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Late developmental dynamics of activity patterns within prefrontal-hippocampal networks in health and a genetic risk model for schizophrenia

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Complex cognitive abilities involve a neural circuitry that extends over much of the brain, yet it is commonly held that the prefrontal cortex (PFC) is a critical hub. PFC provides executive "top-down" control, accounting for attention, salience detection, working-memory and inhibitory control. Most of these abilities are thought to mature in parallel with the development of prefrontal circuits and therefore it is proposed that those emerge relatively late and progressively augment from childhood to young adulthood, and are declining only after middle age. However, some aspects of cognitive flexibility, such as decision-making strategies, have been found to reach a maximal performance at juvenile age. On a flip side, cognitive disruption, which is related to several neuropsychiatric disorders, such as schizophrenia or autism, is firstly detected during late stages of development. This is highlighting the urgent need for understanding the underlying processes in PFC at this age. Our previous studies elucidated the critical role of electrical activity at neonatal age for the adult prefrontal function and cognitive performance. We showed that theta band activity in PFC emerges as result of the excitatory drive from hippocampal CA1 area, whereas gamma oscillations require the local wiring of layer II/III pyramidal neurons. The local and large-scale communication within neonatal prefrontal-hippocampal circuits is impaired in mouse models of schizophrenia. However, the dynamics and the role of electrical activity during the late development of prefrontal-hippocampal circuits is still largely unknown. To fill this knowledge gap, we performed multi-site extracellular recordings to monitor the neuronal and network activity in the PFC and HP of postnatal day (P) 16-60 mice. The activity patterns were compared to those recorded from Df16(A)+/- mice that have a heterozygous 1.3 Megabase long deletion on chromosome 16. The genetic defect mirrors the 22q11.2 microdeletion in humans, which is associated with an enhanced risk to develop schizophrenia during adolescence or early adulthood. The activity patterns are statistically compared over age and between groups with a generalized linear mixed effect model and with multicomparison tests. In wild-type mice, prefrontal gamma oscillations and firing progressively augmented in their frequency and amplitude peaking around P30, and decreased afterwards. These developmental gamma features are impaired in the Df16(A)+/- mice. Similarly, the firing in PFC and HP was abnormally timed by oscillatory rhythms during specific time windows of late development. The power of theta activity progressively augmented with age in both groups, yet the increase was weaker in Df16(A)+/- mice. Moreover, the communication between PFC and HP dynamically evolved with age. The Df16(A)+/- showed a different developmental profile, corresponding to a decreased theta band coupling between the two areas. Thus, we conclude that complex dynamic processes take place during late development and that those processes are disrupted in schizophrenia.
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Manipulating a brain circuit associated with sociability by a two-recombinase system

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Background: Social withdrawal is often diagnosed as an early symptom across various neuropsychiatric diseases and can therefore be considered as a trans-diagnostic marker in patients [1]. Therefore, one goal of the EU IMI project PRISM2 (Psychiatric Ratings using Intermediate Stratified Markers) is to investigate the pathophysiological relationship between the DMN (Default Mode Network) and social dysfunction. Clinical data suggest that people exhibiting low social functioning show reduced white matter integrity, specifically in the Forceps Minor (FM), a fiber tract connecting both orbital frontal areas [2]. As part of this consortium, we aimed to back-translate those findings into preclinical research. Hence, we developed a system to target interhemispheric projection neurons bilaterally. The Two-Recombinase-System, which combines the Cre/loxP system and the Flp/FRT recombination linked to designer receptors exclusively activated by designer drugs (DREADDs), allows us to chemogenetically inhibit the activity of FM projections.
Methods: The specific virus combinations (AAV2-retro-Cre-GFP/AAV8-FRT-hM4Di-mCherry-FRT and AAV2-retro-Flpo-EBFP2/AAV8-loxP-hM4Di-mCherry-loxP) are stereotactically injected into 24 male BL/6 mice (AP: +2.68, ML: ±0.45, DV: - 2.25). Social behavior is monitored and analyzed by using Radio-frequency identification (RFID)-assisted SocialScan, a method that integrates precise identification of individuals in a group of mice by RFID-signaling into video-tracking. During the monitoring period of seven days (24h/day) those mice are injected on three consecutive days either with Clozapine-N-oxide (CNO) (5mg/kg) or NaCl i.p. after a habituation phase of three days. After a washout phase, compound groups chronically receive CNO (1mg/kg) via drinking water for three weeks. The behavior of the CNO groups and control animals is monitored again during their third week of treatment.
Results: Acute experiment: CNO treated animals show an increased preference to stay in the peripheral area compared to controls during the three application days (z-score as mean values for 24-hour frame, group factor $F(1,21) = 53.59$, $p < 0.0001$, two-way ANOVA, Bonferroni post-hoc test). The interindividual distance (> 10 cm) is also enhanced during six-hour period after injection ($p = 0.0409$, unpaired t-test).
Chronic experiment: Mice receiving CNO chronically, show a tendency to increase their distance to other box mates compared to control groups (z-score as mean values of six days for 24-hour frame, group factor $F(1,22) = 7.739$, $p = 0.0109$, two-way ANOVA, Bonferroni post-hoc test).
Conclusions: We show that acute inhibition (hM4Di-DREADDs) of FM-projection neurons by injecting Clozapine-N-oxide (CNO) results in increased preference to stay in the periphery, close to the walls compared to control mice. This behavior could be a sign of anxiety-like and social avoidance behavior, indicated also by the fact that those animals increase their interindividual distances after CNO injection. Additionally, we chronically suppress the activity of these projection neurons for three weeks by administration of CNO via drinking water. During chronic treatment mice also exhibit reduced social dynamics whereby the general locomotor activity is comparable between both groups and therefore unaffected by the treatment. These results indicate that inhibiting the activity of Forceps minor projections results in social withdrawal-like behavior.
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Bate palmas (bapa) mutant mouse, a model of Kabuki syndrome presents behavioral hyperactivity via increased striatal tyrosine hydroxylase expression

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Background: The recessive mutant mouse bate palmas (bapa) arose from N-ethyl-N-nitrosourea mutagenesis. Previous studies of our group revealed some behavioral impairment such as motor incoordination and a mutation in the lysine (K)-specific methyltransferase 2D (Kmt2d) gene. Bapa mouse was proposed as a model of Kabuki syndrome because mutations in the KMT2D gene in humans are the main responsible for Kabuki syndrome. Besides other symptoms, Kabuki syndrome is characterized by speech impairments and variable behavioral issues, usually diagnosed in the early childhood. Unfortunately, there is a limitation of the experimental

models for Kabuki syndrome. Therefore, the objective of this study was to evaluate oral communication, motor, exploratory, and anxiety-like behaviors, as well as pathophysiological and neurobiological processes involved in the murine model of Kabuki syndrome for a better characterization of the model.
Methods: The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number: 035/17). Juvenile male and female BALB/cbapa (bapa) mice (Mus musculus) and respective controls (BALB/c) were studied in the prepubertal period (postnatal day [PND] 30-32). To study oral communication, ultrasonic vocalizations were evaluated. Open-field behavior and striatal tyrosine hydroxylase (TH immunohistochemistry) expression were also studied. Therefore, on PND30-32, mice were evaluated for ultrasonic vocalizations and open-field behavior (simultaneously). Immediately after behavioral tests, mice were euthanized, and their striatum were collected for TH studies.
Results: Only one range of frequency was detected for all evaluated mice: 28–35 kHz. Sex factor affected all the evaluated parameters for 28–35 kHz ultrasonic vocalizations: number of vocalizations, mean duration, maximal duration, total duration, and mean dominant frequency of vocalizations. However, mutant factor and the association of factors (mutant vs. sex) did not affect ultrasonic vocalizations. Control and bapa female mice emitted 31-kHz ultrasounds on prepubertal period when exploring a novel environment, a frequency not yet described for mice, being defined as 31-kHz exploratory vocalizations. Males did not emit these vocalizations. Both male and female mice were included in the behavioral and brain evaluations to identify possible sex-dependent effects of the experimental manipulations. However, initial analyses showed that there were no significant sex-specific effects. Therefore, the data of the two sexes were combined for final analyses. Mutant factor affected locomotion and rearing frequencies and immobility duration. Bapa mice presented increased rearing frequency. None of the anxiety parameters (time spent in the central and peripheral zones) was affected. Thus, bapa mice presented increased motor/exploratory behavior on prepubertal period. Mutant factor affected TH expression. Specifically, bapa mice presented increased striatal TH expression. In all cases, the results were considered significant at $p<0.05$ (Student's t-test and ANOVA followed by Tukey's test).
Conclusions: Control and bapa female mice emitted 31-kHz ultrasounds on prepubertal period when exploring a novel environment, a frequency not yet described for mice, being defined as 31-kHz exploratory vocalizations. Males did not emit these vocalizations. Moreover, juvenile bapa mouse presented behavioral hyperactivity via increased striatal tyrosine hydroxylase expression, revealing striatal dopaminergic system hyperactivity.
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Study the effects of a newly synthesized isoquinoline derivative 1-(2-chlorophenyl)-6,7-dimethoxy-3-methyl-3,4-dihydroisoquinoline on active and passive memory tests in rats

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Background: Isoquinoline derivatives belong to the phosphodiesterase (PDE) inhibitors group and PDEs can increase cellular cGMP levels which may contribute to the enhanced cognitive performance after PDE inhibition [1]. Previously we investigated the synthesis and learning and memory processes in rats for an isoquinoline precursor IQP [2]. Next, we turned our attention toward the cyclization to 3,4-dihydroisoquinoline. Our aim was to synthesized 1-(2-chlorophenyl)-6,7-dimethoxy-3-methyl-3,4-dihydroisoquinoline (DIQ) and find its effect on memory processes in rats.
Material and Methods: The male Wistar rats (170-210 g b.w., 8 per group) were used for studying the effect on learning and memory processes. They were divided into four groups and treated orally once daily: 1st group (control) - Dimethyl sulfoxide 0.1 mg/100g b.w.; 2nd group – DIQ 5 mg/kg; 3rd group - DIQ 10mg/kg; and 4th group - DIQ 20 mg/kg. The animals were tested 60 minutes after drug application in automatic reflex conditioners. In shuttle-box active avoidance test were measured: the number of conditional stimuli responses (avoidances), the number of unconditioned stimuli responses (escapes) and the number of intertrial crossings. The learning session was performed in 5 consecutive days and on 12th day was made a memory retention test. In step-down passive avoidance test learning session was performed in 2 days, and on 6th day was made a memory retention test. A criterion for step-down test was latency of reaction 60 s. The statistic evaluation was done in SPSS (19.0).
Results: We found that in the shuttle-box test the control group significantly increased the number of avoidances during the learning session and memory retention test compared to the first day control. The group with DIQ 10 mg/kg significantly increased number of avoidances on 5th and 12th day compared to the same day controls. The number of avoidances was significantly increased in group with DIQ at a dose of 20 mg/kg on 3rd, 4th, 5th and 12th day. The number of escapes was significantly increased by group with DIQ 10 mg/kg on 5th day and the group with DIQ 20 mg/kg on 12th day compared to the same day controls. The group with DIQ 10 mg/kg significantly increased the number of intertrial crossings on 2nd, 4th and 12th day compared to the same day controls. The animals with DIQ 20 mg/kg showed an increase in the number of intertrial crossings on 3rd, 5th and 12th day compared to the same day controls. In step-down test the animals treated with DIQ 5 mg/kg and DIQ 20 mg/kg significantly increased the latency of reactions on the second day of the learning session. For the long memory retention only the DIQ 20 mg/kg affected the animals significantly compared to the same day controls.
Conclusion: Based on the results obtained, we can conclude that DIQ improve learning and memory processes in rats. The effect is dose-dependent. The highest dose has the most pronounced effect on training and consolidation of memory traces in active and passive avoidance tests. This may lead to the development of a drug for treating cognitive dysfunctions.
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Back translational study: social dysfunction association with Default mode network

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Background: The Psychiatric Ratings using Intermediate Stratified Markers (PRISM) project focuses on understanding the biological background behind social deficits, specifically social withdrawal irrespective of diagnosis. Reduced connectional integrity in fiber tracts such as Forceps minor has been indicated in low social individuals as a part of the PRISM 1 project. These fiber tracts are also involved in the Default Mode Network (DMN) and the Social network and they share a common region, the Orbitofrontal Cortex (OFC).This study aims to back-translate the clinical data to preclinical studies and associate social dysfunction in rodents with DMN and particularly OFC. Parvalbumin interneurons are targeted based on their fundamental role in maintaining Excitatory Inhibitory (E/I) balance in brain circuits. Numerous studies indicate behavioral impairment in rodents by increasing excitability of PV+ interneurons.
Methods: As an initial step, we characterized the population of projection neurons within OFCs by combining Cholera Toxin subunit B (CTB) as a retrograde tracer and In situ hybridization (ISH) technique (RNAscope). We identified the expression of mRNAs marking glutamatergic (vesicular glutamate transporter [VGLUT]) and GABAergic (vesicular GABA transporter [VGAT]) by using Slc17a7 and Slc32a1 probes. CTB was injected unilaterally in the left OFC (AP=2.68, ML=-0.8, DV=2.2). after 10 days mice were perfused and RNAscope assay was performed using RNAscope® Multiplex Fluorescent kit