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EFFECT OF SOCIAL ISOLATION: ANALYSIS OF BEHAVIORAL ACTIVITY AND DEPRESSION LEVELS IN RAT BRAIN

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ABSTRACT

Stress in early life affects the development of the brain and can contribute to mental disease. Social isolation from early stage of life is used to explore many elements of mental illnesses. This isolation can produce long-term molecular expression and behavioral changes in rats. As social segregation simulates serious mental disorders, the general well-being of animal is affected. Therefore, the purpose of this review is to evaluate different techniques on refinement, such as social isolation in adults. We looked into whether alternatives still activated the phenotype essential while reducing animal stress. Interestingly, we have found no lower digging performance in solitary rats related to wellbeing. In rat's subject to adult isolation alone, although re-socializing improves locomotive malformation, the hyperactive phenotype found in socially separated animals was noticed. Some of those impairments were restored upon resocialization; similar, levels of exon VI Brain-derived neurotropic factor messenger ribonucleic acid were decreased exclusively in chronically separated animals. Conversely, Glutamic Acid Decarboxylase 67 and Polyvinyl butyral, two Gamma-aminobutyric acid markers, have not been affected by social deprivation, but alterations in

dopamine d1 and d2 expressions have occurred. As the aggressive phenotypes, reduced neuroplasticity of the medial prefrontal cortex were sufficient to cause isolation in adults, it may be a candidate to develop a strategic refinement in specific study areas. To assess the degree of adult solitary and resocialization, social isolation and alternatives, a more thorough and multi-modal diagnostic strategy is required.

Keywords: Dopamine, mRNA, Serotonin, Social isolation stress

INTRODUCTION:

Prolonged stress is a key threat factor for psychiatric abnormalities on environment.[1] Mood disorders, such as sadness and anxiety, are neurological conditions that result from the interaction of many biological pathways.[2] Depression is a heritable neuropsychiatric disorder marked by very minor cellular and molecular changes spread across a neural substrate circuit.[3] When compared to chronic disorders like diabetes and arthritis, depression causes the biggest decline in health, especially in Covid 19 pandemic situation.[4] Animal stress models have been employed as a useful tool to examine the underlying mechanisms by which stress has negative impacts on brain functions and animal behavior.[5] Social isolation or a deficiency of social support is linked to enhanced risk of a psychiatric disorder such as atypical depression, as well as hyperphagia and weight gain.[6] Social isolation, which is a one-dimensional concept, can be characterized as a lack of social integration.[7] Prolonged social isolation produces depression, cognitive impairment, anxiety as well as major illness and mortality.[8]

Isolation from others affects brain development and can lead to mental illness.[9] Social isolation impairs memory, increases the expression of depression-related proteins, and reduces neuronal formation.[10] Young age social isolation can prevent dendritic formation on the hippocampus and causes limbic system neurological dysfunction.[11] Several brain regions, including the hippocampus, are involved in the structural and functional changes that lead to these consequences. [12]

The animal model of early-life social isolation in rats is frequently used to study many aspects of mental diseases.[13] This isolation in rats can result in long-term changes in functional connections, behavior, and molecular expression.[14] Rodents are long been used to measure developmental changes in stress-related behaviors and fear, they can show a variety of behavioral alterations including diminished cognitive function, anxiety, reduced motor activity, and aggressive behavior.[15]

ANIMALS MODELS:

There were various animal models which are used for social isolation. Each type of model has its unique property while socially isolating. Male Wistar Rats weighing 150–175 g at 5–7 weeks of age. They were given a week to adjust to the laboratory setting and ready access to food and water before the trials. The facility has a 12-hour dark-light cycle, (with lights turned on at 6 a.m.). The temperature and humidity levels in the room were adjusted to 22±1 degrees Celsius and 45±5.0% percent, respectively.[16]

ANIMALS HANDLING:

The rats were kept in acrylic cages (18" x 15" x 12.5") with a constant ambient temperature (25±21° Celsius), relative humidity (50±5.0 percent), and a regulated 12:12 hours dark-light cycle (lights on at 06 hour). Rat's pellets (supply 3.30 kcal g–1 with 72.1 percent carbohydrate, 23.4 percent protein, and 4.5 percent fat) and drinking water were freely accessible.

PARAMETERS:

OPEN FIELD TEST:

In rodents, this test is used for evaluating anxiety along with general locomotor activity.[17] We employed within apparatus 77 X 77 X 25 cm sq arena and walls to restrict escape, as well as a grid that divided the arena into 16 identical squares.[18] The overall number of movements was used to measure normal locomotor activity, while the number of movements in the middle of the cage is used to measure anxiety.[19] The researcher watched the animal individually for 5 minutes and noted when all forepaws of a rat traveled from one area to another.[20]

ELEVATED PLUS-MAZE TEST:

This test was used to measure Anxiety-like behavior using the elevated plus-maze apparatus. Plus, shaped arms (450 mm x 100 mm) of the elevated plus-maze device are linked at the right with two opposing arms of the same length. The junction portion of the middle platform (100 mm x 100 mm) is elevated 650 mm above the ground. The rats are put in the center of the platform, facing the closed arm, and are given 5 mins to freely wander the maze. All four paws were required to enter an arm. The open arms were timed and entries were recorded.[21]

FORCED SWIMMING TEST:

The rats were placed on the Plexiglas cylinder (500 mm tall, 100 mm radius) containing water (25 ±1 ° C) to a depth of 300 mm. Rats were free to swim in the cylinder under the condition that they would not be able to escape. The cylinder will be cleaned after each test. Each test period lasted 5 minutes and was video recorded in a poorly lit room before being graded by two untrained observers. A time-sampling technique is a method of

collecting data over some time was used to score and for every 5 seconds, one of the following behaviors occurs: (a) swimming, characterized as active motions produced by rats to place their nostrils above the water (i.e. swimming); (b) immobility, defined as the least amount of movement generated by rodents to maintain the noses above water (i.e. floating); (c) climbing, defined as vigorous actions conducted on the pool (i.e. navigating around the jar and diving), and (d) swimming.[22] Usually against the walls, with forepaws in and out of the water. A total of 60 counts were gathered in a 5-minute test, including immobility, swimming, or climbing are all options.[23]

MORRIS WATER MAZE TEST:

This test is used to assess spatial working memory. Rats had to learn in their physical area on a concealed platform on a circular pool filled with water (30±1°C) at the tall of 500 mm and a diameter of 1800 mm. In the north quadrant's center, a concealed platform (400 mm tall and 150 mm diameter) was installed 20 mm below the water level and camouflaged as a dark background. This test comprised of trails thrice a day for five days. During every trial, in one of those three quadrants without the platform, the rat was kept on the water facing the wall of the maze. Both swim speed and time spent ascending the platform (escape latency, with a 20-second cutoff period) were also recorded. The "60-second" probing trial, in which the platform was withdrawn, was conducted 24 hours after the completion of the six-day training period. Memory performance can be measured by the proportion of time being spent in the targeted area.[24]

BURROWING:

In Macrolon Type IV cages, we installed gravel-filled (2500 g) plastic burrowing tubes (320 mm long 100 mm, entrance 60 mm above the floor) and determined the time it took for the animal to start digging, as well as the amount of burrowed material that remained in the tube after one hour. On day one in the training, every rat was given one hour to examine an empty cage before being given another one hour to examine an empty tube. Following one-hour habituation to the empty cage, on each of the three training days, a gravel-filled tube was placed in each cage to simulate the test situation. The gravel within the tube was weighed after another one hour, as well as the amount of gravel removed was computed.

DETERMINATION OF ADRENALINE AND CORT:

Separate animals were sacrificed by quick decapitation, i.e. right after stress treatment, for biochemical and molecular examination. The adrenal glands are meticulously removed and the weights of both right and left glands were measured

after sacrifice. The adrenal cortices were removed and weighed with care. At the time of sacrifice, each animal's blood was taken.[25] Centrifugation at 3,000 rpm at "15-minute" was used to prepare the serum. The corticosterone concentration was determined using the OCTEIA Corticosterone Elisa kit, as directed by the manufacturer.[26]

EXTRACTION OF RNA AND mRNA ISOLATION:

The rat's brain tissue is extracted and then stored in liquid nitrogen until it could be processed further. TRI Reagent was used to extract total Ribonucleic acid from the Hippocampus and prefrontal cortex.[27]A Potter-Elvehjem Teflon-glass homogenizer was used to weigh the tissue and homogenize it in 1 milliliter TRI Reagent per 100 milligrams of tissue.[28] Homogenates are maintained at 30° C for 300 seconds, then 0.2 ml chloroform is added, and the homogenate can be rapidly stirred for 15 seconds before being maintained for 3 minutes at 30° C.[29] The RNA-containing aqueous solution was mixed with 0.5 milliliter isopropanol for 10 mins at 3° Celsius, then centrifuged at 12,000 rpm for 10 minutes at 4 ° Celsius. The RNA pellet was reconstituted in 75 percent ethanol and centrifuged. (7,500 rpm, 5 minutes, 4° Celsius), dried in the air, and dissolved in 100 µl 0.1 percent diethyl pyrocarbonate Solution.

MEASUREMENT OF BDNF MRNA LEVELS BY RT-PCR:

The level of BDNF mRNA in the Medial prefrontal cortex and hippocampus was measured using the ABI Prism (7300 sequence) detection system and quantitative RT-PCR.[30] The internal control for normalization was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). "5 - CCATAAGGACGCGGACTTGT3" (BDNF, forward), "5 - AGCCCAGGATGCCCTTTAGT-3" "AGCCCAGGATGCCCTTTAGT-3", "5 - GACATGCCGCCTGGAGAAAC-3" (GAPDH, reverse), "5 - GAGGCTCCAAAGGCACTTGA-3" (BDNF, reverse). All samples were put through three rounds of testing.[31] Denaturation at 95°C for 10 minutes started the thermal cycling process. 0After this, 40 PCR cycles (95° Celcius for 30 secs and 60° Celcius for 1 min) were performed, followed by a single cycle of 95° Celcius for 1 min, 55° Celcius for 30 secs, and 95° Celcius for 30 secs.[32]The suitable amounts of mRNAs in unknown samples were computed to GAPDH mRNA levels. These methods were modified from the preceding research.[33]

WESTERN BLOT ANALYSIS:

In the western blot examination, the hippocampus tissues were harvested and promptly frozen at 70 degrees Celsius.[34] On ice, the hippocampus tissues were

homogenised and lysed in a lysis buffer comprising 50 mM HEPES "pH 7.5", 150 millimeter sodium chloride, 10 percent glycerol, 1 percent Triton X-100, 1 millimeter PMSF, 1 millimeter EGTA, 1.5 millimeter MgCl26H2O, 1 millimeter sodium orthovanadate, and 100 mM sodium fluoride.[35] A Bio-Rad colorimetric protein test kit was used to determine protein concentration. 30 gram of Protein was separated from sodium dodecyl sulphate–polyacrylamide gels electrophoresis before being applied to a nitrocellulose membrane.[36] Secondary antibodies were horseradish peroxidase-conjugated anti-rabbit antibodies for BDNF and Bid [37] and horseradish peroxidase-conjugated anti-mouse antibodies for Bax and Bcl-2.[38] Except for the transplanted membranes, all experiments were conducted out under standard laboratory settings and at room temperature. The membranes were transferred at 4° C using a cold pack and pre-chilled buffer.[39]The enhanced chemiluminescence (ECL) detection kit was used to identify bands (Santa Cruz Biotechnology). The identified bands were computed densitometrically using Molecular Analyst TM.[40]

CORT ENZYME-LINKED IMMUNOASSAY:

Corticosterone was assessed using a corticosterone EIA kit. 10 microliter of plasma is added to 990 microliter of assay buffer to make a hundred-fold dilution of plasma (containing 2.5 percent steroid displacement reagent).[41] Following that, 100 microliters of each diluted sample was put to assay plate wells coated by goat antibody which is specific to the fragment crystallizable region of rat IgG.[42] After that, an Automated Strip Washer was used to wash the sample wells. After three washing, each well was filled with 200 microliters of "p-nitrophenyl phosphate substrate", which was cultured at room temperature for 1 hour. After that, the reaction was stopped by pouring 50 microliter of stop solution into each well. An Automated Microplate Reader was used to obtain each sample's absorbance levels were measured at 405 nanometers (wavelength correction was set at 595 nanometer), and plasma corticosterone levels were measured by projecting absorbance values onto a corticosterone standard curve KC jr software.[43]

DOPAMINE:

Increased levels of dopamine and 5-hydroxyl tryptamine receptors release in male isolation-reared rodents are considered as the neurochemical basis for the development of aberrant behaviors.[44]Through phasic burst firing, social isolation-induced rats release more dopamine in response to salient stimuli. Increased external dopamine levels and changes in dopaminergic activity have been

associated with social isolation in many studies.[45] Isolation from others causes stress. Cocaine-induced dopaminergic efflux in nucleus accumbens is enhanced, although cocaine's efficiency in inhibiting uptake was not affected by rearing conditions.[46] In situ hybridization revealed that isolation rearing increased dopamine release in the certain regions of NAcc in the striatum and Prefrontal cortex owing to a lowering in NMDA receptor 1 A mRNA expression.[47] Dopamine depletion in NAcc reverses isolation-induced prepulse inhibition impairments.[48] The nucleus types core is involved in SI-induced increased dopamine terminal activity generation and intake in the dorsal striatum, as well as increased dopamine uptake in the ventral striatum in rats. (NAc).[49]There is an increase in psychostimulant reinforcement in SI rats. Many investigations found that psychostimulants reinforced striatal dopamine terminals, increased dopaminergic release rate in the NAc, and strong suppression of dopamine intake in the DMS, but had varied effects on release.[50] Extracellular dopamine and 5-hydroxyl tryptamine level in the medial prefrontal cortex brain of the invader male isolation-reared rat were not changed by anesthesia to pentobarbital or a new item, but prefrontal dopamine levels were greater during or after an aggressive interaction, according to some studies.[51]

SEROTONIN:

Serotonin is a neurotransmitter that regulates mood (5- hydroxyl tryptamine) According to research, 5-hydroxytryptamine3 receptors, which are ligand-gated ion channels, that is associated with the development of obstructive systems in the brain throughout maturation.[52] It can be implicated on the pathophysiology mood disorders and anxiety since rats missing these receptors show decreased anxiety-like behaviors.[53] Early social isolation stress affects the development of the neurotransmission systems such as the serotonin system, which controls behavior and mood, and any changes in this system affected by social isolation of stress are reported by many brain areas, including the limbic area.[54] The release of 5- hydroxyl tryptamine in the dorsal hippocampus of social but not isolated rats is increased by “p-chloroamphetamine” and footshock, indicating a significant impairment in serotonin activity in isolates' hippocampus.[55] The usual increase in 5-hydroxyl tryptamine release produced by systemic amphetamine in prefrontal cortex is attenuated on socially isolated rat.[56] In isolation, exposure to inescapable footshock increases 5- hydroxyl tryptamine release from the medial NAcc, as assessed by microdialysis.[57] The synergistic antidepressant effects of sub-effective

dosages of 5- hydroxyl tryptamine receptor antagonists and N-methyl D-aspartate receptor antagonists correspond with the direct interaction of 5- hydroxyl tryptamine 3 receptors with N-methyl D-aspartate receptors.[58] Serotonin (5- hydroxyl tryptamine), a key neuromodulator in the production of depression and aggressiveness, has also been linked to social depression-induced unwanted types of aggression and sadness. In rats, social exclusion is utilized as a depression model, and hypofunction of the serotonergic system may induce severe depression and obsessive-compulsive disorder.[59] Chronic therapy with selective serotonin reuptake inhibitors (SSRIs) inhibits serotonin release in a continously-dependent way, according to researchers.[60] The impact of prolonged social exclusion on anxiety-related behaviors was investigated in the research. Prolong-term social deprivation reduced anxiety-related behavior and lowered serotonin levels, while levels of dopamine and their metabolites DOPAC and 5HIAA, constant.[61] In Prolong social isolation rats, a selective-5- hydroxyl tryptamine reuptake inhibitor (SSRI), fluoxetine, regulates chronic social isolation-induced aggressive behavior. However, according to another research, the 5- hydroxyl tryptamine 3 receptor cannot cause persistent social isolation-induced depressive-like behavior.[62] Tropisetron, a 5- hydroxyl tryptamine 3 receptor antagonist, was studied and to see whether it had any effect on adolescent social isolation stress, which has been linked to the onset of depression. The findings revealed the critical involvement of mitochondrial function in depressive pathophysiology, as well as the importance of 5-hydroxyl tryptamine 3 in the psychosocial stress response during adolescence caused by tropisetron-induced the role of mitochondrial function in the pathophysiology of depression.[63]

AMPA RECEPTOR:

The activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA receptor in the amygdala has been shown to cause anxiety and depression-like behaviors. Furthermore, AMPA receptor protein levels in the amygdala of rats subjected to protracted social isolation were elevated. That is linked to depressive-like symptoms, which was verified by the measuring of AMPA receptor subunits glutamate receptor 1, glutamate receptor 2 protein levels. Ketamine, on the other hand, reduced chronic stress caused by social isolation stress.[64]

Table 1: Consequences of social isolation in rat brain

consequences of social isolation		
Endorinal changes : HPA axis -↑ functions os HPA axis. Glucocorticoids - ↓ Gcs stress response ACTH - ↓ levels of basal ACTH CORT - ↓ levels of basal CORT	5- hydroxytryptamine receptors: socially isolated rats ↓ activity of brain	Neurotrophic factors : BDNF - hippocampal BDNF was decreased in rats during isolation.

Table: 2 Effects of isolation on serotonin and dopamine effects

Area of the brain	Isolation time	strain	Serotonergic effects	Dopaminergic effect
mPFC	**PD21-PD105**	**Wistar rats**	**↑ 5-HT levels**	**↑ DA levels and concentrations**
Hippocampus	**PD21-PD51**	**Wistar rats**	**↑ 5-HT**	**↑ DA**
NAC	**PD51-PD105**	**Wistar rats**	**↓5-HT**	**↑ DA**
Amygdala	**PD21-PD105**	**Wistar rats**	**↑ 5-HT**	**↑ DA**

DISCUSSION:

This aims to ascertain the impact of social isolation on male rats at various developmental stages, such as youth, early adults, or both, as well as the consequences of socialization processes. To analyze, the social isolation stress various tests were conducted to ensure the depression level in rodents. Open field test helps to determine the anxiety level in male Wister rats and an elevated plus-maze is used to determine the behavioral test to measure fear and anxiety.

The Reduced plasma corticosterone levels in social isolation adult rats are mirrored by a smaller weight, where there is a discrepancy among the both right and left adrenal glands, indicating that this hyperfunction exists which is caused by morphological alterations in the hypothalamic-pituitary-adrenal axis. Surprisingly, even decades after the traumatic events, individuals with posttraumatic stress disorder (PTSD) had lower baseline levels of corticosterone. The HPA axis activation is different under short term and long - term stress. As a conclusion, corticosterone levels and GR expression rise steadily during chronic stress. The stress response was affected differently by handling and social isolation.

A few research looking at the impact of social isolation on Bdnf expression on the prefrontal cortex revealed no changes right after adolescence stress

exposure, however returning to the social housing following young social isolation tends in enhancing cortical Bdnf expression. Simultaneously, adulthood social stress resulted in increase in Bdnf expression in the prefrontal cortex. Even though our data contradict previous findings, pathological investigations in schizophrenia and depressed individuals have consistently shown a reduction in cortical Bdnf expression. Furthermore, the separated animals with the lower Bdnf Prefrontal cortex mRNA levels were shown to be in the more active state in the OF tests. According to this data, behavioral disruption in locomotor activity may be linked to a loss of neuroplasticity.

CONCLUSION:

This review shows the effect of handling animals in social isolation in rats to determine the behavior changes and fear, anxiety through corticosterone. Our review shows that the social isolation of rats influences healthy developed brains into mental disordered animals. Here Our main suggestion to the future researchers involves the process of resocialization and to analyze the changes in brain, determine the levels of depression.

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