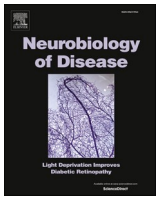
Neurobiology of Disease 194 (2024) 106487

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



# Hippocampal Pyk2 regulates specific social skills: Implications for schizophrenia

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ARTICLE INFO

*Keywords:*
Hippocampus
Psychosis
Social symptoms

ABSTRACT

Pyk2 has been shown previously to be involved in several psychological and cognitive alterations related to stress, Huntington's disease, and Alzheimer's disease. All these disorders are accompanied by different types of impairments in sociability, which has recently been linked to improper mitochondrial function. We hypothesize that Pyk2, which regulates mitochondria, could be associated with the regulation of mitochondrial dynamics and social skills. In the present manuscript, we report that a reduction of Pyk2 levels in mouse pyramidal neurons of the hippocampus decreased social dominance and aggressivity. Furthermore, social interactions induced robust Pyk2-dependent hippocampal changes in several oxidative phosphorylation complexes. We also observed that Pyk2 levels were increased in the CA1 pyramidal neurons of schizophrenic subjects, occurring alongside changes in different direct and indirect regulators of mitochondrial function including DISC1 and Grp75. Accordingly, overexpressing Pyk2 in hippocampal CA1 pyramidal cells mimicked some specific schizophrenia-like social behaviors in mice. In summary, our results indicate that Pyk2 might play a role in regulating specific social skills likely via mitochondrial dynamics and that there might be a link between Pyk2 levels in hippocampal neurons and social disturbances in schizophrenia.

## 1. Introduction

Proline-rich tyrosine kinase 2 (Pyk2) is a $Ca^{2+}$-activated non-receptor tyrosine kinase enriched in adult hippocampal neurons (Giralt et al., 2017). Although it is mainly localized in the cytoplasm, upon neuronal activation, Pyk2 can translocate to different subcellular compartments such as the nucleus (Faure et al., 2007), dendritic spines (Bartos et al., 2010), and mitochondria (López-Molina et al., 2022). Consequently, the main functions described for Pyk2 are related to these localizations, such as the regulation of synaptic plasticity and mitochondrial dynamics (de Pins et al., 2021; López-Molina et al., 2022). Pyk2 is strongly implicated in the pathophysiology of several neurological disorders such as Huntington's disease (Giralt et al., 2017), chronic stress (Montalban et al., 2019), and Alzheimer's disease (Salazar et al., 2019). Interestingly, a common feature of all these conditions is the clinical manifestation of a myriad of social deficits (Braund et al., 2019; Kessels and Elferink, 2021; Mason et al., 2021). However, no research has yet addressed the role of Pyk2 in the modulation of social

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https://doi.org/10.1016/j.nbd.2024.106487
Received 6 February 2024; Received in revised form 14 March 2024; Accepted 25 March 2024
Available online 27 March 2024

behaviors, either in normal or pathological conditions. Recently, a genome-wide association study (GWAS) described *PTK2B* (gene encoding Pyk2) as a gene potentially associated with schizophrenia (Trubetskoy et al., 2022).

Schizophrenia is a multifactorial neuropsychiatric disorder caused by genetic and environmental factors (Pardiñas et al., 2018) that affects about 1% of the worldwide population (Saha et al., 2005). It manifests as positive symptoms (such as hallucinations) and negative symptoms (such as social dysfunctions) (Blokhin et al., 2020; Picchioni and Murray, 2007). While current treatments for this disorder involve antipsychotic drugs to manage the positive symptoms, negative symptoms such as social deficits remain poorly treated, thus hampering the quality of life and relationships of schizophrenic patients (Remington et al., 2016; Tamminga et al., 1998). Social behaviors rely on proper hippocampal function (Montagrin et al., 2018; Schafer and Schiller, 2018). Schizophrenic patients display functional and structural alterations in the hippocampus, including loss of hippocampal volume and aberrant neural activities (Bogerts et al., 1993; Bogerts et al., 1985; Lieberman et al., 2018; Roeske et al., 2020; Wegrzyn et al., 2022). One of the proposed mechanisms for this hippocampal dysfunction is severe synaptic disorganization, including synaptic and dendritic alterations (Harrison and Eastwood, 2001; Matosin et al., 2016; Rosoklija et al., 2000; Toro and Deakin, 2005). Moreover, mitochondrial abnormalities have also been widely reported in schizophrenia, including alterations in metabolism (Lieberman et al., 2018; Schobel et al., 2013), a reduction in mitochondrial density (Kolomeets and Uranova, 2009), and impaired mitochondrial respiration (Altar et al., 2005). Hence, it is conceivable that such mitochondrial deficiencies could be associated with social

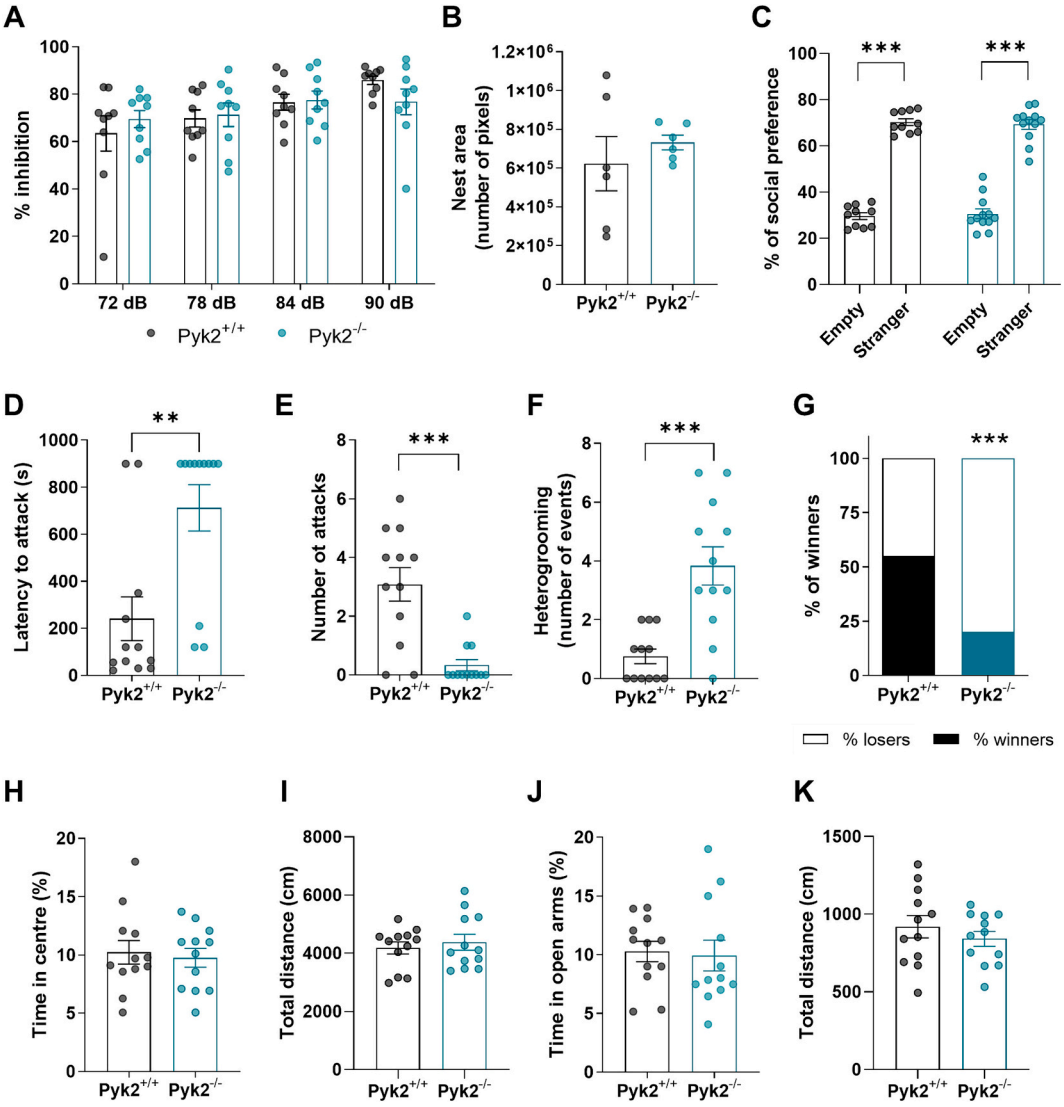


**Fig. 1.** Sensory gating, social behaviors, and anxiety levels in Pyk2$^{-/-}$ mice.
(a) Sensory gating was studied using the pre-pulse inhibition paradigm. (b) Social nesting behavior was also monitored. (c) Sociability was evaluated in the three-chamber social interaction test (TCSIT). (Two-way ANOVA, social preference effect: $F_{(1, 40)} = 440.8$, $p < 0.0001$). (d-f) Resident-intruder test: the latency to attack (d, unpaired *t*-test, $t = 3.486$, df = 22, $p = 0.0021$), the number of attacks (e, unpaired t-test, $t = 4.580$, df = 22, $p < 0.0001$), and heterogrooming (f, unpaired t-test, $t = 4.431$, df = 22, $p = 0.0002$) were monitored. (g) Social dominance test. Dominance was established as a % of winners (chi-squared test, chi = 26.13, $p < 0.0001$). (h) Anxiety levels and (i) exploratory behavior were both determined by evaluating the % of time spent at the center of an open field and the locomotor activity in the open field, respectively. Furthermore, (j) anxiety levels and (k) exploratory behavior were both evaluated by monitoring the % of time spent in the open arms and locomotor activity, respectively, in the elevated plus maze test. Data represent the mean ± SEM. In (c), ***$p < 0.001$ when comparing empty vs stranger (Bonferroni's post-hoc test). In (d-g), **$p < 0.01$ and ***$p < 0.001$ when comparing Pyk2$^{-/-}$ mice with Pyk2$^{+/+}$ mice. Three independent cohorts of mice were used. Cohort 1 was used in a ($n = 9$/group), cohort 2 was used in b (24 mice/group were used, with 4 mice in each cage to give a final $n = 6$ cages/group), and cohort 3 was used in d-k ($n = 12$/group).

deficits (Picard and Sandi, 2021; Ülgen et al., 2023).

As we previously found that Pyk2 regulates mitochondrial function (López-Molina et al., 2022), we hypothesized that Pyk2 could play a role in sociability. We also explored its potential contribution to schizophrenia. In the present work, we demonstrate that hippocampal Pyk2 is a regulator of some social behaviors probably via the regulation of mitochondrial density and that this phenomenon could have implications for psychiatric disorders with social impairments such as schizophrenia.

## 2. Materials and methods

### 2.1. Animals

$Pyk2^{+/+}$ and $Pyk2^{-/-}$ mice (Figs. 1–2 and 4) as well as $Pyk2^{flox/flox}$ mice (Fig. 3), in which the *Ptk2b* exons 15b-18 are flanked by LoxP sequences (Gen-O-way, Lyon, France; (Giralt et al., 2016), were established by our group and described elsewhere (Montalban et al., 2019). Mice were maintained in a C57BL/6 strain background and genotyped from an ear biopsy used for DNA amplification, as previously reported (Giralt et al., 2017). For this study, we also used adult (12 weeks old) C57BL/6 males (MGI catalog #5657800, RRID:MGI:5657800) in the experiments depicted in Fig. 6. Only the male littermates of all the genotype groups and experiments were used since the human samples were all from males. Animal handling and experiments were performed following the guidelines of the Declaration of Helsinki and the NIH (1985 revised publication no. 85–23, European Community Guidelines), as well as of the local animal ethics committee (477/15 at the *Universitat de Barcelona*). The method of euthanasia used was cervical dislocation.

### 2.2. Behavioral characterization

#### 2.2.1. Amphetamine and ketamine treatments

$Pyk2^{+/+}$ and $Pyk2^{-/-}$ mice were treated daily for 8 days with vehicle (0.9% NaCl), ketamine (30 mg/kg; A4393 from Sigma), or D-amphetamine sulfate (3 mg/kg; 2813 from Tocris Bioscience). On days 1 and 7, the locomotor activity of the mice induced by amphetamine or ketamine in an open field (see below for the open field dimensions and characteristics) was evaluated and recorded with a video caption system (SMART junior software, Panlab). On day 8, the mice were sacrificed 15 min after injection and the hippocampus was rapidly dissected out and frozen at −80 °C until use.

#### 2.2.2. The resident-intruder paradigm

Social responses and territorial aggression were assessed by monitoring aggressive, dominant, and interactive behaviors displayed by the resident (mouse subject isolated for 7 days) when a novel age- and gender-matched mouse was placed in the resident's home cage for 20 min. The parameters scored were attack latency (latency to first attack), attack incidence (number of attacks), and heterogrooming behaviors (including licking behavior).

#### 2.2.3. Prepulse inhibition (PPI) of startle response

Mice were placed in isolation boxes inside a clear acrylic tube affixed over a piezoelectric accelerometer (San Diego Instruments) that captured animal movement in response to precisely calibrated white noises of varying intensities emitted from a loudspeaker. Each session consisted of a 5-min habituation period followed by 74 trials of 3 types: (1) no stimulation, (2) 120 dB acoustic stimulus (AS50), and (3) four different prepulse stimuli ranging from 7 dB over background (PP72) to 25 dB over background (PP90). The PPI of the acoustic startle response was measured in the presence of a 65-dB white-noise background. Mice were given three different types of trials. The trial order within each block was pseudorandomized so that no trial type occurred consecutively, and the trials were separated by a variable inter-trial interval (ITI) of 15 s (maximum ITI = 20 s, minimum ITI = 10 s). One trial type consisted of a 40-ms 120-dB white-noise startle stimulus. In the second trial type, the startle stimulus was preceded by a 20-ms prepulse stimulus that was 7, 13, 19, or 25 dB above the white-noise background. In the third trial type, only the prepulse stimuli were presented (no auditory startle stimulus was presented) and these null trials served as controls for the background movements of the animals. The PPI was determined by comparing the percentages of PPI between the groups and the prepulse decibel levels. The percentage of PPI was calculated by the following formula: % PPI = [(mean reactivity of pulse alone trials – mean reactivity of prepulse trials) / (mean reactivity of pulse alone trials)] x 100.

#### 2.2.4. Social dominance test

The tube apparatus used for the social dominance test was a 35-cm long clear tube that was 30 mm in diameter, with a small box on either end. A guillotine door was placed in the middle of the tube. The day after the mice were allowed to freely explore the apparatus for 5 min individually, they were then trained to walk through the tube (with gentle nudges as necessary), with the door opening when their snout came into contact with the guillotine door. On the test day, a pair of mice were simultaneously placed on opposite sides of the tube, allowing either of them to walk to the door, which was open, and interact with the other mouse. The first mouse to retreat and place its two rear paws outside the tube was recorded as the "loser" of the trial (2 min of cut-off time), while the other mouse was the "winner". If two mice withdrew simultaneously, a "tie" was recorded and the trial was repeated. $Pyk2^{+/+}$ and $Pyk2^{-/-}$ mice were always paired with the $Pyk2^{+/+}$ mice that were not involved with the experiment.

#### 2.2.5. Nesting test

Six cages of $Pyk2^{+/+}$ and six cages of $Pyk2^{-/-}$ mice ($N = 4$ mice per cage) were used. Each mouse was considered a replicate of the same cage. Nesting patterns were evaluated. A piece of cotton measuring 5 × 5 cm (Ancare, Bellmore, NY) was placed in each cage as the nesting material. After 45 min, photographs were taken of each nest and the nest area was measured using the ImageJ software.

#### 2.2.6. Three-chamber social interaction test

The apparatus (40 × 40 × 60 cm) consisted of three interconnected lined compartments with open doors. The mouse subjects were habituated to the apparatus for 5 min. After the habituation phase, the subjects were tested in the sociability task for 10 min. An unfamiliar mouse (stranger) was placed in one of the side chambers and enclosed in a small round wire cage that allowed nose contact between the bars but prevented fighting. A second empty round wire cage was placed in the opposite compartment. The mouse subject had a choice between the first unfamiliar mouse (stranger) and the empty wire cage (empty). The time spent exploring each small cage was measured using the SMART junior software (Panlab).

#### 2.2.7. Open field

The apparatus consisted of a pale gray square arena measuring 40 × 40 × 40 cm in length, width, and height. A dim light intensity of 60 lx was present throughout the arena. Animals were placed at the center of the arena and allowed to explore freely for 15 min. Spontaneous locomotor activity was measured. At the end of each trial, any defecation was removed and the apparatus was wiped with 30% ethanol. Animals were tracked and recorded with the SMART junior software (Panlab, Spain).

#### 2.2.8. Elevated plus maze test

Briefly, the elevated plus maze was made of plastic and consisted of two opposite open arms measuring 30 × 8 cm and two opposite closed arms (enclosed by 15-cm-high walls) measuring 30 × 8 cm. The maze was raised 50 cm above the ground and lit by dim light. Each mouse was placed in the central square of the maze, facing an open arm, and its

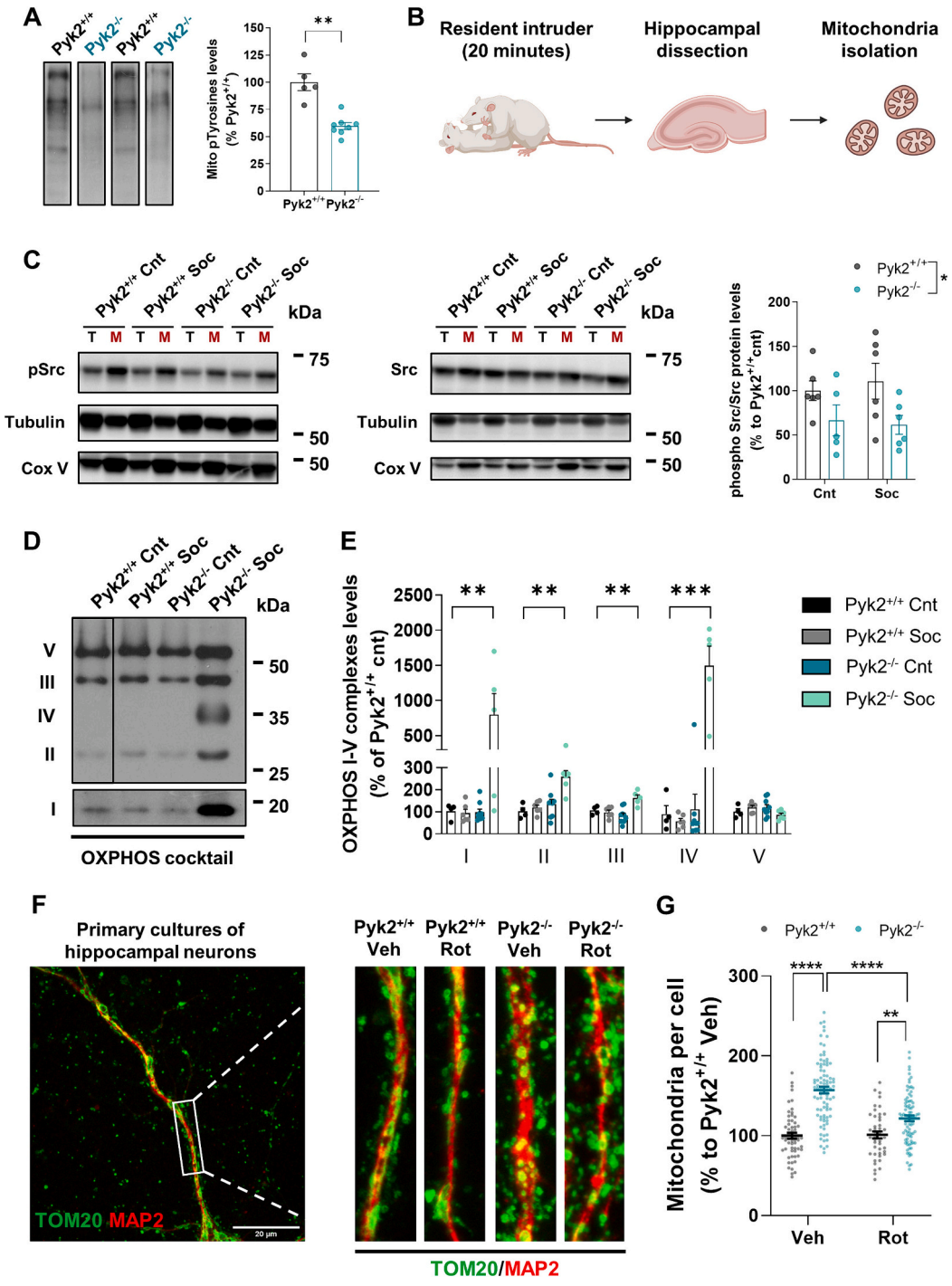


**Fig. 2.** The morphological and molecular changes in the mitochondria that are associated with social interactions in Pyk2 mutant mice.
(a) Phosphorylated tyrosine levels were analyzed in the mitochondrial fraction of the hippocampus from Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice by immunoblotting (unpaired t-test, $t = 5.66$, df $= 11$, $p = 0.0001$). (b) Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice were subjected to a 20-min session of the resident-intruder test (Soc) and compared with naïve mice (control, Cnt). Immediately after the behavioral test, the dorsal hippocampus was dissected out and the mitochondria were isolated to perform immunoblotting. Schematic illustration created using BioRender.com. (c) Protein levels of Src and its phosphorylated form (phosphoSrc$^{Y418}$) were analyzed in the mitochondrial fraction (M) versus total (T) extract (two-way ANOVA; genotype effect, $F_{(1,19)} = 7.33$, $p = 0.014$). Tubulin shows cytosolic enrichment, whereas Cox V shows mitochondrial enrichment. (d-e) Levels of proteins of the OXPHOS complexes were evaluated in the mitochondrial fraction (two-way ANOVA; complex I, interaction effect: $F_{(1,20)} = 7.54$, $p = 0.012$; complex II, interaction effect: $F_{(1,21)} = 5.55$, $p = 0.028$; complex III, interaction effect: $F_{(1,20)} = 12.33$, $p = 0.0022$; and complex IV, interaction effect: $F_{(1,20)} = 28.88$, $p < 0.001$). (f) Hippocampal neurons in culture from Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice at DIV21 were treated with vehicle (DMSO) or rotenone (25 µM) for 2 h. The mitochondria were immunolabelled with TOM20 (green) and the dendrites with MAP2 (red), before quantifying the number of mitochondria per micron (g, two-way ANOVA; treatment effect: $F_{(1,\ 273)} = 16.78$, $p < 0.0001$, interaction: $F_{(1,\ 273)} = 18.89$, $p < 0.0001$). Bonferroni's post-hoc test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Data represent the mean ± SEM. $n = 5$–8 animals/group in a, $n = 4$–9 animals/group in e, and $n = 60$–82 neurons from 4 to 6 different mice per group in g. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

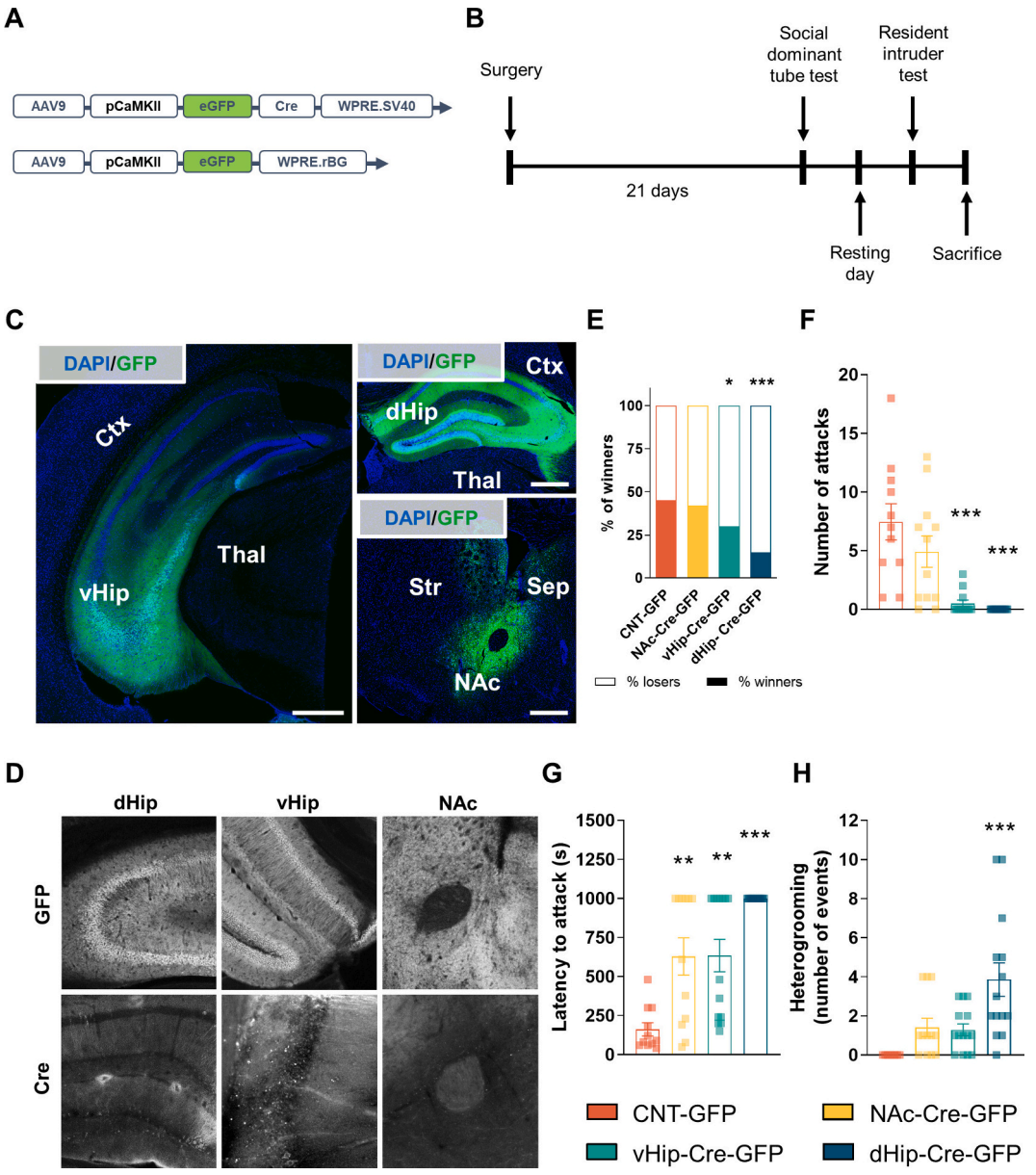


**Fig. 3.** Effects of the specific deletion of Pyk2 from different brain locations on social behaviors.
(a) Schematic illustration of an AAV expressing GFP (AAV-GFP) or GFP-Cre (AAV-Cre). (b) Timeline of the experimental design. AAV-GFP or AAV-Cre was injected into the ventral hippocampus (vHip), the dorsal hippocampus (dHip), or the nucleus accumbens (NAc) of $Pyk2^{flox/flox}$ mice. (c) GFP immunoreactivity in vHip, dHip, and NAc 3 weeks after viral injection. Scale bars, 200 μm. (d) Representative images of each brain region injected with AAV-GFP (upper panels) or AAV-Cre (lower panels). While Pyk2 intensity was decreased by AAV-Cre, AAV-GFP expression did not alter Pyk2 immunoreactivity. NAc, nucleus accumbens; Str, striatum; dHip, dorsal hippocampus; vHip, ventral hippocampus; Sep, septum; Ctx, cerebral cortex; Thal, thalamus. (e) Social dominance test, with dominance measured as a % of winners (chi-squared test; CNT-GFP vs vHip-Cre-GFP: $chi^2 = 4.8$, 1, $p = 0.028$; CNT-GFP vs dHip-Cre-GFP: $chi^2 = 21.43$, 1, $p < 0.0001$). (f-h) Resident-intruder test, in which the latency to attack (f, one-way ANOVA; $F_{(3,47)} = 13.31$, $p < 0.0001$), the number of attacks (g, Kruskal-Wallis test; 26.60, $p < 0.0001$), and heterogrooming (h, Kruskal-Wallis test; 18.30, $p < 0.0001$) were monitored. Data represent the mean ± SEM. $n = 11–14$ animals/group in e-h. In e-f and g-h, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs CNT-GFP.

behavior was monitored for 5 min. At the end of each trial, any defecation was removed and the apparatus was wiped with 30% ethanol. We recorded the time spent in the open arms and locomotor activity. Animals were tracked and recorded with the SMART junior software (Panlab, Spain).

### 2.3. Immunoblot analysis

Immunoblots of hippocampal samples were performed as previously described (Cherubini et al., 2020; López-Molina et al., 2022). Immunoblots were probed with the following antibodies (all diluted 1:1000): Pyk2 (Sigma, #074 M4755), phospho-Y402-Pyk2 (Invitrogen, #44-618G), Src (Abcam, #ab4705), phospho-Y418-Src (Abcam, #ab47411), CoxV (Invitrogen, #A21347), OXPHOS cocktail (Abcam, #ab110413), DISC1 (Proteintech, #15500–1-AP), Mfn2 (Abcam, #ab56889), VDAC1 (Abcam, #ab15895), Grp75 (Abcam, #ab2799), ACSL4 (Proteintech, #22401–1-AP), and phospho-tyrosine (4G10) mouse mAb (Cell Signaling, #96215). The membranes were then incubated with anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibody (1:30,000; Promega #W4021 or #W4011). Secondary antibody binding was detected by the Luminol reagent (Santa Cruz #sc-2048). For loading control, a mouse monoclonal antibody against alpha-

tubulin (1:30,000; Sigma, #083M4847V) or alpha-actin (1:30,000; Sigma #A3854) was used.

### 2.4. Mitochondrial isolation

Hippocampal samples were removed and homogenized in Buffer A (20 mM HEPES, 2 mM EDTA, 1.5 mM $MgCl_2$, 10 mM KCL, pH 7.5, 1 mM PMSF, 10 μg/ml of aprotinin, 1 μg/ml of leupeptin, and 2 mM sodium orthovanadate) using two steps of mechanical disintegration. The homogenates were centrifuged at 500*g* for 5 min, with the resulting pellet (P1) discarded and the supernatant (S1) centrifuged again at 13,000*g* for 20 min. The resulting second supernatant (S2) was discarded and the pellet (P2) was resuspended in Buffer B (250 mM sucrose).

### 2.5. Primary hippocampal neurons culture, cell immunocytochemistry and confocal imaging

Hippocampal neurons were obtained from E18 $Pyk2^{+/+}$ and $Pyk2^{-/-}$ mice by mechanical disaggregation as previously described (López-Molina et al., 2022). Neurons at DIV 21 were exposed to 25 μM rotenone (Sigma R8875) for 2 h at 37 °C. Vehicle group was treated with dimethyl sulfoxide (DMSO). After the treatment, neurons were fixed with 4% paraformaldehyde in PBS and blocked with PBS-0.1 M glycine. Cells were incubated overnight with primary antibodies rabbit TOM-20 (1:250, rabbit; Proteintech, 11,802–1-AP) and MAP2 (1:500, mouse; Sigma-Aldrich, M1406) followed by a 1-h incubation with the secondary antibodies Alexa Fluor 488 goat anti-rabbit IgG (1:100) and Cy3 goat anti-mouse IgG (1:300, Jackson ImmunoResearch). Nuclei were stained with DAPI Fluoromount (SouthernBiotech). Immunofluorescence images were obtained with a Leica Confocal SP5-II system using a 63.0 × 1.40 oil objective with a standard pinhole (1 AU), 0.4 μm thickness, and a digital zoom of 3.0.

### 2.6. Viral constructs and stereotaxic injection

For the specific deletion of Pyk2 in the various brain regions, 8-week-old $Pyk2^{flox/flox}$ mice were stereotaxically injected with an adeno-associated virus (AAV) expressing the Cre recombinase (AV-9-PV2521, AAV9.CamKII.HI.eGFP-Cre.WPRE.SV40, AAV-Cre from Perelman School of Medicine, University of Pennsylvania, USA). As a control, we injected AAVs expressing GFP (AV-9-PV1917, AAV9.CamKII0.4.eGFP.WPRE.rBG, AAV-GFP, from the same source). Mice were anesthetized with ketamine-xylazine (100 mg/kg) and bilaterally injected with AAV-GFP or AAV-Cre (~$2.6 \times 10^9$ GS per injection) in one of the following brain regions: the dorsal hippocampus, − 2.0 mm anteroposterior to the bregma, ± 1.5 mm lateral to the bregma, and (two points) -1.3 mm and − 2.1 mm dorsoventral to the bregma; the nucleus accumbens, 1.3 mm anteroposterior to the bregma, ± 1.2 mm lateral to the bregma, and − 4.5 mm dorsoventral to the bregma; and the ventral hippocampus, − 3.0 mm anteroposterior to the bregma, ± 3.0 mm lateral to the bregma, and - 4.25 mm dorsoventral to the bregma. The AAV injection was carried out in 2 min. The needle was left in place for 5 min for complete virus diffusion before being slowly pulled out of the tissue. After 2 h of careful monitoring, the mice were returned to their home cage for 3 weeks. All mice subjected to surgery that had survived and showed no ethical and health problems (such as a head inclination or body weight loss of >15%) were used for behavioral characterization. Once the behavioral characterization was performed, half of the brain was used to verify the site of injection by immunofluorescence (see "Tissue preparation, immunofluorescence, and confocal imaging" section). Mice that showed no viral transduction in the expected location were excluded from the entire study. To overexpress Pyk2 in the dorsal hippocampus, we used AAV1-CamKIIα (0.4)-GFP-2AmPTK2B (Vector Biolabs Malvern, PA, USA). All AAVs contained the CaMKII promoter to restrict the viral expression to the principal neurons.

### 2.7. Tissue preparation, immunofluorescence, and confocal imaging

Mice were deeply anesthetized (pentobarbital, 60 mg/kg i.p.) and intracardially perfused with 40 g/l of a paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.2. The brains were removed and post-fixed overnight in the same paraformaldehyde solution. The brains (mouse or human) were then sliced in a vibratome (Leica, VT1000). All the following steps were performed with gentle shaking. 40-μm-thick coronal sections were washed three times in PBS, permeabilized in PBS-T containing 3 μl/ml of Triton X-100 and 30 μl/ml of normal goat serum (Pierce Biotechnology, Rockford, IL, USA) for 60 min at room temperature, and washed three times. The brain slices were incubated overnight at 4 °C in the presence of the primary antibody in PBS-T. The primary antibody used was a rabbit anti-Pyk2 antibody (1:500; Sigma, #07 M4755, Chemical Co., St Louis, MO, USA). The sections were then washed three times and incubated for 2 h at room temperature with the fluorescent secondary antibody Alexa Fluor 488 goat anti-mouse IgG (1:200; Jackson ImmunoResearch, West Grove, PA, USA). No signals were detected in the control sections incubated in the absence of the primary antibody. Immunostained sections (40-μm thick) containing tissue from the nucleus accumbens, the dorsal hippocampus or the ventral hippocampus were imaged using a Leica Confocal SP5-II system (20× numerical aperture lens, 1-Airy unit pinhole). Confocal images were taken at a pixel resolution of 1024 × 1024. The characteristics and demographics of the human samples are described below. Both the fixed and frozen tissues came from the same patients and from the same biobank.

### 2.8. Human post-mortem samples

The brain (hippocampus) samples from schizophrenic (SCZ) patients used in this study were provided by the Sant Joan de Déu Brain Bank (Sant Boi de Llobregat, Barcelona, Spain). The donation and procurement of the samples were regulated by the ethics committee of the institution. The sample processing followed the rules of the European Consortium of Nervous Tissues: BrainNet Europe II (BNEII). All the samples were protected in terms of individual donor identification, following the BNEII laws. The clinical diagnosis of SCZ in the donor subjects was confirmed premortem by clinical examiners with the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DMS-IV) and the criteria of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Most donors had been hospitalized for >40 years and were re-evaluated every 2 years to monitor and update their clinical progress. Case information can be found in Supplementary Table 1. All the procedures for the obtention of post-mortem samples followed the guidelines of the Declaration of Helsinki and local ethics committees (Universitat de Barcelona: IRB00003099; Fundació CEIC Sant Joan de Déu: BTN-PSSJD).

### 2.9. Statistical analysis

Statistical analyses were carried out using the GraphPad Prism 8.0 software. All experimental designs were based on power calculations with a power of 98% and an alpha value of 0.05. The experimenter was blinded to all the experiments. Two-tailed Student's *t*-test (95% confidence), one-way ANOVA, or two-way ANOVA with Tukey's or Bonferroni's post-hoc multiple comparison test was performed, as required, if the distributions were normal. In the case of non-normal distributions, the Mann-Whitney *U* test, Dunn's test, or the Kruskal-Wallis test was used, as indicated in the figure legends. Furthermore, linear regression analysis or Fisher's exact test and/or Chi-squared ($\chi 2$) test was used as appropriate and indicated in the figure legends. In the case of non-continuous data, survival analysis was performed with the log-rank (Mantel-Cox) test as a statistical comparison. A $p$ value $<0.05$ was considered significant.

## 3. Results

### 3.1. Pyk2 does not regulate sensitization to ketamine and amphetamine

To explore the potential role of Pyk2 in the schizophrenia-like phenotype in vivo, we used Pyk2$^{-/-}$ mice (Giralt et al., 2016). Since several cohorts of mice were used, a timeline for each cohort is provided for clarity (Suppl. Fig. 1). We subjected these mice to chemically-induced models of agitation by injecting them with amphetamine (3 mg/kg/day) or ketamine (30 mg/kg/day) for 8 days (Powell and Geyer, 2007). On days 1 and 7 of this sub-chronic treatment, the mice were monitored in the open field just after vehicle, amphetamine, or ketamine injection to evaluate locomotor activity (Suppl. Fig. 2a). Both ketamine (Suppl. Fig. 2b) and amphetamine (Suppl. Fig. 2d) induced increased locomotor behavior. In both cases, this increased locomotor behavior was exacerbated on day 7 of ketamine (Suppl. Fig. 2c) or amphetamine (Suppl. Fig. 2e) treatment. However, no significant changes were detected between the genotypes in any condition.

On day 8 of treatment, all the mice were sacrificed 15 min after vehicle, amphetamine, or ketamine injection, and the hippocampal tissue from Pyk2$^{+/+}$ mice was processed for immunoblotting. The total and phosphorylated levels of the Pyk2 protein (phosphorylated at tyrosine 402; phospho-Pyk2$^{Y402}$) were evaluated. We observed that neither the total (Suppl. Fig. 2f-g) nor the phospho-Pyk2$^{Y402}$ (Suppl. Fig. 2f, h) levels were affected by either of the two treatments. Altogether, the data indicated that Pyk2 is unlikely to be involved in the sensitivity to psychostimulants, which are used to produce pharmacological models of the positive symptomatology of schizophrenia (Powell and Geyer, 2007).

### 3.2. Pyk2$^{-/-}$ mice display changes in specific social behaviors

We then evaluated phenotypes related to sensory gating, anxiety, and social skills in new and different cohorts of Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice. In the first cohort of mice, there were no significant differences in the percentage of prepulse inhibition (Fig. 1a) between the two genotypes. Next, in the second cohort of Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice, similar nesting behavior was observed (Fig. 1b). In the third cohort of mice, sociability was evaluated in Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice by using the three-chamber social interaction test, but no differences were observed between the genotypes (Fig. 1c). Conversely, regarding the other social skills, resident Pyk2$^{-/-}$ mice were less dominant than resident Pyk2$^{+/+}$ mice in the resident-intruder test (Fig. 1d-f and Suppl. Fig. 3a). These findings were reinforced by the results of the social dominance test, in which the Pyk2$^{-/-}$ mice were significantly less dominant than their Pyk2$^{+/+}$ counterparts (Fig. 1g). Finally, since the findings of these social tests could be strongly influenced by anxiety levels or locomotor activity (Hollis et al., 2015), we tested these parameters in the elevated plus maze and open field paradigms. There were no differences in the anxiety- and locomotor-related phenotypes between the Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice in both the open field (Fig. 1h-i) and elevated plus maze (Fig. 1j-k) tests. Altogether, the behavioral tests indicated that Pyk2$^{-/-}$ mice were less aggressive and less dominant than the Pyk2$^{+/+}$ mice.

### 3.3. Lack of Pyk2 alters mitochondrial proteins in the resident-intruder paradigm

Since we previously described that hippocampal Pyk2 is a key protein in regulating mitochondrial dynamics and function in neurons (López-Molina et al., 2022), we wondered whether a lack of Pyk2 compromised mitochondrial function during sociability processes. To explore this possibility, we first isolated mitochondria from the hippocampi of Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice. In this subcellular fraction, Pyk2$^{-/-}$ mice showed a reduction in total phosphotyrosine levels compared to Pyk2$^{+/+}$ mice (Fig. 2a), as expected for the Pyk2-loss-of-function experiments (de Pins et al., 2021). We then subjected a new cohort of mice to the resident-intruder paradigm and, again, hippocampal mitochondria were isolated (Fig. 2b). First, we evaluated the levels of phosphorylated Src (phospho-Src$^{Y418}$), a kinase strongly related to the regulation of Pyk2 activity (Walkiewicz et al., 2015; Xu et al., 2012), since it has been previously linked to social skills (Blázquez et al., 2019; Ward et al., 2019) and translocates to the mitochondria (Lurette et al., 2022; Zhang et al., 2022). Phosphorylated Src levels were diminished in the mitochondrial fraction of Pyk2$^{-/-}$ mice compared to Pyk2$^{+/+}$ mice independently of whether the mice socialized or not (Fig. 2c). Since Src can modulate OXPHOS complexes (Hunter et al., 2020), we then evaluated their protein levels in the same samples (Fig. 2d-e). None of the complex protein levels were altered when comparing Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice in control situations. Similarly, complexes I-V remained unchanged in Pyk2$^{+/+}$ mice after social interactions. However, the resident-intruder test in Pyk2$^{-/-}$ mice induced a vast increase in the protein levels of all the complexes except for complex V. These results suggested that the absence of Pyk2 during the resident-intruder test strongly affected the mitochondrial respiratory complexes in the resident mice.

We then evaluated the potential cellular consequences of these changes in the previously reported Pyk2-dependent mitochondrial phenotypes (López-Molina et al., 2022). Given that complex I controls most of the OXPHOS activity (Davey et al., 1998), primary hippocampal neurons were treated with rotenone, a complex I inhibitor. Complex I inhibition in Pyk2$^{-/-}$ neurons rescued the excessive number of mitochondria compared to vehicle-treated neurons (Fig. 2f-g). Overall, these experiments suggested that hippocampal Pyk2 regulates mitochondrial number and possibly some social behaviors via complex I.

### 3.4. Pyk2 in the dorsal hippocampus is essential for the regulation of specific social behaviors

Social behaviors are highly regulated by the hippocampus and the ventral striatum (nucleus accumbens, NAc) (Kohls et al., 2013; Rubin et al., 2014). In mice, Pyk2 is mostly enriched in the hippocampus, but it is also expressed in the NAc (de Pins et al., 2020; Giralt et al., 2016). Therefore, we used the Pyk2$^{flox/flox}$ mice to specifically delete Pyk2 in the principal neurons of the hippocampus (ventral and dorsal separately) and in the medium spiny neurons of the NAc (Fig. 3a-b). We first verified that endogenous Pyk2 protein levels were not affected by the LoxP insertion (Suppl. Fig. 4a). Next, AAVs expressing the Cre recombinase under the control of the CaMKII promoter (AAV-CaMKII-Cre-GFP) were bilaterally injected into the NAc, the ventral hippocampus, or the dorsal hippocampus (the NAc-Cre-GFP, vHip-Cre-GFP, and dHip-Cre-GFP groups, respectively, Fig. 3c and Suppl. Fig. 5a) to delete Pyk2 expression in those brain regions (Fig. 3d). Control mice were injected with AAVs only expressing the GFP protein under the control of the CaMKII promoter (AAV-CaMKII-GFP) in the same three regions (CNT-GFP group).

Next, social behaviors were evaluated based on the positive results observed in Fig. 1d-g with the Pyk2$^{-/-}$ mice. First, in the social dominance test, only the vHip-Cre-GFP and dHip-Cre-GFP groups displayed a significant reduction in the proportion of winners compared to the CNT-GFP group, with the dHipp-Cre-GFP animals having the worst score (Fig. 3e). In the resident-intruder test, the dHip-Cre-GFP mice were consistently the group with the most significant changes when compared to the CNT-GFP mice. Specifically, the dHip-Cre-GFP mice displayed a decrease in the number of attacks (Fig. 3f and Suppl. Fig. 3b) and an increase in both the latency to attack (Fig. 3g) and heterogrooming behaviors (Fig. 3h). Thus, we conclude that the dorsal hippocampus is highly involved with the social parameters evaluated.

We then evaluated whether the aberrant social behaviors displayed by the Pyk2$^{-/-}$ (full KO) mice could be reverted by performing a genetic rescue. Thus, in a new experiment and with a new cohort of Pyk2$^{-/-}$ mice, an AAV was injected into the dorsal hippocampus to recover the expression of Pyk2 in the pyramidal neurons (AAV-CaMKII-GFP-2 A-

*PTK2B*, Fig. 4a-b). These mice (Pyk2$^{-/-}$:Pyk2 group) were compared to the Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice transduced with the control AAV-CaMKII-GFP (Pyk2$^{+/+}$:GFP and Pyk2$^{-/-}$:GFP, respectively) (Fig. 4c-d and Suppl. Fig. 5b). Although the Pyk2 protein levels were only partly rescued (Fig. 4d), the Pyk2$^{-/-}$:Pyk2 mice showed significant restorations in the number of winners in the social dominance test (Fig. 4e) as well as in the latency to attack (Fig. 4f and Suppl. Fig. 3c), number of attacks (Fig. 4g), and heterogrooming events (Fig. 4h) in the resident-intruder test. Overall, these results indicated that Pyk2 in the dorsal hippocampus is crucial in the regulation of the social conduct evaluated.

### 3.5. *Pyk2 is increased in the hippocampus of schizophrenic subjects and is associated with molecular changes involving mitochondrial function*

Since we observed that hippocampal Pyk2 is important for the regulation of social behaviors and that sociability is altered in neuropsychiatric disorders such as schizophrenia (Bobes et al., 2010; Zorkina et al., 2020), we evaluated Pyk2 protein levels in the hippocampal postmortem samples from schizophrenic and matched control individuals. Pyk2 levels were significantly increased in the hippocampus of subjects with schizophrenia compared to controls (Fig. 5a). However, these changes did not correlate with particular PANSS scores potentially related with social skills, including P7 (hostility), N1 (blunted affect), N3 (poor rapport), N4 (passive/apathetic social withdrawal), PG14 (poor impulse control), and PG16 (active social avoidance) (Suppl. Fig. 6a-f). The lack of significant correlations could be due to the low number of samples employed. We then evaluated Pyk2 levels by immunofluorescence in CA1 pyramidal neurons in the fixed tissue from both schizophrenic and control patients. We observed that increased Pyk2 levels were localized in the pyramidal cells from patients with schizophrenia when compared with the control group (Fig. 5b-d). Finally, to test whether changes in Pyk2 levels were associated with changes in mitochondrial function, we looked for potential changes in the direct and indirect regulators of mitochondrial function such as DISC1, Mfn2, and VDAC1 as the direct regulators (Camara et al., 2017; Ghosal et al., 2023; Park et al., 2010) and Grp75 and ACSL4 as the indirect regulators (Radif et al., 2018; Tiwary et al., 2021). Indeed, it is noteworthy that DISC1 (Ma et al., 2018) and Grp75 (Trubetskoy et al., 2022) have been identified as genetic risk factors for schizophrenia. Thus, we found increased hippocampal protein levels of DISC1 (Fig. 5e-

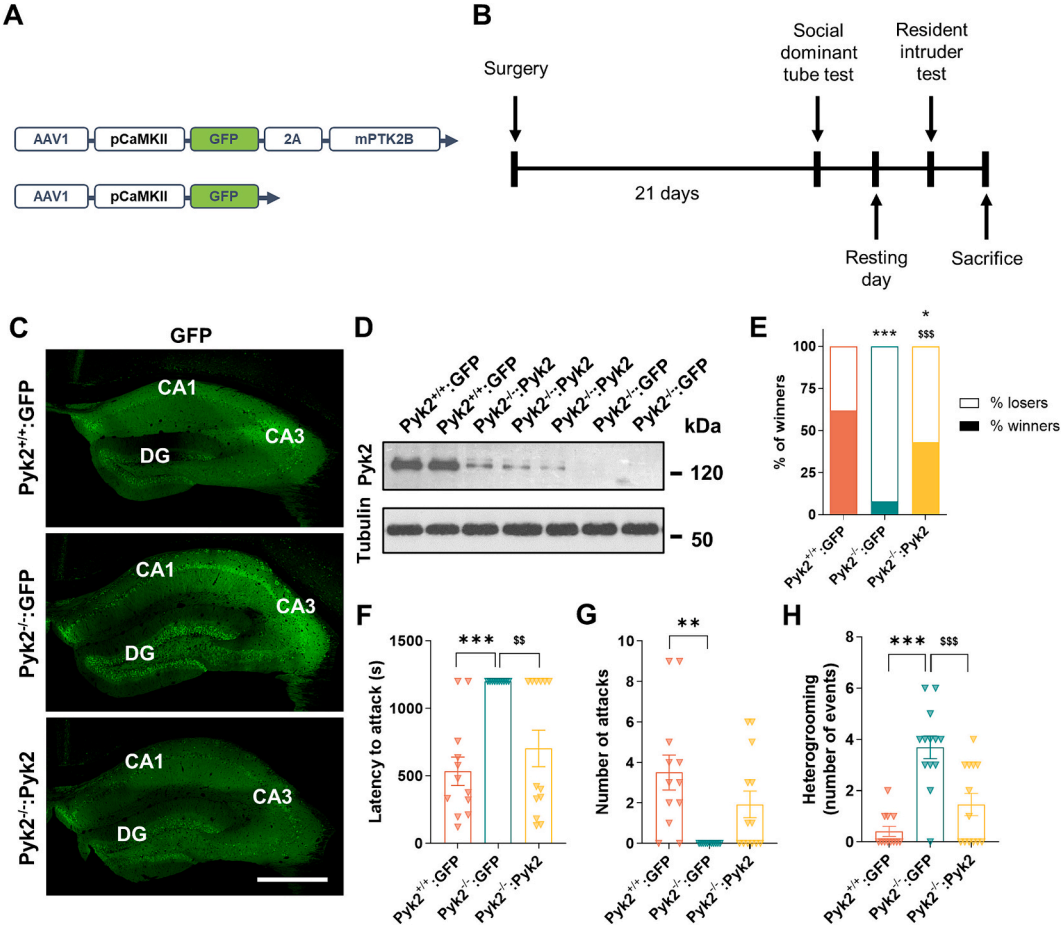


**Fig. 4.** Overexpression of Pyk2 in the hippocampus of Pyk2$^{-/-}$ mice improves alterations in social behavior.
(a) Schematic illustration of AAV-GFP or AAV-Cre. (b) Timeline of the experimental design. An AAV expressing GFP (AAV-CaMKII-GFP) or Pyk2 (AAV-CaMKII-*Ptk2b*-2 A-GFP) was bilaterally injected into the dorsal hippocampus of Pyk2$^{+/+}$ and Pyk2$^{-/-}$ mice to generate the following three groups: Pyk2$^{+/+}$:GFP mice, Pyk2$^{-/-}$:GFP mice, and Pyk2$^{-/-}$:Pyk2 mice. (c) Representative images of GFP immunofluorescence in each of the experimental groups. Scale bar, 200 μm. DG, dentate gyrus. (d) Representative immunoblot of Pyk2 protein levels, with tubulin used as the loading control, in 3 representative mice from the 3 groups. Note that the recovery of Pyk2 protein levels was not complete, but partial. (e) Social dominance test, with dominance measured as a % of winners (chi-squared test; Pyk2$^{+/+}$:GFP mice vs Pyk2$^{-/-}$:GFP mice: chi$^2$ = 64.09, df = 1, p < 0.0001; Pyk2$^{+/+}$:GFP mice vs Pyk2$^{-/-}$:Pyk2 mice: chi$^2$ = 7.238, df = 1, $p$ = 0.007; and Pyk2$^{-/-}$:GFP mice vs Pyk2$^{-/-}$:Pyk2 mice: chi$^2$ = 32.24, df = 1, p < 0.0001). (f-h) Resident-intruder test, in which the latency to attack (f, one-way ANOVA; $F_{(2,35)}$ = 2.007, $p$ = 0.018), the number of attacks (g, Kruskal-Wallis test; 10.39, $p$ = 0.0055), and heterogrooming (h, one-way ANOVA; $F_{(2,35)}$ = 19.05, p < 0.0001) were monitored. Data represent the mean ± SEM. Post-hoc Dunn's test was conducted in g, while post-hoc Bonferroni's test was performed in f and h. *p < 0.05, **p < 0.01, ***p < 0.001 vs Pyk2$^{+/+}$-GFP. $^{\$\$}$p < 0.01 and $^{\$\$\$}$p < 0.001 vs Pyk2$^{-/-}$:GFP. Data represent the mean ± SEM. In e-h, n = 12–13 animals/group.

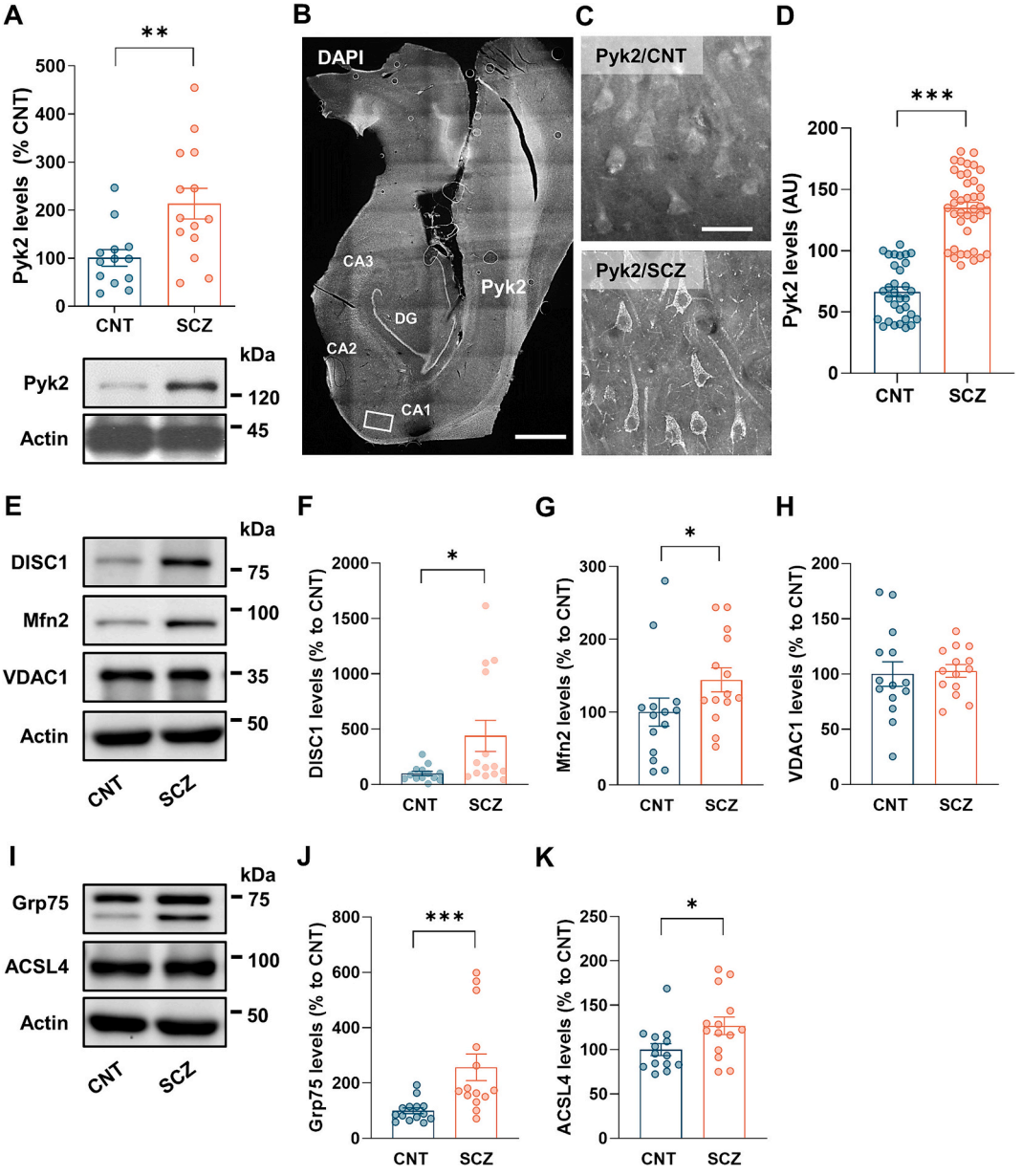


**Fig. 5.** Levels of hippocampal Pyk2 and mitochondrial markers in patients with schizophrenia.
(a) Immunoblot densitometric quantification of Pyk2 levels relative to α-actin levels as the loading control in dorsal hippocampal post-mortem samples from human schizophrenic subjects (SCZ) and matched controls (CNT). (Mann-Whitney *U* test; A, B = 306, 1585, U = 201; $p = 0.0276$). (b-d) Fixed hippocampal tissues from 3 CNT and 4 SCZ were subjected to DAPI staining (b) and Pyk2 immunofluorescence (c). (d) Densitometric quantification of Pyk2 immunofluorescence in the samples in c in arbitrary units (unpaired t-test; $t = 11.25$, df = 70, $p < 0.0001$). A total of 32 CNT and 40 SCZ pyramidal neurons were quantified (10–14 per subject). Immunoblot densitometric quantification of DISC1 (e and f, Mann-Whitney U test; A, B = 135, 243, U = 44; $p = 0.0222$), Mfn2 (e and g; Mann-Whitney U test; A, B = 154, 252, U = 49; $p = 0.0241$), and VDAC1 (e and h) levels relative to α-actin levels as the loading control in dorsal hippocampal post-mortem samples from human schizophrenic subjects (SCZ) and matched controls (CNT). Immunoblot densitometric quantification of Grp75 (i and j; Mann-Whitney U test; A, B = 133, 273, U = 28; $p = 0.0008$) and VDAC1 (i and k; unpaired t-test; $t = 2.243$, df = 26, $p = 0.036$) levels relative to α-actin levels as the loading control in dorsal hippocampal post-mortem samples from human schizophrenic subjects (SCZ) and matched controls (CNT). Data represent the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs CNT. In a, e-k, $n = 14$/group.

f) and Mfn2 (Fig. 5e, g), whereas hippocampal VDAC1 levels remained unchanged (Fig. 5e, h) when comparing schizophrenia samples with control samples. Next, we found that the protein levels of Grp75 (Fig. 5i, j) and ACSL4 (Fig. 5i, k) were increased in the hippocampus of schizophrenic patients compared with controls. These results suggested that increased Pyk2 levels in CA1 pyramidal neurons occurred alongside changes in the direct and indirect regulators of mitochondrial function. Altogether, these changes could contribute to some of the social deficits previously described in subjects with schizophrenia.

### *3.6. Overexpression of Pyk2 in dorsal CA1 pyramidal neurons induces social dominance and prevents heterogrooming behavior in mice*

Our results suggested that the increase in Pyk2 levels in CA1 neurons could be a potential underlying molecular event for some of the social deficits observed in schizophrenia (Dodell-Feder et al., 2015). To test this hypothesis, we overexpressed Pyk2 with an AAV (AAV-CaMKII-GFP-2 A-*Ptk2b*) in the dorsal hippocampal CA1 neurons of wild-type (WT) mice (Fig. 6a-b and Suppl. Fig. 5c). We first verified the increase in Pyk2 levels in the WT-Pyk2 mice (injected with the AAV-CaMKII-GFP-2 A-

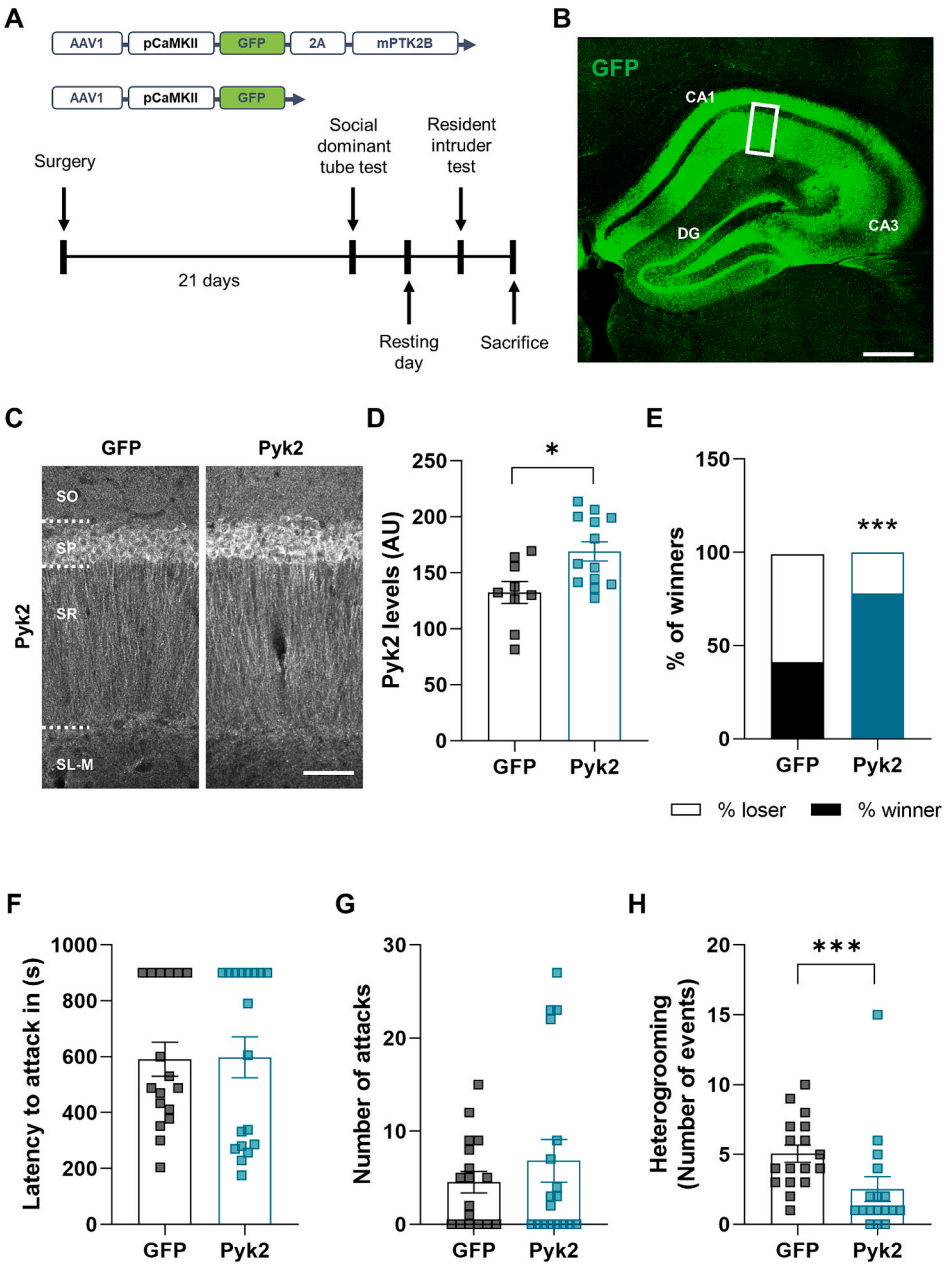


**Fig. 6.** Effects of hippocampal Pyk2 overexpression on social behaviors.
(a) Schematic illustration of AAV-CaMKII-GFP (the WT-GFP group of mice) or AAV-CaMKII-*Ptk2b*-2A-GFP (the WT-Pyk2 group of mice) in the upper panel. Timeline of the experimental design is shown in the lower panel. WT mice are divided into mice injected with AAV-CaMKII-GFP (WT-GFP) or with AAV-CaMKII-*Ptk2b*-2A-GFP (WT-Pyk2). (b) Representative image of the injection site and the corresponding GFP immunofluorescence in the dorsal hippocampus. Scale bar, 200 μm. DG, dentate gyrus. (c) Pyk2 immunofluorescence in both groups in the dorsal CA1. (d) Quantification of the Pyk2-positive intensity and optical density in c (unpaired t-test; $t = 2.79$, df = 20, $p = 0.0114$). (e) Social dominance test, with dominance measured as a % of winners (chi-squared test; $chi^2 = 27.70$, df = 1, $p < 0.0001$). (f-h) Resident-intruder test: the latency to attack (f), the number of attacks (g), and heterogrooming (h, Mann-Whitney U test; U = 52.50, $p = 0.0009$) were monitored. Data represent the mean ± SEM. *p < 0.05 and ***p < 0.001 vs GFP. In d, n = 9–11/group. In e-h, n = 14/group.

*Ptk2b* vector) compared with the WT-GFP mice (injected with the control vector AAV-CaMKII-GFP) (Fig. 6c-d). We then carried out the social dominance test and the resident-intruder test in these groups of mice. In the social dominance test, the WT-Pyk2 mice were significantly more dominant than their WT-GFP counterparts (Fig. 6e). In the resident-intruder test, resident WT-Pyk2 mice displayed no changes in the latency to attack (Fig. 6f and Suppl. Fig. 3d) and number of attacks (Fig. 6g), but showed reduced heterogrooming (Fig. 6h) compared to the WT-GFP mice. These results provided evidence that Pyk2 overexpression in dorsal CA1 pyramidal neurons induced an opposite phenotype to that caused by Pyk2 depletion in the same cells. In addition, Pyk2 overexpression in mouse dorsal CA1 pyramidal neurons mimicked some of the social deficits previously reported in schizophrenic patients.

## 4. Discussion

In the present manuscript, we show that the *Ptk2b* gene (encoding Pyk2), when knocked down in the principal neurons of the dorsal hippocampus, induces a significant reduction in dominant and aggressive behaviors. We also report alterations in Src-related activity as well as in OXPHOS levels in the hippocampal mitochondrial fractions from Pyk2$^{-/}$

$^{-}$ mice during a social task. In contrast to that observed in the Pyk2$^{-/-}$ mice, we provide evidence that Pyk2 is upregulated in the pyramidal CA1 neurons of schizophrenic subjects, occurring alongside increases in the hippocampal levels of the direct and indirect regulators of mitochondrial function such as DISC1, Mfn2, and Grp75. Moreover, Pyk2 overexpression in the dorsal hippocampus of wild-type mice leads to social deficits that could be related to some of those observed in patients with schizophrenia. These results suggest a probable causal relationship between changes in Pyk2 levels in the dorsal hippocampus and social behaviors.

Here, we found a reduction in dominance and aggressiveness and an increase in appeasing behaviors when Pyk2 was deleted in hippocampal neurons. Remarkably, these changes were not associated with anxiety levels or locomotor activity per se (Hollis et al., 2015). We identified the dorsal hippocampus as the brain region where Pyk2 is highly involved with the described social phenotypes. This outcome is consistent with other studies that have described a strong involvement of the hippocampus in social conduct (FeldmanHall et al., 2021; Montagrin et al., 2018; Rubin et al., 2014), including social dominance (Sandi, 2015; Watanabe et al., 2016). Intriguingly, while social appeasing has been well established in relation to olfactory-to-amygdala signaling in rodents (Morozov and Ito, 2019), the role of the hippocampus in that remains unexplored. Thus, our work represents a novel finding that proposes hippocampal Pyk2 as a candidate for the regulation of some social skills related to aggression and dominance. Additionally, despite our findings, we cannot rule out the potential role of other brain regions. Although Pyk2 proteins are principally found in the hippocampus in mice (Giralt et al., 2016) (https://www.proteinatlas.org/ENSG00000120899-PTK2B/brain), Pyk2 is enriched in the hippocampus as well as in the cerebellum in humans (Uhlén et al., 2015) (https://www.proteinatlas.org/ENSG00000120899-PTK2B/brain). Given the emerging role of the cerebellum in both the regulation of social cognition (Hoche et al., 2016) and in the pathological processes associated with schizophrenia (Andreasen and Pierson, 2008), we cannot rule out an additional and unexpected role of Pyk2 in the cerebellum of patients that could have an impact on their social skills.

We also observed that Pyk2-dependent changes in mitochondrial dynamics could be a potential underlying mechanism of social deficits. The mitochondrion has been proposed as a core organelle in social paradigms (Ülgen et al., 2023). Hyperactivation of these organelles may trigger social behavioral deficits (Zhang et al., 2020). While the mitochondria of the nucleus accumbens have been reported to be involved in social behaviors (Picard and Sandi, 2021), the role of hippocampal mitochondria in sociability remains to be elucidated. We observed that mice devoid of Pyk2 showed a reduction of the active form of Src, phosphoSrc$^{Y418}$, which is a major regulator of Pyk2 signaling (Dikic et al., 1996, 1998; Huang et al., 2001) in the mitochondrial compartment. Accordingly, it has been shown that Src translocates to the mitochondria (Djeungoue-Petga et al., 2019; Hebert-Chatelain, 2013; Zhang et al., 2022) and impedes the expression and function of OXPHOS, particularly by phosphorylating complex I (Castellanos and Lanning, 2019). Conversely, the lack of Pyk2 triggered a rise in the levels of OXPHOS complexes specifically during a social behavior test. Similarly, a recent study in *Drosophila* carrying a mutation linked to schizophrenia demonstrated an increase in mitochondrial respiration and the causal role of mitochondria in the regulation of complex social behavior (Kanellopoulos et al., 2020). A different study identified a rise in the expression of genes involved in OXPHOS in subordinate mice compared to their dominant counterparts (Lee et al., 2022). Another potential explanation for the impact of Pyk2 on mitochondrial function could involve glutamate signaling. The function of Pyk2 (phosphorylation or re-localization) is highly dependent on the activation of glutamate receptors through $Ca^{2+}$ influxes via *N*-methyl-*D*-aspartate receptors (NMDARs) (de Pins et al., 2021). In neurons, Pyk2 activation induces its translocation to various subcellular locations or organelles, including the excitatory synapse (Bartos et al., 2010), the nucleus (Corvol et al., 2005), and the mitochondria (López-Molina et al., 2022). Given that social interaction induces changes in hippocampal neuronal activity (FeldmanHall et al., 2021; Xu et al., 2021), such activity could modulate Pyk2 translocation to the mitochondria, influencing its function probably through Src and ultimately impacting social skills. All these observations suggest that the lack of Pyk2 may decrease activated Src in the mitochondria and facilitate an increase in the levels of proteins of OXPHOS complex I when mice are in specific social situations.

We observed that the pharmacological inhibition of complex I with rotenone reverted the increased number of mitochondria in the hippocampal neurons devoid of Pyk2. In line with our results, rotenone treatment has been previously shown to strongly modulate social behaviors such as social dominance (Hollis et al., 2015) and ameliorate social deficits and the related mitochondrial phenotypes (such as increased size and respiration) (Hollis et al., 2015; Kanellopoulos et al., 2020). Overall, we suggest that the hippocampal Pyk2-dependent modulation of social interactions might depend on its function in the mitochondria. Although the direct role of Pyk2 in the mitochondria was not directly addressed in the present work, there is compelling evidence of mitochondrial dysfunction in schizophrenic patients, as an energy deficit could lead to cognitive impairment (Haznedar et al., 2004). Impaired mitochondrial respiration in various brain areas of schizophrenic patients that particularly affects complex I has been previously reported (Ben-Shachar, 2002). In addition, mitochondrial gene expression, including that involved with mitochondrial oxidative metabolism and complex I, II, and IV subunits, has been also reported to be altered in brain samples from schizophrenic individuals (Altar et al., 2005; Karry et al., 2004).

Finally, we observed increased Pyk2 protein levels in the pyramidal cells of post-mortem hippocampal samples from schizophrenic subjects. Concomitant with these changes in Pyk2 levels, we also detected changes in several direct and indirect regulators of mitochondrial function such as Grp75, DISC1, and Mfn2. Interestingly, some of these regulators are, indeed, genetic risk factors for schizophrenia (Ma et al., 2018; Rittenhouse et al., 2021; Trubetskoy et al., 2022). It is well known that schizophrenic patients present impaired social skills such as social withdrawal (Lee et al., 2013) and aggressiveness (Serper, 2011). In contrast to Pyk2-deficient mice, which were dominated in the social dominance test, we showed that Pyk2 overexpression in the hippocampus of wild-type mice enhanced dominant and aggressive conduct while reducing appeasing behaviors. These observations suggest that Pyk2 alterations may contribute to particular social behavior alterations related to schizophrenia.

Importantly, our study has some limitations, primarily in the experiments conducted with human samples. In this regard, the number of samples per group was relatively low, the time of tissue collection varied slightly, and while the control samples were from both men and women, the samples from patients with schizophrenia were only from males. Therefore, the conclusions drawn from these experiments must be approached with caution. Moreover, regarding the studies with mice, although Pyk2-deficient mice did not show sensitization to psychostimulants at the basal state, we cannot rule out that more complex and long-lasting interactions were taking place. Future studies should address whether psychostimulants can induce indirect phenotypes in Pyk2-deficient mice in terms of sociability or even cognitive alterations.

Despite the aforementioned limitations, our data suggest that the Pyk2-dependent control of mitochondrial density and morphology in CA1 pyramidal neurons could be a molecular basis for some specific social skills and that changes in Pyk2 levels could contribute to particular social impairments observed in schizophrenia. Our findings open up potential novel approaches for therapy that warrant further investigation.

## Author contributions

Investigation was conducted by L.L.-M., A.S.-B., O.A.-M., E.M., and

A.G. Conceptualization of the study and the writing of the original draft were conducted by L.L.-M. and A.G. Project administration and supervision were performed by A.G. The writing-review and editing were conducted by J.-A.G. and B.A. Resources were provided by J.A.G. and B. A. Funding acquisition and resources were provided by J.A., J.-A.G., and A.G. All authors have read and approved the final version of the manuscript.

## CRediT authorship contribution statement

**Laura López-Molina:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft, Writing – review & editing. **Anna Sancho-Balsells:** Data curation, Formal analysis, Investigation, Methodology. **Omar Al-Massadi:** Investigation, Methodology. **Enrica Montalban:** Investigation, Methodology. **Jordi Alberch:** Funding acquisition, Resources, Writing – review & editing. **Belén Arranz:** Investigation, Methodology, Resources. **Jean-Antoine Girault:** Resources, Supervision, Writing – review & editing. **Albert Giralt:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation.

## Declaration of competing interest

The authors declare no competing interests.

## Data availability

Data will be made available on request.

## Acknowledgments

This work was supported by grants from the Ministerio de Ciencia e Innovación/ AEI/10.13039/501100011033/ and "FEDER": AG: PID2021-122258OB-I00; JA: PID2020-119386RB-100. OAM was funded by a Miguel Servet Research Contract (CP20/00146) from the ISCIII. We thank Ana López and Maria Teresa Muñoz (María de Maeztu Unit of Excellence, Institute of Neurosciences, University of Barcelona, CEX2021-001159-M, Ministry of Science and Innovation) for their technical support. We thank María Calvo from the Advanced Microscopy Service (Centres Científics i Tecnològics Universitat de Barcelona) for her help in the acquisition, analysis, and interpretation of the confocal microscopy images. Work in the lab of JAG was supported by grants from the *Agence Nationale de la Recherche* (ANR-15-NEUC-0002-02 and ANR-19-CE16-0020) and from the *Fondation pour la recherche médicale* (FRM, EQU201903007844).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2024.106487.

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