characterized by specific facial malformations that resemble to stage makeup used in kabuki, a Japanese traditional theatrical form. Several other symptoms are documented, including cardiac, gastrointestinal, and renal anomalies, motor and cognitive impairments, increased susceptibility to infections, and immune mediators' deficits. It is also reported anxiety and stereotyped behaviors. The hypothesis of the present study is that Kabuki syndrome affects neurobehavioral pathways via neuroinflammation. To answer this question, our experimental model of Kabuki syndrome (bapa mice) was used [1]. The objective was to evaluate if brain of bapa mice presents significant neuroinflammation. Behavioral aspects of Kabuki syndrome were also studied, such as anxiety, motor behavior, and repetitive/stereotyped behaviors. Increased GFAP expression is defined as astrogliosis, a biomarker for neuroinflammation.

Methods: Female adult BALB/c and BALB/c^{bapa} (bapa) mice on luteal phase of the estrous cycle were used (n=7/group, Ethics permit No 035/17). Light-dark test was performed to evaluate anxiety-like behavior and motor/exploratory behavior [2]. Spontaneous self-grooming behavior was evaluated in a glass box. Splash test (induced self-grooming, spraying a 10% sucrose solution on the dorsal coat of a mouse, [3]) was evaluated 24h latter in the same glass box. The parameters were evaluated as described [4]. Immediately after behavioral tests, brains were processed for immunohistochemical analysis using anti-GFAP immunoglobulin [5]. The area of astrocytes and their processes were automatically calculated (index per area). Means values of five photomicrographs/mouse were used as unit. Normality was verified using Shapiro-Wilk (W) or Kolmogorov-Smirnov (KS) tests (alpha=0.05). Student's t-test (unpaired, two-tailed) was used to compare the parametric data and Mann-Whitney U-test for the nonparametric data. The results are expressed as mean±SEM for BALB/c and bapa mice, respectively; the results were considered significant if $p<0.05$.

Results: All parameters passed the normality test, except for one tail/genital grooming time. In the light-dark test, none of the anxiety parameters was altered between groups. Dark side entry latency: 24.69±7.19 and 36.72±12.19, p=0.4117; time spent in the dark side: 186.80±38.79 and 147.70±16.60, p=0.3733; and time spent in the light side: 107.30±39.09 and 148.60±16.75, p=0.3504. However, bapa mice presented affected motor/exploratory behavior, i.e., it increased rearing behavior: 6.86±2.18 and 16.14±2.74, p=0.0210. In the spontaneous self-grooming behavior, all the evaluated parameters were increased for bapa mice. Head washing time: 5.98±1.25 and 16.87±2.64, p=0.0029; body grooming time: 18.15±4.76 and 54.86±8.17, p=0.0022; paw/leg licking time: 67.06±4.68 and 109.70±12.62, p=0.0081; and tail/genital grooming time: 33.69±3.47 and 64.55±4.04, p<0,0001. In the splash test, bapa mice presented increased body grooming and paw/leg licking times. Head washing time: 5.15±0.97 and 4.92±0.47, p=0.8330; body grooming time: 4.51±1.84 and 47.11±8.08, p=0.0002; paw/leg licking time: 29.52±2.62 and 45.56±2.22, p=0.0005; and tail/genital grooming time: 0.56±0.41 and 0.51±0.33, p>0.9999. Bapa mice presented increased striatal GFAP expression: 0.04±0.002 and 0.14±0.003, p>0.1000.

Conclusions: Bapa mice did not presented anxiety behavior, but increased motor/exploratory behavior as well as increased spontaneous and induced self-grooming. Bapa mice also presented striatal astrogliosis. Thus, Kabuki syndrome' neurobehavioral impairments seem to be associated with neuroinflammation.

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NETWORK ANALYSIS OF STRESS RESPONSE VULNERABILITY IN A MOUSE MODEL OF MAJOR DEPRESSIVE DISORDER

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Background: Major depressive disorder (MDD) represents a significant global health burden with diverse manifestations and unclear etiology, impacting millions of people worldwide [1]. While stress is a recognized precursor to MDD [2], the varying responses to stress among individuals underscores the need for predictive markers to identify those at risk. Genetic and molecular biomarkers have limitations, prompting the exploration of behavioral indicators as potential predictors of stress vulnerability. Exploiting the network theory of plasticity [3-5], which posits plasticity levels as a marker for stress susceptibility, we investigated the possibility of identifying stress vulnerability based on individual behavioral network connectivity in a mouse model of major depressive disorder.

Methods: Fifty-five C57BL/6J adult male mice were housed in an automated apparatus (Intellicage system, TSE) for behavioral monitoring throughout the experiment. Following three weeks of standard conditions, mice underwent a two-week exposure to Chronic Unpredictable Mild Stress (CUMS) to induce a depressive-like phenotype. Stress effects were assessed via liking- and wanting-type anhedonia (i.e., saccharine preference and progressive ratio test), and cognitive function using the Novel Object Recognition (NOR) test. Blood samples were collected before and after the stress exposure for transcriptomic analysis. To measure the network connectivity strength, we conducted a longitudinal analysis of individual behavioral data recorded during the standard condition, using Dynamic Time Warping (DTW) implemented in R software. Statistical analyses included one-way ANOVA and Tukey's post-hoc tests.

Results: CUMS induced a depression-like phenotype, as evidenced by increased liking- ($p<0.001$) and wanting-type anhedonia ($p<0.001$). The network analysis performed on the spontaneous behaviors revealed two distinct groups of animals with a significantly different connectivity strength [$F(1,51)=96.58$, $p<0.001$]. The NOR test showed significant differences in time spent in the periphery zone between the two groups ($F(1,51)=4.202$, $p=0.045$), with the low-connectivity group spending more time in the periphery, suggesting a more anxious profile, compared to the high-connectivity group. The low-connectivity mice also showed a significantly lower discrimination index than the high-connectivity, indicating an impaired recognition memory [$F(1,51)=4.065$, $p=0.049$]. No differences between the two groups were found in the delta saccharine preference and progressive ratio measured during the stressful period, while transcriptomic analysis revealed a different expression profile prior to stress exposure between the two groups in blood, with 456 differentially expressed genes (DEGs); 204 genes relatively higher in high-connectivity mice and 252 in low-connectivity mice. A limitation of the study arises from low variability among animals, especially following CUMS exposure, when differences in stress vulnerability became even less pronounced, challenging the identification of distinct response groups.

Conclusions: Our findings suggest that it is possible to stratify individuals according to their level of connectivity, by analyzing spontaneous behavior during standard environmental conditions. From a translational standpoint, quantifying network connectivity as a marker of plasticity facilitates personalized approaches based on individual plasticity levels. Stratifying individuals according to their connectivity levels prior to stress exposure holds promise for improving both preventive and therapeutic interventions.

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APOMORPHINE GENETIC SUSCEPTIBILITY AND PRENATAL INFECTION ALTER EARLY NEURODEVELOPMENT, SYNAPTIC DENSITY, AND ANTICIPATORY BEHAVIOUR IN RATS

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Background: Schizophrenia, a complex psychiatric disorder, emerges from a combination of genetic and environmental influences affecting early brain development, synaptic density, and behaviour. Rather than single genetic mutations, the cumulative impact of multiple genes increases susceptibility, particularly when combined with environmental factors like prenatal infection. Here we investigated whether rats with a polygenetic susceptibility for schizophrenia exhibit early signs of neurodevelopmental changes, altered synaptic density, and behavioural abnormalities in adulthood, and explored whether these effects were aggravated by a prenatal infection.
Methods: To achieve this, we tested male and female rats with a genetic susceptibility to apomorphine (APO-SUS), which exhibits schizophrenia-like features. We investigated separation-induced ultrasonic vocalizations (USVs) and heart rate variability (HRV) on postnatal day (PND) 7 and 14, as they are a well-suited functional marker of neurodevelopmental abnormalities (social communication and autonomic functioning respectively). We also used the locomotor activity during adolescence and adulthood to assess anticipatory pleasure, often disturbed in schizophrenia and other psychiatric disorders. Western blots measured post-synaptic density 95 (PSD95) and synaptophysin levels as indicators of synaptic density. Additionally, we tested whether APO-SUS rats showed altered behavioral response to a prenatal infection on gestational day 15 (polyinosinic:polycytidylic acid). Data was statistically analyzed using the generalized estimating equation model in SPSS.
Results: APO-SUS rats displayed significant alterations in neurodevelopmental markers. Specifically, APO-SUS rats showed significant USVs alterations (reduced USVs quantity [-82%, p=0.008] and average call length [-54%, p<0.001], and higher principal frequency [+25%, p<0.001]) compared to controls, which has been related to a heightened anxiety state, a common comorbidity with schizophrenia. Furthermore, females APO-SUS rats, but not males, showed a reduction in HRV, characterized by a significant reduction in the parasympathetic system (high frequency percentage [-30%, p=0.004], and RMSSD [-17%, p=0.036]), and a significant increase in the sympathetic nervous system (low frequency percentage [+28%, p=0.006], and LF/HF ratio[+75%, 0.007]), when compared to controls. These changes indicate increased sympathetic dominance over the parasympathetic system, a feature often observed in psychiatric disorders. Prenatal infection prevented these HRV changes. During adolescence, but not adulthood, APO-SUS rats (+22%, p<0.001) or male rats exposed to the prenatal infection (+30%, p<0.001) showed anticipatory locomotor behaviour. Only APO-SUS rats with a prenatal injection showed elevated PSD95 and synaptophysin levels in the hippocampus in adolescence and in the frontal cortex in adulthood compared to controls (+150-500%, p<0.01), suggesting a genetic-environmental synergy.
Conclusion: The present findings indicate that rats with a genetic susceptibility for schizophrenia (APO-SUS) show early signs of altered neurodevelopment, which could be a possible indicator of future changes in synaptic density that could be related to cognitive, emotional, and affective disruptions. These alterations were more pronounced in females. Moreover, genetic and environmental risk factors may synergize to modify synaptic density, independent of behavioural effects, and even counteract each other's effects on neurodevelopment.
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TRANSLATING TESTS AND MODELS ACROSS SPECIES, THE CASE OF THE ELEVATED PLUS MAZE IN NOCTURNAL AND DIURNAL RODENTS

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Background: Rats and mice are the frequently used mammals model animals in biomedical research. Research with rats and mice has many advantages, however, for some aspects of physiology and pathology, it had been suggested that there are significant advantages to using diurnal model animals [1]. Utilizing diurnal rodent models comes with difficulties. First, diurnal rodents are uncommon in nature and in laboratories. Furthermore, species that are diurnal in their natural habitat may shift to a nocturnal phenotype under laboratory conditions. Additionally, behavioral tests that were developed for mice and rats may not be appropriate for diurnals. One test that presents a problem is the Elevated Plus Maze (EPM) which tests anxiety-like behavior and is based on an approach-avoidance conflict where aversion from open arms relies on the innate tendency of nocturnal rodents to avoid open, well-lighted spaces [2]. It is a question therefore whether diurnal rodents will also show aversion to the open arms at the same level as nocturnal rodents. The present study explores this question by reviewing previous studies from our group in one diurnal species, the fat sand rat (psammomys obesus), and one nocturnal species, CD-1 (ICR) mice.
Methods: We reviewed open/closed arms ratio in the EPM of control intact animals in 8 studies with sand rats and 7 studies with CD-1 mice. Because studies were conducted by one research group, the EPM protocol was the same across all of them.
Results: The averages of open/closed arms ratios across studies for sand rats ranged between 0.28 and 0.9 with a mean of 0.61. The averages for CD-1 mice ranged between 0.035 and 0.81 with mean of 0.23 [t-test: t(13)=3.06, p<0.01].
Discussion and conclusions: The open/closed time ratio is considered as a measure of anxiety-like behavior with lower ratios indicate higher anxiety-like state. A ratio of 1.0 would suggest indifference regarding the open/closed areas and therefore no aversion from open well-lighted areas. Our results show that both diurnal sand rats and nocturnal mice demonstrate preference towards the closed arms of the maze as the open/closed time ratio is less than 1.0 in all studies. However, the aversion of the mice from open areas is significantly higher compared with the aversion of sand rats. These results suggest that the EPM may still be a valid test even in diurnal animals and it is probable that even without an innate aversion to lighted areas, the sand rats prefer closed, more protected areas but not to the same extent as nocturnal animals. Beyond the issue of circadian preference, it is important to note that we examined only one diurnal species and one mouse strain. Mice strains may be very different from each other, and certainly different diurnal species can be dissimilar in their behavior in the EPM. The critical conclusion is that behavioral tests should not be simply transformed from one species to another but should be re-validated whenever such transition is needed.
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CORTICOTROPIN RELEASING FACTOR RECEPTOR ONE MEDIATES THE EFFECT OF A TRAUMATIC-LIKE STRESS ON THE PERSISTENCE OF MORPHINE SEEKING BEHAVIOUR

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Background: Post-Traumatic Stress Disorder (PTSD) and Substance Use Disorder (SUD), particularly with opioids, are highly comorbid and share common neurological pathways [1]. Codified treatments exist for each disorder but are