group with SI), specifically on social interaction during the first five minutes of the SI test (see Figure 2). Hence, the subjects that benefit the most from the effects of the NH treatment in the social interaction test were also those that presented a specific increase in c-Fos expression in the IL. Thus, this actually points to a specific activation of the IL by NH and SI only in RHA rats, which would be in line with the notion of a different role of IL and PrL in social interaction. While the present procedure and results do not allow further conclusions on the possible complementary (or opposite) role of the PrL and IL on social behavior, they suggest, for the first time to our knowledge, that genetic factors (or genetically-based strain differences) should be more systematically taken into account. In this regard, it is worth mentioning that c-Fos activation in medial PFC (mPFC) differs between the Roman rat strains in response to experimental situations different from the present, although these studies did not differentiate between mPFC subregions (Tapias-espinosa et al., 2019).

There were strain- and treatment-specific increases in c-Fos activation in the subregions of the medial amygdala (MeA). The results show that NH-treated RHA rats submitted to SI exhibit increased c-Fos levels in the MePD without a specific increase in the "control" (untreated) RHA groups or the RLA groups. However, this increase of c-Fos

in the NH+SI RHA group was not observed in the MePV. The MeA receives afferents from the IL, and participates in several social behaviours such as social defeat, social recognition, social categorization, juvenile play, sexual attraction and copulation, maternal behaviour, and dominant/subordinate relations (Adolphs, 2010; Weathington et al., 2012; Zhang et al., 2022). Studies have identified differential functions and projections of the subregions of the MeA (Cádiz-Moretti et al., 2016; Ko 2017; Vertes, 2004). The MePD subregion integrates signals and cues from the environment to coordinate the display of social behaviours, whereas the MePV plays role in the expression of threat-linked defensive reactions (Cádiz-Moretti et al., 2016; Schulz & Sisk, 2016). This may seem to be consistent with the fact that no effects of NH or/and SI have been observed in the MePV in the present study, and with the apparently stronger effects of CTX condition (which is threatening, but not social) on c-Fos expression in the MePV (Figure 4e-f) relative to the MePD (Figure 4c-d) in both strains. Considering the above, and since the MePD receives projections from the IL (Cádiz-Moretti et al., 2016; Ko 2017; Vertes, 2004), it seems plausible that the increase of neuronal activation in the IL of NH-RHA rats under the SI condition may be associated with parallel increases in c-Fos also in the MePD as reflected in the results.

The increase in c-Fos activation in both (control and NH-treated) SI-RHA groups in the BLA deserve some mention. BLA is involved in cognition, motivation and stress responses and is interconnected with many other brain regions, including the mPFC (Ko 2017; Sharp, 2017). Furthermore, it has been reported that inactivation of the BLA increases social behaviour, while BLA activation significantly suppresses social behaviour (Sanders & Shekhar, 1995; Wellman et al., 2016; Yang & Wang, 2017). Therefore, such a negative regulation of social behaviour by BLA does not seem easy to conciliate with the finding that NH-RHA rats (in the SI condition), which constitute the group displaying the highest levels of social interaction (see Figure 2a-b), are those exhibiting the highest c-Fos expression levels in the BLA. Thus, the rats that display higher social interaction and locomotor activity in the SI test (i.e., NH-RHA rats) are those displaying stronger c-Fos activation in this region, for which we have no interpretation at present.

To conclude, the main findings of the present study are that 1) untreated RHA rats exhibit lowered social preference than their RLA counterparts, 2) NH treatment increases social behaviour in RHA rats and 3) in parallel the treatment specifically enhances c-Fos expression in the NH-treated RHA rats relative to their RLA counterparts in some brain areas related to social behaviour, i.e. the IL and the MePD regions. These behavioral results, particularly these of social preference, add further validity to the RHA as a model of schizophrenia-relevant negative-like symptoms. The c-Fos expression results, instead, are more difficult to conciliate with neuro-functional findings from patients with schizophrenia. In fact, neuronal activation of the Cg and PrL was similar between both strains, whereas activation of the IL was specifically higher in NH-treated RHA rats under the social (SI) experience, thus showing no evidence of hypofrontality in RHA rats in the

present experimental conditions (compare with Meyza et al., 2009; Tapias-espinosa et al., 2019). In addition, in two regions of the amygdala, the BLA and MePD, c-Fos activation was higher in RHA than RLA rats (see "Strain x Condition" interaction effects; Figure 4a-d), particularly in the SI condition, which is also partially in contrast with findings from studies comparing RHA vs RLA rats in other testing situations (Meyza et al., 2009; Tapias-espinosa et al., 2019). One possible explanation of these seemingly paradoxical findings between these two reports and the present study would be that these authors used novelty-based non-social testing situations (i.e. PPI testing and novelty open-field-like tests). Whether these discrepancies in strain-related c-Fos activation are due to different testing conditions/situations would deserve further experimental evaluation. Interestingly, however, to the best of our knowledge there has been only one report of c-Fos expression measures in a SI condition that has included both the CTX and HC control conditions. Perkins et al., (2017) compared male and female F344 rats and showed no effects of SI (vs. HC and CTX conditions) in males, whereas SI induced c-Fos elevations in several PFC and amygdala regions in females. It is interesting that females under the SI condition seemingly exhibited higher c-Fos activation in IL, PrL and MeA than males, which was paralleled by higher social behaviour also in females (Perkins et al., 2017). Thus, these findings are partially consistent with the present ones, in that the animals showing higher social interaction (females in Perkins et al., 2017 study, and NH-RHA in the present study) also show higher c-Fos expression levels in the IL and MePD regions.

Finally, future studies should evaluate whether longer habituation periods before the social interaction test may improve discrimination between the effects of CTX and SI conditions on c-Fos expression.

Author contributions

D.S-V., T.C., A.T. and A.F-T. conceived and designed the experiments. D.S-V. and T.C. conducted the behavioral experiments. F.S. and I.O. assisted in the behavioral experiment. A.F-T. and D.S-V. analyzed the data and wrote the original manuscript. A.T., F.S., T.C. and I.O. provided critical review of the original draft. All authors read and approved the manuscript.

Acknowledgments

Supported by grants PID2020-114697GB-I00, PSI2017-82257-P, 2017SGR-1586, "ICREA-Academia 2013" (A.F-T., A.T.). F.S. was the recipient of a PhD fellowship funded by the Italian Ministry of University and Research (MUR). Thank you for the drawings of Figure 1 to F.H-R.

Conflict of interest

The authors present no conflict of interest.

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ESTUDIO 3

Social preference in Roman rats: Age and sex variations relevance for modeling negative schizophrenia-like features

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Artículo publicado

Physiology and Behavior; IF(2021)=3.74

https://doi.org/10.1016/j.physbeh.2022.113722

ABSTRACT

Social withdrawal is one of the most relevant negative symptoms of schizophrenia. Animal models that mimic schizophrenia's symptoms, in general, and negative symptoms, in particular, are difficult to develop because of the high complexity of symptoms and neurochemical disturbances that schizophrenia patients display throughout their lives. In recent years we have shown that Roman High-Avoidance (RHA) rats exhibit some phenotypes that are thought to represent positive symptoms, cognitive/attentional symptoms, as well as some negative symptoms of the disease. In the present study, we aimed at elucidating whether the social interaction (SI) deficits exhibited by adult male RHA rats, compared to their Roman Low-Avoidance (RLA) counterparts, are also present during adolescence, as well as whether there are between-strain differences in adolescent and adult female rats. The results of the present study show that adult male RHA rats exhibited a deficit in social preference compared to their RLA counterparts. Such a deficit was not observed in adolescent RHA rats or female rats of any age. The results also show that the adult male rats of both strains had significant decreases in social preference compared to the adolescent male rats. Additionally, we also show that female adult RHA rats have greater social preference than their male counterparts. These results seem to be in line with previous rodent and human studies and add face validity to the RHA rats as a model of schizophrenia.

Keywords: RHA and RLA rats, Schizophrenia, Social Interaction, Asociality, Neurodevelopment.

1. INTRODUCTION

Social withdrawal is one of the most important negative symptoms of schizophrenia that has been linked to longer and more debilitating prodromal periods [1, 2]. According to Marder and Galderesi [3], asociality in schizophrenia should be defined as a reduction in the motivation to create new relationships with others, and it should not be regarded as if the subjects have or don't have social interactions or close relationships. Thus, animal models that are used to test the different aspects of social engagement may be essential in furthering the knowledge about the neurobiological features that underlie the negative symptoms of schizophrenia.

Negative symptoms are present in one-third of the patients with schizophrenia, and they are associated with the development of the disorder, and the patients' quality of life [4, 5, 6, 7, 8]. Negative symptoms may be secondary to positive symptoms, but there are patients with schizophrenia that primarily present negative symptoms [9].

Regarding the development of schizophrenia's symptoms, there is no clear evidence of a straightforward course of the illness. Nevertheless, several theories have pointed to altered neurodevelopment, where processes related to the molecular as well as anatomical changes during adolescence could have a key role in the emergence of the disorder (see Uhlhaas [10] for review). Although negative symptoms usually appear in the prodromal phase of schizophrenia, it has to be taken into account that they can arise at any point throughout the course of the disorder [11, 12].

Usually, preclinical studies have employed the social interaction test, in which two unfamiliar rodents are placed in an open field-like situation where they are free to interact with each other. This test is an ethologically valid method to assess social behavior (for a review, see Gururajan et al. [13]). Additionally, other social interaction tests can measure social preference/motivation to better separate social behavior from the anxiety originating from being in a new environment with an unfamiliar conspecific [14, 15, 16].

Modeling schizophrenia's symptoms, in general, and negative symptoms, in particular, is a difficult task because of the low level of understanding of the etiopathology of the illness [17, 18, 19]. To overcome this issue several animal models have been developed (for review see [20, 21, 22][23]. The Roman rat strains (Roman high-avoidance rats (RHA) and Roman low-avoidance (RLA) rats) have been extensively studied regarding the schizophrenia-like profile of the RHA rats (see reviews [24, 25]).

In recent years we have shown that RHA rats exhibit some phenotypes that are thought to represent positive symptoms, such as increased baseline and psychostimulant-enhanced locomotor activity [26, 27, 28], cognitive/attentional symptoms such as diminished PPI [29, 30], impaired latent inhibition [31, 32], working memory and reference memory deficits [29], [33, 34].

Regarding the negative symptoms domain, we have recently shown that adult male RHA display deficits in social interaction compared to their RLA counterparts and heterogeneous (outbred) stock rats [16]. In addition, the RHA rats also present some neurobiological alterations in the mesolimbic dopaminergic system, along with some characteristics in the central serotoninergic and glutamatergic function, and in the function of the prefrontal cortex and hippocampus, that resemble the neurochemical and neuroanatomical traits found in patients with schizophrenia (reviewed by Giorgi et al. [24], Fernández-Teruel et al. [25]).

In the present study, we aimed at elucidating whether the social interaction deficits exhibited by the RHA rats are present in their adolescence and whether there are differences between strains and sexes regarding their social behavior during neurodevelopment.

Taking into account previous results in Roman rats [16] we expect to find a social preference deficit in adult male RHA rats compared to their RLA counterparts. Additionally, previous literature suggests that negative symptoms are more prevalent in men [35, 36, 37], thus we expect to find a more robust deficit in male rats. Finally, we also hypothesize that adolescent rats will exhibit increased social preference compared to their adult counterparts since several reports suggest that adolescent animals have increased sensation-seeking and reward-seeking behaviors [38, 39, 40, 41].

2. MATERIAL AND METHODS

2.1 Animals

Roman (RHA and RLA) male and female rats were used. The total number of rats was 96. Each experimental group had 12 rats for each strain (RHA/RLA), age (adolescent/adult), and sex (female/male). All rats came from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine (School of Medicine, Autonomous University of Barcelona, Spain), since 1996. The rats from each experimental group came from 10 to 12 different litters (1–2 rats from each litter). The adolescent rats were approximately 46–54 days old and the adult rats were 5 months old at the beginning of the experiment. Animals were housed in same-sexed pairs in standard (50 × 25 × 14 cm) macrolon cages and maintained under a 12:12 h light-dark cycle (lights on at 08:00 a. m), with controlled temperature (22 + 2 ◦C) and humidity (50–70%). They had food and water available ad libitum.

All testing was carried out between 09:00 and 14:00 h. All procedures were carried out under the Spanish Legislation (Royal Decree 53/ 2013, 1st February 2013) and the current regulation related to "Protection of Animals used for Scientific Purposes" established by the European Union (2010/63/UE, 22 September 2010).

2.2 Social interaction

The social interaction (SI) set-up test was adapted from Gururajan et al., [15]. Two acrylic boxes (65 × 23 × 20 cm) were placed in front of each other at 12 cm (see drawing in [16]), to prevent physical contact between the animals, in a red-lit room. Each box had two holes at the ends of 3 cm diameter. The hole facing the other box was named "social hole", while the opposite (distal) hole was named as "non-social hole". All the procedure was recorded by a camera placed on the roof and connected to a TV monitor outside the experimental room.

The procedure can be divided into 2 phases: habituation and testing. During the habituation phase (30 min, 24 h before SI testing), the four holes were covered with tape and a barrier was placed between the two boxes to limit the exploration activity of the next box. A pair of non- familiar weight-matched animals were placed into the boxes (one rat in each box).

For SI testing phase the holes were opened and two weight-matched non-familiar rats of the same strain were placed (one in each box) into the set-up for a 15-min test. Time spent exploring (i.e. nose poking) the social hole (Social Time) and the non-social hole (Non-Social Time) were measured by two trained observers blind to strain, and from these measures, the percentage (%) time spent in the social hole (Social preference) was calculated according to the following formula:

%Social Time = (Social Time/ (Social Time + Non-social Time)) x 100

The total number of crossings was also scored (each box was divided into three equal squares by lines painted on the floor to measure horizontal activity –crossings-). The boxes were cleaned with a 70% ethanol solution and dried with a paper towel between every successive pair of animals.

2. 3. Statistical analyses

The statistical analyses were carried out using the "Statistical Package for the Social Sciences" (SPSS, version 17). The p-value threshold was set at $p < 0.05$.

Three-way ANOVAs, with strain, sex, and age as independent variables, were employed to analyze social preference and locomotor activity. To analyze social and non-social time we performed a repeated- measures ANOVA with the social and non-social times as within- subjects factor and strain, sex, and age as independent between- subject factors. Then separate ANOVAs or repeated-measures ANOVA for each strain and each sex were employed to explore further interactive effects identified in the full ANOVA. Further, post-hoc Duncan's multiple range tests were used when there were significant effects of the strain, age, or sex, as well as the interactions between these factors in order to elucidate among which groups there were significant differences. Additionally, partial eta squared ($\eta^2 p$) was included as an effect size measure.

3. RESULTS

The results of the three-way ANOVA with the social preference as the dependent variable and with strain (RHA and RLA), age (adolescent and adult), and sex (male and female) as between-subjects factors showed significant effects of the strain and age ($F(1,88)= 21.65$, $p \leq 0.001$, $\eta^2p= 0.20$; $F(1,88)=44.87$, $p \leq 0.001$, $\eta^2p = 0.34$; respectively), as well as, the interactions between strain x age, strain x sex and age x sex ($F(1,88)=4.30$, $p \leq 0.041$, $\eta^2p=0.05$; $F(1,88)=5.10$, $p \leq 0.026$, $\eta^2p=0.06$, $F(1,88) = 11.77$, $p \leq 0.001$, $\eta^2p= 0.12$, respectively) (Figure 1).

Then, we performed separate ANOVAs for each strain. The results for the RHA rats showed a significant age effect ($F(1,44) = 42.06$, $p \leq 0.001$, $\eta^2p= 0.49$) as well as, the interaction between age x sex ($F(1,44)=15.75$, $p \leq 0.001$, $\eta^2p= 0.26$), indicating that adult rats showed reduced levels of social preference and that this reduction was more marked in adult male rats (Figure 1).

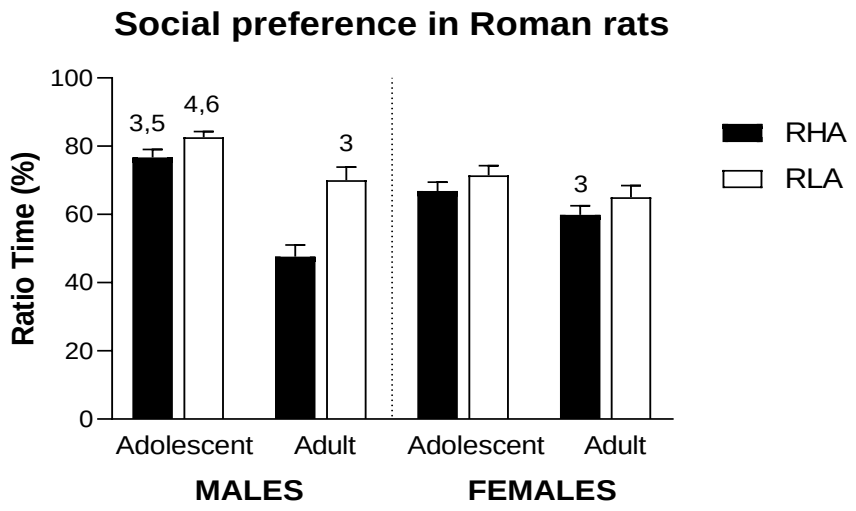

Figure 1.-. Mean social preference (±SEM) in male and female Roman rats. Numbers above the bars indicate significant differences between groups after significant differences strain, age, sex, or any interaction among these factors in the ANOVA. The groups' numbers are as follows: 1-RHA adolescence male, 2-RLA adolescence male, 3-RHA adult male, 4-RLA adult male, 5-RHA adolescence female, 6-RLA adolescent female, 7-RHA adult female, 8-RLA adult female rats. $p < 0.05$ between the indicated groups in the post-hoc Duncan's multiple range tests.

For the RLA rats the results showed that age and sex effects were significant ($F(1,44)=9.86$, $p \leq 0.003$, $\eta^2p=0.18$; $F(1,44)=7.12$, $p \leq 0.011$, $\eta^2p=0.14$, respectively). These results indicate that social preference in RLA rats was higher in adolescent rats compared to their adult counterparts, and they also suggest that social preference was higher in males than in female RLA rats (Figure 1).

We also performed separate ANOVAs for each sex. For the male rats, the results show significant strain ($F(1,44)=23.48$, $p \leq 0.001$, $\eta^2p=0.35$) and age ($F(1,44)=50.45$, $p \leq 0.001$,

η^2p= 0.53) effects, together with the interaction of these factors (F(1,44) =7.91, p ≤ 0.007, η^2p=0.15). These results indicate that compared to the RHA adolescent rats, the social preference levels of the adult RHA rats were reduced; whereas the social preference percentage of the RLA rats was similar in both ages. On the other hand, the results for the female rats only show a significant age effect (F(1,44)=5.43, p ≤ 0.024, η^2p= 0.11), indicating that adolescent animals of both strains showed increased social preference levels compared to their adult counterparts (**Figure 1**).

Further post-hoc analyses revealed there were significant differences between RHA and RLA adult male rats. Regarding the effect of age be- tween the strains, we observed significant reductions of social preference in adult male rats of both strains compared to the adolescent male rats. The sex effect was more apparent in adolescence where male rats of both strains had greater social preference than adolescent female rats. In the adult rats, we found that the social preference of female RHA rats was higher than their male counterparts (**Figure 1**).

Concerning the social time and non-social time (**Figure 2-3**), we performed a repeated-measures ANOVA with the social and non-social time as within-subjects measure and the strain, sex, and age as the between-subjects factors. The results revealed a significant effect of the within- subject factor (F(1,88)=186.44, p ≤ 0.001, η^2p=0.68), as well as the interaction between Social and Non-Social time x age (F(1,88) = 39.88, p ≤ 0.001, η^2p=0.31), the interaction Social and Non-Social time x age x strain (F(1,88) = 4.53, p ≤ 0.036, η^2p=0.05) and the interaction Social and Non-social time x sex x age (F(1,88)=5.91, p ≤ 0.017, η^2p=0.06). The strain and sex effects were also significant (F(1,88)=34.17, p ≤ 0.001, η^2p=0.28; F(1,88)= 15.87, p ≤ 0.001, η^2p=0.15, respectively) Then, we performed separate repeated-measures ANOVAs for each strain. The results for the RHA rats showed a significant effect of the Social and Non-social time (F(1,44)=63.86, p ≤ 0.001, η^2p=0.59) and the interactions Social and Non-social time x age (F(1,44)= 27.29, p ≤ 0.001, η^2p=0.38) and Social and Non-social time x age x sex (F(1,44)=7.17, p ≤ 0.010, η^2p=0.14). The sex effect was also significant (F(1,44)=7.18, p ≤ 0.010, η^2p=0.14). These results indicate that female rats spent more time in both holes compared to male rats. Additionally, the interactions suggest that female RHA rats of both ages spent similar time in both holes whereas adult RHA males increased their non-social time and decreased their social time compared to their adolescent counterparts (**Figure 2-3**).

The repeated-measures ANOVA for the RLA rats showed a significant effect of the Social and Non-social time (F(1,44)=149.27, p ≤ 0.001, η^2p=0.77) and the interaction Social and Non-social time x age (F(1,44)= 12.63, p ≤ 0.001, η^2p=0.22). The sex effect was also significant (F(1,44)=9.14, p ≤ 0.004, η^2p=0.17). These results also indicate that female RLA rats spent more time in both holes than their male counterparts, while the significant interaction seems to suggest that the time spent in the social hole tended to decrease with age whereas the contrary was true in the non-social hole (**Figure 2-3**).

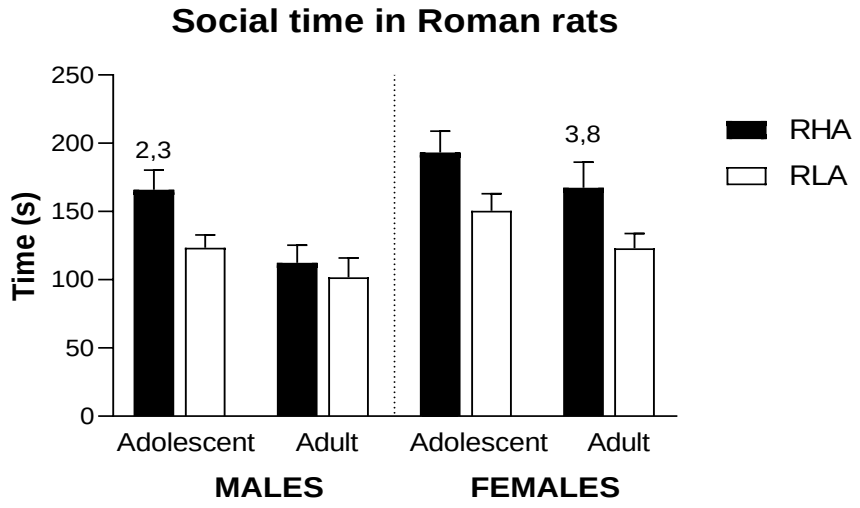

Figure 2.-. Mean social time (±SEM) spent by male and female Roman rats. Numbers indicate significant differences between groups after significant strain, age, sex, or any interaction among these factors in the ANOVA. The groups' numbers are as follows: 1- RHA adolescent male, 2-RLA adolescent male, 3-RHA adult male, 4-RLA adult male, 5-RHA adolescent female, 6-RLA adolescent female, 7-RHA adult female, 8- RLA adult female rats. $p < 0.05$ be- tween the indicated groups in the post-hoc Duncan's multiple range tests.

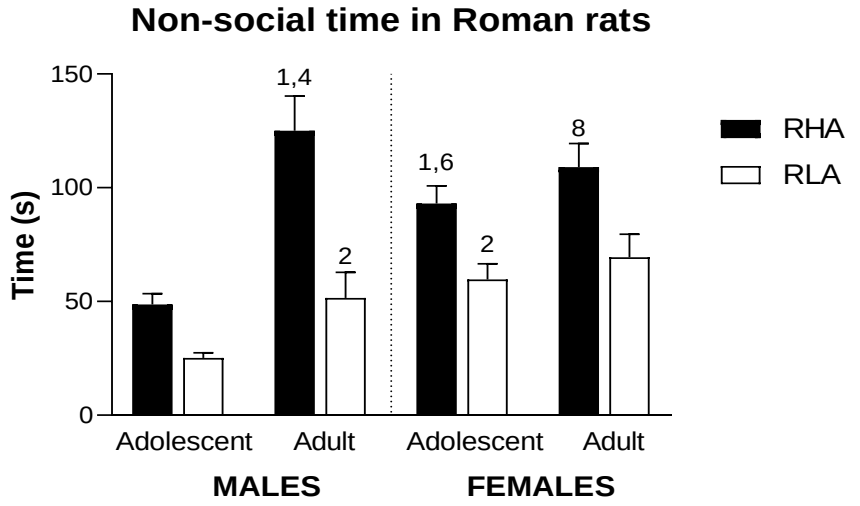

Figure 3.-. Mean non-social time (±SEM) spent by male and female Roman rats. Numbers indicate significant differences between groups after significant strain, age, sex, or any inter- action among these factors in the ANOVA. The groups' numbers are as follows: 1- RHA adolescent male, 2-RLA adolescent male, 3-RHA adult male, 4-RLA adult male, 5-RHA adolescent female, 6-RLA adolescent female, 7-RHA adult female, 8- RLA adult female rats. $p < 0.05$ between the indicated groups in the post- hoc Duncan's multiple range tests.

We also conducted separate repeated-measures ANOVA for each sex. The results of the analysis for the male rats revealed a significant effect of the Social and Non-social time ($F(1,44)=87.60$, $p \leq 0.001$, $\eta^2p=0.67$) and the interactions Social and Non-social time

x age ($F(1,44)= 43.38$, $p \leq 0.001$, $\eta^2p=0.50$) and Social and Non-social time x age x strain ($F(1,44)= 9.18$, $p \leq 0.004$, $\eta^2p=0.17$). The strain effect was also significant ($F(1,44)=16.37$, $p \leq 0.001$, $\eta^2p= 0.27$). These results indicate that overall the RHA rats spent more time in both holes than RLA rats. The second-order interaction seems to indicate that adult RHA rats reduced their social time compared to their adolescent counterparts. In the non-social hole, the adult RHA rats spent more time than their adolescent counterparts. This tendency was not observed in male RLA rats (Figure 2- 3).

The results for the female rats showed a significant effect of the Social and Non-social time ($F(1,44)=98.99$, $p \leq 0.001$, $\eta^2p=0.69$) and the interactions Social and Non-social time x age ($F(1,44)= 6.74$, $p \leq 0.013$, $\eta^2p=0.13$). The strain effect was also significant ($F(1,44)= 17.80$, $p \leq 0.001$, $\eta^2p=0.29$). These results indicate that overall the female RHA rats spent more time in both holes than RLA rats. The interaction seems to indicate that in both strains social time decreased with age whereas non-social time increased with age (Figure 2-3).

Further post-hoc analyses for the social time revealed that adolescent male RHA rats spent more time in the social hole than their RLA counterparts, while there were no differences in the adult male rats. On the other hand, there were significant differences between adult females of both strains as RHA rats spent more time than RLAs in the social hole.

Furthermore, we observed a significant reduction of social time in the adult male RHA rats compared to the adolescent male rats. This reduction was not significant in RLA adult male rats nor in female rats of both strains. We also found significant differences between sexes, since adult female RHA rats spent more time in the social hole than their male counterparts (Figure 2).

Post-hoc analyses for the non-social time revealed a between strain difference in the adult male rats. There were significant between-strain differences in both adolescent and adult female rats. Additionally, we observed a significant increase in the time spent in the non-social hole in the adult male rats of both strains compared to their adolescent counterparts. The effect of sex was only apparent in the adolescent rats, where female rats of both strains spent more time in the non-social hole than their male counterparts (Figure 3).

Finally, we analyzed the locomotor activity of the rats throughout the 15 min of the SI test. The three-way ANOVA revealed significant effects of the strain and sex ($F(1,88)= 68.81$, $p \leq 0.001$, $\eta^2p=0.44$ and $F(1,88)= 10.73$, $p \leq 0.002$, $\eta^2p=0.11$, respectively); the interaction between strain x age was also significant ($F(1,88)= 8.89$, $p \leq 0.004$, $\eta^2p= 0.09$) (Figure 4).

Then, we conducted separate ANOVAs for each strain. The results for the RHA rats revealed significant effect of the interaction sex x age ($F (1,44)= 8.89$, $p \leq 0.004$,

η^2p=0.09). These results indicate that activity increased with age in males while the opposite was true for the female RHA rats. The same analysis for the RLA rats revealed that the age and sex effects were significant ($F(1,44) = 11.18$, $p \leq 0.002$, η^2p=0.20; $F(1,44)$=16.82, $p \leq 0.001$, η^2p=0.28, respectively), indicating that adolescent RLA rats travelled longer distances than their adult counterparts and that female rats also had higher levels of locomotor activity than males.

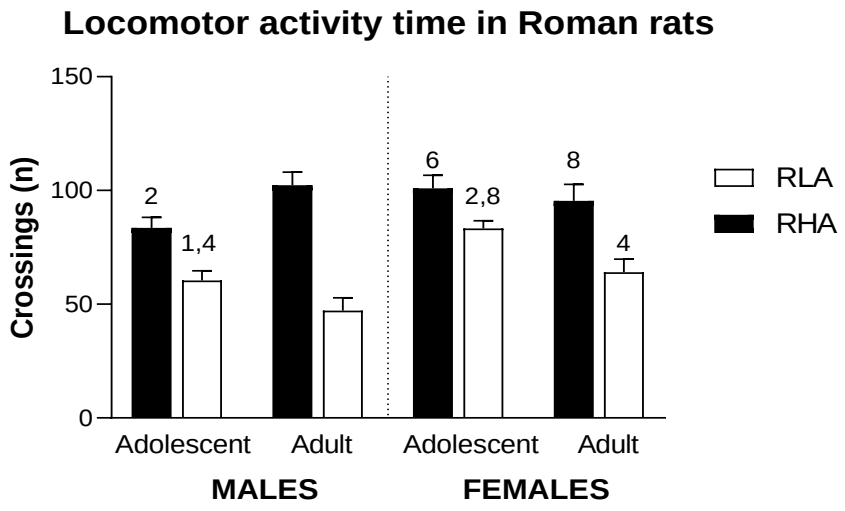

Figure 4.-. Mean locomotor activity (±SEM) of male and female Roman rats. Numbers indicate significant differences between groups after significant strain, age, sex, or any interaction among these factors in the ANOVA. The groups are as follows: 1- RHA adolescent male, 2-RLA adolescent male, 3-RHA adult male, 4-RLA adult male, 5-RHA adolescent female, 6-RLA adolescent female, 7-RHA adult female, 8- RLA adult female rats. $p < 0.05$ between the indicated groups in the post-hoc Duncan's multiple range tests.

After that, we performed separate ANOVAs for each sex. The results for the male rats showed a significant strain effect ($F(1,44)$= 58.08, $p \leq 0.001$, η^2p=0.57), as well as the interaction between strain x age ($F(1,44)$= 9.76, $p \leq 0.003$, η^2p=0.18), indicating that the locomotor activity of the RHA increased with age while the RLA's locomotor activity decreased with age. For the female rats the results show that the strain ($F(1,44) = 18.48$, $p \leq 0.001$, η^2p=0.30) and age ($F(1,44) = 4.68$, $p \leq 0.036$, η^2p=0.10) effects were significant. These results indicate that in female rats, RHAs have high levels of locomotor activity than RLA rats and that adolescent rats traveled longer distances than adult rats.

Further post-hoc analyses revealed significant differences between RHA vs. RLA rats of both ages and both sexes indicating that RHA rats traveled longer distances during the SI test. Adult male RHA rats had a higher number of crossings compared to the male adolescent RHA rats. Conversely, adolescent female RLA rats traveled longer distances than adult female RLA rats. Regarding the differences between sexes, we observed that

female RLA rats of both ages traveled longer distances than their male counterparts. (all $p \leq 0.05$; Figure 4).

4. DISCUSSION

Regarding the main measure of social behavior of the present study, i.e. social preference, we have found that adult male RHA rats exhibit a deficit compared to their RLA counterparts, but this deficit is not observed in adult RHA vs. RLA females or between adolescent RHA vs RLA rats of both sexes. Interestingly, there is an overall "age" effect reflecting that, in general, adolescent rats display higher social preference than adult rats, although this effect is more pronounced in males (leading to an "age x sex" interaction; Figure 1).

With regard to the raw (non-relative) measures of social and non-social behavior, i.e. "social time" and "non-social time", it is observed that RHA rats generally show higher levels of both behaviors ("strain" effect; Figure 2-3) and females also show overall higher levels than males ("sex" effect; Figure 2-3). The most relevant effect to be highlighted here is that, while adult RHA and RLA males show similar levels of social time (Figure 2), adult RHA males show a marked increase of non-social time relative to RLA males (Figure 3); this is the main reason for the deficit in social preference observed in adult RHA males (which actually do not prefer the social over the non-social hole) vs. adult RLA males (Figure 1).

With respect to locomotor activity, we found that in both sexes and both ages the RHA traveled longer distances than their RLA counterparts ("strain" effect, Figure 4), whereas females globally show higher activity levels than males ("sex" effect, Figure 4). The observed "strain x age" effect is due to the fact that RLA rats tend to decrease activity across age, whereas the opposite trend is observed in RHA rats (Figure 4).

The results of the adult male rats in social preference are in accordance with our previous report showing that adult male RHA rats exhibited decreased levels of social preference compared to their RLA counterparts [16]. Similarly, in both studies, adult male RHA's social preference was around 50%, indicating a random exploration of both holes. The present study adds the evaluation of both ages and both sexes of RHA vs. RLA rats. Interestingly, no between-strain significant differences were observed at adolescence or between female rats although the global "strain" trend observed reflects that RLA rats' social preference was generally above the social preference displayed by the RHA rats.

As said above, the deficit of social preference in adult male RHA rats can be explained by the pattern found in the time spent in both holes, namely a reduction in the social time (adult vs. adolescent RHA rats; Figure 2) and an increased time spent in the non-social hole (Figure 3) by that strain. This pattern was not observed in the RLA or females of both strains (see Figure 2-3).

Furthermore, we also found that adolescent male rats preferred the social hole more than their adult counterparts. This result can be explained because novelty-seeking behavior and the motivation for a wide range of rewards are important aspects for the

development of adolescent animals compared to adult animals [38, 39, 40, 41]. Thus, the fact that the social preference is higher in adolescent rats than adult rats has also been found in other studies. For instance, Reppucci et al. [38] found a 183% increase, on average, of social time that adolescent Wistar rats spent investigating a social stimulus compared to adult rats. In another study, Douglas et al. [42] found that isolated male adolescent rats had greater social preference than their adult counterparts. Although there are some important methodological differences between these studies, it seems clear that adolescence is a critical period where social factors have an important role in neurodevelopment (e.g.[39], [40, 41, 43]). Additionally, reward-seeking behavior is a hallmark trait of adolescence in many species [40], hence the increased social preference in the adolescent rats we found in the present study.

Although the social preference of adolescent Roman rats was higher than their adult counterparts in all groups, the difference between adolescent male RHA and adult male RHA rats was the only one that was statistically significant, suggesting altered neurodevelopment of the male adult RHA rats. Indeed, recent results indicate that adult male RHA rats exhibit some neurochemical features that are different from the RLA rats, which in turn suggest a delayed (or immature) cortical development and resemble the characteristics found in schizophrenia patients. For instance, compared to male RLA rats, the RHAs showed increased expression of Homer1, Nrg1, Syp, Bdnf, Grin2B and Drd1, in the pre- frontal cortex (PFC), which is similar to the expression found in child- hood and adolescence in human studies [44]. Hence, the neurodevelopment of the RHA rats leads to an immature PFC, which is a key area involved in the pathophysiology of schizophrenia, as well as in the neurobiology of social behavior [44, 45]. Along with these neuro-chemical features of brain development, other aspects may be behind some of the between-strain differences, for example, compared to the adult male RLA rats, the PFC of the RHA counterparts also presents decreased volume and neuronal activity (c-fos), and increased density of pyramidal dendritic “thin” (immature) spines and astroglia number [30] [46],[47, 48, 49]. Apart from these features of the PFC, we and others have also found that the adult male RHA rats exhibit decreased volume, neuronal density, and function (c-fos) of the hippocampus and the amygdala [30, 34, 46, 47, 48, 50, 51], which also have a fundamental role in the pathophysiology of psychosis [52], and their connectivity with the PFC is important for social behavior (e.g. [53, 54]). Moreover, we have recently found that RHA rats show decreased frontocortical expression of the CD38 gene, which is a regulator of oxytocin release and has a crucial role in social behaviors [55]. Thus, it seems possible that these traits, along with the known between-strain differences regarding dopamine, serotonin and glutamate systems (particularly NMDA and mGlu2 receptors; see [44, 56, 57]; for review see [24, 25]), may have a modulating role on social behavior and thus in the RHA vs. RLA differences seen in the present study [16, 58]. To sum up, the reward-seeking tendency of the adolescent animals along with the neurochemical and neuroanatomical features of the adult male RHA may partly be

the reason for the present results regarding the strain and age effects on social preference.

We also found some differences between the sexes. Regarding social preference, we found that adolescent male rats of both strains had higher social preference than their female counterparts, which is in line with findings from others [42]. Additionally, the fact that in adult male rats there were statistical differences between strains while there weren't significant differences in the adult female rats is consistent with human studies showing that negative symptoms such as social withdrawal are less apparent in women than in men [59, 60, 61]. Along these lines, other studies also show that men with psychotic disorders exhibit more severe negative symptoms, poorer psychosocial functioning, and worse illness trajectory than women [35, 36, 37].

Regarding the social and the non-social time we found that adolescent female RHA rats spent more time in both holes than their male counterparts. The adult female RHA rats spent more social time than males. Concerning the female RLA rats, only the adolescent rats spent more non-social time than their male counterparts. So, the results seem to suggest that a stronger tendency towards reward-seeking and novelty-seeking behavior in adolescent female rats of both strains may underlie their globally augmented exploration of both holes.

Finally, it is worth mentioning that RHA rats display higher locomotor activity than RLAs, while females are globally more active than males in the SI test (see "strain" and "sex" effects, Figure 4), which is consistent with several previous studies also showing these strain (e.g. [25, 62]) and sex (e.g. [62, 63]) differences. Also interestingly, we found a "strain x age" effect on locomotor activity, indicating that RLA rats globally decreased activity across age, whereas the opposite trend was found in RHA rats, particularly in males (Figure 4).

SI tests aimed at measuring social interaction to model "asociality" as a negative symptom of schizophrenia need to be devoid (as much as possible) of influences of novelty or anxiety components. For these reasons, habituation of the animals to the test conditions prior to SI testing (as done in the present study) is necessary for the SI test to be able to measure the motivation (or preference) for social interaction while minimizing anxiety influences or interferences of novelty (or novelty effects on activity) as much as possible. Importantly, in a previous study, we have shown that the present SI procedure indicates motivation for social preference regardless of the effects on anxiety- related measures and the levels of activity. According to Deak et al. [14], the present SI test is able to combine measures of social motivation and measures of exploratory/locomotor activity. To the extent that spontaneous locomotor activity is considered to model some positive symptoms of schizophrenia (see reviews [19][20],[21],[22],[23]), such an increase of activity across age in RHA rats may be interpreted as reinforcing the face validity of this strain as a model of the disorder. This is further supported by evidence

from a variety of different tests, in which RHA rats have consistently shown increased locomotor activity relative to their RLA counterparts [24] [25].

Despite these noteworthy results, the present study has some limitations such as the fact that we only present behavioral data without any accompanying neurobiological factor that could explain (or be related to) the aforementioned social preference differences between groups. Furthermore, as mentioned in the introduction social behavior has a wide range of manifestations that should also be investigated (e.g. maternal behavior, play behavior, etc. But see findings on maternal behavior in Fernandez-Teruel et al., [25]). In the context of this variety of social behaviors, there are other factors such as male-male dominance that could play a role in the interpretation of the results from SI tests, as it has been reported that dominant and subordinate rats have different behavioral maturation [64]. Testosterone and its role in the identification of some odors could also have an impact on social behaviors, as Thor [65] reported that male rats showed more social investigation than females and that testosterone (administered in very high dosage, 5.0 mg/day) improved the performance in an odor-detection task of castrated females, exceeding the performance of intact males or females [65][66]. However, according to Deak et al. [14], in the current experimental setup, which includes a physical separation (preventing physical contact) between the tested rats, animals are prevented from expressing actual dominance/territorial behaviors and sexual behaviors. Furthermore, the present results replicate our previous studies in adult males [16], in which over 80 rats of each strain were compared in three independent experiments. In addition, we have unpublished data indicating that some antipsychotics such as clozapine, ziprasidone, aripiprazole, as well as oxytocin, can partly reverse the hyperlocomotion and the impairment of social preference induced by the NMDA antagonist dizocilpine in RHA rats (for review see [25]). Thus, the present results, together with the above-mentioned findings, seem to indicate that adult male RHA rats display a deficit in social preference that is likely due to a lack of motivation to engage in social investigation [14, 16].

To conclude, the main findings of the present study are that adult male RHA rats exhibit a deficit in social preference compared to their RLA counterparts. The results also show that the adult male rats of both strains have significant decreases in social preference compared to the adolescent male rats. Additionally, we also show that female adult RHA rats have greater social preference than their male counterparts. These results are consistent with previous rodent and human studies showing that men have worse negative symptoms than women. These results add further validity to the RHA as a model of schizophrenia-relevant symptoms. Nevertheless, longitudinal studies including pre- and post- pubertal stages of development to evaluate the emergence and course of the negative symptoms in this model are warranted to gather new data regarding these neurodevelopmental features of the model.

Author contributions

A.F-T., I.O. and A.T. conceived and designed the experiments. I.O. and O.J.S-R. conducted the behavioural experiments, T. C. and D. S-V. assisted and supervised the behavioral experiments, A.F-T. and I.O. analyzed the data and wrote the original manuscript, A.T., O.J.S-R, T. C. and D. S-V. provided critical review of the original draft.

Acknowledgments

The authors and part of the work reviewed herein are supported or have received support from grants PID2020–114697GB-I00, PSI2017–82257-P, 2017SGR-1586 and "ICREA-Academia 2013'' (A.F- T., A.T.), and "Juan de la Cierva" postdoctoral fellowship (FJC2018–038808-I; I.O.).

Conflict of interest

The authors present no conflict of interest.

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ESTUDIO 4

Atypical antipsychotics attenuate MK801-induced social withdrawal in the RHA rat: a model of schizophrenia-relevant features

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Manuscrito en revisión (Psychopharmacology)

ABSTRACT

Rationale: The administration of NMDA receptor (NMDAR) antagonists constitutes a widely used model that produce both positive (e.g., hyperactivity) and negative (e.g., social withdrawal) symptoms relevant for schizophrenia in rodents. These effects can be reversed with the administration of atypical (second and third generation) antipsychotics.

Objectives: In this study we combined the NMDAR-antagonist model with the Roman High-Avoidance (RHA) strain, a psychogenetically selected model of schizophrenia-relevant features. We also studied whether some atypical antipsychotics drugs (clozapine, ziprasidone, and aripiprazole) would be able to attenuate or reverse the behavioural alterations induced by MK801- and whether such effects might be dependent on the rat strain.

Methods: MK801 dose-response study was conducted in RHA and Roman Low-Avoidance (RLA) male rats. After that, the 0.15 mg/kg MK801 dose was selected to carry out pharmacological studies versus atypical antipsychotics.

Results: In the first experiment we establish that MK801 (dizocilpine), a NMDAR antagonist, produces dose-related hyperactivity and social withdrawal, which are more marked in RHA than RLA rats. The administration of the atypical antipsychotics clozapine (2.5. mg/kg) or ziprasidone (2.5 mg/kg) partially reversed or attenuated some of the social behaviour deficits and hyperactivity induced by the administration of MK801. Aripiprazole (3 mg/kg), a third-generation antipsychotic, reversed or attenuated the social preference deficit, the hyperactivity and the impairment of social latency induced by MK801.

Conclusions: These results seem to be in line with previous studies with the NMDAR-antagonist model and add face and predictive validity to the RHA rat strain as a model of schizophrenia-relevant features.

***Keywords**:* Schizophrenia; Negative symptoms; Social withdrawal; NMDAR antagonist; Atypical antipsychotics; RHA and RLA rats

1. INTRODUCTION

The glutamate model of schizophrenia is based on the action of the N-methyl-D-aspartate receptor (NMDAR) antagonists (Aghajanian & Marek, 2000; Krzystanek & Pałasz, 2019; Neill et al., 2010, 2014; Rung, et al., 2005). The administration of those NMDAR antagonists (e.g. ketamine, phencyclidine –PCP- or dizocilpine -MK801-) in rodents is widely used to produce cognitive deficits and social withdrawal relevant to cognitive and negative symptomatology of schizophrenia (Gururajan et al., 2012; Neill et al., 2010; Rung et al., 2005; Wilson & Koenig, 2014). It is well know that NMDAR antagonists also produce psychotic-like symptoms, such as hyperactivity, paranoia and hallucination, and the administration of NMDAR antagonists to patients with schizophrenia exacerbates their symptoms (Malhotra et al., 1997; and see review by Neill et al., 2010).

The relevance of pharmacological models based on the administration of NMDAR antagonists lies in the fact that their effects can be reversed by second-generation (but not first-generation) antipsychotics (APs, such as clozapine, risperidone, olanzapine or sertindole; e.g., Gururajan et al., 2012; Neill et al., 2010).

The Roman high- (RHA) and low-avoidance (RLA) rat strains were developed in Rome in the 1960's trough bidirectional selective breeding of Wistar rats for their rapid (RHA) or extremely poor acquisition (RLA) of the two-way active avoidance –TWAA- task (Bignami, 1965; Fernández-Teruel et al., 2021). Among many other phenotypic strain differences related to anxiety, vulnerability to stress and to drug abuse (see reviews by Fernández-Teruel et al., 2021; Giorgi et al., 2019), it is noteworthy that RHA rats display (compared with RLAs, and also with unselected outbred rats) a number of schizophrenia-relevant phenotypes. Thus, among other psychotic/schizophrenia-relevant traits (reviewed by Fernandez-Teruel et al. 2021), RHA rats present cognitive disfunction, as indicated by impairments of reference and working memory (Oliveras et al., 2015; Río-Álamos et al., 2019), attention-related deficits (e.g. latent inhibition and prepulse inhibition) and hyperactivity (Oliveras et al., 2017; reviewed by Fernandez-Teruel et al., 2021). We have recently reported that drug-free RHA rats also exhibit relative asociality (i.e. lowered preference for social interaction), compared with their RLA counterparts and outbred HS rats (Oliveras et al., 2022; Sampedro-Viana et al., 2021), which is considered to model social withdrawal, a negative symptom of schizophrenia.

At neurochemical and molecular levels, selection for extremely divergent rates of acquisition of TWAA, or comparison of RHA vs RLA rats, has been shown to be associated with differential expression of many genes at the cortical, hippocampal and amygdala levels (Díaz-Morán et al., 2013; Sabariego et al., 2011, 2013). Most interestingly, recent studies have shown that RHA rats present alterations of synaptic markers and trophic factors in the prefrontal cortex (PFC) and/or hippocampus (HPC), such as neuregulin1, homer1, synaptophysin, brain-derived neurotrophic factor (BDNF), and others, that have

been linked with glutamatergic disfunction, PFC maturation and schizophrenia (Elfving et al., 2019; Neill et al., 2010; reviewed by Fernandez-Teruel et al., 2021). Hence, the genetically-based RHA model appears to recapitulate a considerable number of neurobehavioral traits that are relevant for the disorder (Fernandez-Teruel et al., 2021; Giorgi et al., 2019).

In this context, the present study was aimed at expanding the schizophrenia-relevant phenotypic profiling of RHA vs RLA rats by performing a pharmacological characterization of their social behaviour. We first aimed at evaluating the effects of the administration of the NMDAR antagonist MK801 on social behaviour of RHA vs RLA rats. We also studied whether some atypical antipsychotic drugs (clozapine, ziprasidone, and aripiprazole) would be able to attenuate or reverse the behavioural alterations induced by MK801- and whether such effects might be dependent on the rat strain. We hypothesized that, (i) MK801 would lead to more profound social behaviour deficits and enhanced hyperlocomotion in RHA than RLA rats, and (ii) second- (clozapine, ziprasidone) and third-generation (aripiprazole) antipsychotics would reduce MK801-induced impairment of social behaviour and hyperactivity more markedly in RHA rats than in their RLA counterparts.

2. METHODS

2.1 Subjects

Animals used in the present study were naïve male rats from the inbred Roman high- (RHA, n=217) and low-avoidance (RLA, n=222) strains, from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine (School of Medicine, Autonomous University of Barcelona, Spain), since 1996. They were 4-5 months old at the beginning of the experiments with an average weight of 421,50g ±3,60g (mean ± SD).

Animals were housed in macrolon cages (standard size: 50 x 25 x 14cm) in same-sexed pairs. They were maintained under a 12:12h light-dark cycle (lights on at 8:00h) with controlled temperature (22ºC ± 2ºC) and humidity (50-70%). Food and water were available *ad libitum*. All testing was performed in the morning between 8:00 and 14:00h. All the experimental procedures agreed with the Spanish legislation on "Protection of Animal Used for Experimental and Others Scientific Purposes" (RD 53/2013) and the European Communities Council Directive (2010/63/EU).

2.2 Apparatus

The set-up used to test social interaction was based on the one used by Gururajan et al., (2012) which was a modified version of that initially designed by Panksepp et al., (1997). It consists of two acrylic boxes (65 x 23 x 20cm) placed facing one another. The cages were divided by lines, in three equal sectors: social, middle, and non-social. Both boxes had two 3cm-diameter holes on their right and left sides (social/non-social hole). To prevent physical contact, the social holes of both cages were separated by 12cm. Above the test set up, there was a video camera recording the session which was connected to a screen out (TV monitor) of the experimental room where experimenters observed and assessed behaviour *in situ*. Further analysis was performed by visualization of the video tapes on a computer.

2.3 Drug treatment

Dizocilpine (MK801, M107), clozapine (CLZ, C6305), aripiprazole (ARI, SML0935) and ziprasidone (ZPR, Z2777) were purchased from Sigma-Aldrich (St. Louis, MO, USA). MK801 was dissolved in saline (0.9% NaCl). CLZ was dissolved in a small amount of glacial acetic acid and then diluted in distilled water with NaOH to neutralize the acidification. ZPR and ARI were dissolved in 2% of Tween 80 diluted in distilled water.

MK801 and CLZ and their respective vehicles were administered subcutaneously (s.c.) in a volume of 1 ml/kg body weight, 20 min and 60 min before testing, respectively. ARI and ZPR and their vehicles were administered intraperitoneally in a volume of 1 ml/kg body weight, 60 min and 30 min respectively before the test.

To assess the interactions between the propsychotic drug and the antipsychotics, the dose of 0.15 mg/kg of MK801 was selected according to its effects on "Non-social time", "Social time", "Social preference", "Social latency" and locomotion in Study 1. All the solutions were freshly prepared each day. The doses of each antipsychotic were selected according to pilot studies from our laboratory and/or the effective doses used in previous studies from our laboratory and others (Deiana et al., 2015; Oliveras et al., 2017; Ratajczak et al., 2016; Snigdha & Neill, 2008).

See experimental overview in Figure 1.

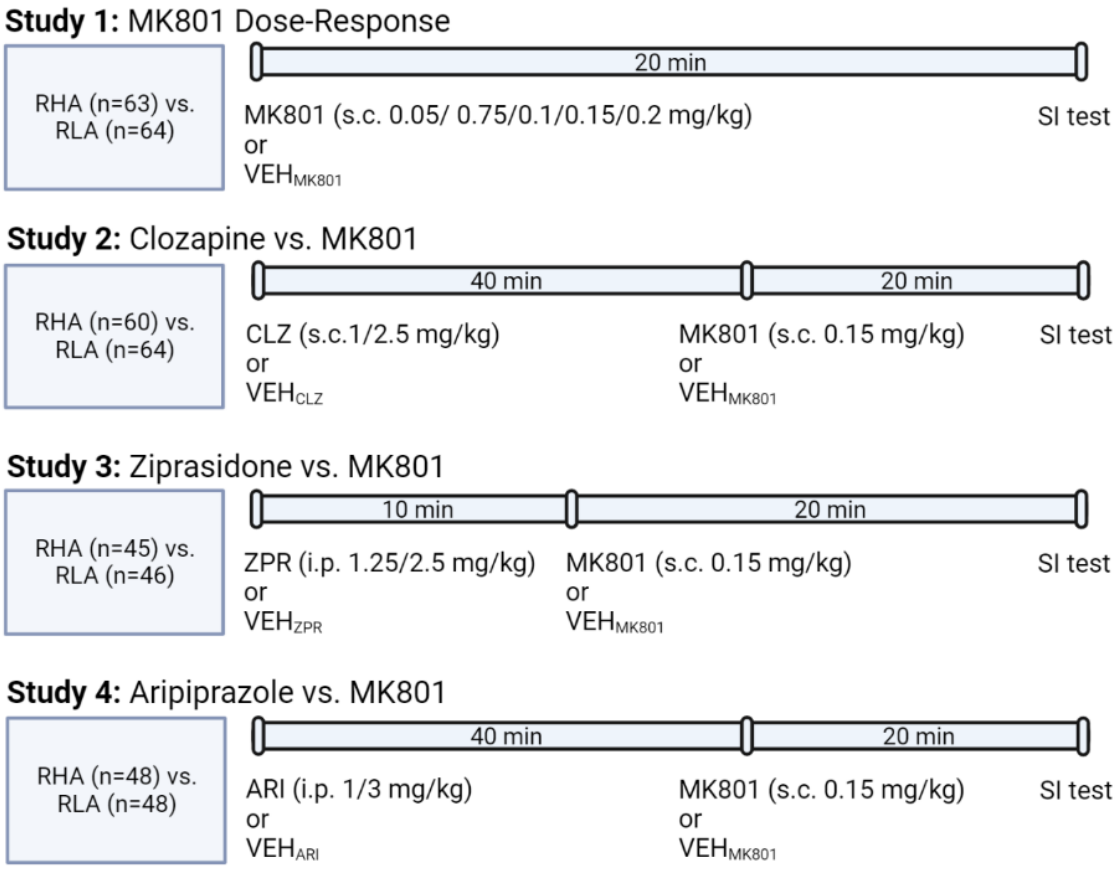

Figure 1. **Overview of the experimental schedules** (see "Materials and methods"). **Study 1:** different doses of MK801 were tested in the social interaction (SI) test. MK801 or its vehicle (VEH_{MK801}) were subcutaneously (s.c.) administered 20 minutes before the SI test. The 0.15 mg/kg MK801 dose was selected for the following studies. **Study 2**: Clozapine (CLZ; 1 or 2.5 mg/kg) or its vehicle (VEH_{CLZ}) were s.c. administered 40 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 min after MK801 administration the SI test was performed. **Study 3:** Ziprasidone (ZPR; 1.25/2.5 mg/kg) or its vehicle (VEH_{ZPR}) were administered i.p. 10 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 after the second administration the SI test was performed. **Study 4:** Aripiprazole (ARI; 1 or 3 mg/kg) or its vehicle (VEH_{ARI}) were intraperitoneally (i.p.) administered 40 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 min after the second administration the SI test was performed.

2.4 Experimental procedure

The experimental procedure was the same as the one used by Sampedro-Viana et al. (Sampedro-Viana et al., 2021). Pairs of unfamiliar weight-matched rats (±25gr.) were placed separately in each box for 30 minutes during the habituation sessions carried out 24h before testing, and then different pairs of unfamiliar weight-matched rats were placed one in each box for the 15-min test. The holding room was slightly illuminated with red light both during habituation and testing sessions. After testing each rat pair, the boxes were wiped clean with 70% ethanol to remove olfactory cues. The variables measured in each experiment were the same: "social latency", measured as the time elapsed until the first exploration of a social hole; "social time", measured by the time animals spend nose-poking at the social hole; "non-social time", measured by the time animals spent nose-poking at the non-social hole; "Social Preference", measured as the following equation: "Social Preference = (Social Time/ (Social Time + Non-social Time)) x 100"

"Locomotor activity" was measured as the number of crossings across the three equal sectors of the cage.

2.4.1 Study 1: MK801 Dose-Response

In study 1, we compared the effects of different MK801 (0.05, 0.075, 0.1, 0.15 and 0.2 mg/kg) doses. A total of 64 RLA rats and 64 RHA were tested in this study.

2.4.2 Antipsychotics vs. MK801

This study encompasses three experiments, in which the effects of MK801 on social behaviour and their possible reversal or attenuation by three different antipsychotics drugs, i.e., clozapine (CLZ), ziprasidone (ZPR) or (ARI), were evaluated.

Animal's matching, time of testing, room conditions and variables measures were the same as described above.

Study 2: Clozapine vs. MK801

In study 2, the effects of the pro-psychotics MK801 (0.15 mg/kg) and the antipsychotic CLZ (1 and 2.5 mg/kg), were assessed on both rat strains in a 2 x 2 x 3 factorial design (i.e., "2 strains" x 2 "MK801 levels" x "3 CLZ levels"). A total of n=64 RLA and n=60 RHA were used.

Study 3: Ziprasidone vs. MK801

In study 3, the effects of MK801 and ZPR (1.25 and 2.5 mg/kg) were tested in a 2 x 2 x 3 factorial design (i.e., "2 strains" x "2 MK801 levels" x "3 ZPR levels"). A total of n=46 RLA and n=45 RHA were tested in this experiment.

Study 4: Aripiprazole vs. MK801

In study 4, the effects of MK801 and ARI (1 and 3 mg/kg), were evaluated on both strains in a 2 x 2 x 3 factorial design (i.e., “2 strains” x “2 MK801 levels” x “3 ARI levels”). A total of n=48 RLA and n=48 RHA were used

2.5 Statistical analysis

Statistical analysis was performed using the *Statistical Package for Social Science 17 (*SPSS, Inc., Chicago, IL, USA). The data from Study1 were evaluated with two-way ANOVAs (2 “Strain” x 6 “Treatment”). If the two-way ANOVA revealed “Strain”, “Treatment” or “Strain x Treatment”, post hoc contrasts with Duncan’s multiple range tests to explore differences between groups were performed. The data from studies 2, 3 and 4 were evaluated with a three-way ANOVA (2 “Strain” x 2 “MK801” x 3 “APs” levels). If the ANOVA revealed “Strain”, “MK801”, “Clozapine”/ “Ziprasidone”/ “Aripiprazole”, or interactions between these factors post hoc Duncan’s multiple range tests to explore differences between groups were performed. All the results are expressed as mean ± SEM. The threshold for statistical significance was set at $p< 0.05$ for all measures.

3. RESULTS

Study 1: MK801 Dose-Response

The results of study 1 are presented in Figure 2. Factorial ANOVA (2 "strains" x 6 "Treatment") revealed a "Treatment" ($F_{(5,116)}$=15.641, p<0.001) effect on "Non-social Time", indicating a global decrease in this measure by MK801 administration. It also revealed a "Strain x Treatment" ($F_{(5,116)}$=2.943, p<0.05) effect, as the MK801-induced reduction of non-social time in RHA was higher than in their RLA counterparts (Figure 2a, and Duncan's test).

Regarding "Social time", there were "Strain" ($F_{(1,116)}$=6.503, p<0.05), "Treatment" ($F_{(5,116)}$=17.558, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=3.280, p<0.01) effects, the latter being due to the stronger effect of MK801 on social time in RHA rats compared with their RLA counterparts (Figure 2b, and Duncan's test).

ANOVA also revealed "Strain" ($F_{(1,116)}$=44.669, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=4.201, p<0.001) effects on the "Social preference" measure. Duncan's post hoc comparison revealed a MK801-induced deficit in social preference by the highest doses of MK801 (0.15 and 0.2 mg/kg) in RHA rats (Figure 2c, and Duncan's test).

As for "Social Latency", there were "Strain" ($F_{(1,116)}$=43.257, p<0.001), "Treatment" ($F_{(5,116)}$=23.864, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=7.941, p<0.001) effects, reflecting the increase of social latency due to MK801 treatment and the stronger effect on the RHAs compared with RLA rats (Figure 2d, and Duncan's test).

With regard to "Locomotor Activity", there were "Strain" ($F_{(1,116)}$=65.284, p<0.001) and "Treatment" ($F_{(5,116)}$=65.284, p<0.001) effects (Figure 2e).

Study 2: Clozapine vs. MK801

The results of study 2 are presented in Figure 3 and Figure 4. Since in this study there were no significant effects of MK801 (0.15 mg/kg; see comparison of VEH-VEH and VEH-MK groups in RHA rats, Figure 3e) on "Social preference" considering the whole 15-min SI test, the first 5-min period of the test was also statistically analysed.

Factorial ANOVA (2 "Strain" x 2 "MK801 dose" x 3 "CLZ dose") revealed a "Clozapine" ($F_{(2,112)}$=7.85, p<0.001) and "MK801" ($F_{(1,112)}$=36.987, p<0.001) effects on "Non-social time" (Figure 3a, and Duncan's test). Similar "Clozapine" ($F_{(2,112)}$=11.834, p<0.001) and "MK801" ($F_{(1,112)}$=72.423, p<0.001) effects on that variable were found during the first five minutes of the test (Figure 3b).

As for "Social time", there were "Clozapine" ($F_{(2,112)}$=6.931, p<0.001) and "MK801" ($F_{(1,112)}$=37.041, p<0.001) effects (Figure 3c, and Duncan's test), which were also present during the first five minutes of the test ("Clozapine" effect $F_{(2,112)}$=5.148, p<0.05,; "MK801"effect $F_{(1,112)}$=50.511, p<0.001) (Figure 3d).

ANOVA also revealed "Strain" ($F_{(1,112)}$=16.108, p<0.001), "Clozapine" ($F_{(2,112)}$=4.822, p<0.001), "Strain x MK801" ($F_{(1,112)}$=13.209, p<0.001) and "Strain x MK801 x Clozapine" ($F_{(2,112)}$=3.779, p<0.05) effects on "Social preference" (Figure 3e, and Duncan's test). Moreover, "Strain" ($F_{(1,112)}$=30.167, p<0.001), "Clozapine" ($F_{(2,112)}$=5.593, p<0.001), "MK801" ($F_{(1,112)}$=7.212, p<0.01) and "Strain x MK801" ($F_{(1,112)}$=15.804, p<0.001) effects were observed during the first five minutes of SI testing. The above interaction effects indicate, first, that MK801 effects are more marked in RHA than RLA rats, and second, the highest dose of clozapine (2.5 mg/kg) reversed the relative deficit in social preference showed by MK801-treated RHA rats (see comparisons between the RHA CLZ2.5-MK vs VEH-MK and CLZ1-MK groups, in Figure 3e and 3f, and Duncan's tests). Remarkably also, the RHA VEH-MK801 group was different (i.e. decreased social preference) from RHA VEH-VEH group during the first five minutes of testing (Figure 3f, and Duncan's test).

With respect to "Locomotor Activity", there were "Strain" ($F_{(1,112)}$=30.562, p<0.001), "Clozapine" ($F_{(2,112)}$=9.666, p<0.001), "MK801" ($F_{(1,112)}$=77.073, p<0.001) and "Clozapine x MK801" ($F_{(2,112)}$=4.383, p<0.05) effects. This interaction indicates that only the highest dose of clozapine (2.5 mg/kg) reversed the MK801-induced hyperactivity in both rat strains (Figure 4a, and Duncan's test). Similarly, "Strain" ($F_{(1,112)}$=45.807, p<0.001), "Clozapine" ($F_{(2,112)}$=9.148, p<0.001), "MK801" ($F_{(1,112)}$=70.155, p<0.05) and "Strain x MK801" ($F_{(1,112)}$=21.596, p<0.001) effects were observed during the first five minutes of SI testing, reflecting that MK801-induced hyperactivity was higher in RHA rats than in their RLA counterparts (Figure 4b and Duncan's test).

As for "Social latency", "Strain" ($F_{(1,112)}$=29.888, p<0.001), "MK801" ($F_{(1,112)}$=51.931, p<0.001) and "Strain x MK801" ($F_{(1,112)}$=22.431, p<0.001) effects were observed, indicating that MK801 increased social latency more markedly in RHAs than in RLA rats (Figure 4c, and Duncan's test).

Study 3: Ziprasidone vs. MK801

The results of study 3 are presented in Figure 5. Factorial ANOVA (2 "strain" x 2 "MK801 dose" x 3 "ZPR dose") revealed "Strain" ($F_{(1.79)}$=5.105, p<0.05), "MK801" ($F_{(1,79)}$=47.151, p<0.001) and "Strain x MK801" ($F_{(1,79)}$=9.591, p<0.05) effects, reflecting a reduction of "Non-social time" in RHA rats compared with their RLA counterparts (Figure 5a, and Duncan's test).

As for "Social Time", there were "MK801" ($F_{(1,79)}$=110.649, p<0.001) and "Strain x MK801" ($F_{(1.79)}$=17.143, p<0.001) effects, the interaction being due to the stronger effect of MK801 on RHA than RLA rats (Figure 5b, and Duncan's test).

Regarding "Social preference", there were "Strain" ($F_{(1,79)}$=20.528, p<0.001), "Strain x MK801" ($F_{(1,79)}$=9.646, p<0.05) and "Strain x MK801 x Ziprasidone" ($F_{(1,79)}$=3.345, p<0.05) effects, the latter being due to the specific effect of ZPR (2.5mg/kg) to reverse the

MK801-induced deficit in social preference in RHA, but not in RLA rats (Figure 5c, and Duncan's test).

Related to the "Social latency" variable, "Strain" ($F_{(1,79)}$=15.406, p<0.001), "MK801" ($F_{(1,79)}$=43.695, p<0.001) and "Strain x MK801" ($F_{(1,79)}$=17.730, p<0.001) were observed, reflecting that the MK801-induced increase of social latency was more marked in RHA rats compared with their RLA counterparts (Figure 5d, and Duncan's test).

ANOVA also revealed "Strain" ($F_{(1,79)}$=9.336, p<0.05), "Ziprasidone" ($F_{(2,79)}$=3.451, p<0.05) and "MK801" ($F_{(1,79)}$=98.090, p<0.001) effects on "Locomotor activity" (Figure 5e, and Duncan's test).

Study 4: Aripiprazole vs. MK-801

The results of study 4 are presented in Figure 6. Factorial ANOVA (2 "strain" x 2 "MK801 dose" x 3 "ARI dose") revealed an "MK801" effect on "Non-social time" ($F_{1,84)}$= 75.194, p<0.001), indicating a global decrease in this measure in all groups. It also revealed a "Strain x MK801" effect ($F_{(1,84)}$=11.110, p<0.001), as the reduction of non-social time in RLA rats was lower than in their RHA counterparts (Figure 6a, and Duncan's test).

Regarding "Social time", there was an "MK801" effect ($F_{(1,84)}$= 73.952, p<0.001), reflecting that the drug decreased social behaviour in all groups, and a "Strain x MK801" ($F_{(1,84)}$=13.315, p<0.001) effect, as the reduction of social time was more marked in RHA rats (Figure 6b, and Duncan's test).

As for "Social Preference", there were "Strain" ($F_{(1,84)}$=10.534, p<0.05), "MK801" ($F_{(1,84)}$=7.747, p<0.05) and "Strain x MK801" ($F_{(1,84)}$=6.331, p<0.05) effects, the latter being due to the reversal of the MK801-induced reduction of social preference in the RHA rats by the highest ARI dose (Figure 6c, and Duncan's test).

ANOVA also revealed "Strain" (F(1,84)=29.991, p<0.001), "MK801" (F(1,84)=79.711, p<0.001) and "Strain x MK801" (F(1,84)=10.716, p<0.05) effects on "Social latency". Duncan's post hoc comparison revealed a difference between the highest dose of ARI (3 mg/kg) and "VEH-MK" group in RHA rats, meaning that the MK801-increased social latency was partially reversed by ARI (Figure 6d, and Duncan's test).

Related to the "Locomotor activity" variable, results showed a "Strain" effect ($F_{(1,84)}$=29.991, p<0.001), as RHA rats present global higher levels of activity than RLA rats. Moreover, an "MK801" effect ($F_{(1,84)}$=79.711, p<0.001) was found, reflecting an overall increase in locomotion in all groups administered with MK801 (Figure 6e and Duncan's test). A significant "Strain x MK801" interaction ($F_{(1,84)}$=10.716, p<0.01) was also observed, indicating that a more marked effect of MK801 in RHA rats (Figure 6e, and Duncan's test).

4. DISCUSSION

The main findings of the present study may be summarized as follows: (1) The dose-response experiment (study 1) shows that MK801 0.15 and 0.2mg/kg doses induced a similar significant decrease of non-social behaviour ("Non-social time") in both Roman rat strains, but the drug reduced "Social time" and especially "Social preference" specifically in RHA rats. (2) Moreover, MK801 was more effective in increasing "Social latency" and hyperlocomotion in RHA rats. (3) The higher doses of ARI and ZPR attenuated MK801-induced hyperlocomotion in the RHA but not in RLA rats. This effect was also observed with CLZ, although this drug reduced activity globally and in both rat strains in parallel to its reversal of MK801-induced hyperactivity. (4) ARI 3 mg/kg and ZPR 2.5 mg/kg reversed the MK801-induced impairment of social preference of RHAs, being devoid of effects in RLA rats. (5) We analysed the first 5 min in the CLZ study (Study 2) because MK801 detrimental effects on social behaviour (specifically on "social preference") were more clear during that initial interval than during the whole 15-min test. Thus, only during the first 5 min of the SI test CLZ 2.5 mg/kg reversed the MK801-impaired social preference in RHA rats. (6) Among the three APs tested, ARI appears to be the most potent in reducing MK801-induced deficits, since apart from reversing MK801 effects on "Social Preference", ARI was the only drug that attenuated the effect of MK801 on "Social latency" (see Figure 6d) and produced a net increase of "Social time" in RHA rats (see Figure 6b).

It is worth to highlight here that measuring "Social preference", a parameter that has not been used in previous pharmacological studies carried out with the present SI procedure (e.g. Gururajan et al., 2010, 2012), enables the detection of some specific drug effects that do not arise when measuring just "Social time" and "Non-social time". The effects observed with MK801, and with the combination of this drug and the antipsychotics, suggest that social preference may better reflect, at least in some instances (e.g. following some particular drug treatments), the motivation of the animals for social interaction with relative independency of the absolute levels of social or non-social time. On the other hand, it is also worth mentioning that vehicle-treated (RLA vs. RHA) rats did not show differences in social preference in any of the present studies. This contrasts with our earlier (and replicated) findings indicating that treatment-naïve RLA rats consistently exhibit higher social preference than their RHA counterparts (Oliveras et al., 2022; Sampedro-Viana et al., 2021). These apparently contrasting findings open the reasonable possibility that the mild stress involved in handling and vehicle injection a few minutes before SI testing may lead to (slight) changes in the behavioural SI profiles of the rats. This possibility will certainly deserve further study.

The relevance of this study lies on the fact that this is the first time that: (i) Strain-related NMDA-antagonist (MK801)-induced impairments in social preference are reported in RHA vs. RLA rats and, (ii) attenuation of MK801-induced deficits in social preference (and locomotion) by atypical antipsychotics is shown specifically in RHA rats.

We have previously reported that RHA rats exhibit, compared with RLAs and other rat strain/stocks, several schizophrenia-relevant phenotypic traits related with positive and attentional/cognitive deficits of the disorder (Esnal et al., 2016; Fernández-Teruel et al., 2006; Giorgi et al., 2019; Oliveras et al., 2015; Río-Álamos et al., 2017, 2019; Tapias-Espinosa et al., 2018, 2019). Our present results go along with these findings and give further support to the schizophrenia-like profile of RHA rats by adding NMDA-antagonist detrimental effects on social behaviour (which is considered to model negative symptoms –asociality- of the disorder) as a new phenotype differing in the Roman rats.

Pharmacological models have already shown effectiveness of NMDAR antagonists to mimic not only positive and cognitive but also negative symptoms of schizophrenia on standard laboratory rat strains. Specifically, the most used NMDAR antagonists which have shown to induce social withdrawal are PCP, ketamine and MK801, being the latter more potent than the former ones (Gururajan et al., 2010; Neill et al., 2010). Hence, our results with MK801 administration confirm what has already been shown in other rat strains (not genetically selected). Importantly, using both Roman rat strains allowed us to add the novel finding that RHA rats are clearly more affected by MK801 than their RLA counterparts, in line with what we could expect from a rat strain presenting a schizophrenic-relevant profile. In line with the fact that negative symptoms of schizophrenia are thought to be related to frontal cortex and hippocampal dysfunction, RHA rats also present decreased prefrontal cortex and hippocampus (HPC) activity and reduced volume of both regions compared with their RLA counterparts (Meyza et al., 2009; Río-Álamos et al., 2017, 2019; Tapias-Espinosa et al., 2019). Administration of NMDA receptor antagonists is thought to reproduce these cortical and hippocampal dysfunctions, as the reduction of glutamatergic activity produces a disinhibition of mesolimbic dopaminergic function and an inhibition of mesocortical dopaminergic activity (e.g. Fernández-Teruel et al., 2021; Neill et al. 2014). RHA and RLA rats are known to differ in dopaminergic function in several brain regions, so that these divergences could underlie the between-strain differences in MK801 effects observed in the present work (e.g. Fernández-Teruel et al., 2021; Giorgi et al., 2007, 2019).

Differences in glutamatergic NMDA-receptor-mediated transmission might also be involved in the strain-related (RHA vs RLA) effects of MK801 on social interaction (e.g., Elfving et al. 2019). Recent studies have been devoted to elucidate whether RHA and RLA rats differ in parameters related to neurotransmitter receptor alterations in PFC and HPC, as well as pre- and post-synaptic markers of neural activity. Thus, RHA rats exhibit an increase of *Grin2b* (glutamate receptor NMDA2B) mRNA expression in PFC and enhanced NMDA2B in the HPC (Elfving et al., 2019; Klein et al., 2014). In addition, RHA rats exhibit enhanced 5-HT2A mRNA expression in the HPC and increased 5-HT2A receptor binding in the PFC (Elfving et al., 2019; Klein et al., 2014). Related to that, in particular in the context of the involvement of the 5-HT2A/mGlu2 receptor complex in schizophrenia (e.g. González-Maeso et al. 2008), it is worth to highlight that RHA rats exhibit a dramatic

deficit of mGlu2 receptors (mGlu2R) and *Grm2* expression (the gene for mGlu2R) in PFC, HPC and striatum (Elfving et al., 2019; Fomsgaard et al., 2018; Klein et al., 2014), due to a stop codon mutation in that receptor that makes RHA rats a naturally-occurring knock-out for it (Wood et al., 2017).

Comparing the effects of the three APs on MK801-induced alterations of social behaviour, it is worth highlighting that both CLZ and ZPR produced global significant effects on activity (see ANOVA main drug effects). In particular, CLZ dose-dependently reversed MK801-induced hyperactivity in both rat strains (see Figure 4a,b), which coheres with our previous findings using a specific activity test procedure (Oliveras et al., 2017). Conversely, ARI did not produce a global effect on locomotion (i.e., as also shown by the absence of significant main factor "ARI" effects on that measure), but its effects on activity were restricted to the attenuation of MK801-induced hyperlocomotion in RHA rats, but not in their RLA counterparts. Thus, it seems that CLZ and ZPR produce stronger and more general effects on activity than ARI. MK801 hyperlocomotor effects have been related to the increase of mesolimbic dopaminergic activity induced by NMDA receptor antagonism (e.g. Meltzer et al., 2011a, 2011b; Oliveras et al., 2017). Since RHA rats have a higher functional tone of the mesolimbic DA system than RLA rats, this could be the reason for the stronger hyperlocomotor effects of MK801 in the former strain (Giorgi et al., 2019). D2 receptor antagonism, which reduces mesolimbic dopaminergic activity, has in turn been related to the reduction of locomotor activity produced by antipsychotic drugs (e.g. Oliveras et al., 2017, and references therein). Hence, the global effects on activity observed with CLZ and ZPR might be related to their D2 antagonist properties. Conversely, since ARI acts as a partial D2 receptor agonist (Mauri et al., 2014) this could be the reason for the absence of global effects of ARI on activity while it retains a specific capacity to attenuate MK801-induced hyperactivity in RHA rats.

Moreover, the three antipsychotics have in common that they act as a antagonists of 5-HT2A receptors, and they also have partial agonist activity at 5-HT1A receptors, with CLZ showing the weakest and ARI showing the most potent partial agonist activity of the three drugs (e.g., see Odagaki and Toyoshima 2007). 5-HT1A partial agonist activity has been proposed as a key neurochemical mechanism in the attenuation or reversal of PCP-induced deficits on social behaviour (Snigdha & Neill, 2008). In fact, the three APs attenuate the deficits of "Social Preference" induced by MK801. It is nevertheless noteworthy that CLZ and ZPR appear to produce that effect by a trend to a relative reduction of "Non-Social Time" (relative to "Social Time"), whereas ARI is the only drug able to specifically and dose-dependently increase the "Social Time" (see Figure 6b).

Importantly, in addition, ARI is the only of the three APs that significantly reduces the "Social Latency" (Figure 6d) of MK801-treated RHA rats (i.e., a reduction of >200s in "ARI3+MK" group vs. "VEH-MK" group). Collectively, these findings of ARI attenuation of MK801 effects on social time, hyperlocomotion and social latency, besides its effects on social preference, confirm that ARI is the most potent of all three APs drugs under the

present conditions. This differential profile of effects of ARI (vs. CLZ and ZPR) might suggest that its more potent partial agonist effect at 5-HT1A receptors, relative to CLZ and ZPR (Odagaki & Toyoshima, 2007), and perhaps its D2 partial agonist activity, might underlie those effects on social behaviour. Since it has been shown that antagonism of 5-HT1A receptor prevents the ARI attenuation of PCP-induced deficits on social behaviour in rats (Snigdha & Neill, 2008), the observed ARI specific effects give support to the notion that they might be predominantly mediated by its potent partial agonism at 5-HT1A receptors.

5-HT1A receptors have been implicated in the cognitive/affective anomalies present in schizophrenia (e.g. Aznar and Hervig 2016; Ohno 2011) and, in line with that, previous reports have shown enhanced 5-HT1A receptor levels in the PFC of schizophrenic patients (Abi-Dargham, 2007; Burnet et al., 1996; Joyce et al., 1993; Meltzer & Sumiyoshi, 2008; Selvaraj et al., 2014). It has been proposed that postsynaptic 5-HT1A receptors tonically inhibit cholinergic and glutamatergic neuronal activity in septo-hippocampal/cortical areas, so that 5-HT1A-receptor partial agonists (such as atypical antipsychotics) might ameliorate some of the above symptoms due to a disinhibition of these cholinergic/glutamatergic neurons or systems (e.g. Ohno 2011). Consistent with the above findings from patients, rats showing deficits of prepulse inhibition, such as the Low-PPI-stratified outbred HS rats and RHA rats, which are known to also display correlated cognitive deficits (Oliveras et al., 2015), exhibit increased mRNA expression or binding of 5-HT1A receptors in the PFC (Elfving et al., 2019; Klein et al., 2014; Oliveras et al., 2017; Østerbøg et al., 2020). In addition, we have shown that prepulse inhibition of the startle response (which is impaired in patients with schizophrenia) is negatively correlated with mRNA expression of 5-HT1A receptors in PFC in a mixed sample of RHA, RLA and HS rats (Oliveras, 2017). Hence, the aforementioned profile of 5-HT1A receptors in RHA rats might also underlie the specific effects of ARI in this rat strain observed in the present study.

To conclude, the main finding of the present study is that, in line with the literature, MK801 induces social withdrawal in the Roman rats, but this is clearly more marked in RHA rats than in their RLA counterparts. The results also show that the three atypical antipsychotics used here can reverse the MK801-induced impairment in "Social preference" in the RHA rats, whereas ARI presents the most potent "therapeutic" profile of the three APs. These results are consistent with previous human and rodent studies showing that NMDAR antagonists induce some negative symptomatology of schizophrenia and hyperactivity, and these effects could be reversed by the administration of second and third generation antipsychotics (Abel et al., 2003; Deakin et al., 2008; Neill et al., 2014). The present findings add further evidence that RHA rats may be a valid model of schizophrenia-relevant symptoms. Further studies, using sub-chronic or chronic administration of MK801 and/or other NMDAR antagonists, in combination

with atypical antipsychotics and different cognitive/attentional tests/tasks, are warranted to extend the present findings to other symptom-related phenotypes.

Author contributions

D.S-V., T.C., C.R-A., A.T. and A.F-T. conceived and designed the experiments. D.S-V., T.C. and I.O. conducted the behavioural experiments. F.S., V.L., P.T., S.C., A.S-G., C.T-E. and L.M. assisted in the behavioural experiments. A.F-T. and D.S-V. analyzed the data and wrote the original manuscript. A.T., T.C., I.O., C.R-A., C.T-E and A.S-G. provided critical review of the original draft. All authors read and approved the manuscript.

Acknowledgments

Supported by grants PID2020-114697GB-I00, PSI2017-82257-P, 2017SGR-1586, "ICREA-Academia 2013" (A.F-T., A.T.) and "Juan de la Cierva" postdoctoral fellowship (FJC2018-038808-I; I.O.). F.S. was the recipient of a PhD fellowship funded by the italian Ministry of University and Research (MUR). C.R-A receives support from project "FONDECYT INICIACIÓN" ref. 11190240 (Chile; C.R-A).

Conflict of interest

The authors declare that they have no conflict of interest.

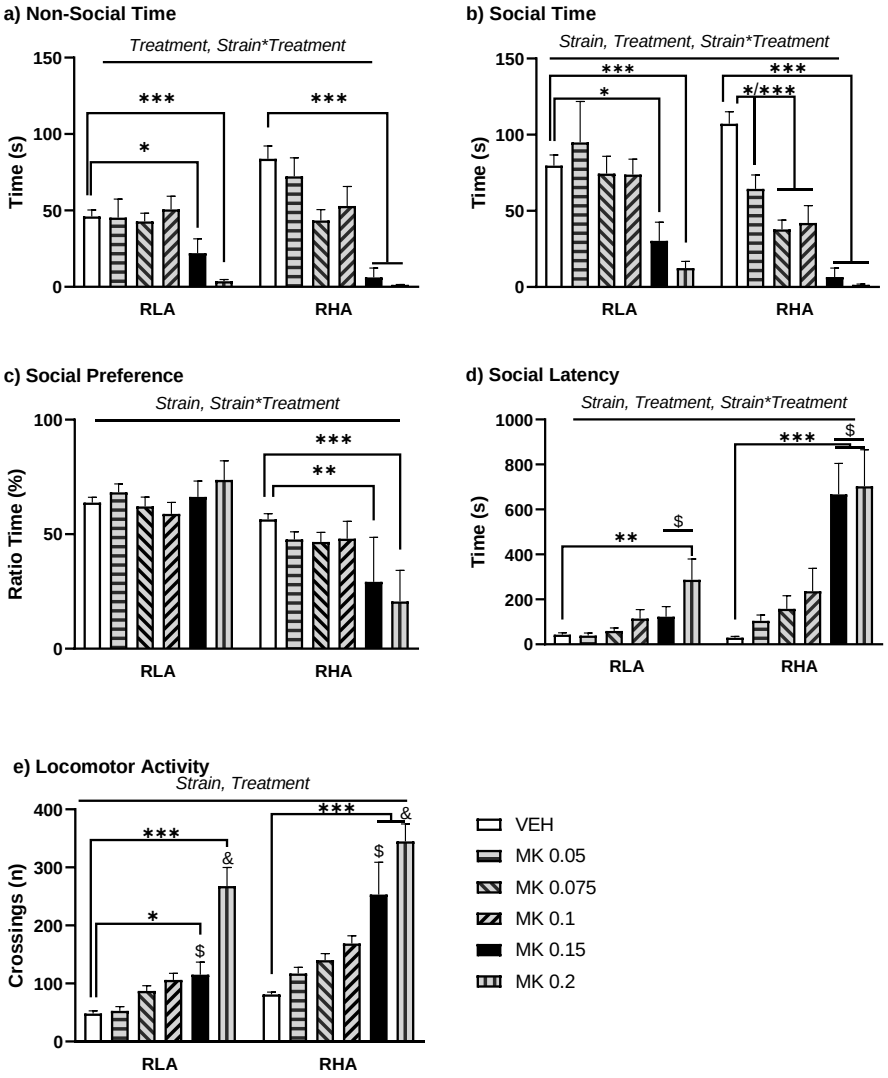

Figure 2. Dose-response effects of MK801 in the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for all MK801 doses. d) Mean social latency (±SEM) of RHA and RLA rats is shown for each MK801 dose. d) Social latency time (±SEM) of RHA and RLA rats is shown for each MK801 dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all the MK801 doses. RLA groups: VEH n=19; MK 0.05 n=10; MK 0.075 n=10; MK 0.1 n=10; MK 0.15 n=9; MK 0.2 n=6. RHA groups: VEH n=20; MK 0.05 n=10; MK 0.075 n=10; MK 0.1 n=10; MK 0.15 n=5; MK 0.2 n=8. "Strain", "Treatment" and "Strain*Treatment" effects (ANOVA). * p<0.05; ***p<0.001, between the groups indicated (Duncan's multiple range test). $, p<0.001 between the groups with the same symbol; &, p<0.01 between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

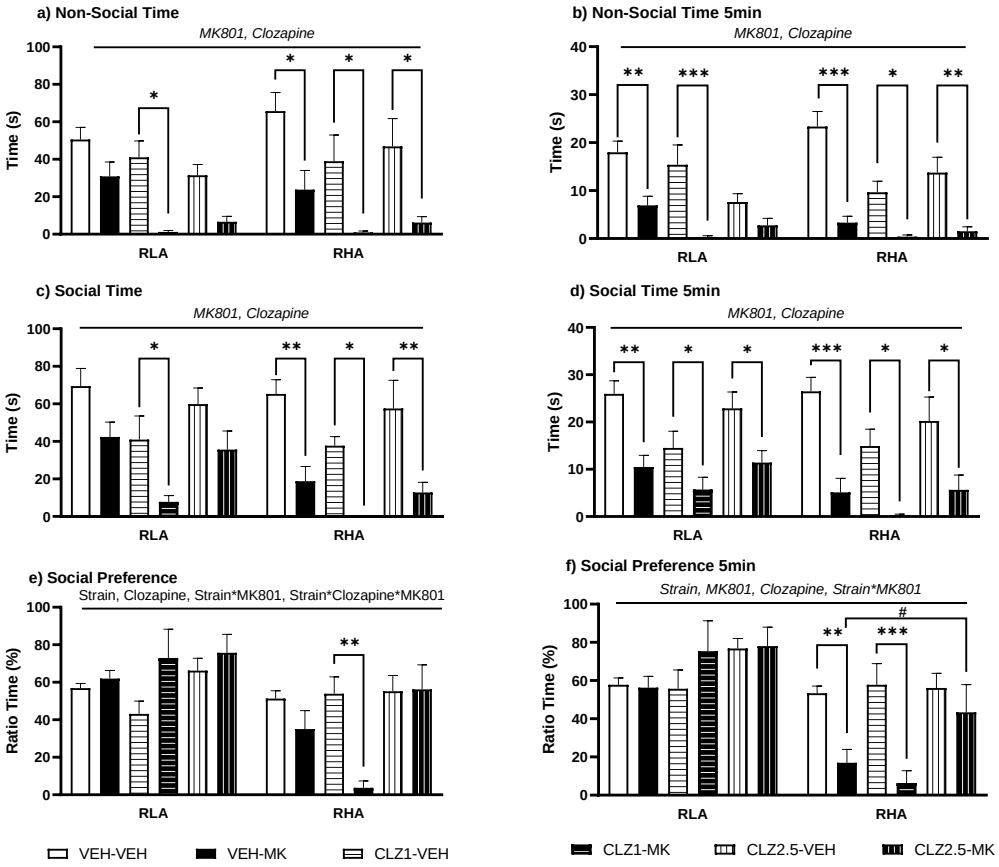

Figure 3. Clozapine (CLZ) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. b) Mean non-social time (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. c) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. d) Mean social time (±SEM) of the first five minutes of RHA and RLA is shown for each MK801 and CLZ dose. e) Mean social preference (±SEM) of RHA and RLA rats is shown for all MK801 and CLZ doses. f) Mean social preference (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. RLA groups: VEH-VEH n=16; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. RHA groups: VEH-VEH n=12; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. "Strain", "MK801", "Clozapine", "Strain*MK801", "Clozapine*MK801" and "Strain*Clozapine*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated (Duncan's multiple range test).

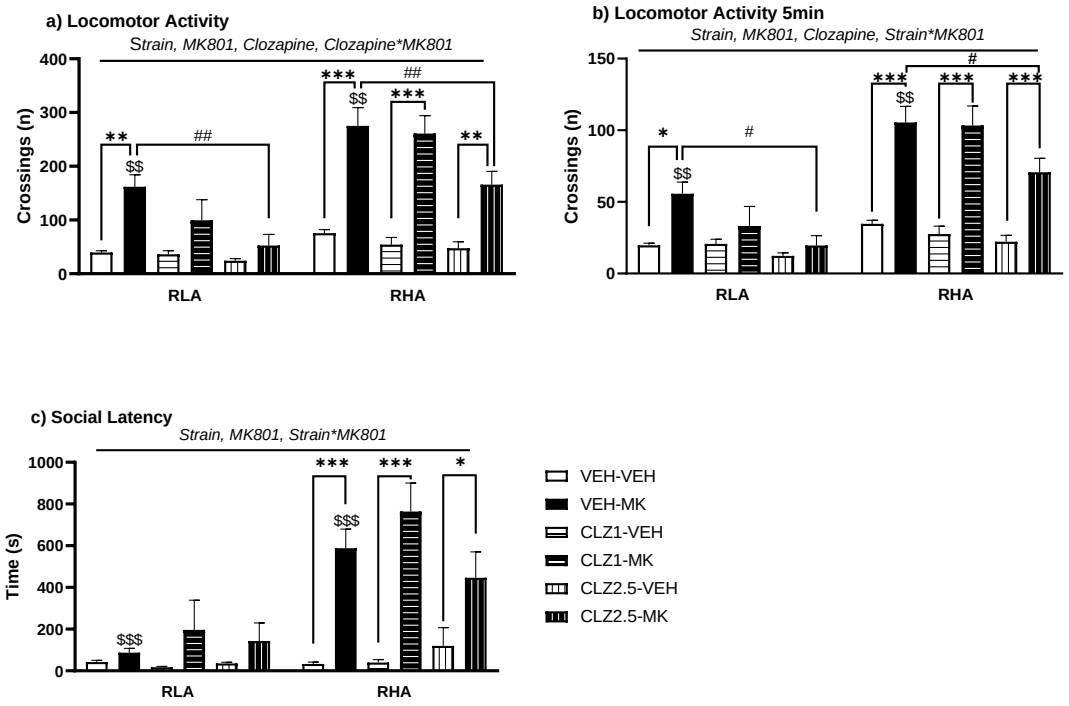

Figure 4. Clozapine (CLZ) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean number of crossings (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. b) Mean number of crossings (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. c) Mean social latency time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. RLA groups: VEH-VEH n=16; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. RHA groups: VEH-VEH n=12; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. "Strain", "MK801", "Clozapine", "Strain*MK801", "Clozapine*MK801" and "Strain*Clozapine*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05; $$ p<0.01; $$$ p<0.001 between the groups indicated. $$, $$$, p<0.01, p<0.001 respectively, between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test)

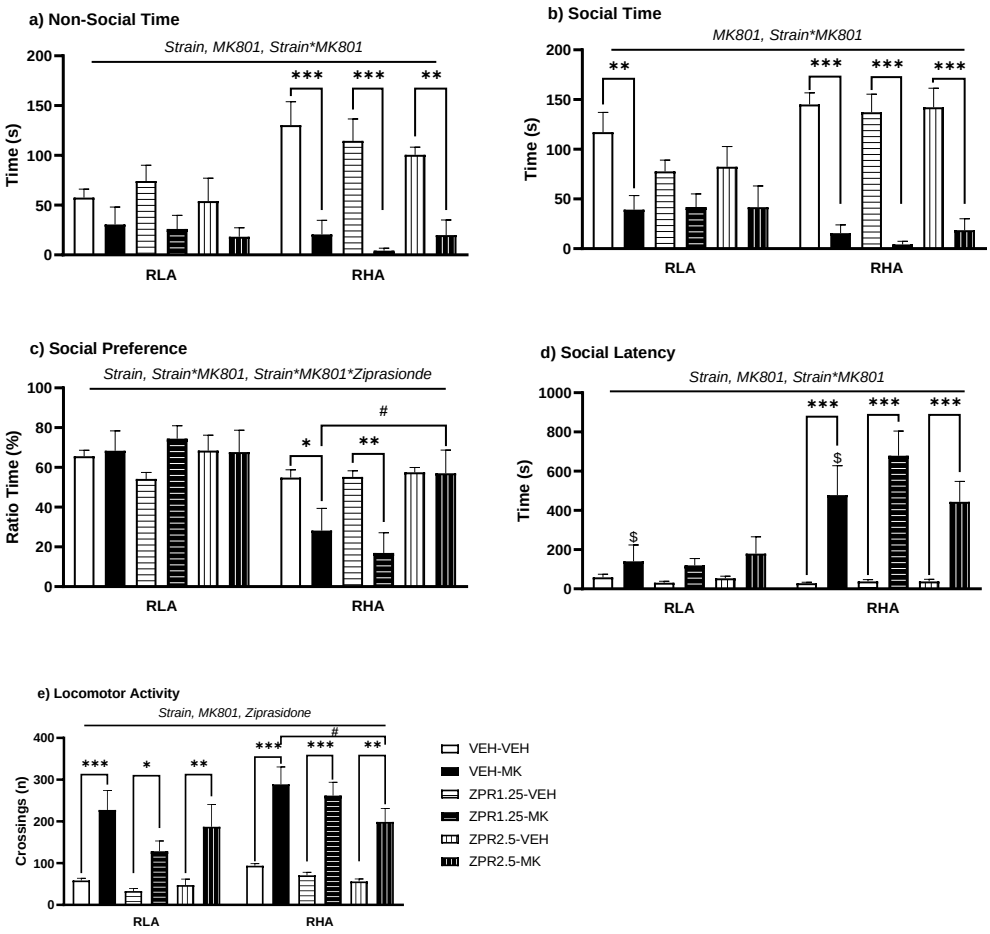

Figure 5. Ziprasidone (ZPR) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. d) Mean Social latency (±SEM) of RHA and RLA is shown for each MK801 and ZPR dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all MK801 and ZPR doses. RLA groups: VEH-VEH n=8; VEH-MK n=6; ZPR1.25-VEH n=10; ZPR1.25-MK n=8; ZPR2.5-VEH n=8; ZPR2.5-MK n=6. RHA groups: VEH-VEH n=8; VEH-MK n=7; CLZ1-VEH n=6; CLZ1-MK n=8; CLZ2.5-VEH n=6; CLZ2.5-MK n=10. "Strain", "MK801", "Ziprasidone", "Strain*MK801", and "Strain*Ziprasidone*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated. $, p<0.05 between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

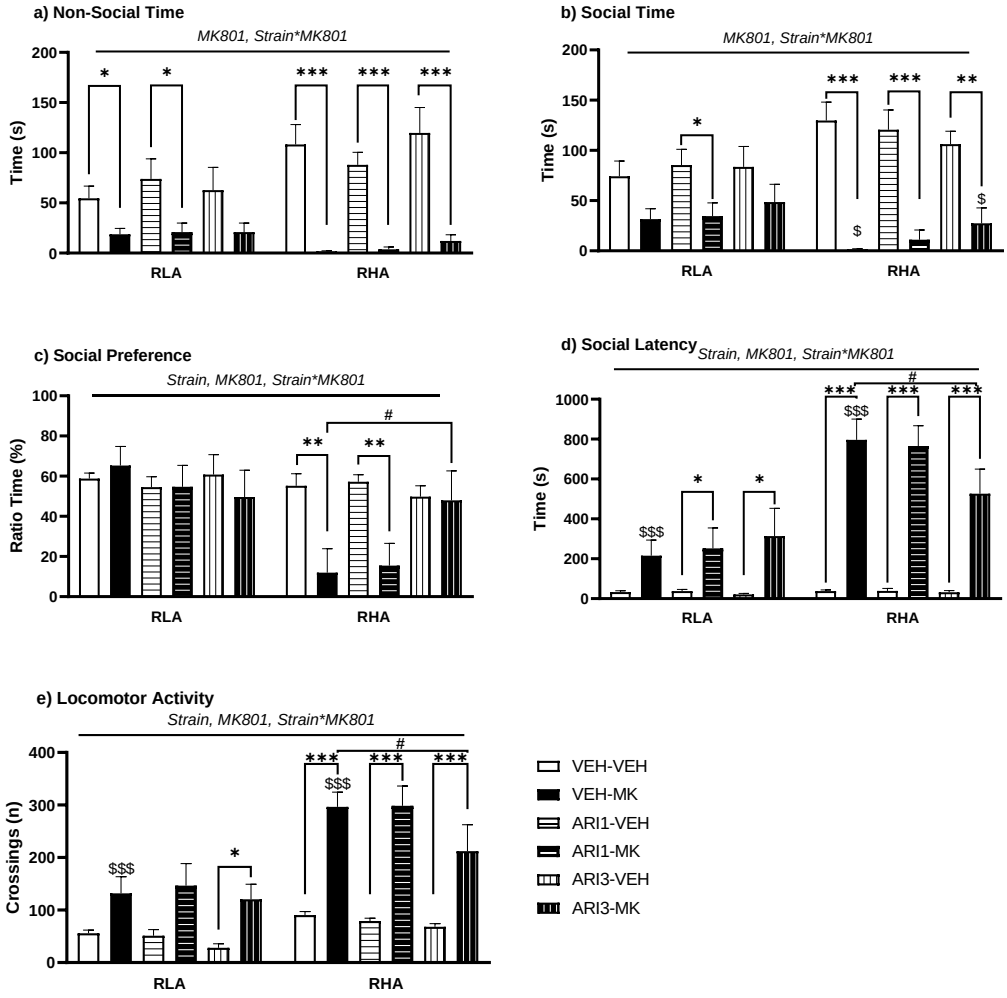

Figure 6. Aripiprazole (ARI) vs. MK801 results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. d) Mean social latency time (±SEM) of RHA and RLA is shown for each MK801 and ARI dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all MK801 and ARI doses. RLA n=8/group. RHA n=8/group. "Strain", "MK801" and "Strain*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated; $, $$$, p<0.05, p<0.001 respectively, between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

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ESTUDIO 5

Effects of oxytocin on dizocilpine-induced impairment of social behavior and hyperactivity in the Roman rat strains

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Manuscrito pendiente de publicar

ABSTRACT

Social withdrawal in rodents is a measure of asociality, an important negative symptom of schizophrenia. The inbred Roman high-avoidance (RHA) rats have shown lowered social behavior compared to their low-avoidance (RLA) counterparts. This investigation was focused on the study of these two rat strains on a social interaction task, under the effects of dizocilpine (MK801, an NMDAR antagonist), a pharmacological model of schizophrenia used to produce asociality and hyperactivity. Also, as oxytocin has been proposed as a natural antipsychotic and a potential adjunctive therapy for social deficits in schizophrenia, we focused on the effects of oxytocin administration and its ability to reverse the MK801-impairing effects.

MK801 administration produced hyperlocomotion and a decrease of social and non-social behavior in both strains, but in RHAs these effects were more marked. These deficits were attenuated with 0.04 mg/kg and 0.2 mg/kg oxytocin doses.

The alterations produced by MK801 and their attenuation by oxytocin occur preferentially in RHA rats, supporting previous findings of reversal of MK801 impact on social interaction by atypical antipsychotics. Besides, these results give further support to the anti-schizophrenic properties of oxytocin and to RHA rats as a model of some of the negative symptomatology of schizophrenia.

Keywords: Schizophrenia, Social Interaction, NMDA antagonist, MK801, Oxytocin, RHA and RLA rats.

1. INTRODUCTION

Negative symptomatology of schizophrenia are those which involve disrupted emotions, motivation, affect and social behaviors (Marder & Cannon, 2019; McCutcheon et al., 2020; Mitra et al., 2016). Social withdrawal is one of the most important negative symptoms an should be defined as a reduction in social initiative due to decreased interest in forming close relationship with others (Marder & Galderisi, 2017). These symptoms (along with the cognitive symptomatology) have become a focus of preclinical research in an effort to find new treatments that can also improve them (Simpson et al., 2010).

The N-methyl-D-aspartic acid (NMDA) receptor antagonists, such as dizocilpine (MK801) or phencyclidine, can produce schizophrenic-like psychotic (positive symptoms) as well as negative and cognitive symptoms, which make them a useful tool for research on the neurobiology of the disease focusing on the glutamatergic hypothesis of schizophrenia (Deiana et al., 2015; Neill et al., 2010; Rung et al., 2005; Uno & Coyle, 2019; Vales & Holubova, 2021). The administration of an NMDA antagonist to induce social withdrawal (negative symptoms) and hyperlocomotion (positive symptoms) in rodents is widely used and is currently considered a valid pharmacological model for the disease (Rung et al., 2005; Gururajan et al., 2010; Neill et al., 2010, 2014; Winship et al., 2019). Furthermore, it has been shown that the impairments induced by the administration of an NMDA antagonist can be reversed by administration of atypical antipsychotics (Abdul-Monim et al., 2003; Gururajan et al., 2012), though it may produce severe side effects (X.-F. Huang et al., 2019; Stępnicki et al., 2018).

Synthesized in the paraventricular and supraoptic nuclei of the hypothalamus, the cross-species-conserved neuropeptide oxytocin (OXT), has been proposed as an alternative natural antipsychotic both in some animal models and in humans (MacDonald and Feifel, 2012; Feifel et al., 2015; Shilling and Feifel, 2016; Ettinger et al., 2018; Kohli et al., 2019; Goh et al., 2021). Some studies have reported that the administration of OXT modulates social behavior and improves social deficits in patients with schizophrenia (Woolley et al., 2014; Shilling and Feifel, 2016; Zimmermann et al., 2016).In addition, it has been reported that low levels of oxytocin in blood plasma are related to several psychiatric conditions, such as autism spectrum disorders, depression, and schizophrenia (Cochran et al., 2013).Of note, the administration of OXT has been shown to reverse phencyclidine-induced social deficits in rodents (Lee et al., 2005; Kohli et al., 2019).

The inbred Roman high-avoidance (RHA) rat strain/line exhibits, in comparation with the Roman low-avoidance (RLA) strain/line, a wide range of schizophrenia-related phenotypes: e.g. (i) enhanced novelty-induced locomotor activity (Tapias-Espinosa et al., 2018), (ii) impaired latent inhibition (Esnal et al., 2016), (iii) impaired prepulse inhibition (PPI) (Río-Álamos et al., 2019; Tapias-Espinosa et al., 2019), (iv) poorer maternal/nesting and social preference behavior (Río et al., 2014; Sampedro-Viana et al., 2021; Oliveras et

al., 2022), (v) enhanced locomotor and mesolimbic (dopaminergic) sensitization following chronic administration of psychostimulant drugs, and many other relevant behavioral, pharmacological and neurobiological phenotypes related to schizophrenia (Giorgi et al., 2019; Fernández-Teruel et al., 2021). Some neuroanatomical and molecular studies have revealed that the RHA strain displays brain anomalies (e.g. prefrontal cortical and hippocampal function) that are similar to schizophrenia (Fernández-Teruel et al., 2021). Interestingly, in this regard, it has been reported that OXT administration attenuates the deficits of prepulse inhibition of the startle response (PPI) shown by RHA rats , which in turn present a lowered expression of CD38 gene (which regulates OXT secretion) in prefrontal cortex relative to their RLA counterparts (Tapias-Espinosa et al., 2021). Also, we have found that the MK801 (NMDA-receptor antagonist)-induced social withdrawal and hyperactivity is enhanced in RHA rats relative to RLAs, and both MK801 effects are selectively attenuated by atypical antipsychotics in the former strain (Sampedro-Viana et al., 2023 under review).

Taking into account the above previous results (Sampedro-Viana et al., 2023 under review; Tapias-Espinosa et al., 2021) we expected to find that: (1) MK801 would decrease social behavior/preference more markedly in RHA rats, in comparison to RLAs; (2) RHA rats would display more marked hyperactivity after MK801 administration than RLAs; and, (3) the attenuation of MK801-impaired social preference and hyperactivity by OXT would be more marked in RHA rats.

2. MATERIALS AND METHODS

2.1. Subjects

RHA and RLA male rats were used. The total number of rats was 153. All the animals came from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Department of Psychiatry and Forensic Medicine (Autonomous University of Barcelona, Bellaterra, Spain). Animals were 3-4 months old at the beginning of the experiment with an average weight of 317,5 g for RLAs and 333,6 g for RHAs. They were housed in same-sexed pairs in macrolon cages (standard size) and maintained under a 12:12h light-dark cycle, with controlled temperature (22 + 2 ºC) and humidity (50-70%). They had water and food available *ad libitum*.

All testing was carried out between 9:00 and 13:30h. Naïve rats were used for each of the 6 groups/strain. All the procedures were in accordance with the Spanish legislation on "Protection of Animals Used for Experimental and Other Scientific Purposes" and the European Communities Council Directive (86/609/EEC) on those subjects.

2.2. Social Interaction

The social interaction (SI) set-up test was adapted from Gururajan et al., (2012) and described by Sampedro-Viana et al., (2021). Two transparent acrylic boxes (65 x 23 x 20 cm) placed facing one to another at 12 cm, to prevent physical contact between the animals. Each one of the boxes has a 3 cm diameter hole on the ends corresponding to the social and the non-social holes. All the procedure were recorded and scored later on by a trained observer who was blind to strain and treatment conditions of the animals.

One day before the behavioral test, a 30-min habituation session was carried out where the four holes were covered with tape and a barrier was placed between the two boxes to prevent exploratory activity. A pair of non-matched weight-matched animals were placed into the boxes (one rat for each box). In the SI testing day, different pairs of unfamiliar animals were individually placed in the set-up for ten minutes with the holes uncovered, to allow exploration through them (one animal per each SI box). The experimental room (in the habituation and the experimental sessions) was slightly illuminated with a red light. After habituation and experimental session, each box was cleaned with 70% ethanol solution.

Variables measured in this experiment were: "Social time", the time that a subject spent nose-poking at the social hole; "Non-social time", the time spent nose-poking at the non-social hole; "Social preference", the percentage preference for the social hole, calculated with the following formula *"Social preference = ((Social time)/ (Social time + Non-social Time)) x 100"*. "Locomotor activity", the number of crossings through the 3 sectors marked on the testing cage.

2.3. Drug Treatment

After a random assignment of the subjects to the different experimental groups, RHA and RLA rats received a subcutaneous injection thirty minutes before the start of the SI test, of either sterile 0.9% saline vehicle, 0.04 mg/kg or 0.2 mg/kg of OXT (Oxytocin 96%, J63421, Thermo Fisher Scientific) dissolved in sterile saline. Ten minutes after the first injection (twenty minutes before the SI task) the subjects received a subcutaneous injection of MK801 (M107, Sigma-Aldrich; St. Louis, MO, USA) 0.015 mg/kg or sterile 0.9% saline vehicle.

2.4. Statistical Analysis

The statistical analyses were carried out using the "Statistical Package for the Social Science" (SPSS, version 17). The p-value threshold was set at $p < 0.05$.

To evaluate the independent effects of MK801 and OXT, and the interaction between them on the behavioral dependent variables, factorial ANOVAs (2 "strain" x 2 "MK801" x 3 "OXT" levels) were performed. Tukey's and Duncan's (between the groups indicated) multiple range test was performed after significant results were obtained with the ANOVA.

3. RESULTS

The results of the present study are presented in Figure 1 and Figure 2. Factorial ANOVA (2 "strains x 3 "OXT doses" x 2 "MK801 doses"), revealed "Strain" ($F(1,141)=5.04$; $p<0.05$) and "MK801" ($F(1,141)=123.47$; $p<0.001$) effects on "Non-social Time", which is explained by the globally lower non-social behavior in RHA than RLA rats and by the global effect of MK801 in decreasing non-social behavior. ANOVA also revealed a "Strain x MK801" ($F(1,141)=18.66$; $p<0.001$) interaction, indicating a greater MK801-induced reduction of non-social time in RHA rats than in RLA groups (Figure 1A, Tuckey's test).

Regarding "Social time", there was an "MK801" ($F(1,141)=222.75$; $p<0.001$) effect, which reflects the decrease of the global social activity after the administration of MK801. The interaction "Strain x MK801" ($F(1,141)=30.77$; $p<0.001$) indicates that MK801-induced impairment in social behavior is more marked in RHA rats than in their RLA counterparts. ANOVA also revealed an "Oxytocin x MK801" ($F(2,141)=5.12$; $p<0.01$) interaction, indicating that oxytocin tends to attenuate the MK801-reducing effect on social time (Figure 1B, Tuckey and Duncan's tests).

As for "Social preference", there were "Strain" ($F(1,141)=16.84$; $p<0.001$), "MK801" ($F(1,141)=18.25$; $p<0.001$) and "Strain x MK801" effects ($F(1,141)=7.92$; $p<0.01$). This interaction effect indicates that MK801 has profound effects in RHA but not in RLA rats. Notably, in RHA rats, the marked and significant difference seen between VEH-VEH and VEH-MK groups (see Tukey test in Figure 2C) is not observed between OXT0.04-VEH and OXT0.04-MK groups or between OXT0.2-VEH and OXT0.2-MK groups (Tukey tests in Figure 1C), indicating an attenuation of MK801 effects by OXT.

With regard to "Locomotor activity", there were "Strain ($F(1,141)=24.64$; $p<0.001$), "Oxytocin" ($F(2,141)=7.98$; $p<0.01$) and "MK801" ($F(1,141)=121.85$; $p<0.001$) effects, as well as a "Strain x MK801" ($F(1,141)=18.55$; $p<0.001$) interaction, indicating that MK801-induced more marked hyperactivity in the RHA rats compared with their RLA counterparts. Also, an "Oxytocin x MK801" ($F(2,141)=7.32$; $p<0.001$) interaction was revealed, indicating that OXT attenuates the effect of MK801 on locomotion. Finally, we observe differences between groups that were shown by Tukey Post Hoc analysis (see Tukey's comparisons, Figure 3). Importantly, in RHA rats, there is a marked and significant difference seen between VEH-VEH and VEH-MK groups in locomotor activity (see Tukey's test in Figure 2), whereas OXT0.04-MK and OXT0.2-MK groups show a significant decrease of activity compared to VEH-MK groups (Tukey's tests in Figure 2), indicating an attenuation of MK801 effects by OXT administration.

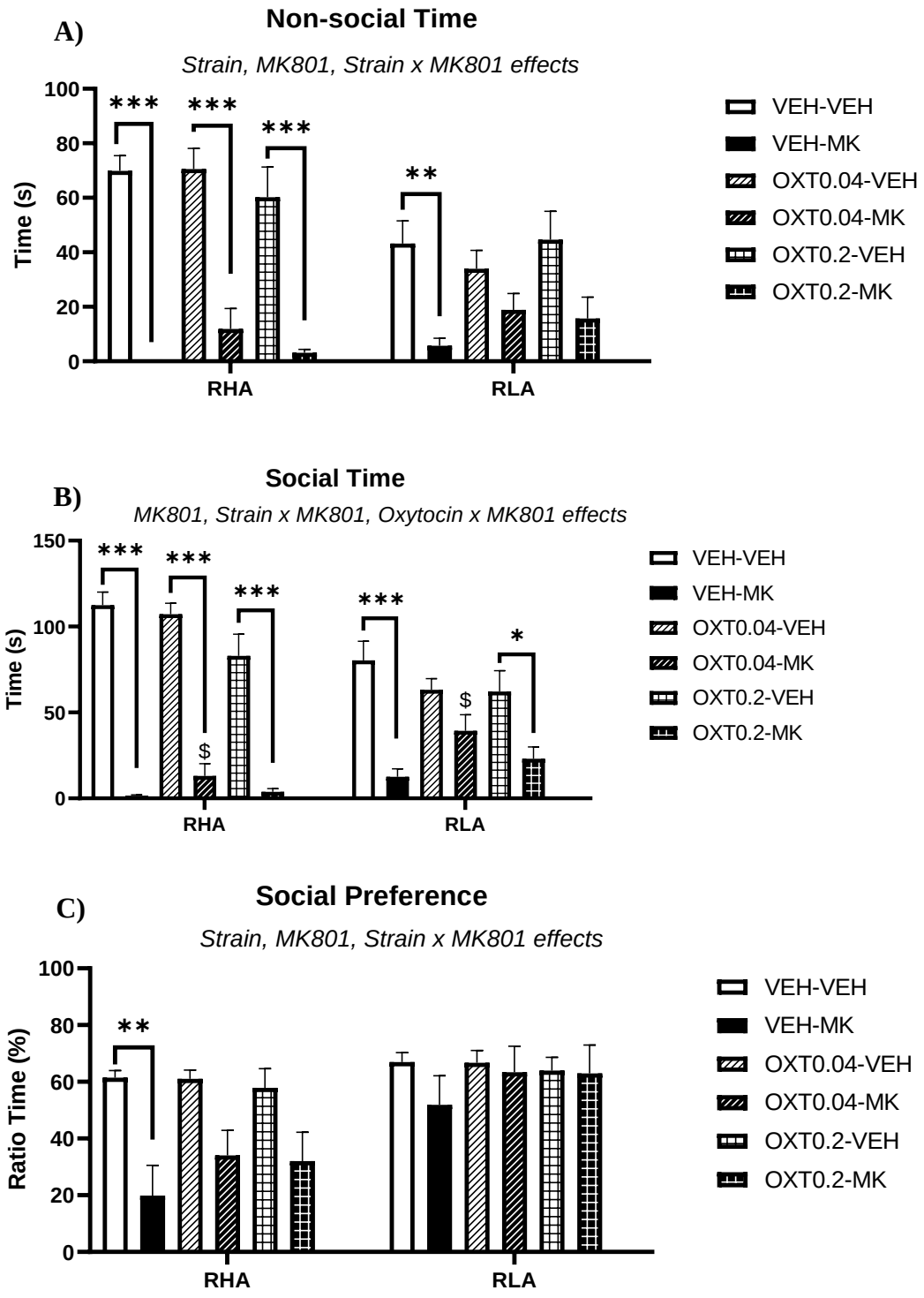

Figure 1. Oxytocin (OXT) vs. MK801 (0.15 mg/kg) results for the social interaction test in the Roman rat strains. (A) Mean non-social time (± SEM) of RHA and RLA rats is shown for each MK801 and OXT dose. **(B)** Mean social time (± SEM) of RHA and RLA rats is shown for each MK801 and OXT dose. **(C)** Mean social preference (± SEM) of RHA and RLA rats is shown for each MK801 and OXT dose. RHA groups: VEH-VEH n=14; VEH-MK n=14; OXT0.04-VEH n=12; OXT0.04-MK n=14; OXT0.2-VEH n=12; OXT0.2-MK n=12. RLA groups: VEH-VEH n=14; VEH-MK n=14; OXT0.04-VEH n=12; OXT0.04-MK n=12; OXT0.2-VEH n=12; OXT0.2-MK n=11. "Strain", "MK801", Strain x MK801" and "Oxytocin x MK801" effects (ANOVA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's test) \$ $p < 0.05$ between groups (Duncan's test).

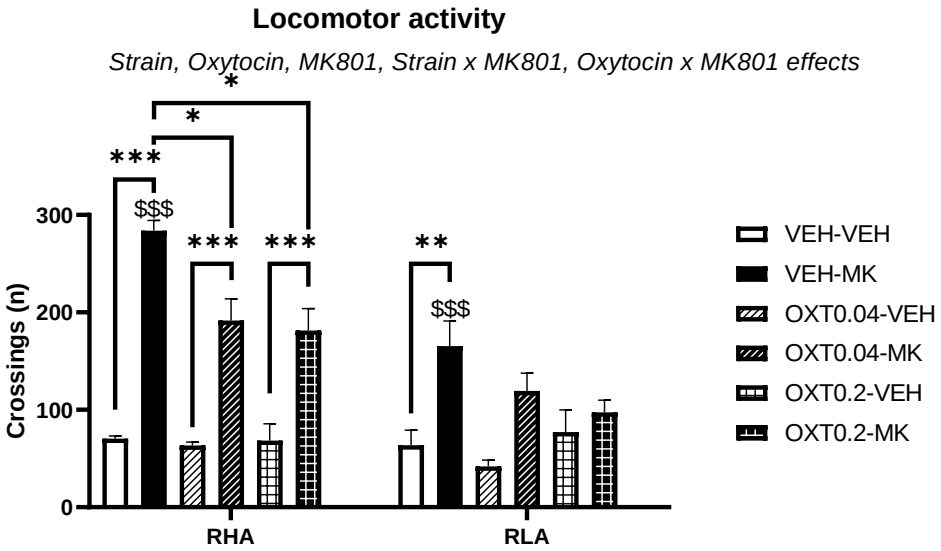

Figure 2. Mean locomotor activity (±SEM) of RHA and RLA rats is shown for each MK801 (0.015 mg/kg) and oxytocin dose. RHA groups: VEH-VEH n=14; VEH-MK n=14; OXT0.04-VEH n=12; OXT0.04-MK n=14; OXT0.2-VEH n=12; OXT0.2-MK n=12. RLA groups: VEH-VEH n=14; VEH-MK n=14; OXT0.04-VEH n=12; OXT0.04-MK n=12; OXT0.2-VEH n=12; OXT0.2-MK n=11. "Strain", "Oxytocin", "MK801", Strain x MK801" and "Oxytocin x MK801" effects (ANOVA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Tukey's test) $$$ $p < 0.001$ between groups (Duncan's test).

4. DISCUSSION

Schizophrenia is a disease characterized by an important deficit in social cognition related to social dysfunction, which constitutes a major disability factor (Pedersen et al., 2011; Khan et al., 2015). Different studies on the levels of OXT and their relation with schizophrenia have found evidence that patients suffering this disease had a perturbation of the oxytocinergic system (Goh et al., 2021; MacDonald & Feifel, 2012), and it is known that OXT could increase the interest of these patients for social interaction (Woolley et al., 2014).

Previous studies from our laboratory have shown that the two OXT doses used here improve PPI in RHA rats and have central effects, as they induce increases in the expression of the OXT receptor gene in prefrontal cortex of RHA and RLA rats (Tapias-Espinosa et al., 2021).

The present results have shown that MK801 decreases the time spent in both social and non-social holes more markedly in RHA than RLA rats (see the "strain x MK801" interactions from ANOVA analyses), and also reduces social behavior preference –i.e. % preference for the social hole- in RHA rats (see also the "strain x MK801" interaction –ANOVA- in this measure), thus indicating a very specific effect of this drug on social behavior in the RHA strain (Sampedro-Viana et al., 2023, under review). Likewise, the results show a marked increase in locomotor activity in MK801-treated animals, which is also much more pronounced in RHA than RLA rats (see also the "Strain x MK801" interaction –ANOVA) as observed in our previous studies (Sampedro-Viana 2023, under review).

These findings, in particular the MK801 effects on both the "Social preference" and "Locomotor activity" in RHA rats, add support to the predictive and face validity of the RHA model as an analogue of some social deficits and positive-like symptoms (i.e. MK801-induced hyperactivity) found in schizophrenia, and in turn point to central glutamatergic transmission as a system that may be altered and may underlie some of the phenotypic schizophrenia-relevant traits of the RHA rat strain. In this context, previous work from our laboratory indicates that glutamatergic transmission is indeed altered in RHA compared with their RLA counterparts (Klein et al., 2014; Woolley et al., 2014; Fomsgaard et al., 2018; Elfving et al., 2019). Importantly, the present results also suggest that when the treatment of MK801 is combined with OXT, the MK801-induced hyperlocomotion and decrease of "Social preference" are attenuated by OXT more clearly in RHA rats.

The attenuation or reversal of MK801-induced impairment of PPI by OXT has already been reported (Feifel & Reza, 1999). It has also been shown that OXT antagonizes the phencyclidine (PCP) - and MK801-impairing effects on SI in rats and zebrafish (Lee et al., 2005; Zimmermann et al., 2016), as well as PCP-induced hyperactivity in rats (Kohli et al., 2019), which suggests that the oxytocinergic system interacts with glutamatergic

transmission to modulate some schizophrenia-linked phenotypes (e.g. Qi et al., 2012; Zhou et al., 2015). The present work adds to these previous reports that alterations induced by NMDA receptor antagonism on social preference and hyperactivity are preferentially attenuated by OXT in RHA rats, which are also more vulnerable to the "pro-schizophrenic-like" MK801 effects than their RLA counterparts. This seems to be in line with the above-mentioned evidence that RHA rats have alterations of central glutamatergic transmission (Elfving et al., 2019; Fernández-Teruel et al., 2021; Fomsgaard et al., 2018; Giorgi et al., 2019c; Klein et al., 2014) and oxytocinergic function when compared with RLA rats (Giorgi et al., 2019; Tapias-Espinosa et al., 2021).

There are some areas related to schizophrenia where we can find OXT receptors. Some of these areas are subregions of the basal ganglia, the substantia nigra, the central nucleus of the amygdala, the lateral septal nucleus (Grinevich et al., 2016; Shilling and Feifel, 2016), the hippocampus and the nucleus accumbens (Feifel & Reza, 1999). Also, it is known that the interaction of OXT and serotonin in the nucleus accumbens is related with the rewarding properties of SI, and the interaction of OXT with the dopaminergic system eases social-cognitive functions (Shilling and Feifel, 2016).

Thus, the differences between RHA and RLA rats in OXT systems (Tapias-Espinosa et al., 2021), together with those found in mesolimbic and mesocortical dopaminergic, glutamatergic and serotoninergic systems (Fernández-Teruel et al., 2021; Giorgi et al., 2019) may explain the differential effects of MK801 and OXT on social behavior and activity in RHA compared with the RLA rats. Thus, RHA rats, which show lowered social behavior preference than RLAs (see also Sampedro-Viana et al., 2021; Oliveras et al., 2022), have also been found to exhibit decreased expression levels of the CD38 gene, which regulates OXT secretion (Tapias-Espinosa et al., 2021). RHA rats show higher expression of glutamate NMDA receptor subunits (Elfving et al., 2019), as well as increased 5-HT2A receptors and dramatically reduced glutamate mGlu2 receptors (which normally regulate glutamate availability at the synapses) in the PFC (Fomsgaard et al., 2018; Klein et al., 2014). These alterations may compromise the excitatory/inhibitory balance -shifting it to a behavioral disinhibition outcome- in this region, and thus lead (or contribute) to the altered social and locomotor behavior in the RHA model.

In fact, previous findings indicate that the RHA rats could have an excessive glutamatergic and dopaminergic tone in the PFC, striatum and mesolimbic system that could drive an imbalance between excitation and inhibition, compared to RLAs (Elfving et al., 2019; Fernandez-Teruel et al., 2021; Giorgi et al., 2019). Accordingly, since OXT administration has been associated with a reduction in glutamate and an increase in GABA release (Qi et al., 2012; Zhang et al., 2015; Zhou et al., 2015), it is possible that OXT reduces dopamine transmission by increasing inhibition in PFC neurons (see also Tapias-Espinosa et al., 2021), a mechanism that could be involved in enhanced behavioral inhibition and reflected by better social discrimination (and preference) and lowered

hyperactivity after administration of OXT against NMDA receptor antagonism (e.g. by MK801 administration) (Shilling and Feifel 2016).

Although we have mostly dealt with the relation of OXT and animal models of schizophrenia, there are several studies carried out in humans. In one of these experiments, the authors found that the use of OXT as a treatment to improve social cognition in schizophrenia patients, one of the major disability aspects of this disease, is possible (Pedersen et al., 2011). Besides, other studies had determined that a brief period of intranasal OXT administration on patients in treatment, reduces psychotic symptoms (Feifel et al., 2010). Also, other studies had demonstrated that the intranasal administration of OXT improves complex social perception and social cognition (Fischer-Shofty et al., 2013).

The impairment of social preference induced by NMDA receptor antagonists (PCP, ketamine, MK801) is considered to be a valid model of asociality (or social withdrawal) seen in schizophrenia as part of the negative symptom domain, whereas the hyperactivity induced by these NMDA antagonists is considered to model some positive symptoms of schizophrenia (Lee et al., 2005; Rung et al., 2005; Gururajan et al., 2012; Krzystanek and Pałasz, 2019). Compared with RLA rats, the RHA model shows enhanced detrimental effects on social behavior (i.e. preference) after MK801 administration, as well as much more pronounced MK801-induced hyperactivity (see also Sampedro-Viana et al., 2023, under review, and Oliveras et al., 2017). These MK801 effects have been shown to be attenuated or reversed in RHA rats, more clearly than in their RLA counterparts, either by different atypical antipsychotics (Sampedro-Viana et al., 2023 under review) or by OXT (present study). Altogether the present and the above mentioned findings, and the evidence of hypofrontality in RHA rats (Meyza et al., 2009; Río-Álamos et al., 2019; Tapias-Espinosa et al., 2019), tend to give support to the anti-schizophrenic properties of OXT, and in turn provide consistent support to the RHA model as an analogue of schizophrenia-relevant traits (in this case, in the negative symptom domain) with considerable predictive and construct validity.

Author contributions

D.S-V., T.C., A.T. and A.F-T. conceived and designed the experiments. D.S-V., T.C. and P.A. conducted the behavioral experiments. A.F-T., P.A. and D.S-V. analyzed the data and wrote the original manuscript. P.A. and A.T. provided critical review of the original draft. All authors read and approved the manuscript.

Acknowledgments

Supported by grants PID2020-114697GB-I00, PSI2017-82257-P, 2017SGR-1586, "ICREA-Academia 2013" (A.F-T., A.T.).

Conflict of interest

The authors present no conflict of interest.

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04 / Discusión General

El objetivo principal de la presente Disertación Doctoral era abordar, profundizar y continuar con la validación del perfil conductual y neurobiológico de un modelo animal de síntomas relevantes de esquizofrenia, la cepa de ratas RHA, así como qué efectos tendría la estimulación postnatal (NH) sobre su perfil de conducta social y sobre la actividad de regiones cerebrales "sociales". Los trabajos y estudios que aquí se han presentado se focalizaban en el aspecto de sintomatología negativa del modelo, hasta ahora no estudiado. Para lograr estos objetivos se han realizado varios estudios centrados en aspectos conductuales, neuroanatómicos y farmacológicos de las cepas de ratas *Roman*, así como estudios con manipulación ambiental y estudios de dimorfismos sexuales y de ontogenia.

Un breve resumen de los resultados principales sería:

En el Estudio 1 mostramos por vez primera que la cepa de ratas RHA presenta una disminución de la preferencia social comparada con la cepa RLA. También observamos como el tratamiento ambiental de estimulación neonatal (NH) incrementa la interacción social en ambas cepas, si bien su efecto en los primeros cinco minutos de prueba es más marcado en la cepa RHA.

Siguiendo los resultados del primer estudio evaluamos si el aumento de preferencia o conducta social observado tras el tratamiento de NH podría traducirse también en un aumento de la actividad neuronal de las áreas cerebrales que constituyen el llamado "cerebro social". Por ello, en el Estudio 2 confirmamos que la estimulación neonatal aumentaba la preferencia social y, que había un incremento de la expresión de c-Fos en áreas como el córtex infralímibico (IL) y la región medial posterodorsal (MePD) de la amígdala. Este aumento lo observamos específicamente en la cepa RHA y solo en los grupos con tratamiento NH tras el test de interacción social.

Llegados a este punto, nos preguntamos si el déficit de conducta social presentada por los machos de la cepa RHA también se observa en edad juvenil y en hembras de ambas edades. Así, en el Estudio 3 observamos que los animales adultos de ambas cepas muestran una reducción significativa de la preferencia social comparada con los adolescentes. Además, las hembras adultas de ratas RHA presentan una mayor preferencia social que los machos de la misma cepa. Estos resultados son consistentes con resultados previos obtenidos de roedores y humanos, que indican que hombres que padecen esquizofrenia tienen peores síntomas negativos que las mujeres con el mismo trastorno.

En el Estudio 4 quisimos comprobar si podíamos añadir validez predictiva y de constructo a las ratas RHA como modelo animal de síntomas relacionados con la esquizofrenia. Para ello administramos un antagonista del receptor NMDA (el MK801) y observamos que este fármaco induce una disminución de la conducta social en ambas cepas, pero su efecto es mucho más marcado en la cepa RHA que en la RLA. Los resultados también nos mostraron que los tres antipsicóticos atípicos administrados

(clozapina, ziprasidona o aripiprazol) fueron capaces de atenuar los efectos inducidos por el MK801 y que, de entre ellos, el aripiprazol presentaba el efecto "terapéutico" más potente.

Finalmente, considerando las propiedades antipsicóticas de la oxitocina, quisimos comprobar sus efectos sobre los ya observados del antagonista NMDA. En el **Estudio 5** mostramos como la cepa RHA presentaba mayor sensibilidad a los efectos "terapéuticos" de la oxitocina, capaz de atenuar en mayor medida los déficits inducidos por el MK801 en las ratas RHA en comparación con la cepa RLA.

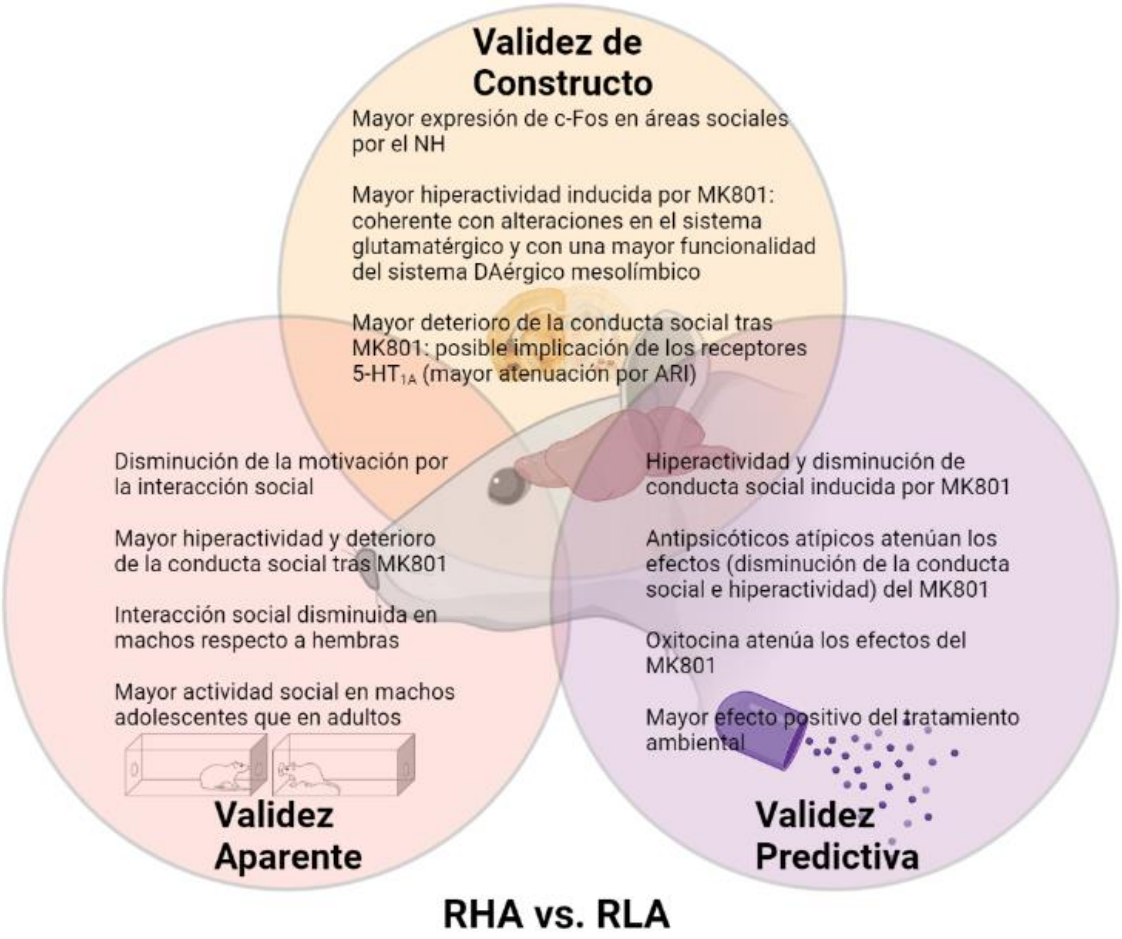

Figura 5: Resumen de los principales resultados obtenidos en esta Disertación Doctoral. Dividido según los criterios de validez de los modelos animales (validez aparente, validez de constructo y validez predictiva), se muestran los principales resultados obtenidos a lo largo de los diferentes estudios que forman el cuerpo de la presenten Disertación Doctoral. 5-HT_{1A}=subtipo de receptor de serotonina; ARI=aripiprazol; DA=dopamina; MK801=dizocilpina; NH=estimulación neonatal. x

Sintomatología negativa en la cepa RHA: asocialidad desde una perspectiva conductual

Primeramente, empezamos el relato de la presente disertación estableciendo que se han estudiado en profundidad varios aspectos del perfil conductual relacionados con la sintomatología positiva y cognitiva de esquizofrenia de la cepa RHA. Se han investigado fenotipos conductuales, fenotipos neuro- y psicofarmacológicos y fenotipos neuroanatómicos, moleculares y funcionales de la cepa de ratas *Roman* RHA (Fernández-Teruel et al., 2021; Giorgi et al., 2019). Sin embargo, muy pocos son los trabajos donde se ha abordado la sintomatología negativa en la cepa de ratas RHA. Hasta la fecha, sólo algunos estudios donde se observaba la conducta maternal en relación al cuidado de las crías ha podido establecer perfiles conductuales que se puedan asociar con la sintomatología negativa de esquizofrenia (Driscoll et al., 1979, 1991; Río et al., 2014). Por lo tanto, llegados a este punto, el grupo de investigación quiso indagar y continuar con la validación del modelo focalizándose en la sintomatología negativa.

Basados en la metodología usada en los estudios de Gururajan et al., (2012), que a su vez se basaban en los trabajos de Deak et al., (2009), nos planteamos empezar con la investigación de los aspectos de asocialidad mediante la prueba de interacción social que en ambos trabajos se describe. Usando este procedimiento pretendíamos establecer una separación física para prevenir el posible contacto entre los sujetos de investigación, evitando así expresiones de dominancia, conducta territorial o conducta sexual.

Observando los resultados pudimos demostrar por primera vez, y así se ha ido replicando a lo largo de todos los estudios, que la cepa de ratas RHA presenta una menor preferencia social comparada con la cepa RLA. Para una mejor comparación, también se realizó un estudio con la cepa de ratas genéticamente heterogéneas NIH-HS, cuya caracterización conductual (para otros fenotipos) se puede observar en los trabajos de Hansen y Spuhler, (1984), López-Aumatell et al., (2009) y Díaz-Morán et al., (2012). Los resultados mostraron que la preferencia social entre las cepas RLA y NIH-HS era prácticamente la misma y, que los niveles mostrados de preferencia social por las RHA conformaban la anormalidad. Los valores de preferencia del 50% de la cepa RHA nos habla de una conducta indiscriminada entre el agujero social y el no social, frente a los valores de 70% tanto de las RLA como de las NIH-HS, mostrando claramente una preferencia por el agujero social respecto al no social. Estos resultados son coherentes con la validez aparente del modelo animal de ratas RHA como un análogo de rasgos/síntomas relevantes de la esquizofrenia, ya que agrega un fenotipo similar a los observados en la sintomatología negativa, como es la asocialidad (o retraimiento social).

Siguiendo con el perfil conductual de asocialidad de la cepa, y relacionado con los dimorfismos sexuales y la ontogenia, mostramos que el déficit observado de las RHA respecto a las RLA en preferencia social no se observaba entre hembras adultas ni entre adolescentes de ambos sexos. Sin embargo, observamos que los adolescentes machos prefieren más el agujero social que los machos adultos en ambas cepas. Esto podría explicarse en función de que la conducta de búsqueda de sensaciones nuevas y la

motivación por la búsqueda de recompensa/reforzamiento son aspectos importantes para el desarrollo de los animales adolescentes en comparación con los animales adultos (Fernández-Teruel, 2021; Walker et al., 2017). A pesar de este aumento de preferencia social en los adolescentes, solo en la cepa RHA observamos diferencias significativas entre los adultos y los jóvenes, sugiriendo quizá alteraciones en el neurodesarrollo de los machos adultos de la cepa RHA. Otros estudios han mostrado que existen diferencias neuroquímicas entre las cepas *Roman* que sugieren una cierta inmadurez o cierto retraso en el desarrollo cortical de las RHA, similares a las observadas en los pacientes con esquizofrenia (Sánchez-González et al., 2021). Las RHA muestran mayor expresión de ciertos marcadores sinápticos en la corteza prefrontal, similar a los niveles observados en la infancia y adolescencia en estudios con humanos (Elfving et al., 2019; Hagihara et al., 2014). Relacionado con la inmadurez de las RHA, también hemos observado una mayor densidad de espinas dendritas piramidales del tipo "delgadas" (*thin*) o inmaduras (Sánchez-González et al., 2021), así como una disminución de la expresión cortical del gen CD38, que regula los niveles de oxitocina liberada y tiene un papel crucial en la conducta social (Tapias-Espinosa et al., 2021).

Así mismo, la ausencia de diferencia entre las hembras adultas de las cepas *Roman* va en concordancia con el hecho de que estudios realizados en humanos muestren que la sintomatología negativa se manifiesta en menor medida en mujeres que en hombres (Li et al., 2016; Shalev & Weiner, 2001).

Efectos de una manipulación ambiental, la estimulación neonatal, sobre la conducta social y la expresión de c-Fos en áreas "sociales" del cerebro.

A la luz de los resultados obtenidos en relación con la conducta social y con los aspectos de asocialidad observados en la cepa de ratas RHA, nos preguntamos si podría haber una mejora de este aspecto con una intervención ambiental temprana. Para ello usamos el tratamiento ambiental de estimulación neonatal (*NH,* del término inglés, *neonatal handling*) descrito previamente en el trabajo de Río-Àlamos et al., (2015, 2017, 2019). El tratamiento, que consiste en una separación individual de las crías de la madre (por un total de 8 minutos), seguido de unos masajes gentiles (suaves) de pocos segundos con la mano desnuda por el dorso de la rata, fue administrado por la mañana y por la tarde durante los primeros veintiún días de vida de las crías (hasta el destete).

Los resultados mostraron, primeramente, que las RLA presentan mayores niveles de conductas asociadas con la ansiedad que las ratas RHA en la prueba de la exploración del objeto novedoso (*NOE,* por sus siglas en inglés, *Novel Object Exploration*) y, que este comportamiento podría verse beneficiado por el tratamiento ambiental del NH. El NH fue capaz de reducir drásticamente el tiempo de latencia de exploración del objeto novedoso en la cepa RLA e incrementar el tiempo de exploración del mismo en ambas cepas. Aunque la prueba de NOE no es el principal objeto de investigación del estudio, sí que sirve como un indicador (o "marcador") que confirma que el tratamiento ambiental fue administrado de forma correcta y presentaba los efectos esperables. Los resultados obtenidos concuerdan con los ya conocidos efectos ansiolíticos y antiestrés a largo plazo que provoca el tratamiento de NH (Fernández-Teruel et al., 2002; Levine, 1956; Río-Álamos et al., 2015, 2017, 2019). En relación con la prueba de interacción social, los resultados mostraron que le tratamiento de NH es capaz de incrementar la conducta social en ambas cepas, pero de forma más clara en la cepa RHA.

Aunque otros estudios ya han mostrado efectos del tratamiento NH sobre la conducta social, ninguno lo había hecho desde una perspectiva de la asocialidad como análogo de sintomatología negativa de la esquizofrenia, sino desde la perspectiva de una medida o indicador de ansiedad (Raineki et al., 2014), lo que implica que en esos otros trabajos se han utilizado procedimientos de interacción social que implicaban posibles elementos de confusión, como la interferencia de la actividad locomotora o el efecto de la novedad de la situación-test, por ejemplo. Así mismo, las diferencias en los tiempos de administración del tratamiento y en el tipo particular de tratamiento NH en sí mismo, hacen difícil la comparativa entre los estudios. Volviendo a los efectos observados, hay que remarcar como importante el hecho de que el tratamiento de NH no incrementa la exploración/actividad general de los animales, sino que específicamente actúa aumentando la conducta social en ambas cepas de ratas, pero de manera más marcada en las ratas RHA.

Por otro lado, se han observado alteraciones de los sistemas dopaminérgicos y serotoninérgicos cerebrales provocados por el tratamiento de NH (Fernández-Teruel et

al., 2002; Panagiotaropoulos et al., 2004), ambos sistemas implicados en la etiopatogenia de la psicosis y la esquizofrenia. A su vez, el tratamiento de NH también produce cambios en la densidad de receptores de glutamato AMPA y NMDA en regiones corticales y límbicas en roedores (Stamatakis et al., 2009). Estos sistemas, que se hallan alterados en las ratas RHA (ver Fernández-Teruel et al., 2021), podrían tener un papel modulador de los efectos del NH sobre el comportamiento social observado en el presente estudio.

Parece oportuno discutir y clarificar el efecto del NH sobre la conducta social y su independencia de la reducción en ansiedad que también produce el tratamiento. Como se ha mencionado, la cepa RHA presenta menores niveles de ansiedad que las RLA (resultados observados en el NOE, y en muchas otras pruebas; Fernández-Teruel et al., 2021; Río-Álamos et al., 2015) pero mayor sensibilidad al efecto "terapéutico" del tratamiento respecto a la interacción social. Si la prueba de SI aquí utilizada estuviese contaminada o influenciada por la ansiedad, esperaríamos que los animales "control" de la cepa RLA (con mayores niveles de ansiedad) presentaran menor conducta/preferencia social que las RHA "control", y que las ratas RLA tratadas con NH presentaran mayor preferencia social y/o mayor tiempo social, pero dichos efectos no se observan. Por tanto, se puede proponer que el efecto del tratamiento NH es genuinamente sobre la conducta social y que la ansiedad no parece interferir en el presente procedimiento de interacción social.

Profundizando en los efectos del tratamiento de NH, quisimos observar cual era el efecto del NH sobre la expresión de un gen de expresión temprana, como es el c-Fos, en distintas áreas del cerebro relacionadas con la conducta social. El c-Fos ha sido descrito como un indicador de la activación transcripcional, activándose en las neuronas después de la estimulación sináptica, proporcionando una lectura indirecta de la activación neuronal (Hudson et al., 2018).

Los resultados mostraron que el tratamiento de NH era capaz de incrementar la conducta social en la cepa RHA y paralelamente incrementar de forma específica la expresión de c-Fos proteico en comparación con las ratas RLA. Este incremento de c-Fos era específico de ciertas áreas del cerebro relacionadas con el llamado "cerebro social", concretamente en la región IL de la corteza prefrontal medial y la MePD de la amígdala. En relación con la mPFC, cabe destacar que en el estudio aquí presentado la corteza PrL no fue sensible a la interacción social, ya que la condición de SI no mostró niveles de expresión de c-Fos superiores a los observados en la condición CTX en ninguna de las cepas. Sin embargo, este aumento en la expresión de c-Fos sí fue observado para la corteza IL en las ratas RHA tratadas con NH. Estas diferencias entre el PrL y el IL podrían ir en consonancia con el modelo "*PrL-go/lL-stop*", en cuanto que aparentemente existe una evidencia de roles funcionales opuestos entre el PrL y el IL para la misma respuesta conductual (Gourley & Taylor, 2016; Minami et al., 2017).

Así mismo, otra diferencia en expresión de c-Fos inducida por el tratamiento de NH se halló en las subregiones de la amígdala medial (MeA). Los resultados mostraron que el tratamiento de NH incrementa la expresión de c-Fos en la cepa de ratas RHA en la

subregión de la MePD, sin observar dicho aumento en el grupo control o en la cepa RLA. Este aumento tampoco lo observamos en la subregión de MePV. Esto podría ser debido a que la MePD integra señales del ambiente para coordinar la respuesta de la conducta social, mientras que la MePV juega un papel en la expresión de reacciones defensivas vinculadas a amenazas (Cádiz-Moretti et al., 2016; Schulz & Sisk, 2016). Esto parece ser consistente con el hecho de que no se ha observado un incremento de c-Fos en la MePV en la condición SI, pero sí que se ha observado un efecto considerable en la condición CTX, en la que existe "novedad" (y, por tanto, cierto nivel de "amenaza") sin haber conducta social. Así mismo, se ha descrito que la MePD recibe proyecciones del IL (Ko, 2017), por lo que parecería plausible que el incremento de células positivas para c-Fos de la corteza IL en los animales RHA tratados con NH pueda estar asociado con el incremento paralelo observado en MePD del mismo grupo experimental.

Parece oportuno comentar el incremento que se observa en todos los resultados de expresión de c-Fos en la condición CTX, comparado con la condición HC. La habituación al contexto y la condición CTX para el análisis de c-Fos fueron añadidas al diseño experimental para discriminar el efecto de la exposición a un ambiente novedoso (y por consiguiente, un efecto del estrés) sobre la conducta social. No parece descabellado, inferir que con una mayor habituación al contexto (sala experimental y aparato de experimentación) observaríamos una reducción en la expresión de c-Fos en la condición CTX, quizá permitiendo así observar con mayor claridad diferencias (si las hubiese) entre los grupos CTX y SI. Hasta donde sabemos, solo se ha reportado un estudio de expresión de c-Fos en un contexto de interacción social que también incluya las condiciones CTX y HC, el trabajo de Perkins et al. (2017). En este trabajo compararon machos y hembras de ratas de la cepa F344, y observaron un incremento de los niveles de c-Fos en la condición SI, en distintas regiones de la PFC y de la AMY, pero solo en hembras. Es interesante observar, comparando nuestro estudio con el de Perkins et al., (2017), que los animales que presentan mayor conducta social (hembras F344 en el estudio de Perkins et al., 2017, y las ratas RHA tratadas con NH en el presente trabajo) también presentan mayores niveles de expresión de c-Fos en las subregiones IL y MePD.

En síntesis, las medidas de activación celular cerebral por expresión de c-Fos nos han permitido observar, por vez primera, que existen efectos específicos, dependientes de la cepa de ratas (es decir, de sus características genéticas y/o epigenéticas diferenciales), del NH sobre la conducta social y sobre la activación celular en regiones implicadas en el "cerebro social", como la IL y la MePD. Las ratas RHA, que responden más al tratamiento NH en términos de incremento de conducta social, son las que muestran mayor activación de ambas regiones como consecuencia de la exposición a SI y al tratamiento NH. El análisis de expresión de c-Fos en otras regiones cerebrales que también han sido implicadas en aspectos de conducta social, tales como el hipocampo, el núcleo del lecho de la estria terminal o el núcleo accumbens, cabe esperar que arroje más luz sobre las implicaciones (y quizá generalización a otras regiones cerebrales) de los presentes resultados.

Efectos de la administración de un antagonista del receptor NMDA (MK801), y atenuación con antipsicóticos atípicos y oxitocina

Una vez observado el perfil conductual de la cepa RHA frente a una prueba de interacción social y, una vez estudiados los efectos de la manipulación ambiental (NH) sobre la conducta social y la expresión de c-Fos, nos preguntamos si podríamos añadir mayor validez predictiva al modelo desde una aproximación farmacológica.

En base a la teoría glutamatérgica de la esquizofrenia, muchos modelos farmacológicos ya han demostrado la eficacia de la administración de antagonistas NMDA (como el MK801, la PCP o la ketamina) para mimetizar no solo la sintomatología positiva y cognitiva de la esquizofrenia sino también la sintomatología negativa (Neill et al., 2014; Wilson & Koenig, 2014).

Los resultados obtenidos en los experimentos farmacológicos han mostrado por primera vez los efectos de la administración de MK801 en las cepas de ratas *Roman*. La disminución de conducta no social por la administración de MK801 es similar en ambas cepas, pero la afectación de la conducta social y de la preferencia por el agujero social es claramente mayor en la cepa RHA que en la RLA. Además, el MK801 resultó mucho más efectivo para aumentar el tiempo de latencia de exploración del agujero social y para producir hiperactividad en la cepa RHA que en la RLA. En estudios previos de Elfving et al., (2019) y de Klein et al., (2014) se han observado diferencias en la transmisión glutamatérgica entre las cepas de ratas *Roman*. La cepa RHA muestra mayores niveles de ARNm para el gen *Grin2b* (receptor de glutamato NMDA2B) en la corteza prefrontal y un aumento de NMDA2B en el hipocampo. Además, las ratas RHA muestran mayor expresión de ARNm para el 5-HT2A en el hipocampo y una mayor unión al receptor 5-HT2A en la corteza frontal. También hay que remarcar que las ratas RHA tienen una mutación en el codón de parada (*stop codon*) para el receptor mGlu2 y su gen (*Grm2*) que las convierte en un *knock-out* natural para este gen, disminuyendo así su expresión (Fomsgaard et al., 2018; Wood et al., 2017). Estos resultados brindan un mayor respaldo al perfil de síntomas relacionados con la esquizofrenia de las ratas RHA, añadiendo los efectos perjudiciales del antagonista NMDA en el comportamiento social y la hiperactividad como un fenotipo diferencial de las cepas *Roman*.

Cuando exploramos los efectos de los distintos antipsicóticos atípicos usados (clozapina, ziprasidona y aripiprazol) obtuvimos una atenuación de los efectos inducidos por el MK801 en preferencia social y actividad locomotora, atenuación que fue más marcada en la cepa RHA. De entre todos los antipsicóticos, el ARI parece tener el efecto "terapéutico" más potente, ya que no solo es capaz de atenuar el incremento de latencia social provocado por el antagonista NMDA, sino que es el único capaz de producir un incremento neto (modesto, aunque significativo) de conducta social en las ratas RHA (véase *"Social Time"* en Figura 6b del Estudio 4). Los resultados muestran una alteración general de la conducta provocada por los antipsicóticos CLZ y ZPR. En cambio, no hay un efecto global del ARI en actividad locomotora, sino que su efecto se limita a atenuar la

hiperactividad inducida por el MK801 en las ratas RHA, pero no en las RLA. Este hecho podría estar relacionado con la naturaleza de agonista parcial del receptor de dopamina D2 del ARI, en comparación con la actividad antagonista que presentan tanto la CLZ como la ZPR, que sí que reducirían el tono funcional del sistema dopaminérgico mesolímbico.

Además, los tres antipsicóticos comparten la actividad antagonista del receptor 5-HT2A, y agonista parcial para el 5-HT1A (siendo la CLZ la que menor actividad de agonista parcial 5-HT1A presenta y ARI el que más). Este último receptor se ha propuesto como un mecanismo neuroquímico clave en la atenuación o reversión de los déficits inducidos por otros antagonistas NMDA (como la PCP) en conducta social (Snigdha & Neill, 2008). En coherencia con lo anterior, en las ratas RHA se ha observado un aumento en la expresión de ARNm y de unión del receptor 5-HT1A en corteza prefrontal, en línea con los estudios realizados con pacientes humanos con esquizofrenia (Aznar & Hervig, 2016; Elfving et al., 2019). Por tanto, el perfil del receptor 5-HT1A en las RHA podría estar relacionado con el efecto específico del ARI en los animales tratados con MK801, puesto que, a diferencia de la CLZ y la ZPR, el ARI disminuye la "latencia social" y aumenta la conducta social neta en esta cepa de ratas.

Una vez realizados los estudios con diferentes antipsicóticos, quisimos comprobar si podría haber una mejora en la interacción social usando un neuropéptido endógeno con capacidad antipsicótica natural. Teniendo en cuenta que los pacientes que padecen esquizofrenia muestran una perturbación en el sistema oxitocinérgico, y que la oxitocina (OXT) puede incrementar la conducta social (Fischer-Shofty et al., 2013; Goh et al., 2021; Woolley et al., 2014), quisimos estudiar este fenómeno con el modelo animal de las RHA.

Los resultados obtenidos en el último estudio muestran que, tal y como ya habíamos observado, el MK801 disminuye la conducta social en ambas cepas siendo su efecto más marcado en la cepa RHA. De igual manera, la administración del antagonista NMDA induce una hiperactividad mayor en la cepa RHA que en la RLA. También hemos observado que esta hiperactividad y este déficit en la conducta social (inducidos por el MK801) resultan atenuados con la administración de OXT, y de forma aparentemente más pronunciada en la cepa RHA.

En estudios previos realizados por el grupo de investigación, ya se habían mostrado los efectos beneficiosos de la OXT en la cepa RHA en relación con la PPI y el incremento del gen para el receptor de la OXT después de su administración. De igual manera, se había observado una alteración en el sistema oxitocinérgico basal en las RHA, que muestran una menor expresión de la proteína CD38 (encargada de la regulación en la liberación de la oxitocina) en corteza prefrontal medial comparadas con las RLA (Tapias-Espinosa et al., 2021). En la bibliografía también se puede observar la reversión de la hiperactividad inducida por MK801 y por PCP mediante la administración de OXT en roedores (Kohli et al., 2019; Lee et al., 2005).

Este efecto “terapéutico” de la OXT podría estar relacionado con el hecho de que su administración se ha asociado con una reducción de glutamato y un aumento en la liberación de GABA en corteza prefrontal (Qi et al., 2012; Zhang et al., 2015). Es posible que la OXT reduzca la transmisión de DA al aumentar la inhibición en las neuronas corticales, un mecanismo que podría estar involucrado en una mayor inhibición del comportamiento y reflejado en una mejor discriminación o preferencia social y una menor hiperactividad después del antagonismo del receptor NMDA (Shilling & Feifel, 2016).

Los resultados de la bibliografía relevante, en conjunto con los resultados previos del grupo y con los aquí presentados, respaldan las propiedades antipsicóticas (moderadas) de la OXT y, a su vez, brindan respaldo al modelo de las RHA como un análogo de rasgos relevantes para la esquizofrenia con una considerable validez predictiva y de constructo.

Aportaciones, limitaciones y futuros estudios

Es prácticamente imposible mimetizar y obtener un modelo animal exactamente igual a la esquizofrenia, dada la complejidad del trastorno y la diversidad de síntomas. Pese a todo, podemos considerar que los resultados obtenidos en la presente Disertación Doctoral contribuyen a añadir mayor validez predictiva, aparente y de constructo al modelo animal de síntomas relacionados con la esquizofrenia utilizado, la cepa de ratas *Roman* RHA, concretamente en lo relacionado a la sintomatología negativa (asocialidad, desinterés social) del trastorno.

Los resultados discutidos en las secciones anteriores confirman que la cepa de ratas RHA presenta aspectos de asociabilidad que se pueden relacionar con la sintomatología negativa de la esquizofrenia (McCutcheon et al., 2020b). Los resultados también han mostrado que, al igual que se observa en humanos, esta disminución en conducta o preferencia sociales es más marcada en machos que en hembras (Li et al., 2016; Riecher-Rössler et al., 2018) y que los adolescentes de ambas cepas presentan mayor actividad social que los adultos (Walker et al., 2017).

En relación con la estimulación neonatal (NH), los resultados aportan que el tratamiento ambiental de NH es eficaz para aumentar la conducta y preferencia social de las ratas *Roman*, siendo su efecto más evidente en la cepa RHA que en la RLA. Además, el tratamiento es capaz de aumentar la expresión de c-Fos en áreas asociadas con el "cerebro social" de forma más específica en la cepa RHA.

Finalmente, los resultados aportan evidencias de la disminución en conducta social e incremento de la actividad inducidas por la administración de un antagonista del receptor NMDA, mostrando a su vez que la afectación es claramente mayor en la cepa RHA, como es esperable de un modelo animal de aspectos/síntomas relacionados con la esquizofrenia. También se aportan resultados favorables a la validez predictiva del modelo, por cuanto la administración de antipsicóticos atípicos y de oxitocina atenúan la asociabilidad e hiperactividad inducidas por el MK801 de manera más pronunciada en las ratas RHA (Kohli et al., 2019; Neill et al., 2010; Shilling & Feifel, 2016; Uno & Coyle, 2019).

A pesar de las aportaciones en los distintos aspectos de validación del modelo animal hay que reconocer y aceptar ciertas limitaciones experimentales que no se han podido eludir. Dos grandes limitaciones de la presenten Disertación Doctoral son la limitación de edad y la limitación de sexo en los distintos estudios.

Por un lado, en el **Estudio 3** se han abordado aspectos relacionados con la ontogenia y se han observado diferencias en conducta social entre los adolescentes y los adultos. Serán necesarios futuros estudios longitudinales para estudiar procesos de maduración cerebral y cambios en los sistemas de neurotransmisión a fin de explorar posibles alteraciones en la expresión de factores neurales relacionados con la sincronía neuronal fronto-cortical durante la adolescencia, que se han relacionado con la aparición de

episodios de psicosis, con la esquizofrenia y con los déficits cognitivos (Millan et al., 2016). Estudios longitudinales, con varios puntos de análisis durante el desarrollo de los animales, servirían para observar procesos de integración funcional de la corteza frontal en las redes corticales y así poder abordar con más detalle si las manifestaciones de los síntomas característicos del modelo RHA estarían causados por (o asociados a) una excesiva eliminación sináptica durante la adolescencia o a otros procesos neuromadurativos. Aun con todas las limitaciones, ya se ha avanzado en esa dirección con los estudios de Sánchez-González et al., (2021) y Elfving et al., (2019) (ver revisión de Fernández-Teruel et al., 2021), que apuntan a un endofenotipo inmaduro de la corteza frontal en ratas adultas de la cepa RHA, que puede ser la base de su perfil neuroconductual.

Por otra parte, y en línea con lo hallado en Sánchez-González et al., (2021) y Elfving et al., (2019), con respecto a la sincronía de la actividad fronto-cortical y al balance excitatorio/inhibitorio (E/I) en esta región (procesos cuyas alteraciones son consideradas cruciales en la esquizofrenia), estudios de nuestro laboratorio han mostrado que las ratas (adultas) RHA presentan probables alteraciones en dichos procesos, evidenciadas por déficits en la activación (tras una tarea sensoriomotora, la PPI) de las interneuronas de parvalbúmina (PV; GABAérgicas) (Tapias-Espinosa et al., 2023). Una activación reducida de las interneuronas PV podría conducir a la desinhibición de las neuronas piramidales que provoca un aumento de la dopamina mesolímbica y síntomas similares a la esquizofrenia en las ratas RHA. Los resultados de Tapias-Espinosa et al., (2023) indican que la menor actividad neuronal fronto-cortical durante la prueba de PPI (ver también (Tapias-Espinosa et al., 2019), mostrada por las ratas RHA en relación con las RLA, podría explicarse por las diferencias en la activación de las interneuronas PV. Este resultado apunta a una inhibición cortical reducida (es decir, a una alteración del balance E/I) como un posible candidato para explicar las características similares a las de la esquizofrenia observadas en las ratas RHA, al tiempo que respalda el papel de la inhibición cortical alterada en la esquizofrenia (Tapias-Espinosa et al., 2023). La oxitocina muestra un perfil inhibitorio sobre el balance glutamato/GABA (balance E/I) fronto-cortical (revisado en Tapias-Espinosa et al., 2021). El hecho de que la administración de OXT atenúe tanto el déficit natural de PPI en ratas RHA (Tapias-Espinosa et al., 2021) como los efectos del MK801 sobre la preferencia social y la hiperactividad en dicha cepa (resultados presentes), parece también coherente con la mencionada alteración del balance E/I en las RHA.

Las limitaciones de añadir ambos sexos en los estudios se han empezado a abordar en el Estudio 3. El aumento en conducta social o, dicho de otro modo, la menor asociabilidad observada en las hembras de la cepa RHA es coherente con los resultados obtenidos en los estudios clínicos con hombres y mujeres. Sin embargo, hay que discutir un aspecto importante. Está ampliamente aceptado que los antipsicóticos de segunda y tercera generación han sido clínicamente útiles para tratar pacientes con esquizofrenia (los

resultados del Estudio 4 son coherentes con ello). Sin embargo, en la mayoría de las investigaciones preclínicas (donde nos incluimos) no se abordan las múltiples diferencias sexuales en la eficacia y en las dosis de los fármacos, así como otras consideraciones específicas del sexo (Crawford & DeLisi, 2016). Futuros estudios del grupo de investigación deberían apuntar a responder este tipo de preguntas en relación con el modelo animal e incluir hembras en los estudios farmacológicos.

Otra limitación por comentar, que puede encontrarse en el Estudio 4 y en el Estudio 5, es el hecho de que, en pacientes con esquizofrenia, los antipsicóticos de cualquiera de las tres familias principales deben administrarse durante diversos días, e incluso semanas, para obtener el efecto terapéutico óptimo. (Białoń & Wąsik, 2022; Ellenbroek & Karl, 2016). Así pues, el efecto "terapéutico" descrito en estudios realizados tras una administración aguda de antipsicóticos podría tener ciertos problemas de "translabilidad" o generalización desde la experimentación animal hasta la práctica clínica. Esta limitación es común con la mayoría de los estudios farmacológicos preclínicos y siempre hay que tenerla en cuenta.

Debido a la dificultad de modelar un trastorno tan complejo como es la esquizofrenia con modelos animales, es natural que surjan limitaciones en los estudios de investigación básica preclínica. Sin embargo, los modelos animales permiten, 1) comprender mejor la etiopatología de las enfermedades, o al menos los mecanismos neurobiológicos subyacentes a síntomas o fenotipos concretos que tengan relación con trastornos mentales/neurológicos específicos, y 2) del mismo modo facilitan la comprensión de los mecanismos intervinientes en los tratamientos al uso, así como (en parte gracias a ellos) el establecimiento de nuevos tratamientos.

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Conclusiones

Las principales conclusiones que se derivan de la presente Disertación Doctoral son:

1. La cepa de ratas RHA muestra una menor preferencia social, independientemente de las condiciones previas de habituación a la situación-test, que las ratas RLA y ratas genéticamente heterogéneas (HS).

2. El tratamiento de estimulación neonatal (NH) reduce las respuestas relacionadas con la ansiedad en ambas cepas, pero con un efecto más marcado en la cepa RLA.

3. El tratamiento NH incrementa la conducta social en ambas cepas, pero el efecto es mayor en la cepa RHA.

4. Respecto a la cepa RLA, los machos adolescentes de la cepa RHA no presentan diferencias de interacción social. Dicha diferencia tampoco se observa en las hembras de ninguna edad. Los machos RHA y RLA adolescentes muestran mayor preferencia social que los respectivos machos adultos de ambas cepas.

5. El tratamiento de NH incrementa la conducta social de la cepa RHA en mayor medida que en las RLA y, paralelamente, incrementa la expresión de c-Fos proteico en las subregiones del infralímbico (IL) y de la amígdala medial posterodorsal (MePD) en las ratas RHA.

6. La administración del antagonista del receptor NMDA (MK801) induce una disminución en la conducta no social en ambas cepas de ratas *Roman*. No obstante, la reducción de conducta social y, en concreto, la reducción de preferencia social es específica para la cepa RHA.

7. La administración de MK801 es más eficaz en incrementar la latencia social y la actividad locomotora en la cepa RHA que en la cepa RLA.

8. Las dosis altas de aripiprazol (ARI) y ziprasidona (ZPR) atenúan la hiperactividad inducida por MK801 en la cepa RHA, pero no en la cepa RLA. Este efecto también se observó con la administración de clozapina (CLZ) pero en menor medida.

9. ARI y ZPR revierten la disminución inducida por MK801 sobre la preferencia social en la cepa RHA, sin afectar a la cepa RLA. Este efecto también se observa con CLZ en los primeros cinco minutos del test de interacción social.

10. De entre los tres antipsicóticos atípicos, el ARI ha resultado ser el más potente en la reducción de los déficits inducidos por M801, puesto que es el único de los tres fármacos que atenuó el incremento de “latencia social” inducido por MK801 y que produjo un incremento neto de tiempo social en la cepa RHA.

11. La administración del neuropéptido oxitocina (OXT) fue capaz de atenuar los efectos inducidos por la administración del antagonista NMDA, por lo que respecta a la hiperactividad y a la disminución en la preferencia social, siendo este efecto aparentemente más marcado en la cepa RHA que en la RLA.

12. Teniendo en cuenta los resultados presentados en esta Disertación Doctoral, podemos seguir considerando que el modelo constituido por la cepa RHA es útil para la investigación de ciertos aspectos relevantes para la esquizofrenia, incluidos algunos rasgos/fenotipos relacionados con la sintomatología negativa del trastorno.

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Apéndice:
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