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PhD THESIS

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STRESS IN CRITICAL PERIOD OF EARLY LIFE INFLUENCES GLUTAMATE EXCITATORY NEUROTRANSMISSION: IMPLICATION FOR NEUROPSYCHIATRIC DISORDERS

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// ABSTRACT

Early adverse events occurring during critical periods of brain development may shape the individual's developmental trajectory and increase vulnerability to stress-related disorders across lifespan. The aim of my PhD thesis was to investigate the role of glutamatergic neurotransmission in the early-life stress induced impairments of emotional, cognitive, and social scaffolding in functionally involved brain regions. The study was realized by using the model of postweaning social isolation (PWSI) in mice and the model of perinatal stress (PRS) in rat. Firstly, I compared the behavioral and biochemical profiles of PWSI-induced inbred C57BL/6 N mice to those of BTBR mice, a rodent model used to study autism spectrum disorders in humans. Male C57BL/6 N mice were socially housed at weaning (postnatal day 21) or isolated for four weeks before being subjected to experimental analysis at 48 days of age. Male BTBR mice were socially housed at weaning and subjected to analysis at the same age. Metabotropic glutamate receptor of type 2 (mGluR2), glucocorticoid and mineralocorticoid receptors levels were all decreased in the hippocampus of PWSI and BTBR animals. Moreover, both PWSI mice and BTBR mice displayed decreased social behavior (social investigation and ultrasonic vocalizations), demonstrating that the lack of social stimuli throughout adolescence causes an endophenotype that mirrors some behavioral features of autism spectrum disorders. Secondly, I investigated the possible corrective role exerted by resocialization on the PWSI-induced hippocampal glutamatergic disequilibrium. Results showed that PWSI-induced reduction of hippocampal mGluR2 was not recovered by one week of resocialization. I have also investigated the effect of PRS in adult and aged rats of both sexes

on the expression of AMPA and $GABA_A$ receptor subunits in the hippocampus (ventral and dorsal) and prefrontal cortex in relation to emotional and cognitive behaviors. PRS induced sex-dimorphic and age-dependent effects on some receptor subunits and behavior. Particularly, in both adulthood and ageing, PRS reduced open-arm exploration and recognition score in males, while it improved them in females. Interestingly, in the dorsal hippocampus, PRS reduced the expression of the GluA2 subunit in adult male rats and increased the expression in adult female rats. Moreover, PRS reduced the expression of the GluA3 AMPA receptor subunit in the prefrontal cortex and in dorsal hippocampus of adult male rats, an effect which was limited to the prefrontal cortex of adult female rats. Remarkably, changes in GluA2/GluA3 subunits and behavior induced by PRS persisted in aged male rats, but not females. The $\alpha 1$ subunit of the pentameric $GABA_A$ receptor was also studied in adult and aged rats of both sexes. PRS enhanced the expression of the $\alpha 1$ subunit of $GABA_A$ in the dorsal hippocampus of both sexes and reduced it in the prefrontal cortex exclusively in females. In aged PRS subjects a reduction of $GABA_A$-$\alpha 1$ subunit protein levels in the prefrontal cortex was observed in the male gender. Extending the analysis to synaptic vesicle proteins we found reduction of synaptophysin, syntaxin and rab3a levels in the ventral hippocampus of PRS males, but not females, at adulthood and ageing. Interestingly, control male rats are characterized by a greater density of hippocampal levels of syntaxin, munc-18, synapsin IIa, VAMP, synaptophysin and rab3a with respect to female rats. This difference was abolished by PRS, suggesting that PRS caused dysmasculinization across lifespan. These findings suggest that changes in the expression levels of AMPA and $GABA_A$ receptors contribute to the sex-divergent behavioral

phenotype induced by PRS in adult and aged rats. Collectively, my research shows that early-life stress-induced alterations converge to severe impairment of glutamatergic neurotransmission, underscoring its key role in determining and maintaining the proper developmental trajectory across the lifespan.

RIASSUNTO

Gli eventi avversi che si verificano durante i periodi critici dello sviluppo cerebrale possono modellare la traiettoria di sviluppo dell'individuo e aumentare la sua vulnerabilità a diversi disturbi durante l'intero arco della vita. L'obiettivo della mia tesi di dottorato è stato quello di studiare il ruolo della neurotrasmissione glutammatergica nelle alterazioni cognitive e sociali indotte dallo stress nei primi anni di vita in regioni cerebrali potenzialmente coinvolte. Lo studio è stato realizzato impiegando i modelli murini dell'isolamento sociale post-svezzamento (PWSI) e dello stress perinatale (PRS). Inizialmente, ho condotto un'analisi comparativa tra i profili comportamentali e biochimici del topo C57BL/6 N in isolamento sociale post-svezzamento e del topo BTBR, modello murino validato per studiare alcuni aspetti fenotipici dei disturbi dello spettro autistico nell'uomo. Topi maschi C57BL/6 N sono stati stabulati in gruppo o isolati allo svezzamento (giorno postnatale 21) per quattro settimane prima di essere sottoposti alle analisi sperimentali a 48 giorni di età. I topi maschi BTBR sono stati stabulati in gruppo allo svezzamento e sottoposti alle analisi alla stessa età dei topi PWSI. Entrambi i gruppi sperimentali erano caratterizzati da una ridotta densità dei recettori metabotropici del glutammato di tipo 2 (mGluR2), dei glucocorticoidi (GR) e dei mineralocorticoidi (MR) selettivamente nell'ippocampo. Inoltre, sia i topi PWSI che BTBR hanno mostrato una diminuzione del comportamento sociale (indagine sociale e vocalizzazioni ultrasoniche), evidenziando che la mancanza di stimoli sociali durante l'adolescenza causa un endofenotipo che rispecchia alcune caratteristiche comportamentali dei disturbi dello spettro autistico. Ho inoltre studiato il potenziale ruolo riparatore della

risocializzazione nei topi PWSI. I risultati hanno evidenziato che una settimana di risocializzazione non era in grado di ripristinare i ridotti livelli di espressione dell'mGluR2 nell'ippocampo dei topi PWSI. Nel prosieguo delle mie ricerche, ho studiato nei ratti adulti e anziani di entrambi i sessi l'effetto dello stress perinatale sull'espressione delle subunità dei recettori AMPA e $GABA_A$ nell'ippocampo (ventrale e dorsale) e nella corteccia prefrontale in relazione ai comportamenti emotivi e cognitivi. Il PRS ha indotto effetti sessualmente-dimorfici ed età-dipendenti su alcune subunità recettoriali e sul fenotipo comportamentale. In particolare, sia nell'età adulta che anziana, il PRS ha gravemente compromesso la reattività alla novità e la performance cognitiva nei maschi, migliorandole invece nelle femmine. Nell'ippocampo dorsale il PRS ha ridotto l'espressione della subunità GluA2 nei maschi adulti, aumentandola invece nelle femmine della stessa età. Inoltre, il PRS ha ridotto l'espressione della subunità GluA3 del recettore AMPA nella corteccia prefrontale e nell'ippocampo dorsale dei maschi adulti e questo effetto è stato osservato solo nella corteccia prefrontale delle femmine adulte. È stato interessante osservare che i cambiamenti indotti dal PRS nei livelli di espressione delle subunità GluA2/GluA3 e nel fenotipo comportamentale fossero persistenti nei ratti maschi ma non nelle femmine nell'invecchiamento. Inoltre, nei ratti adulti ed anziani di entrambi i sessi è stato analizzato il profilo di espressione della subunità $\alpha 1$ del recettore $GABA_A$. In particolare, il PRS ha aumentato l'espressione della subunità $\alpha 1$ nell'ippocampo dorsale di entrambi i sessi e l'ha ridotta nella corteccia prefrontale esclusivamente nelle femmine. In età anziana, è stata osservata una riduzione dei livelli proteici della subunità $\alpha 1$ nella corteccia prefrontale dei maschi. Estendendo le analisi biochimiche alle proteine associate alle vescicole sinaptiche, abbiamo riscontrato una

riduzione dei livelli di sinaptofisina, sintaxina e rab3a nell'ippocampo ventrale dei maschi PRS, ma non delle femmine, in età adulta e senile. È stato interessante notare che rispetto alle femmine i maschi del gruppo di controllo erano caratterizzati da una maggiore densità dei livelli ippocampali di sintassina, munc-18, sinapsina IIa, VAMP, sinaptofisina e rab3a. Questa differenza è stata abolita dal PRS, suggerendo che il PRS ha causato una demascolinizzazione durante l'intero arco della vita. Questi risultati suggeriscono che i cambiamenti nei livelli di espressione dei recettori AMPA e $GABA_A$ contribuiscono agli effetti sesso-dipendenti del PRS sulla manifestazione del fenotipo comportamentale dei ratti adulti e anziani. Nel complesso, la mia attività di ricerca dimostra che le alterazioni indotte dallo stress nelle prime fasi della vita convergono verso una grave compromissione della neurotrasmissione glutammatergica, sottolineandone il ruolo chiave nel determinare e mantenere la corretta traiettoria di sviluppo nell'arco della vita.

INTRODUCTION

1. CRITICAL PERIODS IN BRAIN DEVELOPMENT

Neurobiological background can orient individuals toward certain types of experience, and experience can shape, at the same time, an individual's neurobiological substrate throughout life. There is a close correlation between an individual's neurobiological background and experience that, for specific brain areas, is particularly pronounced during specific developmental windows known as critical periods (Larsen and Luna, 2018). Critical periods are unique time windows in an organism's life in which the interplay of genetic, neurobiological, and environmental factors influence brain patterning, diversity, connectivity, and functional maturation of neurons (Levitt and Veenstra-VanderWeele, 2015). Specifically, the normal development of the structural and functional scaffolding of the brain is the result of a conserved sequence of developmental processes (cell division, neuronal migration, network formation, and maturation of brain circuits) within which the triggering of appropriate stimuli can induce events of increased plasticity necessary to permanently determine an individual's behavior in adulthood (Dehorter and Del Pino, 2020). Indeed, critical periods are phases characterized by high synaptic plasticity and during which brain cell connections, being more sensitive and receptive to environmental influences or stimuli, can form, strengthen, stabilize, and mature (Erzurumlu and Gaspar, 2012; Levelt and Hübener, 2012). Notably, these periods of high plasticity are typically preceded by intense axonal proliferation activity, which is the cellular mechanism underlying the experience-dependent process of neuronal circuit formation (Knudsen,

2004; Nelson and Gabard-Durnam, 2020). In addition, while the onset of critical period is triggered by changes in NMDA glutamatergic signaling and dopaminergic receptor density, increased levels of brain-derived neurotrophic factors (BDNF) and maturation of GABAergic inhibitory circuits, leading to the achievement of the balance between excitation and inhibition (E/I), their closure is marked by cellular changes affecting major cellular matrix components (e.g., chondroitinsulfated proteoglycans) and receptor systems involved in myelination processes (Sengpiel, 2007; Larsen and Luna, 2018). However, the lack of certain experiences in early life may have a profound effect on the development of neural connections and next exposure to such experiences may not compensate for their previous absence (Sengpiel, 2007; Nelson and Gabard-Durnam, 2020). This concept was well evidenced by the elegant experiment conducted by Nobel laureates Wiesel and Hubel, who demonstrated how early sensory deprivation dramatically affects the anatomy and functional organization of the visual cortex, thus defining the perinatal period as a crucial phase of life during which visual experience may shape synaptic connections in the primary visual cortex (Wiesel and Hubel, 1963; Cioni and Sgandurra, 2013). Specifically, the authors have reported that occlusion of one eye (monocular deprivation) by suturing the eyelids in the early stages of kitten development (from birth to 2-3 months of age) results in a dramatic decrease in the number of visual cortical cells and consequently functional blindness in the occluded eye, although the state of retinal function was not found to be altered after the animal's eyelids were reopened (Wiesel and Hubel, 1963; Cioni and Sgandurra, 2013). In humans, optimal development of specific brain functions (e.g., binocular vision or language acquisition) occurs at particular critical periods (Sengpiel, 2007). For example,

adolescence is a critical developmental period that lies between childhood and adulthood, characterized by unique neurobiological, social, and cognitive development (Crews and Hodge, 2007; Larsen and Luna, 2018). Neuroimaging studies conducted in humans have shown that the associative cortical areas (prefrontal cortex (PFC), posterior parietal cortex, and superior temporal cortex) involved in the planning of higher-order cognition functions undergo continuous experience-dependent morphological and cellular changes during adolescence, both progressive and regressive (e.g., gray matter volume, neuronal connectivity, overproduction and/or pruning of axons and synapses), thus providing the neurobiological basis for adolescents' unique behaviors and the functional and structural substrate necessary for maturation to adulthood (Crews and Hodge, 2007; Luna et al., 2015; Larsen and Luna, 2018).

2. FACTORS INFLUENCING BRAIN DEVELOPMENT: IMPLICATION FOR NEUROPSYCHIATRIC DISORDERS

Perinatal life, infancy, childhood, and adolescence are critical periods of life that are particularly sensitive to stressors (McEwen, 2008, 2012; Maccari et al., 2017). During these life stages, adverse experiences, including malnutrition, stress, and exposure to environmental factors (e.g., toxins and pathogens), can trigger maladaptive responses that alter the normal developmental trajectory of individuals and provide fertile ground for the onset of chronic diseases (Maccari et al., 2017; Fitzgerald et al., 2020). A growing number of studies have shown that exposure to a negative environment during critical periods of life is a risk factor for the onset of cardiovascular and metabolic diseases (e.g., type 2 diabetes and

hypertension) and is associated with long-term consequences on neurodevelopmental processes in offspring, increasing the risk of neuropsychiatric disorders across the lifespan (Jiang et al., 2013; Suglia et al., 2020; Waters and Gould, 2022). One consequence of this evidence was the development of the Developmental Origins of Health and Disease (DOHaD) theory (Barker et al., 1993; Wadhwa et al., 2009), which, over the past three decades, has been supported by numerous studies in humans and rodents (Nemeroff, 2004; Seckl, 2008; Maccari et al., 2017). For instance, a growing body of evidence has well documented the increased incidence of chronic heart disease, type 2 diabetes, depression, and schizophrenia in adult offspring exposed in the fetal period to an unbalanced maternal diet (Barker et al., 1993; Dana et al., 2019; Stein et al., 2019). Preclinical studies have characterized the long-term adverse effects of in utero unbalanced nutrition on fetal neurodevelopment (Barker and Clark, 1997). In particular, exposure of pregnant mice to a low-protein diet has been shown to dramatically alter the differentiation status of neural progenitor populations in both the cortex and ganglionic eminence of offspring, which showed short-term memory deficits in adulthood (Gould et al., 2018; Fitzgerald et al., 2020). In addition to malnutrition, pathogens are also factors that can program detrimental changes in fetal neurodevelopment (Jash and Sharma, 2022). Some studies have reported a close association between maternal hospitalization for influenza and/or bacterial infections during the first two trimesters of pregnancy and increased incidence of psychiatric disorders (e.g., autism spectrum disorder (ASD)) in adult male offspring (Atladóttir et al., 2010; Lee et al., 2015). Additionally, studies in pregnant rodents have shed light on mechanisms by which maternal viral and bacterial infections influence the abnormal neurological development

of the fetus (Estes and McAllister, 2016; Fitzgerald et al., 2020). For example, maternal cytokine release (e.g., IL-6) has been reported to mediate the effects of Poly I:C-induced maternal immune activation on offspring behavior, which exhibits impaired communication and social interaction and stereotyped and repetitive behaviors in adulthood (Smith et al., 2007). Furthermore, several rodent studies have shown that the offspring prenatally exposed to lipopolysaccharide (LPS) display deficits in memory and learning processes, increased depressive- and anxiety-like behaviors, extensive microglia activation, oxidative stress and alterations in dopaminergic neurotransmission (Zhu et al., 2007; Hao et al., 2010; Kirsten et al., 2012; Al-Amin et al., 2016). Finally, chronic stressful events during critical periods of life can also lead to impairment of the body's coping capacity and alter the developmental trajectory of individuals over the life course (Maccari et al., 2017). Indeed, exposure to stress in early childhood (e.g., perinatal stress, poor maternal care, abuse, childhood trauma, and social isolation) alters children's neural plasticity and function, and these changes have been associated with an increased likelihood of developing various neuropsychiatric and neurodevelopmental disorders, including mood and anxiety disorders, post-traumatic stress disorder (PTSD), substance use disorders, personality disorders, and autism spectrum disorders (ASD) (Agorastos et al., 2019; Waters and Gould, 2022). In particular, it has been shown that alterations in emotional behavior and cognitive domains (e.g., spatial memory and social behavior) that characterize prenatally stressed adult offspring (Maccari et al., 2014; Weinstock et al., 2016) have, among their biochemical correlates, increased catecholamines and glucocorticoids in the fetal circulation, changes in oxytocin signaling neurotransmission, and alterations in the maternal

microbiome (Fitzgerald et al., 2020). Interestingly, the programming of the developmental trajectory of individuals is determined not only by prenatal factors, but also by postnatal factors, among which the quality of maternal care in the first days after birth is worth mentioning. Some rodent studies have examined the effects of maternal care (e.g., licking, grooming, and arc feeding) on the programming of stress responses and behavioral background of offspring (Caldji et al., 1998; Gatta et al., 2018). In particular, it has been observed that rat pups exposed to patchy maternal care exhibit increased expression of corticotropin-releasing hormone (CRH) receptors in the hippocampus and subsequent deficits in memory performance (Ivy et al., 2010). In addition, cross-caring of offspring of stressed mothers with an unstressed control mother or father at birth has been observed to reduce peak corticosterone secretion and altered behavioral phenotype (i.e., anxious behavior) in adult rats stressed early in life (Maccari et al., 1995; Barros et al., 2006). Several studies have shown that the early postnatal environment influences the health of individuals through the induction of changes in epigenetic markers, suggesting the important role of epigenetic modifications (e.g., DNA methylation, acetylation of specific lysine sites) in programming the developmental trajectory induced by early stress (Bockmühl et al., 2015; Alyamani et al., 2018; Fitzgerald et al., 2020). For example, it has been observed that individuals exposed to adverse events early display an increased hypermethylation status of the neuron-specific glucocorticoid receptor promoter (NR3C1) positively correlating with pronounced deregulation of the HPA axis, and greater susceptibility of same individuals to the development of stress-related disorders (i.e., depression) in adulthood (Li et al., 2020).

3. BRAIN AREAS INVOLVED IN NEUROPSYCHIATRIC DISORDERS

The brain is organized into a complex set of neural networks consisting of distinct areas with relatively specialized functions. Adaptive behavior results from the coordination and integration of neural activity across well-distributed brain regions (Sigurdsson and Duvarci, 2016). In the brain, the synaptic transmission between the hippocampus, prefrontal cortex and amygdala plays a key role in behavioral functions (e.g., executive function, working and long-term memory, emotion) and in determining peripheral responses to stress (McEwen et al., 2016; McEwen, 2017; Yavas et al., 2019). The hippocampus is a brain structure located deep in the medial temporal lobe and organized into several subfields, including the dentate gyrus (DG) and cornum ammonis areas 1 (CA1) and 3 (CA3) (Anand and Dhikav, 2012; Sigurdsson and Duvarci, 2016). It is present in all mammalian species and plays a key role in spatial navigation, emotional behavior, regulation of hypothalamic functions and neurogenesis (Fanselow and Dong, 2010; Koehl and Abrous, 2011; Anand and Dhikav, 2012). In addition, the hippocampus is involved in various forms of learning and memory, and several studies have shown that the impairment of hippocampal synaptic plasticity mechanisms can result in compromised mnemonic processes (i.e., spatial memory) (Ivy et al., 2010; Wang et al., 2011). Overall, the hippocampus is arranged along a longitudinal axis that in rodents stretches between a dorsal and ventral pole corresponding respectively to the posterior and anterior poles in primates (Fanselow and Dong, 2010; Sigurdsson and Duvarci, 2016). Particularly, it has been highlighted the existence of a specific and selective dichotomy of gene expression, anatomical connectivity as well as physiological and behavioral functions

along the hippocampal longitudinal axis (Lee et al., 2017). Specifically, the dorsal hippocampus has been shown to be primarily involved in learning and memory processes (i.e., spatial memory), while the ventral sub-region is intimately related to the regulation of stress response and emotional behavior (Fanselow and Dong, 2010). In contrast to the hippocampus, the prefrontal cortex is a neocortical region phylogenetically more divergent and it is implicated in a broad range of behaviors including decision making, attention, working memory, memory consolidation, executive function, self-regulatory behaviors and social behavior (Benchenane et al., 2011; Bicks et al., 2015; McEwen et al., 2016; Sigurdsson and Duvarci, 2016). In primates, the prefrontal cortex is highly interconnected with other brain structures and can be subdivided into two large areas each having different functions: the dorsolateral area, located anterior to the premotor region and involved in various cognitive functions (e.g., executive control, attention and working memory), and the ventromedial area, involved in the regulation of emotional behaviors (Koenigs and Grafman, 2009; Gamo and Arnsten, 2011; Xu et al., 2019). The prefrontal cortex and hippocampus share extensive connections with the amygdala (Kong et al., 2013; Yavas et al., 2019), which is located in the dorsomedial part of the cerebral temporal lobe. The amygdala is a nuclear complex usually divided into basolateral amygdala (BLA), medial amygdala (MeA) and central nucleus of amygdala (CeA) and is involved in processing physiological and behavioral responses to stress and in the regulation of emotional behaviors (i.e., fear, aggressivity) (Zhang et al., 2018; Haller et al., 2018). Several studies in animals and humans have shown that adverse events occurring during critical periods affect the structural and functional development in the hippocampus, PFC and amygdala (Chen and Baram, 2016; McEwen et al.,

2016; VanTieghem and Tottenham, 2018). In particular, rodents exposed to different stress experiences (i.e., maternal separation, fragmented maternal behaviors, chronic restraint stress) show, at the level of the three regions of the limbic system, an alteration of the dendritic arborization process that has been associated to increased anxiety-like and depressive-like behaviors observed in stressed animals (Vyas et al., 2002; Hill et al., 2019; Monroy et al., 2010; Smith and Pollak, 2020). Humans' studies have shown that one of the most widely reported findings in children exposed to abuse, neglect, or an adverse family environment is reduced volume of hippocampus and prefrontal cortex, which has been associated with deficits in learning processes and increased vulnerability to the development of neuropsychiatric disorders in adulthood (Frodl et al., 2010; McEwen et al., 2016; Smith and Pollak, 2020). Moreover, some evidence has reported that stress in early childhood determines an atypical trajectory of PFC-amygdala connectivity, which results in changes in the reactivity and sensitivity of both regions to emotionally salient stimuli (VanTieghem and Tottenham, 2018; Smith and Pollak, 2020). Overall, these pieces of evidence show that the structural and functional disruption of the hippocampus, prefrontal cortex and amygdala play an important role in the relationship between stress in early life and its effects on developmental trajectory of individuals (Smith and Pollak, 2020), representing an important pathophysiological mechanism in various neuropsychiatric (i.e, schizophrenia and autism spectrum disorders) and neurological diseases (i.e., Alzheimer's and Parkinson's diseases) (Baron-Cohen et al., 2000; Rasetti et al., 2009; Colgin, 2011; Li et al., 2015; Sigurdsson and Duvarci, 2016).

4. EXPERIMENTAL MODELS FOR STUDYING NEUROPSYCHIATRIC DISORDERS

4.1 Postweaning social isolation

Early studies conducted in the 1960s and 1970s have shown that mice and rats housed in individual cages for extended period exhibited increased general reactivity to environmental stimuli and an anxiogenic and overly emotional profile (Koch and Arnold, 1972; Sahakian et al., 1977, Fone and Porkess, 2008). These behavioral alterations characterizing rodents reared in social isolation has represented the crucible of so called "isolation-induced stress syndrome'' (Hatch et al., 1965; Valzelli et al., 1973). Over the last few years, most laboratories have adopted a social isolation paradigm consisting of housing male or female rodent (rat or mouse) pups in individual cages from the first day of weaning until the day of experimental tests, typically performed in late adolescence or adulthood (Lukkes et al., 2009; Toth and Neumann, 2013). The day of weaning is determined by the experimenter and might be between postnatal day (PND) 21 and PND 28 (Fone and Porkess, 2008; Lukkes et al., 2009). The post-weaning period can be particularly susceptible to disturbances in the social environment because social interaction during this critical window time is necessary for the proper developmental trajectory across lifespan (Hermes et al., 2011; Adams and Rosenkranz, 2016). This period corresponding to adolescence beginning in rodents is critical for the development of social behavior (Lukkes et al., 2009; van Kerkhof et al., 2013; Toth and Neumann, 2013). For instance, it has been observed that both male and female rats socially isolated between PND 22 and 35 show impaired social interaction (Toth and

Neumann, 2013). Conversely, the same procedure of isolation does not induce reduction of social interaction when applied in adulthood (Ferdman et al., 2007; Caruso et al., 2022a). Therefore, postweaning social isolation determines a comprehensive behavioral change that can be observed only if the intervention is commenced during critical window time of puberty (Einon and Morgan, 1977). The period of social isolation can range between 4 to 8 weeks (Chang et al., 2015) and during this period isolation-reared rodents are completely deprived of social contact with littermates (Tanaka et al., 2019); however, they still have visual, auditory and olfactory contacts with other animals raised in isolation and in group of three to five per cage and both groups are kept in the same rearing conditions (Lapiz et al., 2003; Fone and Porkess, 2008; Caruso et al., 2022a). Any confounding effect of litter is prevented selecting the animals for equal size and dividing them equally from the same dam into isolation and group reared conditions. Particularly, the isolation procedure is followed by the same operator who does not handle housed rodents more than once a week (to change bedding material). Moreover, each source of noise is carefully monitored and reduced in the animal room (Fone and Porkess, 2008). Since the housing environment may affect the precise nature of the final behavioral and biochemical outcome (Akinbo et al., 2022), the standard isolation procedures do not normally include the use of environmental enrichment (Fone and Porkess, 2008; Lukkes et al., 2009). Indeed, several studies have reported that environmental enrichment reverse the behavioral and biochemical alterations induced by post-weaning social isolation (Hellemans et al., 2004; Hoffmann et al., 2009). To date, the majority of laboratories have adopted chronic postweaning social isolation (PWSI) procedure, rearing rodents in individual cages for 4-6 weeks or more during

the postweaning development (Lapiz et al., 2003; Fone and Porkess, 2008; Lukkes et al., 2009) and testing behaviorally them in a state of total social contacts deprivation. Generally, it has been observed that PWSI in rodents elicits a large array of long-lasting behavioral and biochemical alterations which have translational relevance to neuropsychological and developmental disorders including attention-deficit hyperactivity disorder, autism spectrum disorder, schizophrenia and depression (Lapiz et al., 2003; Fone and Porkess, 2008; Mumtaz et al., 2018; Matsumoto et al 2019; Tanaka et al., 2019). Therefore, PWSI represents a nonpharmacological animal paradigm that could be useful in understanding the molecular and neuronal etiology of neuropsychiatric and developmental disorders, allowing the discovery and assessment of new treatment drugs (Fone and Porkess, 2008).

4.2 Perinatal stress

Noise, social isolation, repeated electric shocks to the tail, immobilization have been the most common paradigms adopted to stress female rats throughout gestation (Weinstock, 2001; Weinstock, 2016). An additional well-established animal model of early-life stress is represented by the perinatal stress model (PRS) in the rat. PRS consists of restraining pregnant females rats in a transparent cylinder under bright light for three daily sessions lasting 45 minutes each, from day 11 of pregnancy until the pups are born (Maccari et al., 1995; Maccari and Morley-Fletcher, 2007). Gestational restraint stress induces an alteration in maternal behavior that leads to the establishment of an adverse environment in the postnatal period, in which the developmental trajectory of the offspring may not take the correct physiological direction (Maccari et al., 2017; Gatta et al., 2018).

Given the importance of mother-infant interaction for the physiological and psychological homeostasis of the offspring (Granata et al, 2021), it is believed that the PRS paradigm combines both prenatal and postnatal stress, as newborns of the stressed mother are continuously exposed to disturbed maternal care (e.g., inappropriate sucking behaviors, grooming, licking and petting of pups) in the early postnatal period (Maccari et al., 1995; Morley-Fletcher et al., 2007; Gatta et al., 2018). Interestingly, PRS can be used to study the long-term effects of early-life adverse events on offspring and the subsequent shaping of emotional and cognitive processes in response to environmental challenges across the lifespan (Maccari et al., 2017).

4.3 Mouse model of BTBR

Autism spectrum disorders (ASD) are the most commonly diagnosed neurodevelopmental disorders and represent a heterogeneous set of disorders with an unclear etiology (Salari et al., 2022). The number of epidemiological studies on ASD has increased in recent years, and current estimates indicate that more than 1% of children worldwide are affected (Meyza and Blanchard, 2017). Genetic, epigenetic, and environmental factors contribute to the formation of a highly heterogeneous group of patients who share only the behavioral phenotype characterized by social impairment, communication abnormalities, and restricted and repetitive behavior patterns (Meyza and Blanchard, 2017; Masini et al., 2020). To date, preclinical research models are essential to study underlying pathophysiology of altered behavioral phenotype and for developing new therapy strategies. However, the challenge in modeling autism spectrum

disorders lies in the absence of clear molecular, neurobiological and structural mechanisms involved (Meyza and Blanchard, 2017). Indeed, the present etiopathogenetic study of ASD relies on numerous mouse models that are verified on the basis of the two main behavioral features of ASD patients: i) difficulties in social behavior and communication; ii) excessive repetitive behavior (Meyza and Blanchard, 2017). To this regard, Black and Tan BRachyury T + Itpr3 tf/J (BTBR) mouse, which was once used for studies on insulin-resistance, is, at present, one of the most commonly used animal models in pharmacological research in order to find and develop efficient treatment modalities. The reasons lie in the robust behavioral phenotype characterizing this mouse strain (i.e., reduced social interactions, impaired play behavior, low tendency to explore, unusual vocalizations, and high levels of anxiety compared with other inbred strains) and recapitulating the behavioral deficit observed in autistic individuals (Careaga et al., 2015; Meyza and Blanchard, 2017). BTBR mice have a complex background characterized by multiple neuroanatomical, genetic, epigenetic, molecular and physiological alterations. They show a picture of aberrant brain connectivity, characterized by complete agenesis of the corpus callosum (aCC), profound alterations in the morphology of the cerebral ventricles, hippocampus, and amygdala, and reduction of the hippocampal commissure (Mercier et al., 2012; Jones-Davis et al., 2013). Interestingly, it has been shown that part of this neuroanatomical profile is similar to that observed in some subpopulations of patients with ASD (Ellegood and Crawley, 2015). Recently, molecular genetics and proteomics studies have reported that several groups of genes and proteins associated to the social behavior and involved in key processes of the development and maintenance of proper brain connectivity (e.g., neurogenesis,

myelination, axonal development and activity, regulation of actin cytoskeleton) are differentially regulated in BTBR mice compared with strain C57BL6/J (B6) (Daimon et al., 2015; Provenzano et al., 2016; Wei et al. 2016a). For instance, it has been observed that BTBR mice exhibit several changes in brain function-related genes, including down-regulation of the brain-derived neurotrophic factor (BDNF) in the hippocampus and cerebral cortex (Scattoni et al., 2013); changes in DNA methylation process of several oxidative stress-related proteins in the cerebellum (Shpyleva et al., 2014); disruption of several inter- and intracellular signaling pathways components (i.e., pathway of MAPK) (Faridar et al., 2014). Among the molecular aberrations characterizing the BTBR mouse background are reported impairment of monoaminergic (5HT, DA) and cholinergic transmission as well as imbalance of excitatory/inhibitory neurotransmission, which have been suggested as key mechanisms associated, respectively, with symptom severity and behavioral deficits in ASD patients (Muller et al., 2016; Gangi et al., 2016; Uzunova et al., 2016; Meyza and Blanchard, 2017). Interestingly, in agreement with such an imbalance, it has been reported that the function of glutamate release from excitatory synapse is abnormal in adult and aged BTBR mice (Wei et al., 2015, 2016b). Particularly, it has been observed that the baseline and KCl-evoked glutamate release from the cerebral cortex glutamatergic synapse in BTBR mouse of both ages is significantly lower than B6.

5. STRESS

5.1 Physiology of adrenocortical activation: the hypothalamic-pituitary-adrenal axis

The organism responds to environmental disturbances with a stress response enabling physiological adjustment to the stressor in order to preserve homeostasis (McEwen et al., 2015b, 2016). An important neuroendocrine mediator of the organism's response to stress is the hypothalamic-pituitary-adrenal (HPA) axis, which is involved in the regulation of several bodily processes (i.e., digestion, the immune system, mood and emotions and energy storage and consumption) (Sheng et al., 2021). HPA axis activation determines a cascade of endocrine signaling responding to specific negative feedback circuits in which the hypothalamus, anterior pituitary gland, and adrenal gland are intrinsically involved (Herman et al., 2016; DeMorrow, 2018). HPA axis activity is regulated by a specific population of neurons located in the medial parvocellular region of the paraventricular nucleus (PVN) of hypothalamus (Arnhold et al., 2007). When a stressor is detected, the activation of the HPA axis is initiated by stimulation of parvocellular neurons in the hypothalamic PVN stimulating the synthesis and release of corticotropin-releasing hormone (CRH) and arginine vasopressin (APV) (de Kloet et al., 2005; Franklin et al., 2012). CRH derives from a 192-amino-acid preprohormone and activates type-1 CRH receptors coupled to G_S proteins, while AVP activates V1b receptors coupled to G_q proteins (Van Pett et al., 2000; Caruso et al., 2022b; Mantsch, 2022). Once released from the hypothalamus, CRH and APV reach the anterior pituitary through the hypothalamic-pituitary

portal vessel network of the median eminence (Stephens and Wand, 2012; Sheng et al., 2021). In the anterior pituitary, CRH and APV act on the corticotropic cells, stimulating the synthesis of pro-opiomelanocortin (POMC), which is cleaved into bioactive peptides (i.e., β-lipotropin, β-endorphin and melanocortin peptides) and adrenocorticotropic hormone (ACTH). Once synthesized by the anterior pituitary gland, ACTH is released into the systemic circulation, which, in turn, induces the fasciculate and reticulate portions of the adrenal cortex to produce and release glucocorticoids (cortisol in humans and corticosterone in rodents) and androgens, respectively (Stephens and Wand, 2012). This cascade is transient, and when the stressful input is interrupted or removed, the HPA axis can return to its baseline state by the intervention of several negative feedback loops. The main important mediators of these loops are glucocorticoids (GCs) acting by two different mechanisms: a) a direct mechanism, in which GCs shut down the response of the hypothalamus and pituitary and consequently the release of CRH and ACTH; b) an indirect mechanism, in which GCs activate glucocorticoids receptors (GRs) in the hippocampus and prefrontal cortex, that project back to neuronal population located in the PVN of hypothalamus (Franklin et al., 2012).

5.2 Mineralocorticoid and glucocorticoid receptors

Adrenal glucocorticoid hormones are essential for the maintenance of homeostasis and adaptive response to stress. They exert physiological effects by binding two specific receptors: the mineralocorticoid receptors (MR) and the glucocorticoids receptors (GR) (Koning et al., 2019). MRs and GRs are members of the nuclear hormone receptor superfamily, and they

are ubiquitously expressed in the body. In the brain, MRs and GRs are present in various regions, with the highest expression in humans and rodents observed in the corticolimbic structures (i.e., hypothalamus, hippocampus, prefrontal cortex, amygdala) (Gray et al., 2017). Although MRs and GRs are encoded by two different genes (*NR3C2* and *NR3C1*, respectively), their structure and function are closely related. These receptors are localized in the cytosol and composed of four distinct protein regions, including an N-terminal region, a DNA-binding domain, a nuclear localization signal and a C-terminal hormone-binding region (Meijer et al., 2019). In the brain, MRs and GRs bind the same hormone (primarily cortisol in humans and corticosterone in rodents) with a different affinity and after ligand binding translocate into the nucleus, where they form heterodimers on the DNA at glucocorticoid response elements (GREs) (Kitchener et al., 2004; de Kloet et al., 2005; Mifsud and Reul, 2018). Particularly, MRs have a higher affinity for glucocorticoids compared to GRs. Indeed, MRs are partially occupied and activated by corticosterone even when concentrations of corticosterone are low (i.e., basal levels), while the GRs activation occurs at the circadian peak of glucocorticoids or in response to stressful events (Kitchener et al., 2004; de Kloet et al., 2008; Herman et al., 2012; Caruso et al., 2019). Thus, MRs define the threshold of stress response by sustaining basal activity, while GRs are important mediators of stress response extinction (de Kloet and Reul, 1987; de Kloet et al., 1998, Herman et al., 2012; de Kloet et al., 2019). GRs and MRs are implicated in controlling a variety of processes (i.e., neuronal differentiation, excitability, mood and cognition) that are crucial for facilitating the adaptation of the body to acute or persistent stressful events (Koning et al., 2019). For instance, during the early stages of acute stress, MRs activation serves as an important mediator

event of memory appraisal and recovery processes, while the GR activity has complementary function facilitating memory consolidation and storage (de Kloet et al. 2005; Koning et al., 2021). However, in addition to complementary actions, it has been observed that MRs and GRs can also exert opposing and distinct effects in the brain. Particularly, it has been demonstrated that MRs activation stimulates the excitability of hippocampal CA1 neurons and enhances long-term potentiation (LTP), whereas the activation of GRs suppresses the excitability of the same neuronal population and induces long-term depression (LTD) (Pavlides et al., 1995; Joels et al., 2006; Koning et al., 2021). Particularly, several hippocampal functions (i.e., learning, memory, emotional regulation, and stress response) are dependent by MRs and GRs activity that can exert their actions either through slow genomic mechanisms, involving the interaction of this receptors with transcription factors, and through rapid nongenomic mechanisms which do not depend by gene expression regulation processes (Finsterwald and Alberini, 2014; Gray et al., 2017). For instance, some studies have reported an enhanced presynaptic glutamate release in the hippocampus, medial prefrontal cortex and amygdala through the nongenomic effects mediated by activation of MRs and GRs (Venero and Borrell, 1999; Finsterwald and Alberini, 2014); in other studies it has been instead observed that genomic effects mediated by GRs activation represent the mechanism by which corticosteroid hormone positively regulates fast glutamatergic neurotransmission in the hippocampus, increasing GluA2 containing AMPA receptors (Martin et al., 2009). Changes in the expression or function of glucocorticoid receptors have been associated with a large number of pathological situations (Koning et al., 2021). Interestingly, the clinical study of Patel and Colleagues (2016) has demonstrated that the

subjects with ASD display a significant decrease in mRNA levels of mineralocorticoid and glucocorticoid receptors in the middle frontal gyrus. Particularly, the altered expression profile of GRs and MRs could be responsible for a reduced sensitivity to glucocorticoids, insufficient negative feedback mechanism and HPA axis hyperactivity observed in ASD patients (Patel et al., 2016).

6. GLUTAMATERGIC NEUROTRANSMISSION AND STRESS

Chronic stress induces an excessive increase in glucocorticoid levels causing alterations in glutamatergic neurotransmission (Lowy et al., 1993; Popoli et al., 2011), which has been deeply implicated in the manifestation of the aberrant emotional and cognitive phenotypes observed in stress-related disorders (Maccari et al., 2014; Kadriu et al., 2019; Nasir et al., 2020). It has been reported that glucocorticoids affect the basal release of glutamate in the hippocampus, prefrontal cortex and amygdala (Lowy et al., 1993, 1995; Masneuf et al., 2014). Particularly, studies using patch-clump recordings have shown that the application of corticosterone to hippocampal slices significantly enhance the amplitude and frequency of excitatory postsynaptic potential in CA1 pyramidal neurons (Karst and Joëls, 2005). Rodents studies have also demonstrated that modulation of glucocorticoid levels following exposure to various acute stress manipulations (e.g., tail-pinch, forced swimming, or restraint stress) can rapidly increase extracellular glutamate levels in the prefrontal cortex and hippocampus (Moghaddam, 1993, 2002; Madalena and Lerch, 2017), leading to excitotoxic neuronal damage due to overstimulation of ionotropic glutamate receptors

(i.e., NMDARs, AMPARs) (Dong et al., 2009). Extracellular glutamate levels are determined by the exocytotic release mechanism, which is tightly regulated by presynaptic vesicles-associated proteins that, in response to stimulation, mediate neurotransmitter storage and vesicles fusion with the presynaptic membrane (Popoli et al., 2011). Some authors have investigated the modulatory role of presynaptic exocytotic machinery in the detrimental effects of acute and chronic stress on glutamate release. In principle, acute stress-induced potentiation of stimulus-induced glutamate release can be obtained through increasing the size of the readily releasable pool of synaptic vesicles docked to the active zone of presynaptic membrane and available for subsequent release of excitatory neurotransmitter (Lonart and Südhof, 2000; Matz et al., 2010). For instance, it has been observed that foot shock stress induces, at the level of the presynaptic machinery, a marked increase in the number of SNARE complexes bound to the presynaptic membrane of prefrontal cortex neurons (Musazzi et al., 2010). Extracellular glutamate levels are also finely modulated by membrane-bound transporters located on both on glial cells (EAAT1 and EAAT2) and on neurons (EAAT3 and EAAT4), which control the temporal dynamics of excitatory neurotransmission, quickly removing glutamate released during synaptic activity and thus avoiding the phenomenon of excitotoxicity (Pinky et al., 2018). For instance, it has been shown that adult rats subjected to different chronic stress protocols (i.e., noise, restraint stress, forced swimming, flashing light, isolation or immobilization) display a reduction of glutamate clearance in the hippocampus and prefrontal cortex (Olivenza et al., 2000; de Vasconcellos-Bittencourt et al., 2011). In addition to the impairment of glutamate release and reuptake processes, it has also been observed that stressful events exert their negative effects on ionotropic

glutamate receptors-mediated responses, inducing changes in the number, trafficking and mobility of these receptors at the level of postsynaptic membrane (Popoli et al., 2011). For instance, a growing body of preclinical evidence has shown that male rats subjected to chronic unpredictable stress (CUS) display a reduction in the cellular membrane expression of AMPA receptor GluA1 and GluA2 subunits in the hippocampus and prefrontal cortex and that injection of GR antagonist (RU486) can reverse the altered biochemical profile of AMPA receptor subunits (Li et al., 2011; Yuen et al., 2012, Kallarackal et al., 2013). Since AMPA glutamate receptors are the main fast transduction elements at synapses and are critical for the expression of the synaptic plasticity underlying at the memory and learning processes (Greger et al., 2017), it is not surprising that Caudal et al. (2010) have reported in rat that acute stress-induced disruption of long-term potentiation (LTP) in the dorsal hippocampus and medial prefrontal cortex is biochemically determined by the reduction of Ser831-GluA1 phosphorylation, thus decreasing the AMPAR channel conductance and potentiation of GluA1-mediated current (Caudal et al., 2010). In the last years, it has also been investigated stress-induced detrimental effects on metabotropic glutamate receptors-mediated excitatory neurotransmission. Particularly, it has been shown that in adult C57BL/6 mice, chronic (21-day) application of restraint stress (CRS) produces a reduction in hippocampal mGlu2 receptor expression in association with an increase in immobility time in the tail suspension test (Nasca et al, 2015b). Moreover, behavioral alteration and the status of hippocampal glutamatergic disequilibrium induced by chronic application of restraint stress (CRS) are abolished by treatment with acetyl-L-carnitine (LAC), which determines a rapid up-regulation of mGluR2 via modulation of histone acetylation of H3K27

linked to the Grm2 gene promoter (Nasca et al., 2013, 2017). Other study has also demonstrated that treatment with LY487379 -a selective positive allosteric modulator of mGluR2- is able to abolish the altered electrophysiological (i.e., increased frequency of excitatory postsynaptic current (EPSC) and behavioral (i.e., increased immobility time in the tail suspension test) effects observed in C57/BALB6 mice subjected to CRS (Mango et al., 2019). Therefore, mGluR2 has been identified as a marker of stress susceptibility (Nasca et al., 2015a, 2015b) and promoted to a molecular target of choice for various pharmacological strategies (i.e., acetylating agents, positive allosteric modulators) aimed at restoring the biochemical, behavioral, and electrophysiological alterations induced by stress paradigms. Overall, the stress-induced impairments of glutamate release, reuptake, clearance strategies and glutamate receptor's function could provide a pathophysiological mechanism at the core of the maladaptive responses observed in individuals with stress-associated psychiatric disorders (i.e., mood and anxiety disorders) (Popoli et al., 2011).

6.1 Glutamatergic system

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system (CNS) and it is generally recognized as the most important neurotransmitter for normal brain function (Zhou and Danbolt, 2014; Ribeiro et al., 2017; Crupi et al., 2019). Learning, memory, mood, neuronal maturation, and synaptogenesis are functions specific to the brain in which it has been shown to be directly and indirectly involved (Peng et al., 2011; Willard and Koochekpour, 2013; Jun et al., 2014; Ohgi et al., 2015). Recently, its potential function in the peripheral nervous system (PNS) has

also been considered, but there is much less knowledge currently accessible. (Chen and Kukley, 2020). Glutamate is widely and evenly distributed in the CNS and can be found in both glial and neuronal cells. Particularly, it is estimated that this neurotransmitter is released by more than half of all brain synapses (Purves et al., 2001). Indeed, almost all excitatory neurons are glutamatergic and most cells express at least one type of glutamate receptors (Purves et al., 2001; Pal, 2021). Particularly, hippocampus, prefrontal cortex, nucleus accumbens, striatum, thalamus are examples of brain regions with a predominant glutamatergic component (Garin et al., 2022). Since glutamate is unable to cross the blood-brain barrier, it must be produced directly in nerve tissue in order to exert its effects in CNS (Hawkins, 2009; Zhou and Danbolt, 2014; Andersen et al., 2021). Glutamate synthesis involves mainly the following metabolic pathways: i) glutamine deamination ii) glycolysis and the subsequent Krebs cycle allowing the conversion of glutamate and α-ketoglutarate occurring primarily by transamination reaction (Marmiroli and Cavalletti, 2012; Hertz, 2013; Andersen et al., 2021). Glutamate receptors, exchangers, and transporters are the main mediators of the glutamatergic neurotransmission system and work in concert within a tripartite synapse that is a point of communication between the presynaptic terminal, postsynaptic specialization, and the supporting glia that surround the synapse (Popoli et al., 2011; Nasca et al., 2017). Specifically, basal and stimulated presynaptic glutamate release, pre- and postsynaptic receptor trafficking and activity, transporter-mediated uptake, and glutamate recycling through the glutamate-glutamine cycle represent the main regulatory processes underlying the maintenance of glutamatergic synapse homeostasis (Popoli et al., 2011; Nicoletti et al., 2011; Moretto et al., 2018). Generally, glutamatergic neurotransmission is

mediated by two receptors classis: ionotropic receptors, which act as ion channels, and metabotropic receptors, which are linked to intracellular second messenger systems (Nicoletti et al., 2011; Marmiroli and Cavalletti, 2012; Moretto et al., 2018).

6.2 Ionotropic glutamate receptors

Ionotropic receptors are polymeric complexes resulting from the combination of 4 subunits that participate in forming an ion channel opened by interaction with glutamate (Traynelis et al., 2010). Particularly, they are divided into three functional subclasses based on their pharmacological profile and selective agonist: the N-methyl-D-aspartate receptors (NMDARs), a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs), and kainate receptors (KRs) (Krogsgaard-Larsen in 1980; Watkins and Evans, 1981; Marmiroli and Cavalletti, 2012). Over the past three decades, NMDARs have been the focus of much basic neuroscience research because of their key roles in the development and function of CNS. They are crucial mediators of the neuronal communication and processes underlying learning, memory, and neuroplasticity via the induction of long-term potentiation and long-term depression (Hunt and Castillo, 2012; Lüscher and Malenka, 2012; Paoletti et al., 2013; Li et al., 2016). NMDARs are glutamate-activated calcium ionophores, localized both on excitatory postsynaptic specializations and on presynaptic membranes where they may facilitate glutamate release (Köles et al., 2016). NMDARs are hetero-tetrameric protein complex deriving from arrangement of related pore-forming and auxiliary subunits encoded by seven homologous genes: GluN1, GluN2A-GluN2D and GluN3A-GluN3B

(Sanz-Clemente et al., 2013; Lee et al., 2014; Glasgow et al., 2015; Kumar and Foster, 2019; Zoicas and Kornhuber, 2019**)**. The expression and composition of these subunits determine the functional properties of native receptors and depend on the developmental age, brain region and disease states (Sanz-Clemente et al., 2013; Lee et al., 2014; Zoicas and Kornhuber, 2019). Since the activation of NMDARs involves the binding of two co-agonists, glutamate and glycine (or D-serine), most of these receptors consist of two GluN1 subunits, which are required for the formation of the binding domain for glycine and D-serine, and two GluN2 subunits, which are instead involved in the formation of the binding domain for glutamate (Hansen et al., 2018; Zoicas and Kornhuber, 2019). Moreover, NMDARs ion channel is highly permeable to Ca2+ and subject to a voltage-dependent block by extracellular Mg2+ (Hansen et al., 2018). NMDARs are considered important therapeutic targets for many CNS disorders. Indeed, NMDARs abnormal expression levels and altered function have been shown to be involved in numerous neurological and psychiatric disorders (Zhou and Sheng, 2013; Iadarola et al., 2015). Particularly, it has been observed that NMDARs hypofunction can result in cognitive defects in ageing brain, whereas their overstimulation induced by high Ca2+influx to the neuronal cell and increased glutamate extracellular, causes cell death, excitotoxicity and subsequent neurodegeneration (Blanke and VanDongen, 2009; Lin et al., 2014). AMPA receptors (AMPARs) are glutamate-gated ion channels responsible for the fast and immediate postsynaptic response to glutamate release (Diering and Huganir, 2018). AMPARs play an important role in neural circuit activity, synapse stabilization and long-term forms of synaptic plasticity (i.e., LTP and LTD), which are central events for the normal functioning of the brain (Katz and Shatz, 1996; Man, 2011; Gasbarri

et al., 2014). The AMPARs family is composed of four genes encoding the GluA1, GluA2, GluA3 and GluA4 subunits (Chater and Goda, 2022), which are widely distributed in different regions of the CNS such as nucleus accumbens, dorsal striatum, prefrontal cortex and hippocampus. These subunits can assemble in different combinations, giving rise to tetrameric receptor complexes of homomeric or heteromeric nature (Reimers et al., 2011; Gan et al., 2015). Specifically, GluA4 subunit is characterized by different distribution pattern with respect to other AMPARs subunits because it is primarily expressed in the early stage of the development and it is absent from the majority excitatory pyramidal neurons of adult brain (Zhu et al., 2000; Diering and Huganir, 2018; Chater and Goda, 2022). Electrophysiological, genetic and immunoprecipitation studies have demonstrated that hippocampal CA1 neurons are mainly enriched with combinations of GluA1/2 and GluA2/3 subunits, with a minor contribution of GluA1 homomers and GluA1/3 heteromers (Wenthold et al., 1996; Lu et al., 2009). Each AMPA receptor subunit has a modular structure comprising approximately 900 amino acids and containing four distinct domain layers which impact tetrameric assembly of AMPARs: i) large amino-terminal domain; ii) extracellularly ligand-binding domain; iii) a transmembrane domain consisting of four hydrophobic segments and iv) an intracellular carboxy-terminal domain (Gan et al., 2015; Yadav et al., 2016). Particularly, the N-terminal domain accounts for up to 45% of the mature polypeptide and is involved in the regulation of several receptor functions such as assembly, allosteric modulation and the desensitization state of the receptor. Normally, the activity of AMPARs results from the balance between several molecular processes including transcription, translation, mRNA level, protein stability, posttranscriptional and posttranslational

modifications (Yadav et al., 2016). It has been hypothesized that alternative splicing contributes to the functional heterogeneity of AMPA receptors-mediated fast synaptic transmission. Indeed, AMPA receptor subunits undergo, at the level of the extracellular ligand-binding domain, the process of alternative splicing, by which splice flip and flop variants are generated (Pei et al., 2009). In the brain, these variants have a differentiated cell- and age-dependent expression profile and influence the kinetic process of channel opening, desensitization properties as well as pharmacological profiles of AMPA receptors (Pei et al., 2009; Acosta et al., 2012). In addition, the subunit composition of AMPAR influences not only the affinity and kinetic properties, but also the ionic permeability of the channel, thus leading to receptors with *sui generis characteristics* (Guo and Ma, 2021). Interestingly, the permeability of AMPARs subunits to calcium ions is a consequence of the ADAR-dependent RNA editing process involving the Q/R site (Q607) of the GluA2 subunit. Specifically, GluA2 undergoes RNA editing at the Q/R site (Q607) that, leading to the conversion of a glutamine codon to an arginine codon, results in the selectivity of the pore to calcium ions (Diering and Huganir, 2018; Guo and Ma, 2021). Particularly, it has been observed that blockade of the GluA2 Q/R site editing causes the increased channel permeability to calcium, high Ca2+influx to the neuronal cell and, consequently, increased extracellular glutamate, neuronal cell death, excitotoxicity and neurodegeneration. Therefore, the altered RNA editing process involving the Q/R site (Q607) of the GluA2 subunit has a detrimental effect on neuronal function and this mechanism has been implicated in different neurological and neurodegenerative disorders, including epilepsy, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, ischemia (Costa Cruz and Kawahara, 2021; Guo and Ma,

2021). In addition to the alternative splicing involving the extracellular ligand-binding domain, AMPA receptor subunits undergo also alternative splicing also at the level of the intracellular C-terminal domains (tails), bringing to the formation of long and short tailed subunits that are crucial for the assembly, regulation of the trafficking and postsynaptic cellular surface expression of receptors (Henley and Wilkinson, 2013). Overall, AMPARs cannot be considered static components of glutamatergic neurotransmission, and this is evidenced by their continuous externalization/internalization at the level of postsynaptic specialization under basal conditions and in response to glutamate release-dependent neuronal activity (Hanley, 2018). Particularly, the trafficking of AMPARs complex is highly regulated and represents a key mechanism underlying activity-induced changes in fast glutamatergic neurotransmission (Anggono and Huganir, 2012). The receptors complex assembly in the endoplasmic reticulum (ER) is the first step of this complex process that is regulated by several auxiliary proteins such as the transmembrane AMPAR regulatory proteins (TARPs), γ-2 (stargazin), γ3-8 and the cornichon like proteins CNIH2/3. Moreover, the externalization/internalization and consequent postsynaptic cellular surface expression levels of AMPARs are controlled by important molecular mechanisms, including PDZ domain-mediated interactions between channel subunits and synaptic scaffolding proteins and clatrin-dependent endocytosis regulated by phosphorylation (Wang, 2008; Milstein and Nicoll, 2009). Particularly, phosphorylation of AMPARs is an important protein kinases (Ca2+/calmodulin-dependent protein kinase II (CaMKII), PKC) activity-dependent post-translational modification modulating the electrophysiological, morphological (externalization and internalization trafficking and clustering), biochemical

(synthesis and subunit composition) properties of the receptor, protein-protein interactions between the AMPA receptor subunits and various intracellular interacting proteins as well as the AMPARs-dependent synaptic plasticity mechanisms (Wang et al., 2005; Chater and Goda, 2014). Generally, decreased activity of AMPARs results in the long-term depression (LTD) of glutammatergic synaptic strength, whereas their increased activity leads to long-term potentiation (LTP) at glutammatergic synapses (Anggono and Huganir, 2012; Niescier and Lin, 2021). KRs are a subfamily of ionotropic glutamate receptors mediating excitatory fast neurotransmission and playing an important role in regulating neuronal networks excitability (Contractor et al., 2011). Five subunits of KRs has been identified (Gluk1, GluK2, GluK3, GluK4 and GluK5) and they can be co-assembled in different combinations, giving rise to tetrameric receptor complexes of homomeric or heteromeric nature (Contractor et al., 2011; Lauri et al., 2021). KRs are expressed in different regions of the CNS such as striatum, globus pallidus, substantia nigra pars compacta, cortex, hippocampus, cerebellum and, depending on the cellular type considered, they can be positioned at distinct sites and specifically to presynaptic, postsynaptic and extrasynaptic sites, regulating different aspects of excitatory synaptic transmission and neuronal plasticity (Jin and Smith, 2011; Evans et al., 2019). For instance, presynaptic KRs are important modulators of the release activity of both excitatory (i.e., glutamate) and inhibitory (i.e., gamma-aminobutyric acid, GABA) neurotransmitters (Evans et al., 2019). In this regard, it has been shown that presynaptic KRs activation elicits a decrease in glutamate release from rat hippocampal synaptosomes and induces depression of GABA release from the CA1 region of the hippocampus, reducing, respectively, excitatory and

inhibitory postsynaptic currents (Chittajallu et al., 1996; Frerking et al., 2001; Rodriguez-Moreno and Lerma, 1998; Evans et al., 2019). Instead, postsynaptic KRs are implicated in the induction of synaptic plasticity mechanisms at the core of learning and memory processes. Particularly, it has been observed that postsynaptic KRs activation elicits postsynaptic AMPARs-dependent LTP on CA1 hippocampal neurons via a metabotropic PKC and PLC dependent pathway (Petrovic et al., 2017; Evans et al., 2019).

6.3 Metabotropic glutamate receptors

The discovery of metabotropic glutamate receptors dates back more than three decades through the pioneering work of the research groups of Sladeczek, Nicoletti and Sugiyama (Sladeczek et al., 1985; Nicoletti et al., 1986a, 1986b; Sugiyama et al, 1987). mGluR1 was the first metabotropic glutamate receptor (mGlu1 or mGluR1 receptor) to be characterized and to date 8 metabotropic glutamate receptors have been identified (Masu et al, 1991; Crupi et al., 2019). mGluRs are G-protein coupled receptors (GPCRs) playing a key role in the modulation of neuronal excitability, synaptic transmission and plasticity and being categorized into three groups based on their sequence homology, second messenger signaling pathways and pharmacological profiles (Nicoletti et al., 2011; Peterlik et al., 2016). The first subgroup (Group I) includes mGluR1 and mGluR5 and is predominantly coupled to Gq/G_{11} proteins and inositol cycle activation; the second subgroup (Group II) includes mGluR2 and mGluR3, whereas the third group is composed by mGluR4, mGluR6, mGluR7 and mGluR8. Both group II and group III receptors are coupled to Gi proteins (Wang and Zhuo, 2012; Crupi et al., 2019). mGluRs have a complex modular structure consisting of

the following domains: (1) a large extracellular N-terminal domain, called the Venus flytrap domain (VFD), which contains the recognition site for glutamate and other orthosteric ligands; (2) a domain rich in cysteine residues that connects the extracellular N-terminal portion to the transmembrane region; (3) highly conserved seven-pass transmembrane domain (TMD) that is involved in receptor assembly and activation processes; (4) an intracellular carboxy-terminal region, which is important for modulating G protein coupling and for the regulation of receptor structure and activity (Niswender and Conn, 2010; Nicoletti et al., 2011; Thibado et al., 2021). All receptor subtypes can form dimeric structures of homomeric or heteromeric nature having distinct functions and properties (Doumazane et al., 2011; Levitz et al., 2016; Bodzęta et al., 2021). Particularly, mGluRs subtypes of the same group and coupled to the same G proteins can form heterodimers that are required for glutamate-driven G-protein activation. mGluRs are expressed in different regions of the CNS such as cerebellar cortex, olfactory bulb, striatum, hippocampus and cerebral cortex and the same neuronal population may contain different receptor subtypes (Ferraguti and Shigemoto, 2006; Crupi et al., 2019). Particularly, mGluRs are positioned at distinct subcellular sites and specifically to presynaptic, postsynaptic and extrasynaptic sites of the neuronal and glial membranes and the final outcome of their modulation activity can be either activation or inhibition of neuronal excitability pattern (Nicoletti et al., 2011; Maksymetz et al., 2017; Bodzęta et al., 2021). For instance, group I mGluRs are associated with postsynaptic specialization of excitatory synapses and their activation usually leads to intracellular Ca^{2+} release-dependent depolarization and an increase of the neuronal excitability; conversely, group II and group III mGluRs are respectively

localized on presynaptic terminals and active zone of neurotransmitter release and their stimulation results in the inhibition of cAMP, voltage-gated calcium entry into the cell and consequent release of glutamate the synaptic cleft (Mannaioni et al., 2001; Byrnes et al., 2009; Nicoletti et al., 2011). Given their wide distribution in the CNS and their involvement in the pathophysiology of stress-related disorders, the pharmacology of mGluRs has been the subject of intense research in recent years. Indeed, current preclinical pharmacological studies targeting specific subtypes of mGluRs have identified promising and effective pharmacological tools for restoring normal behavioral and synaptic functions and neuronal activity that are impaired in animal models of neuropsychiatric and stress-related disorders (Nasca et al., 2017; Bruno et al., 2017; Witkin et al., 2022).

6.4 Transporters system of glutamate

In the cytoplasmic compartment of glutamatergic neurons glutamate concentrations are extremely high (5-10 mM), however in the extracellular spaces of the CNS this amino acid is present at much lower concentrations (Featherstone, 2010; Moussawi et al., 2011; Andersen et al., 2021). One of the distinctive characteristics of the glutamatergic system is the lack of enzymes that can significantly breakdown glutamate, avoiding its accumulation in the extracellular spaces and consequent excitotoxic neuronal death (Nasca et al., 2017; Pajarillo et al., 2019). Indeed, the maintenance of glutamatergic homeostasis can largely be attributed to the presence of active glutamate transport system localized on glial cells and neurons (Nasca et al., 2017; Andersen et al., 2021). Glutamate transporters are members of the Large Solute Carrier (SLC) family, playing an important

role in the mechanisms of quenching glutamatergic signaling at excitatory synapses in the CNS (Cesar-Razquin et al., 2015; Andersen et al., 2021). Indeed, they are important regulators of extracellular glutamate concentration since under physiological conditions they are capable of accomplishing the glutamate transferring from extracellular space to inside the neuronal cell using a secondary active transport mechanism (Andersen et al., 2021). To this aim, these transporters use the energy produced by Na^{+}-K^{+} ATPase and that stored in the ion gradients on either side of the plasma membrane (Guskov et al., 2016; Ruan et al., 2017). Between 1992 and 1997, through molecular biology techniques it has been possible to identify five different isoforms of glutamate transporters (or excitatory amino acid carriers) in the mammalian forebrain: GLT-1 (SLC1A2), GLAST (SLC1A3) and EAAT3 (SLC1A1) EAAT4 (SLC1A6) and EAAT5 (SLC1A7) (Andersen et al., 2021). Glutamate transporters are membrane-bound trimeric proteins and exhibit a highly specific pattern of expression and distribution within the CNS. Indeed, GLT-1 and GLAST are primarily expressed in glial cells (i.e., astrocytes, oligodendrocytes), whereas EAAT3 and EAAT4 are present primarily in neurons (Perego et al., 2000; Karki et al., 2013). Particularly, GLT-1 is the major glutamate transporter of the brain and is highly expressed in astrocytes, in excitatory presynaptic terminals (Rothstein et al., 1994; Chen et al., 2004) and together with GLAST in perisynaptic astrocytic processes (Lehre et al., 1995). The diversity and pleiotropic functions of these astrocytic glutamate transporters are determined by several molecular mechanisms, including epigenetic modifications, transcriptional regulation, RNA splicing and posttranslational modifications (PTMs) (Pajarillo et al., 2019). Finally, EAAT3 is expressed in neurons with a somadendritic distribution, while EAAT-4 and EAAT-5 are predominantly

expressed in Purkinje cells in the cerebellum, and in the retina, respectively (Andersen et al., 2021). It has been demonstrated that the astroglial GLT-1 and GLAST transporters play a determinant role in maintaining extracellular glutamate levels in the physiological ranges and, consequently, their expression and activity must be strictly regulated in order to avoid excitotoxicity phenomenon (Perego et al., 2000). Indeed, aberrant changes in their expression or function have been associated with excitotoxic neuronal damage and several pathological conditions (i.e., schizophrenia, autism spectrum disorders, cerebral ischemia, epilepsy) (Wilson et al., 2003; Pampliega et al., 2008; Parkin et al., 2018; Pajarillo et al., 2019). Therefore, these transporters can be considered an important target for active drugs in CNS pathology. For example, current pharmacological agents (i.e., ß-lactam antibiotics, estrogens, growth factors, and translational activators) that increase the uptake of glutamate from extracellular spaces by boosting the expression of GLAST/GLT-1 have been shown to be effective in preventing the neuropathology induced by excitotoxic neuronal damage (Pajarillo et al., 2019). In this context, it is important to point out that glutamate concentrations in the extracellular spaces of the CNS are also finely regulated by the cystine-glutamate exchanger (xc-), which is mainly expressed in astroglial cells. Specifically, the catalytic subunit xCt of the xc- system functions as a regulator in the homeostatic control of extracellular glutamate (Kalivas, 2009; Mahler et al., 2014; Massie et al., 2015; Reissner et al., 2015; Nasca et al., 2017). Indeed, xCT mediates the non-vesicular release of glutamate from glial cells (i.e., astrocytes and microglia), ensuring the maintenance of glutamate concentration in the physiological ranges, in a 1:1 exchange of cystine for glutamate (Nasca et al., 2017). Particularly, glial glutamate released from xc- to the periterminal axon activates a complex

network composed by Glt-1 transporters and mGlu2 receptors, which work in concert to ensure the maintenance of glutamatergic synapse homeostasis and, consequently, prevent damage resulting from elevated glutamate levels in extracellular spaces (Nasca et al., 2017). In the presynaptic terminal glutamate transported or synthesized within the cell is stored in neurons in special secretory vesicles in which it reaches particularly high concentrations (up to 60 mM) (Burger et al., 1989). It is thought that the glutamate transport into these vesicles is a crucial step in switching glutamate from its metabolic activity to its neurotransmitter function. Particularly, this process involves an energy-dependent system coupling glutamate accumulation with ATP hydrolysis and it is realized by the activity of specific transporters located on the membranes of synaptic vesicles: the vesicular glutamate transporters (vGLUTs) (Andersen et al., 2021). vGLUT 1, 2 and 3 are the three isoforms of vesicular transporters identified in the CNS of mammals, where they are widely distributed (Eriksen et al., 2020). Particularly, vGlu1 and vGlu2 are expressed in different brain regions such as hippocampus, cerebral cortex, amygdala, cerebellum, putamen, substantia nigra and thalamus (Du et al., 2020). Since the quantity of glutamate that is packed into the vesicles and released in the synaptic cleft to initiate excitatory neuronal activity depends on the expression levels of vGLUTs, it has been suggested that their activity is essential for normal synaptic function (Du et al., 2020). Indeed, the up- or down-regulation of their activity or expression has been implicated in the pathophysiological mechanisms of several neurological and psychiatric diseases (e.g., schizophrenia, Alzheimer's disease, Parkinson's disease, and epilepsy) (Martineau et al., 2017; Du et al., 2020).

7. GABAergic neurotransmission

Proper brain function is determined by the important modulation of glutamate in combination with gamma-aminobutyric acid (GABA) presiding over the neuronal membrane stability and the maintenance of inhibitory-excitatory balance. Particularly, the intimate interconnection between glutamate and GABA is evidenced by synthesis process of GABA, which occurs from glutamate through a decarboxylation reaction operated by the enzyme glutamate decarboxylase (GAD) (Li and Xu, 2008; Ghit et al., 2021). In the adult mammalian brain, GABA is the main inhibitory neurotransmitter and its signaling system has been implicated in several developmental processes and functions specific to the brain, including cellular proliferation, neuronal migration, synaptogenesis, learning, memory and regulation of mood (Petty, 1995; Wang and Kriegstein, 2009; Heaney and Kinney, 2016). GABA is widely distributed in cerebellum, thalamus, cerebral cortex, hippocampus, basal ganglia and it has been estimated that this neurotransmitter is released by about 25%-40% of the brain synapses (Young and Chu, 1990; Li and Xu, 2008). Depolarization of the presynaptic neuron triggers a series of reactions that lead to the release of GABA from the nerve terminal to the synaptic cleft, where it diffuses to the postsynaptic membrane, binding to GABA receptors (Ghit et al., 2021). Generally, GABAergic neurotransmission is mediated by the following receptor classis: ionotropic receptors ($GABA_A$ and $GABA_C$), which act as ion channels, and metabotropic receptors ($GABA_B$), which are heterodimers formed by GABAB1 and GABAB2 subunits and linked to intracellular second messenger systems (Li and Xu, 2008; Heaney and Kinney, 2016). Specifically, $GABA_A$ receptors are ligand-gated anion channels of a hetero-pentameric nature, arising from the arrangement of the following subunits:

α1-6, β1-3, γ1-3, δ, ε, θ, π, and ϱ1-3 (Rudolph and Knoflach, 2011). The integral channel of $GABA_A$ receptors is permeable to chloride ion, the neuronal electrochemical gradient of which, regulated tightly by the K^+/Cl^- symporter (KCC2) and the $Na^+/K^+/Cl^-$ cotransporter (NKCC1), influences the excitatory-inhibitory switch of GABA during CNS development (Li and Xu, 2008; Rudolph and Knoflach, 2011). In the CNS, the subunit composition of $GABA_A$ receptors changes depending on their subcellular localization, which can be postsynaptic or extrasynaptic in nature. Particularly, postsynaptic $GABA_A$ receptors, which are sensitive to the action of benzodiazepines, mediate phasic synaptic inhibition and are mainly composed by two α (1-3) subunits, two β subunits and one γ subunit (typically the γ2 subunit) (Rudolph and Knoflach, 2011). Conversely, extrasynaptic $GABA_A$ receptors, composed by α (4-6) and δ subunits, are important regulators of tonic inhibitory response and are mainly targeted by ethanol, neurosteroids, and general anesthetics. Dysfunctions of the GABAergic neurotransmission system may contribute to the initiation of the pathophysiological mechanisms underlying the onset of neuropsychiatric and neurological disorders (Ghit et al., 2021). Therefore, the development of drugs targeting specific GABA receptor subtypes could offer a promising pharmacological tool able to restore the altered activity of neuronal inhibitory circuitries observed in these disorders.

EXPOSURE TO STRESS DURING CRITICAL PERIODS

8. Postweaning social isolation (PWSI)

8.1 Postweaning social isolation and social interaction

Social interaction during adolescence is crucial for the proper development of competent social behavior during adulthood (Goodell et al., 2017). Social behaviors represent a range of behaviors that take place when two or more members of the same species interact with each other (Wilson and Koenig, 2014) and depend on a variety of variables such as the individual's social background, traits of social partners and the testing environment (Goodell et al., 2017). Rodents used in preclinical research are considered highly social animals and generally prefer group life. Particularly, in the stage between weaning and early adulthood, there is generally a marked increase in social interaction among conspecifics, the most characteristic expression of which is social play (van Kerkhof et al., 2013). Over the course of development, this particular form of socialization shows a profile characterized by an inverted U-shaped curve that peaks during the juvenile/early adolescent stage and tends to gradually decrease after sexual maturation (van Kerkhof et al., 2013; Tanaka et al., 2019). When two or more conspecifics are introduced to a new environment, they interact with each other, performing a range of social behaviors (i.e., pouncing, wrestling, biting anogenital sniffing and social grooming) that may be measured quantitatively (Wilson and Koenig, 2014). Social interaction in rodents can be analyzed using several experimental tests that measure social affiliation behaviors as indicators of sociality (Matsumoto et al., 2019; Kim et al., 2019).

Tests include a modified version of the novel object recognition test, the 3-chamber test, the open field social interaction test, and a social interaction test based on resident intrusion (Matsumoto et al., 2019; Kim et al., 2019). To date, studies conducted in rodents have not conclusively clarified the effect of PWSI on social interaction behaviors. Lukasz and Colleagues (2013) have reported the lack of effect of PWSI on social interaction behaviors (Lukasz et al., 2013), while other authors have observed the impairment of social interaction in rodents reared in social isolation during adolescence period. In this regard, it has been demonstrated that in male ICR mice (4 weeks old) PWSI for 2-4 weeks induces social interaction deficit (Koike et al., 2009; Okada et al., 2015; Fujiwara et al., 2017). In contrast to the studies just mentioned (Koike et al., 2009; Okada et al., 2015; Fujiwara et al., 2017), Ferdman and Colleagues (2007) have observed that social isolation induces an increase of social interaction in male Wistar rats and this effect was majorly considerable whether the isolation period early started (i.e., PND 21) (Ferdman et al., 2007). The duration of the isolation period, the strain of the rodent, as well as the variety of experimental tests employed to assay social interaction could be the variables that generate the lack of concordance observed among the mentioned studies. However, some studies have considered the PWSI dependent-increased social interaction as a consequence of accentuated levels of aggressive behavior (Wongwitdecha and Marsden, 1996; Liu et al., 2019), which was one of the first behavioral alterations observed in socially isolated male mice (Valzelli, 1973). Specifically, it was observed that during the period of social isolation, male mice of different strains (i.e., C57BL/6J, CD-1) showed significantly higher levels of aggression toward the male intruder in the novel environment, as evidenced by the short attack latency, increased tail biting, aggressive

grooming assessed in the social interaction test (Ibi et al., 2008; Dang et al., 2015; Liu et al., 2019).

8.2 Postweaning social isolation and resocialization

Social isolation induces a range of physiological, neuroendocrine, and behavioral alterations in both human and nonhuman species (Beery and Kaufer, 2015; Mumtaz et al., 2018). Since social buffering plays a key role in psychological and physiological well-being (Taylor et al., 2007; Silk et al., 2010), resocialization represents an important nonpharmacological strategy to ameliorate or reverse social isolation-induced disorders (Tulogdi et al., 2014; An et al., 2017). Over the past decade, several rodent studies have investigated the effects of resocialization on behavioral alterations observed in the socially isolated animal during the postweaning period (Tulogdi et al., 2014; Seffer et al., 2015; Makinodan et al., 2017; Rivera et al., 2020a, 2020b; Begni et al., 2020; Kim et al., 2021). Particularly, resocialization, which is a laboratory analogue of behavioral therapy, has been observed to restore anxiety-like behavior, depressive-like behavior, sleep-related huddling, and spatial memory impairment in socially isolated rodents (Kokare et al., 2010; Tulogdi et al., 2014; An et al., 2017; Rivera et al., 2020a, 2020b). However, some studies have also reported that increased levels of aggression, reduced social interaction, and hyperlocomotion observed in socially isolated rodents are resilient to the resocialization procedure (Tulogdi et al, 2014; Begni et al, 2020; Kim et al., 2021), while in other works it has been demonstrated that resocialization is non pharmacological strategy able to alleviate these behavioral deficits (An et al., 2017; Makinodan et al., 2017). These conflicting results could be explained in part

by differences in experimental conditions, including the severity of the social isolation procedure, duration, and mode of resocialization (Seffer et al., 2015; Makinodan et al., 2017), and also suggest that different behavioral deficits induced by post-weaning social isolation may respond differently sensitivities to the same treatment (Tulogdi et al, 2014).

8.3 Postweaning social isolation and adrenocortical stress activity

Several studies have investigated the effects of PWSI on HPA axis functioning by measuring basal and stress-induced release of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (Rivera et al., 2020a). Most early experiments in male rats showed that basal corticosterone levels are not affected by social isolation, regardless of animal strain, starting point, and duration of social isolation (Moore, 1968; Morinan and Leonard, 1980; Gentsch et al, 1981; Viveros et al, 1988; Schrijver et al 2002; Scaccianoce et al, 2006); moreover, these findings have also been confirmed in more recent studies (Pereda-Perez et al, 2013; Rivera et al, 2020a). Other works have reported that PWSI produces alterations in the functioning of the HPA axis by increasing (Hatch et al., 1965; Gamallo et al., 1986; Gambardella et al., 1994) or decreasing basal levels of CORT and/or ACTH (Sanchez et al., 1998) and increasing the stress-induced release of the two hormones (Weiss et al., 2004). The behavioral alterations observed in rodents reared in social isolation during the postweaning period have not always been associated with alterations in the HPA axis functioning (Weiss et al., 2004; Rivera et al., 2020a). However, in some cases, where observed, the picture of altered basal and stress-induced HPA axis

reactivity has also been indirectly associated with several behavioral alterations observed in socially isolated rodents (Fone and Porkess, 2008).

8.4 Postweaning social isolation and glutamatergic neurotransmission

Over the past 14 years, there has been an increasing amount of works characterizing the effects of social deprivation on the glutamatergic neurotransmission system (Fone and Porkess, 2008; Mumtaz et al., 2018). Particularly, it has been well documented that postweaning social isolation increases excitatory synaptic neurotransmission by altering the expression and function of glutamatergic receptors in the CNS (Mumtaz et al., 2018). Several studies have characterized the impact of chronic social isolation (2-3 months) on the genic and protein expression profile of ionotropic glutamate receptors (i.e, NMDA and AMPA receptors) (Shimizu et al., 2016). For instance, it has been reported that socially isolated mice display the up-regulation of AMPA receptor subunits GluA1 and GluA2 protein levels in the amygdala (Shimizu et al., 2016). Since in AMPA-type glutamate receptor GluA1 subunit-deficient mice a reduction in aggression levels has been observed (Vekovischeva et al., 2004), the biochemical outcome reported by Shimizu and Colleagues (2016) could partially explain the increase in aggressive behavior characterizing mice reared in isolation (Valzelli et al., 1973; Fone and Porkess, 2008). In addition, it has been observed that PWSI rats show alterations of NMDA receptors composition and expression profile, increasing the mRNA and protein density of NR2A subunit in the prefrontal cortex (Turnock-Jones et al., 2009) as well as the mRNA levels of NR2A and NR2B subunits in the ventral hippocampus

(Zhao et al., 2009; Chang et al., 2015; Chang et al., 2018). However, in contrast to the results above mentioned (Turnock-Jones et al., 2009; Zhao et al., 2009; Chang et al., 2015; Chang et al., 2018), it has also been reported that social isolation induces an alteration of glutamatergic neurotransmission tone by down-regulation of NMDA receptors (Mumtaz et al., 2018). Particularly, in situ hybridization analysis reported in the study of Hall and Colleagues (2002) have shown that chronic social isolation (8-9 weeks) induces a reduction of hippocampal GluN1 mRNA levels of Fawn hooded rats (Hall et al., 2002). In addition, other authors have studied the effects induced by social isolation on the expression profile of metabotropic glutamate receptors, which have recently been implicated in the regulation of social behavior (Zoicas and Kornhuber, 2019). For example, Melendez and Colleagues (2004) have shown that PWSI induces reduced efficacy of group I and II mGluRs in regulating extracellular glutamate levels in the PFC, and this reduction is accompanied by impaired working memory performance of socially isolated rats in a delayed alternation task in the T-maze (Melendez et al., 2004). In other study, Ieraci and Colleagues have shown that the 28-day social isolation paradigm induces a reduction of mGluR1 and mGluR2 in the prefrontal cortex but not in the total hippocampus of adult male mice (Ieraci et al., 2016). In particular, considering the role of group II mGluRs in locomotor activity, the authors have hypothesized that under-regulation of mGluR2 levels in the PFC of socially isolated mice may contribute to the hyperactivity observed in socially isolated mice (Ieraci et al., 2016). Taken together, these results provided partial elucidation of the potential role of glutamatergic neurotransmission in the molecular mechanisms underlying some PWSI-induced behavioral disorders in rodents.

9. Perinatal stress (PRS)

9.1 Perinatal stress and reactivity to novelty

Several rodents studies have investigated the effects of perinatal stress on offspring emotionality by using different behavioral analysis approaches, including the open field test (OFT) and elevated plus maze (EPM) (Weinstock, 2016; Roshan-Milani et al., 2021). Both tests make it possible to assess the emotional reactivity and exploratory motivation of the animal and, consequently, characterize the risk-taking behavior, i.e. the conflict between the animal's desire to explore a novel environment and its aversion to open/well-lit spaces (Seibenhener and Wooten, 2015; Kraeuter et al., 2019). It has been reported that young and adult PRS males display an increased incidence of defecation (Weinstock et al., 1992) and exhibit reduced exploratory behavior, spending more time in the corners and less time along the walls and the center of the OF (Weinstock et al., 1992; Van den Hove et al., 2005; Laloux et al., 2012). Generally, the reduced risk-taking behavior of the male offspring PRS observed in the OFT has been also confirmed by using the EPM. Indeed, several studies have shown that male PRS rats spend less time in the open arms of EPM with respect to controls (Vallée et al., 1997; Zuena et al., 2008; Laloux et al., 2012; Marrocco et al., 2012; Glombik et al., 2015; Gatta et al., 2018). However, in contrast to the previous studies reporting reduced exploratory behavior of male PRS offspring (Weinstock et al., 1992; Vallée et al., 1997; Van den Hove et al., 2005; Zuena et al., 2008; Laloux et al., 2012; Marrocco et al., 2012; Glombik et al., 2015; Gatta et al., 2018), some studies have demonstrated that adult male rats prenatally stressed show behavioral hyperactivity to novelty, as

evidenced by increased locomotor activity in a circular corridor (Deminiere et al., 1992) or by the increased number of visits in the Y-maze test during the first five minutes (Vallée et al., 1997). Since Y maze and circular corridor are environments characterized by an absence of animal refuge, it has been suggested that the hyperactivity to novelty of adult male PRS rats actually indicates increased escape behavior (Vallé et al., 1997). In addition, PRS has been observed to induce pronounced impairment of emotional state in offspring not only in infancy and adulthood but also in ageing, underscoring its detrimental programming activity on the developmental trajectory of offspring (Zuena et al., 2008; Marrocco et al., 2012; Laloux et al., 2012; Verhaeghe et al., 2022). The majority of early experiments investigating the impairment of emotional reactivity induced by PRS has been conducted in males and has excluded females (Weinstock, 2016), partly due to the potential estrogen interference (Hossuini et al., 2022). Indeed, the effect of PRS on emotional reactivity appears more controversial to define in female animals and these discrepancies make difficult the understanding of the sex-dependent impact of PRS on the offspring emotional reactivity (Roshan-Milani et al., 2021). Particularly, some works have reported that PRS reduces risk-taking behavior in females (Zohar and Weinstock, 2011; Zohar et al., 2015), while in other studies it has been observed that females are unaffected (Van den Hove et al., 2014; Iturra-Mena et al., 2018) or more protected by detrimental effects of PRS (Zuena et al., 2008; Darnaudery and Maccari, 2008; Verhaeghe et al., 2022). Of note, Zuena and Colleagues have shown a substantial sex effect in the outcome of PRS on emotional reactivity assessed in EPM, with females showing increased risk-taking behavior and males reduced risk-taking behavior in adulthood (Zuena et al., 2008). Interestingly, this sexually-dimorphic

behavioral profile persists until ageing (Verhaeghe et al., 2022), evidencing the long-term programming action of PRS on the offspring and its amplifying power of age-related dysfunction (Maccari and Morley-Fletcher, 2007).

9.2 Perinatal stress and cognitive behavior

Several rodents studies have investigated the effects of prenatal stress on offspring cognitive performance by using the Morris water maze test (Weinstock, 2016). Particularly, it has been observed that PRS impairs the spatial learning and memory retention in adult male rats in the MWM test (Lemaire et al, 2000; Weinstock, 2008; Schulz et al., 2011; Golubeva et al., 2015) and this altered behavioral profile has been associated with a decrease in hippocampal neurogenesis and long-term potentiation (LTP) as well as with an increase in long-term depression (LTD) (Lemaire et al, 2000; Yeh et al., 2012). In contrast to the findings above mentioned (Lemaire et al, 2000; Weinstock, 2008; Schulz et al., 2011; Golubeva et al., 2015), other Authors have shown that PRS fails to affect learning of adult male offspring by using the same test (Bowman et al., 2004; Wu et al., 2007; Zuena et al., 2008). These conflicting results could be explained by the difference in experimental conditions, such us type, timing, duration and intensity of the stressor as well as the period of behavioral assessment (Weinstock, 2008; Roshan-Milani et al., 2021). Moreover, the majority of the early experiments have been performed in males and only a few of them have assessed the effect of prenatal stress on cognitive performance of females (Weinstock, 2016), where the results have been appeared more controversial. Indeed, some works have reported that PRS impairs spatial learning and spontaneous

spatial recognition in juvenile and adult females (Wu et al., 2007; Gue et al., 2004), while in others no effect has been found in adult females (Bowman et al., 2004; Weinstock, 2011). Only few researchers have assessed the effect of PRS on cognitive performance in both sexes. Particularly, Palacios-Garcia and Colleagues have reported that PRS determines a deficit in memory consolidation in the passive avoidance test in rats of both sexes (Palacios-Garcia et al., 2015); conversely, Zuena and Colleagues have revealed that adult PRS females show improved learning with respect to control group, arising the same levels of performance than male controls (Zuena et al., 2008) and displaying greater resilience to the effects of perinatal stress with respect to males (Weinstock, 2016). Moreover, most of rodents studies have focused on the PRS-induced cognitive deficits in adulthood and only few of them have also analyzed the old animals (Vallée et al., 1999; Verhaeghe et al., 2022). Interestingly, Vallée and Colleagues have shown that PRS has little effect on cognitive performance of adult males in the test of spontaneous space recognition (Y maze) but exacerbates the cognitive deficits of male rats during ageing (Vallée et al., 1999).

9.3 Perinatal stress and adrenocortical stress activity

It is well known that maternal glucocorticoid hormone exerts an important role in shaping the neurochemical and behavioral profile of the adult progeny (Angelucci et al., 1985). Therefore, changes in the activity of the HPA axis may represent the biological substrates of the detrimental effects of certain perinatal events. Particularly, high maternal corticosterone levels of pregnant stressed dams contribute to the long-term biochemical and behavioral alterations observed in the PRS offspring (Barbazanges et al.,

1996; Maccari and Morley-Fletcher, 2007). Prenatally stressed offspring is characterized by prolonged corticosterone secretion in response to stressful stimuli (Henry et al., 1994; Koehl et al., 1999; Maccari et al., 1995, 2003; Morley-Fletcher et al., 2003) that has been associated with reduced levels of both mineralocorticoid (MRs) and glucocorticoid (GRs) receptors in the hippocampus (Henry et al., 1994; Maccari et al., 1995; Van Waes et al., 2006). This altered activity pattern of HPA has been observed in infant (Henry et al., 1994), adolescent (Morley-Fletcher et al., 2003), adult (Maccari et al., 1995) and aged (Vallee et al., 1999) PRS animals. These results indicate an impairment of negative feedback control of HPA axis being MRs and GRs receptors the principal substrate of the control mechanism of glucocorticoids secretion (Reul and de Kloët, 1985; Sapolsky et al., 2000). HPA axis dysfunctions of PRS offspring can be reversed by an early postnatal manipulation such as enrichment (Morley-Fletcher et al., 2003) and early adoption of PRS offspring by unstressed mothers (Maccari et al., 1995). Indeed, it has been observed that adoption is able to reverse the effects of prenatal stress, increasing maternal behavior and decreasing the stress-induced corticosterone secretion peak in the adult PRS offspring (Maccari et al., 1995).

9.4 Perinatal stress, glutamatergic and GABAergic neurotransmission

In recent years, several studies have been conducted to shed light on the effects of PRS on glutamate release, clearance, and receptor system in rat offspring (Roshan-Milani et al., 2021). In particular, it has been observed that adult male PRS show selective reduction of glutamate release evoked

by depolarization in the ventral hippocampus, but not in the dorsal hippocampus and perirhinal cortex. No effects of PRS on spontaneous glutamate release have been found (Marrocco et al., 2012; Mairesse et al., 2015). Furthermore, it has been shown that PRS-induced abnormalities in glutamate release activity are a consequences of a selective reduction in presynaptic vesicle-proteins in the ventral hippocampus of adult males (Marrocco et al., 2012). In agreement with these findings, it has been reported that the glutamatergic signaling shutdown system is also affected in prenatally stressed offspring in a brain region-specific manner. Specifically, Zhang and Colleagues (2013) have shown that PRS significantly reduces mRNA levels of excitatory amino acid transporters in the PFC and hippocampus of adult males (Zhang et al., 2013). In addition to the PRS-induced alterations on the presynaptic exocytotic machinery and glutamate release, Maccari and Morley-Fletcher's research group have shown a marked sex-dependent difference in the effects of PRS on the expression profile of group I metabotropic glutamate receptors. Specifically, adult PRS females have shown to exhibit an increased expression profile of mGluR1/5 in the ventral and dorsal hippocampus, whereas adult PRS males have exhibited, at the level of the same brain region, reduced protein levels of mGluR1/5 (Morley-Fletcher et al., 2011). Furthermore, the reduced mGluR5 protein levels of male PRS have been observed not only in adulthood but also in infancy (i.e., PND 14 and PND 22) and in ageing (Zuena et al., 2008; Laloux et al., 2012; Verhaeghe et al., 2022). In contrast to the expression profile of group I mGluRs, no sex-dependent effects of PRS on the protein levels of group II metabotropic glutamate receptors have been shown. However, similar to what has been observed for mGluR1/5, alterations in the expression profile of mGluR2 and

mGluR3 have been observed throughout the lifespan of prenatally stressed rats. In particular, mGlu2/3 receptors have been shown to be decreased in the ventral hippocampus of PRS pups at PND22 (Laloux et al., 2012) and in adult and aged PRS rats of both sexes (Zuena et al., 2008; Verhaeghe et al., 2022). Taken together, these results suggest that PRS-induced programming effects could be driven by the profound impairment of glutamatergic neurotransmission observed in prenatally stressed offspring. Indeed, it has been shown chronic treatment of PRS male rats with antidepressants, oxytocin and S 47445 (a positive allosteric modulator of AMPAR) enhance mGlu2/3 receptor protein expression and glutamate release in the ventral hippocampus by concomitantly correcting emotional, cognitive and social alterations (Morley-Fletcher et al., 2011; Marrocco et al., 2014; Mairesse et al. 2015; Morley-Fletcher et al., 2018). However, in this context it is important to point out that the proper brain function is determined by the maintenance of inhibitory-excitatory balance as well as by the modulation of glutamate in combination with GABA (Wu and Sun, 2015). Few works have explored the effects of perinatal stress on GABAergic system of the offspring. In contrast to the profound PRS-induced detrimental effects on glutamate release in the ventral hippocampus, PRS does not change the release activity of GABA in the same brain region of males (Marrocco et al., 2012; 2014, Morley-Fletcher et al., 2018). Moreover, the unchanged GABA release has also been observed in dorsal hippocampus, prefrontal and perirhinal cortex and striatum (Marrocco et al. 2012). However, the absence of effect of PRS on GABA release is not disregarded since there are no differences in ionotropic $GABA_A$ subunit expression in preweanling PRS rats in the hippocampus, despite an increased number of vocalizations in response to maternal separation (Laloux et al. 2012). Conversely to the

ionotropic GABA$_A$ subunit expression profile observed in the hippocampus, PRS males on PND22 display a reduced expression of the $\gamma 2$ subunit of GABA$_A$ in the amygdala, in which it has been also observed an increased expression of mGluR5. PRS-induced disruption of excitatory/inhibitory signaling balance has been associated with increased reactivity to novelty of male PRS rats in the OFT and EPM tests and might represent the biochemical mechanism at the core of the increased risk of PRS offspring to develop neurobehavioral disorders (Roshan-Milani et al., 2021). Moreover, in this context it is important to point out that GABA is an inhibitory neurotransmitter in the adult brain, but it exerts an excitatory action during the perinatal period (Roshan-Milani et al., 2021). Normally, GABA$_A$ receptors activation induces neuronal membrane depolarization and triggers calcium influx, regulating several important developmental processes, including cell proliferation, differentiation, synaptic maturation and cell death (Cherubini et al., 1991; Owens and Kriegstein, 2002; Wu and Sun, 2015). Particularly, it has been observed that perinatal stress affects early developmental processes by modulating negatively GABAergic neurotransmission, delaying GABAergic progenitors' migration to the developing cerebral cortex and methylation in cortical interneurons (Roshan-Milani et al., 2021).

AIM OF THE THESIS

The aim of my PhD project was to investigate the role of glutamatergic neurotransmission in the early-life stress induced impairments of emotional, cognitive, and social scaffolding. The study has been realized by using the models of postweaning social isolation (PWSI) in mice and perinatal stress (PRS) in rat. In the first part of my PhD project, I discuss the results obtained by a direct behavioral and biochemical comparison between PWSI C57BL/6N mice and inbred mouse strain BTBR, which is considered a putative model for the study of autism spectrum disorders (ASD). Particularly, the following experimental endpoints were the focus of my investigation: i) social behaviors (ultrasonic vocalizations and social investigation), which are not as well documented in PWSI mice as in BTBR mice; ii) hippocampal type-2 metabotropic glutamate (mGlu2) receptors for their involvement in the pathophysiology of stress-related disorders and the mechanisms of resilience to stress; iii) hippocampal glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) that are key regulators of corticosteroid signaling and cognitive and social domains (Caruso et al., 2022). Finally, since some studies have reported that resocialization induces recovery of some behavioral and biochemical alterations induced by PWSI (Makinodan et al., 2017), I have also investigated the potential recovery effect of resocialization on the biochemical profile impairments observed in PWSI mice. In the second part of my PhD project, I have employed the model of perinatal stress which recapitulates the behavioral features of stress-related disorders (Maccari et al., 2017). Given the tight association between stress-related disorders and perturbation in receptor systems mediating effects of excitatory and

inhibitory neurotransmission, I have explored the sex- and age-dependent effects of PRS on AMPA receptor GluA2/GluA3 and GABAergic $\alpha1$ subunits in the hippocampus (ventral and dorsal) and prefrontal cortex in association with the spatial memory and risk-taking behavior.

10. Postweaning social isolation and autism-like phenotype: a biochemical and behavioral comparative analysis

**Adapted by Caruso, A., Ricceri, L., Caruso, A., Nicoletti, F., Gaetano, A., & Scaccianoce, S. (2022a). Postweaning social isolation and autism-like phenotype: A biochemical and behavioral comparative analysis. Behavioural brain research, 428, 113891. doi: 10.1016/j.bbr.2022.113891*

10.1 INTRODUCTION

Social isolation in children is considered a predisposing risk factor for the development of midlife pathologies such as coronary heart disease, depression, and type-2 diabetes (Lacey et al., 2014; Holt-Lunstad, 2018). Animal studies, many of them performed in rodents, have investigated the biochemical correlates responsible for altered brain development and adult behavior which occur in response to postweaning social isolation (PWSI). Most of the effects of social isolation have been observed when isolation starts early (i.e., at weaning), and not when a social isolation procedure of comparable length is applied in the adult life (Ferdman et al., 2007). However, while most of the research has used postweaning as the starting point of the social isolation, duration has varied a lot across studies, ranging from 2 weeks to several months. Two weeks of PWSI in mice affected oligodendrocytes morphology in the medial prefrontal cortex, neuregulin (NRG) expression in the microglia (Makinodan et al., 2012; Ikawa et al., 2017), as well as social exploration (Makinodan et al., 2012), suggesting that the absence of direct social contact with conspecifics for a few weeks during the juvenile period may have profound long-term consequences on brain development. The translational value of PWSI for psychiatric disorders is

highlighted by a series of studies showing explorative hyperactivity, anxiety-like behavior, abnormal sensory gating (prepulse inhibition) (Fone and Porkess, 2008; Lukkes et al., 2009; Day-Wilson et al., 2006; Dalley et al., 2002), and impaired spatial learning and cognitive flexibility (Lu et al., 2003). The pharmacological and non-pharmacological interventions which can be offered to the disabilities related to neurodevelopmental disorder are limited and poorly functioning. Thus, as highlighted by Matsumoto and Colleagues (Matsumoto et al., 2019), the postweaning social isolation model in mice offers valuable insights into the pathophysiology of these disorders and paves the way to possible therapeutic strategies. In addition, neuroimaging studies have shown a reduced functional connectivity in frontal brain regions and an increased connectivity in the posterior brain region in PWSI rats 9 weeks after isolation recapitulating the core imaging alterations observed in neurodevelopmental disorders, such as schizophrenia, autism, and attention-deficit/hyperactivity disorders (Reinwald et al., 2018). It is well known that the hippocampus exerts a central role in learning and cognition. Moreover, its high levels of activity-dependent synaptic plasticity (Bannerman et al., 2014) and constant neurogenesis (Kuhn et al., 2018), confer to this brain structure sensitivity to experience as well as vulnerability to injury and disease (Bartsch, 2012). This scenario constitutes the rationale of studies aimed to elucidate the various aspects of hippocampal impairment in animal models of neurodevelopmental disorders (Li et al., 2019). The use of validated animal models of neurodevelopmental disorder is of remarkable importance when studying potential pharmacotherapeutic agents. This also applies to autism spectrum disorder (ASD). Among the several animal models used in ASD-related research, the BTBR T + tf/J mouse is a widely used one (Haratizadeh

et al., 2021; Meyza and Blanchard, 2017). In this study, we thought it could be meaningful to compare the neurobehavioral profile of PWSI C57BL/6 N mice with that of group-housed BTBR inbred mouse strain. As already mentioned, BTBR mice are considered a putative model of ASD because they show low levels of social approach associated with uncommon patterns of ultrasonic emission in response to social cues, and high repetitive self-grooming (Bolivar et al., 2007; McFarlane et al., 2008; Moy and Nadler, 2008; Roullet et al., 2011; Scattoni et al., 2008; Wöhr et al., 2011). The evidence that PWSI and BTBR mice show a similar profile in brain microglial markers, namely NRG expression (Ikawa et al., 2017), further encouraged a comparison between the two groups of mice. Unfortunately, at present, there is still no evidence-based pharmacological treatment that can be used for the core behavioral symptoms of ASD (i. e., impairments in social communication and restricted/repetitive behavior), and pharmacotherapy offers some efficacy in the control of associated comorbidities but not to treat core deficits (Eissa et al., 2018). In the pathophysiology of ASD, many neurotransmitters systems have been investigated. Among them, the glutamatergic system has received considerable attention (Fung and Hardan, 2015; Horder et al, 2018; Rojas, 2014; Zhang et al., 2020). The hippocampal contribution to the core symptoms of ASD has been hypothesized since this structure exerts a prominent role in social interaction, memory and spatial reasoning, functions that are impaired in ASD (Banker et al., 2021). In the present study, we have focused our attention on the putative presynaptic type-2 metabotropic glutamate (mGlu2) receptors in the hippocampus, on the status of adrenal steroid receptors in this structure of the limbic system, on plasma corticosterone concentrations, on the level of social interaction and

on ultrasonic vocalization pattern. The rationale for selection of experimental endpoints are: (1) mGlu2 receptors play a key role in the regulation of glutamate homeostasis by negatively modulating glutamate release from presynaptic terminals (reviewed by Nicoletti et al., 2011); hippocampal mGlu2 receptors have been linked to mechanisms of resilience to stress (Nasca et al., 2015b; McEwen et al., 2015a) and ligands for this class of receptors have demonstrated potentiality as treatment agents for neuropsychiatric disorders (Nicoletti et al., 2019); (2) hippocampal glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) mediate the negative feedback control of glucocorticoids on the hypothalamic-pituitary-adrenal axis, as well as the effects of glucocorticoids on cognition and emotion (Gray et al., 2017); (3) deficits in social behavior and ultrasonic vocalization are well established in BTBR mice (Scattoni et al., 2008; Scattoni et al., 2011), but less systematically documented after PWSI (Seffer et al., 2015; Keesom et al., 2017), and, therefore, a direct behavioral comparison between the two models cannot be drawn from the existing literature.

10.2 MATERIALS AND METHODS

Animals

Male C57BL/6N mice aged 21 days were purchased from Charles River (Italy). Upon arrival, mice were randomly separated into group housing (GH, 4 mice per cage) or individual housing (one mouse per cage) for 27 days. The socially isolated mice were not handled more than once a week and were kept in the same husbandry conditions as the GH mice. Isolation-reared mice had visual, auditory and olfactory contact with other isolation

reared and group-housed mice kept in the same husbandry conditions. Male BTBR male mice, born in the BTBR colony at Istituto Superiore di Sanità (ISS, Rome, Italy) were weaned at the age of 21 days and socially housed (4 males per cage); for this strain, as controls male C57BL/6J mice (4 mice per cage) of similar age housed in the ISS facilities, were used. Female C57BL/6N and C57BL/6J mice (4 mice per cage) were used as partners for behavioral observations. In the same experiments, mice socially isolated from PND 21 to PND 48 were subjected to one week of resocialization (from PND48 to PND55) according to the experimental protocol of Makinodan and Colleagues (Makinodan et al., 2017). The resocialization procedure was performed by transferring mice isolated from the day of weaning to a cage containing 3 mice of the same age and housed together since weaning (day 21). Mice were kept on a 12 h/12 h dark/light cycle with a room temperature of 21±2 °C and humidity of 55 ± 5% and were given free access to water and food. All the biochemical and behavioral studies we performed in subjects at 48 days of age and conducted from 9.00 to 12.00. All studies were performed in agreement with the European and Italian guidelines on animal care (2010/63/EU; DL 26/2014).

Behavioral observations: male-female social interaction test

Two separate groups of 14 C57BL/6N mice socially isolated from weaning and 14 C57BL/6N mice GH from weaning were evaluated in a male-female encounter; as partners sexually-receptive C57BL/6N female mice aged 13 weeks were used. Behavioral tests were conducted in a standard polycarbonate cage (42 cm×27 cm×14 cm) under red light, video recorded using a Sony Handycam and subsequently analyzed with Noldus Observer X software (Noldus Information Technology). The same protocol was

applied to a separate group of 7-week-old BTBR male mice and C57BL/6J sexually receptive females. The day of male-female testing, vaginal estrous condition was assessed as previously described (Rugh, 1990); only those that were in estrus were used as stimulus mice. Each female was placed into the cage (sawdust 1.5 cm deep) of the male mouse (previously familiarized to the cage for 45 minutes) for a 3-min test session of male-female interactions. The video camera was mounted facing the side of the cage, to record the session for subsequent behavioral scoring. The ultrasonic microphone (Avisoft UltraSoundGate condenser microphone capsule CM16, Avisoft Bioacoustics) was mounted 20 cm above the cage, to record the session for subsequent ultrasonic vocalizations analysis. The ultrasonic microphone was sensitive to frequencies between 10 to 180 kHz. Vocalizations were recorded using Avisoft Recorder software version 3.2. Settings included sampling rate at 250 kHz; format 16 bit. For acoustical analysis, recordings were transferred to Avisoft-SASLab Pro (Version 4.40) and a fast Fourier transformation (FFT) was conducted as previously described (Scattoni et al., 2011). Spectrograms were generated with an FFT-length of 512 points and a time window overlap of 75% (100% Frame, Hamming window). The spectrogram was produced at a frequency resolution of 488 Hz and a time resolution of 1 ms. A lower cut-off frequency of 20 kHz was used to reduce background noise outside the relevant frequency band to 0 dB. The quantitative analysis has been performed by visual inspection of the spectrogram using the interactively (section labels) function of the Avisoft software manually inserting section labels. Number of calls was computed by visual analyzing each spectrogram. Social interactions were scored from the video files for the frequencies and durations of the following behavioral responses performed

by the male mouse: anogenital sniffing (direct contact with the anogenital area), body sniffing (sniffing or snout contact with the flank area), nose to nose sniffing (sniffing or snout contact with the head/neck/mouth area), wall rearing up against the wall of the home cage, rearing, digging in the bedding, and grooming (self-cleaning, licking any part of its own body). No events of mounting, fighting or tail rattling were observed.

Western blot analysis

Mice were killed by decapitation and the hippocampi (whole, ventral and dorsal portions) and prefrontal cortices were dissected and stored at −80 °C. For western blot analysis, tissue was homogenized at 4 °C in 0.1% SDS-lysis buffer containing 1 mM of a cocktail of protease inhibitors (Sigma), 1 mM sodium orthovanadate, 50 mM sodium fluoride, and 10 mM β-glycerophosphate, pH 7.4 with an Ultra-turrax homogenizer. Homogenates were centrifuged at 13,000 × g at 4 °C for 20 min and the supernatant was used for protein determinations. Thirty μg of protein were resuspended in SDS-bromophenol blue reducing buffer containing 40 mM dithiothreitol and separated by electrophoresis on 8% SDS polyacrylamide gels, and later transferred to nitrocellulose membranes (Bio-Rad). Transfer was performed at 4 °C for 2 h in a buffer containing 25 mM TRIS, 192 mM glycine, and 20% methanol, at 360 mA. Filters were blocked 10 min at 4 °C in TTBS buffer (100 mM Tris–HCl; 0.9% NaCl; 0.1% Tween 20; pH 7.4) containing 10% non-fat dry milk and then incubated with gentle shaking with primary antibodies directed against mGlu2 receptor (rabbit monoclonal, 1/50,000, overnight at 4 °C; Abcam, cat. num. 150387), or β-actin (mouse monoclonal, 1/5,000, 1 h at room temperature; Sigma–Aldrich, cat. num. A5441), in TTBS buffer. After three washes with TTBS buffer, blots were incubated for 1 h at

room temperature with peroxidase-conjugated secondary anti-rabbit (Merck, cat. num. 12-348) or anti-mouse antibodies (Merck, cat. num.12-349). Immunostaining was revealed by enhanced chemiluminescence (Amersham Biosciences). Densitometric analysis of the immunoreactive bands was performed by Image J (NIH, Bethesda, MD, United States).

RNA isolation, reverse transcription and quantitative real-time PCR

Total RNA was extracted from tissues with TRI reagent (Sigma Aldrich) according to manufacturer's instructions and quantified by spectrophotometric analysis. RNA samples were digested with DNAse (Promega) and single strand cDNA was synthesized from 1 μg of total RNA using Superscript II (Promega) and random hexamers. Real-time PCR was performed on 20 ng of cDNA by using specific primers and SYBR Green Master Mix (Bioline) on an iCycler Biorad instrument (Hercules). Thermal cycler conditions were as follows: 10 min at 95 °C (polymerase activation) followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 15 s, extension at 72 °C for 15 s. Primers used were as follows:

- mGlu2 receptor forward 5′-TGGACCGCATCAACCGCGAC- 3′ and reverse 5′- CCACGGCTGAGTGAGGCACG-3′;
- xCT forward 5′-CATCTGCCCAGGATTGAGAT-3′ and reverse 5′-CTGTGAGCTTGCCTCACTGT-3′
- Glt-1 forward 5′-GGAAGATGGGTGAACAGGC-3′ and reverse 5′-TTCCCACAAATCAAGCAGG—3′
- GR forward 5′- AACTGGAATAGGTGCCAAGG - 3′ and reverse 5′-AGGAGAACTCACATCTGGT-3′;

- MR forward 5′- ATGGAAACCACACGGTGACCT - 3′ and reverse 5′-AGCCTCATCTCCACACACCAAG-3′;
- β-actin forward 5′- GTTGACATCCGTAAAGACC - 3′ and reverse 5′- TGGAAGGTGGACAGTGAG-3′.

The amount of mRNA was calculated from serially diluted standard curves simultaneously amplified with the samples and normalized with respect to β-actin mRNA levels.

Plasma corticosterone levels

Trunk blood was collected after decapitation and samples were centrifuged at 1900 g for 20 min at 4 °C. Plasma was stored at −80 °C and analyzed for corticosterone using ELISA kits (Arbor Assays) according to the manufacturer’s instructions.

Statistical analysis

Biochemical data were analyzed by T-test after Bartlett's test for the homogeneity of variances and Kolmogorov-Smirnov's test for the Gaussian distribution and One-way Anova followed by Tukey-Kramer Multiple Comparisons Test. Behavioral data were analyzed by non-parametric Mann-Whitney test. The results were considered to differ significantly at $p < 0.05$.

10.3 RESULTS

Behavioral analysis

Whereas PWSI and GH mice did not differ in the total time spent interacting with the female partner during the social interaction test ($U=64$, $n_1=n_2=14$, $p=0.1182$), the number of social investigation events was significantly lower in PWSI mice as compared to GH controls ($U=48$, $n_1=n_2=14$, $p=0.0216$). In addition, PWSI mice spent less time in investigating the head of the female partner (duration: $U=40$, $n_1=n_2=14$, $p=0.0077$; frequency: $U=22$, $n_1=n_2=14$, $p=0.0005$), and they emitted less ultrasonic vocalizations than GH mice during interaction ($U=38$, $n_1=n_2=14$, $p=0.0005$) (Fig. 1). Exploratory responses not directed to the social stimulus did not differ between the two groups of mice (data not showed). BTBR mice behaved similarly to PSWI mice showing reductions in head sniffing ($U=18.5$, $n_1=n_2=12$, $p=0.0020$) and ultrasonic vocalizations ($U=22$, $n_1=11$ $n_2=12$, $p=0.0068$) in the social interaction test, as compared to control mice (Fig. 2).

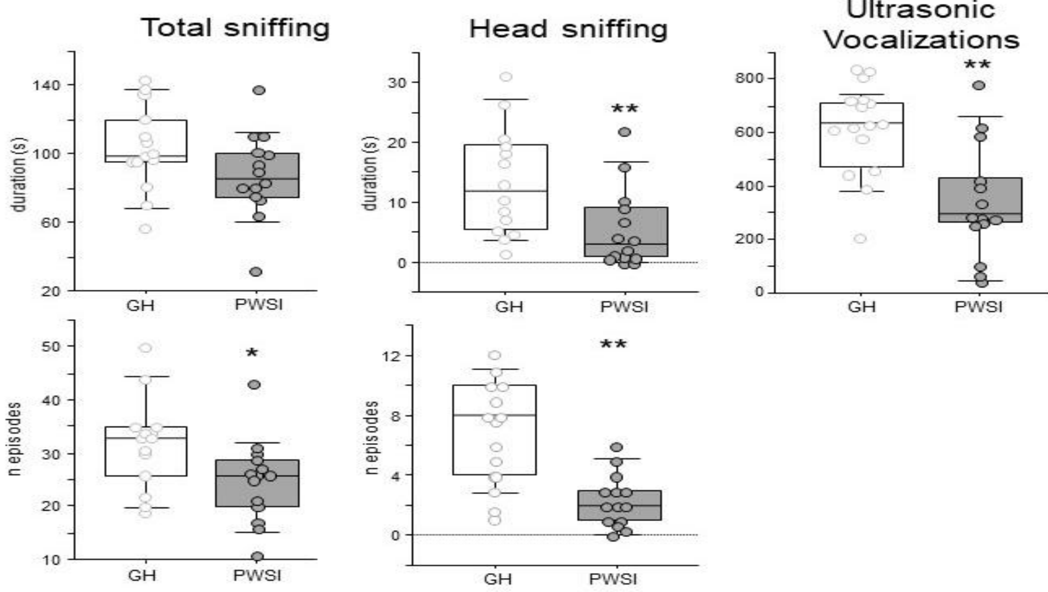


Figure 1: Male-Female social interaction test (total duration 3-min) in Group-housed (GH) and Post Weaning Social Isolation (PWSI) C57BL/6N mice. Duration and number of episodes of sniffing (total sniffing and head sniffing) and total number of ultrasonic vocalizations. Data are box-plots (25th-75th percentiles), with individual data. ** p<0.01, * p<0.05 after Mann-Whitney test.

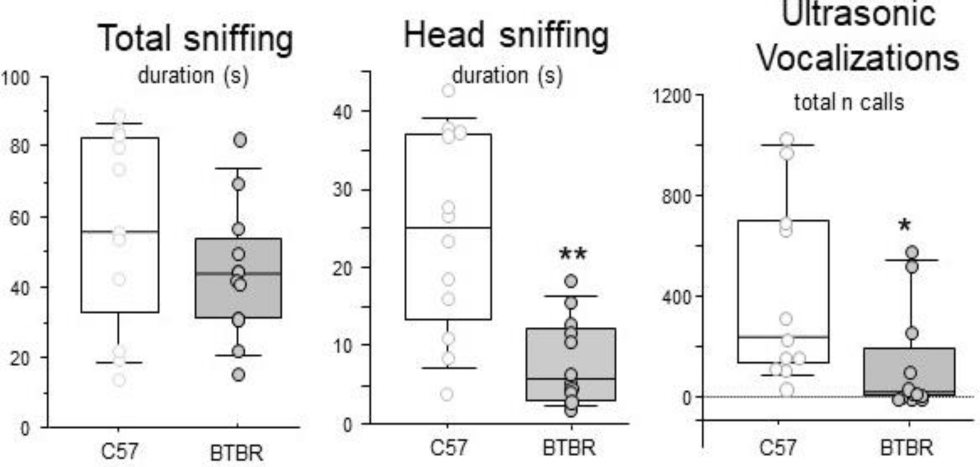


Figure 2: Male-Female social interaction test (total duration 3-min) in C57BL6/6J (C57) and BTBR mice. Duration of total sniffing and head sniffing and total number of ultrasonic vocalizations. Data are box-plots (25th-75th percentiles), with individual data. ** p<0.01, * p<0.05 after Mann-Whitney test.

Biochemical analysis

Western blot analysis of mGlu2 receptors showed the expected band at about 100 kDa corresponding to receptor monomers, and a higher molecular size band (not showed) corresponding to receptor dimers. A significant reduction in hippocampal mGlu2 receptor protein levels was found in PWSI mice and in BTBR mice (Fig. 3A and 3B, respectively), as compared to respective controls. Similar findings were obtained by measuring the transcript encoding mGlu2 receptors by quantitative mRNA analysis (Fig. 3C, 3D). We also measured mGlu2 receptor mRNA and protein levels in the prefrontal cortex, where we found no difference between PWSI or BTBR when compared to respective control mice (data not showed). Hippocampal receptors for glucocorticoid hormones were evaluated in the two experimental groups. As reported in fig. 4A and 4B, quantitative mRNA analysis of MR showed in both experimental groups a significant reduction when compared to respective control mice. Results of the same profile were obtained from quantitative mRNA determination of GR (Fig. 4C, 4D).

WHOLE HIPPOCAMPUS

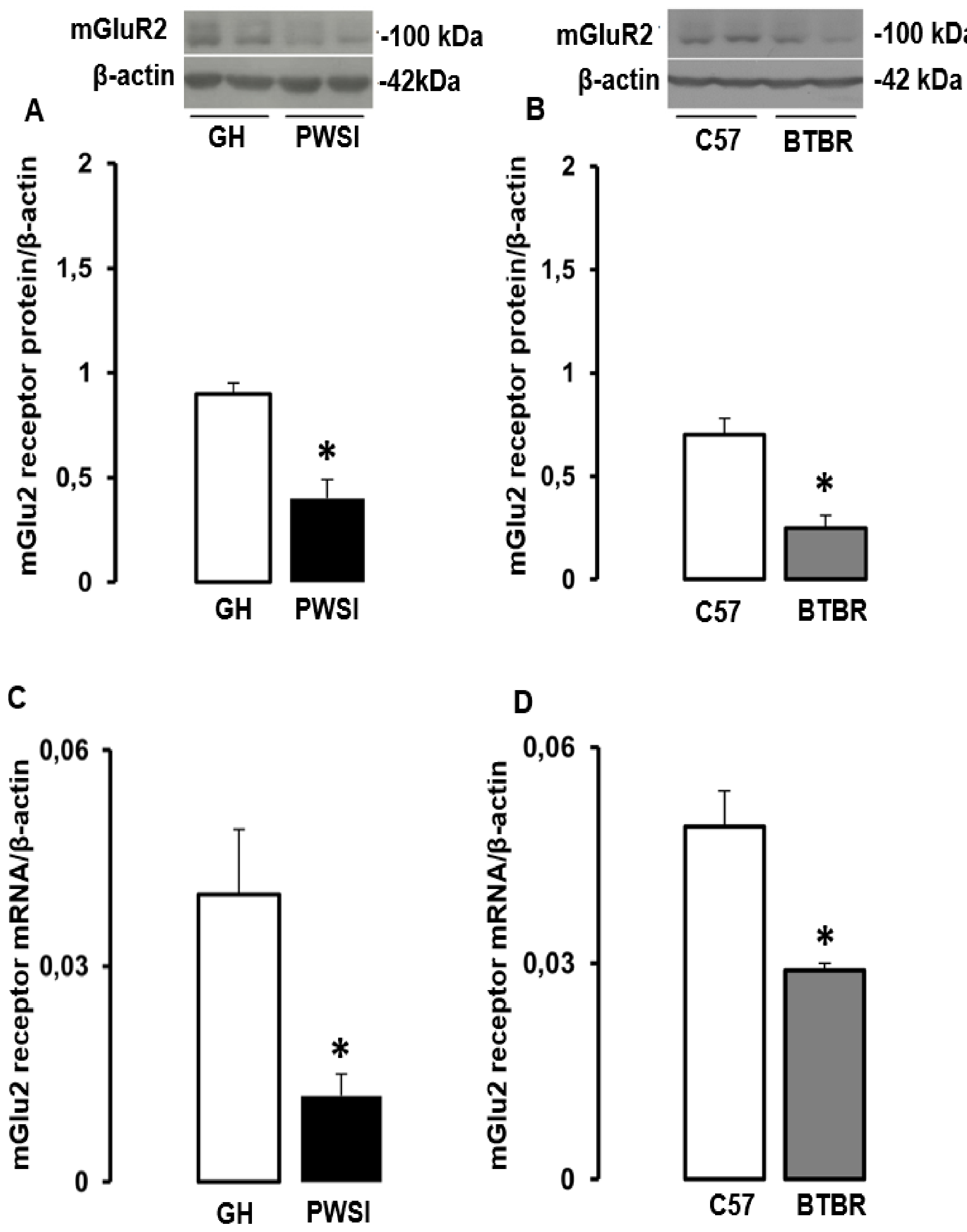


Figure 3: Results from group-housed (GH) and Post Weaning Social Isolation (PWSI) C57BL/6N mice are reported in panels A and C; results from C57BL/6J (C57) and BTBR mice are illustrated in panels B and D. (A, B) western blot analysis of hippocampal mGlu2 receptors; (C, D) mRNA levels of mGlu2 receptors in hippocampus. Data are expressed as mean ± S.E.M., (n = 5-6). $^{*}p<0.05$, $^{**}p<0.01$ after T test.

WHOLE HIPPOCAMPUS

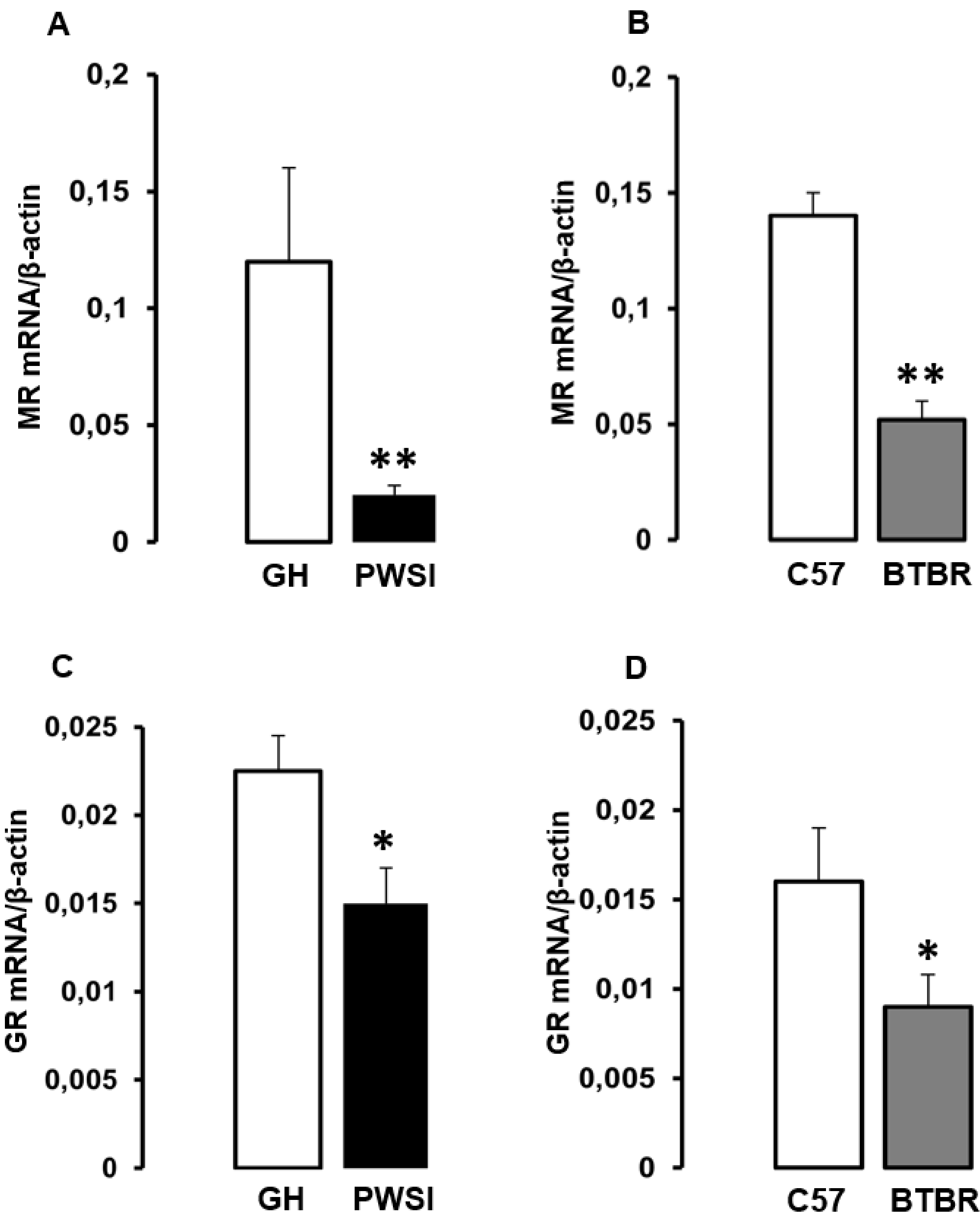


Figure 4: Results from group-housed (GH) and Post Weaning Social Isolation (PWSI) C57BL/6N mice are reported in panels A and C; results from C57BL/6J (C57) and BTBR mice are illustrated in panels B and D. (A, B) mRNA levels of MR in hippocampus; (C, D) mRNA levels of GR in hippocampus. Data are expressed as mean ± S.E.M., (n = 5-6). *p<0.05, **p<0.01 after T test.

Analysis of plasma corticosterone levels in basal conditions indicated no differences between group housed C57BL/6N and PWSI mice. On the contrary, in comparison to group housed C57BL/6J, BTBR mice were characterized by an increased concentration of glucocorticoid hormone (Fig. 5A, 5B).

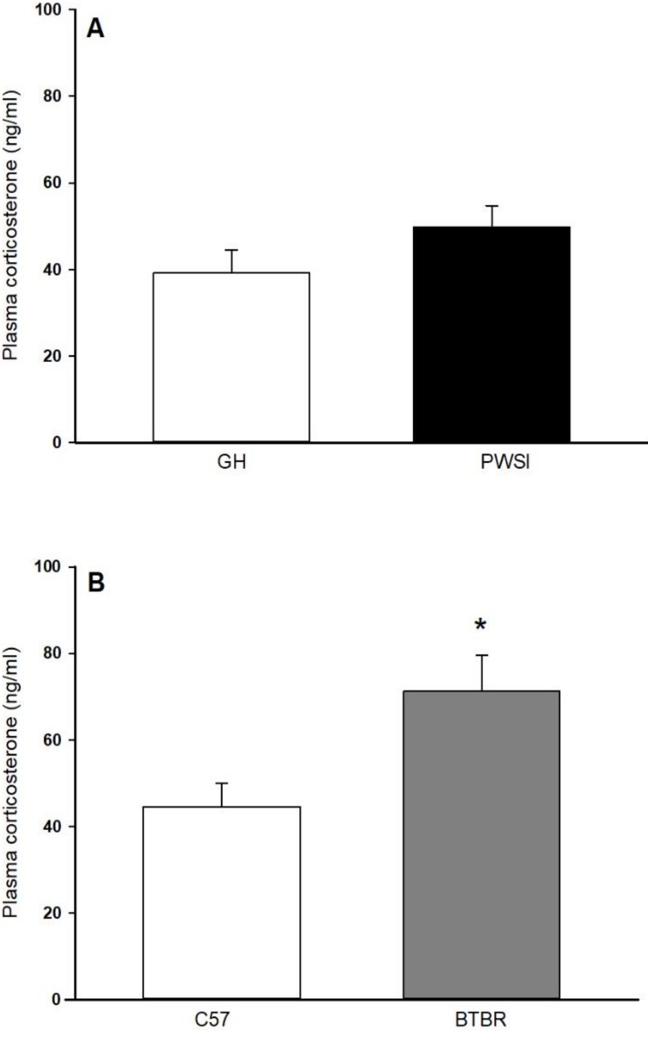


Figure 5: Plasma corticosterone levels in group-housed (GH) and Post Weaning Social Isolation (PWSI) C57BL/6N mice (panel A); C57BL/6J (C57) and BTBR mice (panel B). Data are expressed as mean ± S.E.M., (n = 6). *p<0.05 after T test.

Moreover, we extended our study by taking into consideration the two hippocampal subfields, dorsal and ventral, in evaluating the possible

reparative role exerted by resocialization on the status of hippocampal glutamatergic disequilibrium induced by postweaning social isolation in male C57BL/6 strain mice. Biochemical analysis showed that mGlu2 receptor protein and mRNA levels were not altered by PWSI in the dorsal hippocampus (Fig. 6a: n=7, $F_{(2,18)}$=0,7194, p=0,5005; Fig. 6b: n= 7, $F_{(2,18)}$= 0,4863, p= 0,6227). In contrast, we observed that PWSI induced a selective reduction in mGlu2 receptors protein expression in the ventral portion of the hippocampus (Fig. 7a: n=7, $F_{(2,18)}$= 22,014, p= 0.00001). In the same brain region, similar results were obtained by measuring the transcript encoding mGlu2 receptors by quantitative mRNA analysis, indicating agreement between mRNA and protein levels (Fig. 7b; n= 7; $F_{(2,18)}$= 9,705; p= 0,0014). Moreover, we observed that resocialization is unable to restore the reduced protein and mRNA expression levels of mGluR2 in the ventral hippocampus (Fig. 7a: n=7, $F_{(2,18)}$= 22,014, p= 0.00001; Fig. 7b; n= 7; $F_{(2,18)}$= 9,705; p= 0,0014). We also measured mRNA expression levels of glutamate/cystine antiport xCt and glutamate transporter Glt-1 in the dorsal and ventral hippocampus, where we found no differences in PWSI mice compared with control mice (Fig. 6c: n= 5-7, $F_{(2,16)}$= 1,371 p= 0,2820; Fig. 6d: n=5-7, $F_{(2,16)}$= 2,161, p=0,1476; Fig. 7c: n=7, $F_{(2,18)}$= 0,2034, p= 0,8178; Fig. 7d: n= 7, $F_{(2,18)}$= 0,1499, p= 0,8618). Receptors for glucocorticoid hormones were assessed in the two hippocampal subregions of PWSI mice. Quantitative mRNA analysis revealed that PWSI induced a strong decrease in MRs density in the ventral hippocampus (Fig. 7e: n= 6-7, $F_{(2,17)}$= 7,923 , p= 0,0037), with no change observed in the dorsal subregion (Fig. 6e: n=5-7, $F_{(2,16)}$= 0,6076, p= 0,5568). Results of the same profile were obtained by quantitative determination of GRs mRNA, the expression levels of which are reduced in the ventral hippocampus of PWSI mice compared with the control group

(Fig. 7f: n=7; $F_{(2,18)}$=8,094, p= 0,0031); in contrast, no changes in GRs transcript density were observed in the dorsal hippocampus of PWSI mice (Fig. 6f; n= 5-7, $F_{(2,16)}$=0,6813, p=0,9344). Moreover, we observed that resocialization is unable to restore the reduced mRNA expression levels of MRs and GRs in the ventral hippocampus of PWSI mice (Fig. 7e: n=6-7, $F_{(2,17)}$=7,923, p= 0,0037; Fig. 7f: n= 7; $F_{(2,18)}$= 8,094, p= 0,0031).

DORSAL HIPPOCAMPUS

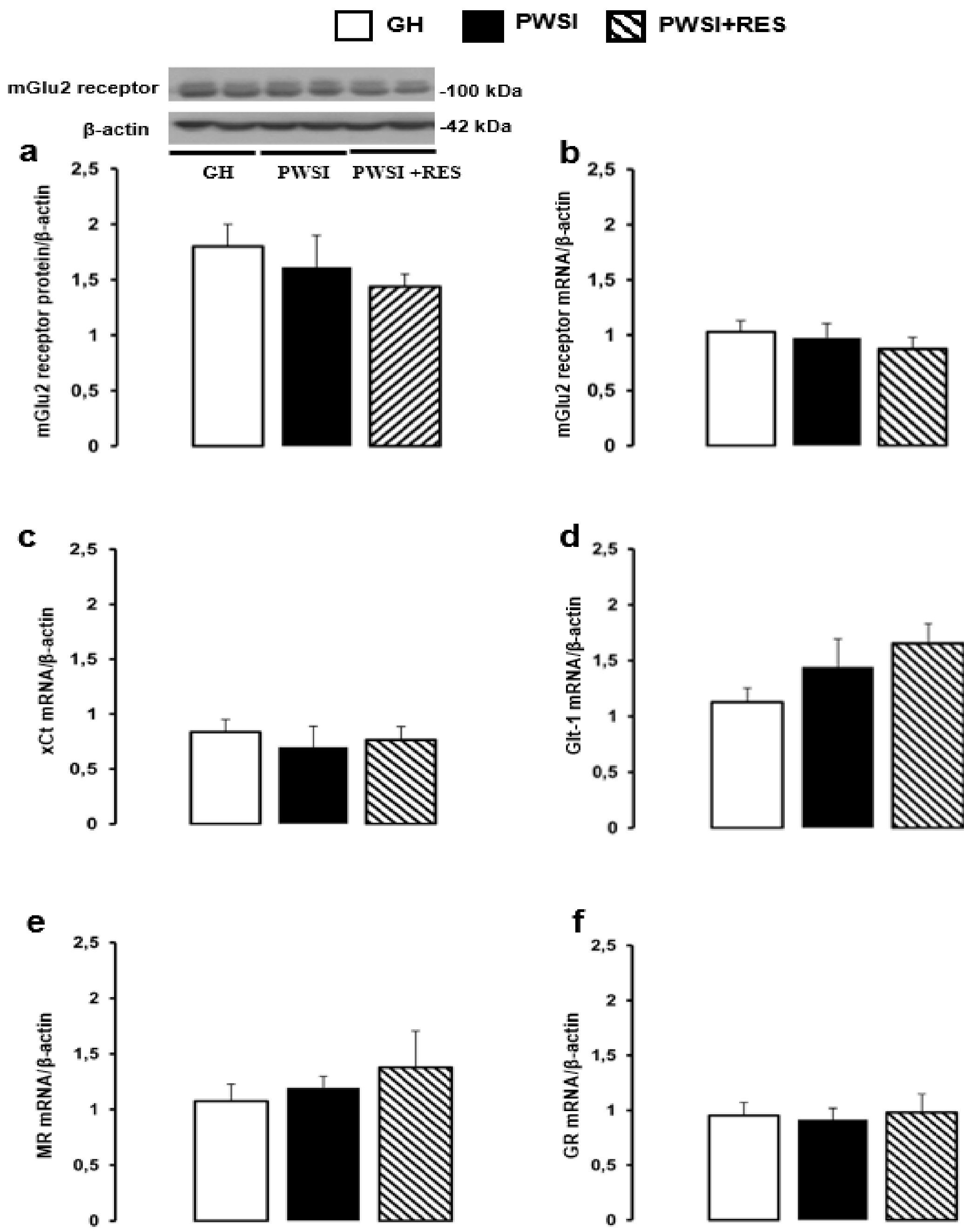


Figure 6: Western blot analysis of mGlu2 receptors (a), mRNA levels of mGlu2 receptors (b), xCt (c), Glt-1 (d), MRs (e) and GRs (f) in the dorsal hippocampus of Group-housed (GH), Post Weaning Social Isolation (PWSI) C57BL/6N mice and PWSI mice in resocialization from PND 48 until PND 55. Data are expressed as mean ± S.E.M., (n=6-7). *$p<0.05$, **$p<0.01$, ***$p<0.001$ after One-way Anova followed by Tukey-Kramer Multiple Comparisons Test. Data not published.

VENTRAL HIPPOCAMPUS

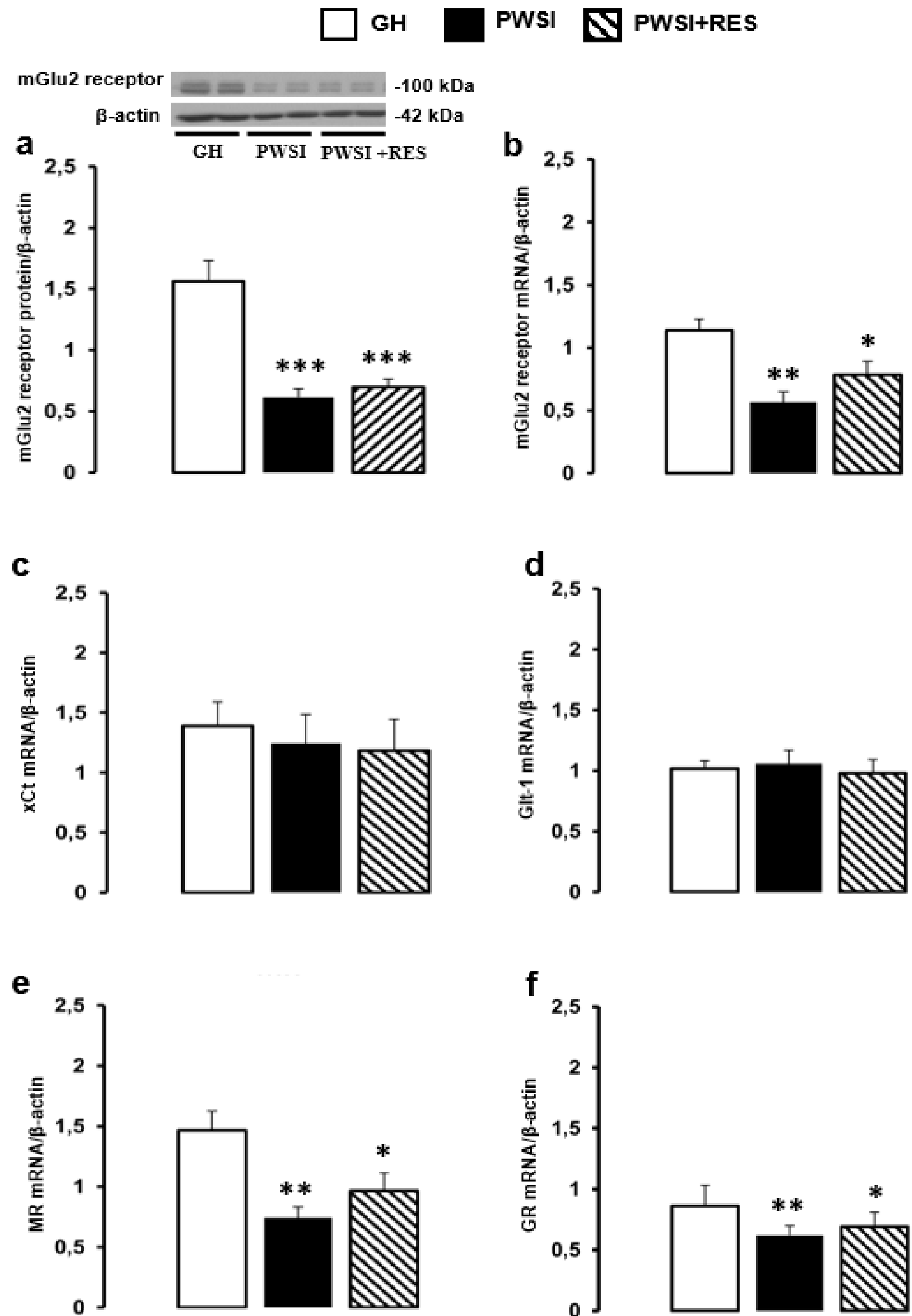


Figure 7: Western blot analysis of mGlu2 receptors (a), mRNA levels of mGlu2 receptors (b), xCt (c), Glt-1 (d), MRs (e) and GRs (f) in the ventral hippocampus of Group-housed (GH), Post Weaning Social Isolation (PWSI) C57BL/6N mice and PWSI mice in resocialization from PND 48 until PND 55. Data are expressed as mean ± S.E.M., (n=6-7). *p<0.05, **p<0.01, ***p<0.001 after One-way Anova followed by Tukey-Kramer Multiple Comparisons Test. Data not published.

10.4 DISCUSSION

Here, we offered a head-to-head comparison between PWSI mice and BTBR mice, showing that they exhibited similar behavioral and biochemical abnormalities with respect to control mice. These include the effect of postweaning isolation on male-female interaction, which has not been investigated so far in spite of the numerous behavioral studies exploring the effect of isolation on social interaction (Koike et al., 2009; Fone and Porkess, 2008). The social interaction test allows to assess social investigation and/or sniffing as well as vocalizations: both aspects appear altered. In both PWSI and BTBR mice we focused our attention on male subjects on the male-female social interaction test on the bases of the following considerations: i) the reproductive period and estrous cycle of mice commences about the 26th day after birth (Ajayi and Akhigbe, 2020), thus variations on the stage of the estrous cycle of subjects performing the female-female social interaction test could represent a factor inducing a high font of variability conferring to the results a difficult interpretation; ii) although the social interaction test has been validated behaviorally, physiologically, and pharmacologically in male rats, it is well known that efforts to develop an analogous test in mice have been limited because of the tendency of mice to produce aggression and the avoidance of interaction by the stimulus mouse (de Angelis and File, 1979); iii) regarding the use of male BTBR mice, it has been reported that children with autism have a sex ratio of 4:1 (male to female), and, accordingly, behavioral phenotyping of animal models ASD has primarily focused on male mice and the male-female interaction has been therefore the most popular interaction test to detect communication deficits in ASD mouse models at adulthood (Radyushkin et al., 2009; Bozdagi et al., 2010; Scattoni et al., 2011; Brielmaier

et al., 2012; Malkova et al., 2012; Rotschafer et al., 2012; Schmeisser et al., 2012; Yang et al., 2012). We observed a clear reduction of social investigation and vocalization rate in BTBR inbred male mice in agreement with previous studies (Scattoni et al. 2011; Scattoni et al. 2013); very similar alterations, including decreased vocalization rates, were also evident in PWSI mice. These findings strengthen the value of postnatal social isolation in mice as a model for neurodevelopmental disorders (reviewed by Mumtaz et al., 2018), and provide a remarkable example of how exposure to an adverse environment within a sensitive time window in juveniles, may lead towards a socially altered phenotype which includes a reduced social interaction resembling what is considered part of the core behavioral symptoms of ASD. We focused the biochemical analysis on corticosteroid and mGlu2 receptors expression. Compared to respective controls, hippocampal MR and GR mRNA levels were reduced in both PWSI and BTBR mice. The reduction in MR transcript found in PWSI is in agreement with data obtained in male Sprague-Dawley rats isolated after weaning for 30 days (Boero et al., 2018). In addition, a down-regulation of GRs in the hippocampus has been observed in 7-week-old male BALB/cJ mice isolated for 3 weeks (Liu et al., 2013). Changes in hippocampal glucocorticoid receptors have not been extensively studied in BTBR mice. Silverman and Colleagues (Silverman, 2010b) have shown an increased density of GR mRNA levels in the CA1 (but not CA2) hippocampal region of adult male BTBR mice, as detected by in situ hybridization. Methodological differences, as well as the different age of BTBR mice, may explain the discrepancy with our findings. Interestingly, reduced GR and MR mRNA and protein levels were observed in the middle frontal gyrus of individuals affected by autism spectrum disorder (Patel et al., 2016). The role of

hippocampal glucocorticoid receptors in mechanisms of neuronal plasticity linked to stress-related disorders has been the subject of excellent reviews (McEwen, 2004; Joëls, 2018). The emerging picture is that glucocorticoid receptors play a key role in promoting long-lasting adaptive protecting mechanisms, such as strategic planning and memory encoding and consolidation; moreover, homeostatic regulation of corticosteroid signaling exerted by glucocorticoid receptors, is critical for mental health. Regarding cognitive performance, it has been hypothesized that MRs are involved in the choice of coping style, appraisal processes, encoding, and retrieval, while GRs on memory consolidation (de Kloet et al., 2018). In line with this hypothesis, it has been demonstrated that an imbalance in the expression and/or function of glucocorticoid receptors are linked to the pathophysiology of psychiatric or neurodegenerative disorders (McEwen, 2005; Anacker et al., 2011; de Kloet et al., 2016; Caruso et al., 2019). In our study, the picture emerging from both experimental conditions (i.e., BTBR and PWSI) is of a strong reduction of both MRs and GRs ranging from a -32% (GRs in PWSI) to a -86% (MRs in PWSI). Regarding the BTBR strain, the reduced availability of these receptors, which mediate a negative feedback control on the hypothalamic-pituitary-adrenal axis activity and reactivity, could be responsible for the increase in basal plasma corticosterone levels observed in the present study, a finding in agreement with previous reports (Benno et al., 2009; Silverman et al., 2010; Frye and Llaneza, 2010). Moreover, it could be suggested that these hippocampal glucocorticoid receptors status is responsible for the reported increase in glucocorticoid secretion after novelty exposure (Gould et al., 2014). Plasma corticosterone levels in basal conditions in PWSI were not different from control mice, a finding in agreement with Schipper and Colleagues

(Schipper et al., 2020) and with Farbstein and Colleagues (Farbstein et al., 2021). However, other studies have reported a reduction of basal plasma glucocorticoid concentrations (Ros-Simó and Valverde, 2012; Ieraci et al., 2016). A question remains to be solved: why the reduction of both MRs and GRs are supposedly affecting adrenocortical activity in BTBR mice and not in PWSI mice? We do not have a clear explanation for this discrepancy; it is, however, tempting to suggest that in PWSI other factors acts to maintain an unchanged level of activity and reactivity of the adrenocortical axis. In this line, it is worth noting that isolating preadolescent female mice decreased the excitability of CRH neurons buffering the HPA axis activity (Senst et al., 2016). At hippocampal level, we also examined mGlu2 receptors expression because these receptors have been related to psychiatric disorders and mechanisms of resilience to stress (Nasca, 2015a; Nasca, 2015b). mGlu2 receptors are preferentially localized in presynaptic terminals, where they negatively regulate glutamate release and thus maintain homeostasis (Schoepp, 2001). Several studies have investigated the potential therapeutic role of mGlu2 receptor ligands in psychiatric disorders, such as schizophrenia and major depression (reviewed by Nicoletti et al., 2015 and Bruno et al., 2017). Exposure to chronic unpredictable stress (CUS) reduces hippocampal mGlu2 receptor protein levels in mice that are not resilient to stress, and mice lacking mGlu2 receptors are more vulnerable to CUS (Nasca, 2015a). As for potential pharmacotherapeutic interest, it has been demonstrated that the acetylating agent, L-acetylcarnitine, displays antidepressant-like activity by epigenetically up-regulating mGlu2 receptors in both mice and rats (Nasca et al., 2013; Nasca et al., 2017). Along this line, the down-regulation of mGlu2 receptors found in PWSI mice might be linked to the stress condition derived from social isolation, and

causally related to the defect in social interaction found in these mice. While mGlu5 receptors have been extensively investigated in BTBR mice (Yang et al., 2015; Seese et al., 2014; Burket et al., 2011; Silverman et al., 2010a; Silverman et al., 2012), there are no studies on mGlu2 receptors in these mice or in other mouse models of autism spectrum disorder, with the exception of a study showing that pharmacological blockade of mGlu2/3 receptors corrects abnormalities in hippocampal activity-dependent synaptic plasticity in mice modeling Fragile-X syndrome (Choi et al., 2011). Whether, and to what extent, the observed down-regulation of hippocampal mGlu2 receptors contributes to the abnormal behavioral phenotype of BTBR mice remains to be determined. During development, early experiences shape the architecture of the brain, and derangements of this developmental process can affect the capacities for learning and relating to others (Brown et al., 2009; Kessler et al., 2010; Friedman et al., 2015). The severity of behavioral (reduced social interaction) and biochemical (impairment of glutamatergic synapses) alterations induced by PWSI is such that the identification of potentially restorative intervention strategies is necessary. In this regard, one nonpharmacological approach aimed at restoring the effects produced by social isolation is resocialization. Recent studies have shown that resocialization causes reversion of some of the effects induced by social isolation such as, for example, reduction of myelination at the level of the prefrontal cortex (Makinodan et al., 2017). Therefore, we went to evaluate the possible reparative role exerted by resocialization on the state of glutamatergic imbalance in the hippocampus induced by postnatal social isolation in male C57BL/6 strain mice. Specifically, this procedure was performed according to the experimental protocol reported in the work of Makinodan and Colleagues (2017), transferring the isolated mouse from the

day of weaning to a cage containing 3 mice of the same age and housed together since weaning (day 21). In addition, we extended our study by considering the two subfields of the hippocampus, dorsal and ventral. The distinction of this brain region into the two sub-regions is now unavoidable for an extensive brain sub-localization of the molecular targets of the neurochemical changes induced by the mechanisms of perturbation of the body's biological homeostasis (Nasca et al., 2017). Particularly, results obtained from western blot and qPCR analysis confirmed that postweaning social isolation reduces expression levels of mGlu2 receptors (mRNA and protein), MRs and GRs (mRNA) showing, for the first time, that this reduction occurs selectively in the ventral hippocampus. With respect to the effects induced by resocialization, the data produced indicated that one week of resocialization is unable to restore the PWSI-induced reduction in hippocampal mGluR2, MRs and GRs, underscoring the severity of the glutamatergic neurotransmission and corticosteroid signaling impairments produced by 4 weeks of social isolation. However, the resocialization-induced recovery absence is not entirely unexpected as different biochemical deficits induced by post-weaning social isolation may show different sensitivities to the same treatment. Ongoing studies will define the ways and timing of resocialization needed to reverse the profound neurochemical consequences induced by lacked social interaction. Overall, our findings strengthen the value of postnatal social isolation in mice as a model for psychiatric disorders (reviewed by Mumtaz et al., 2018), and provide a remarkable example of how genetics (in BTBR mice) and exposure to an adverse environment at the time of weaning may converge into a common phenotype. Moreover, it is conceivable to hypothesize that the effects observed at hippocampal level after PWSI (i.e., reduced density

of mGlu2 receptors, MR and GR), are in some way responsible for the induction of a behavioral profile which is similar to what confers to the BTBR strain validity as a model for ASD. In conclusion, we found that mice subjected to social isolation after weaning and BTBR mice share some behavioral and biochemical features in their phenotypes. This is a further demonstration that social interaction immediately after weaning (i.e., when the animal becomes autonomous in its relationship with the environment) is a key determinant in the developmental trajectory of the CNS.

11. Early life stress induces a sex-dimorphic programming of AMPA/GABA$_A$ receptors balance in emotional and cognitive behaviors

11.1 INTRODUCTION

Early-life stress events occurring during the critical period of brain development profoundly shape the health of an individual and causes lifelong alterations in brain programming (Barker, 1995; Maccari et al., 2017). In humans and animal studies, there is increasing evidence for early-life stress induced acceleration of brain ageing and consequently associated cognitive decline (Li et al., 2010; Danese and McEwen, 2012; Haapanen et al., 2018 and Marrocco et al., 2020, respectively), which in turn is a marker of neuropsychiatric diseases mediated by glutamate NMDA receptors (Näätänen et al., 2011). Furthermore, exposure to chronic stress causes changes in glutamatergic neurotransmission (Lowy et al., 1993; Popoli et al., 2011), with glutamatergic pyramidal neurons being more vulnerable to ageing (Morrison and Hof, 1997; Morrison and Baxter, 2012). Indeed, glutamate, the most abundant excitatory neurotransmitter in the central nervous system (CNS), is deeply involved in emotional cognitive alterations observed in stress-related disorders (Kadriu et al., 2019; Nasir et al., 2020). The rat model of PeRinatal Stress (PRS), which combines prenatal and postnatal stress, is a valuable model for studying the impact of early-life stress events on the individual's neurobehavioral adaptations to environmental challenges during the entire life span (Maccari et al., 2017). Indeed, PRS rats are characterized by hyper-reactivity and impaired feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Liu

et al., 2023). Interestingly, glutamate transmission is at the core of the PRS-induced programming of HPA axis hyperactivity and related neurobehavioral alterations (Maccari et al., 2017). Thus, PRS decreases depolarization-evoked glutamate release in the ventral hippocampus (Marrocco et al., 2012), a brain region that is specifically related to HPA axis stress response and emotions but not in the dorsal hippocampus, which is more involved in memory and cognition (Fanselow and Dong, 2010). Particularly, the abnormalities in the exocytosis of glutamate are associated with reduced expression of presynaptic vesicles-related proteins such as synapsins, rab3a and munc-18, as well as syntaxin, VAMP and synaptophysin (Marrocco et al., 2012), together with reduced density of the glutamatergic metabotropic (mGluR) type I and 2/3 receptors. This occurs specifically in the ventral hippocampus of adult (Zuena et al., 2008; Mairesse et al., 2015; Morley-Fletcher et al., 2018) as well as aged rats (Verhaeghe et al., 2022), but not in the dorsal hippocampus, excepted for mGlu5 and synapsin1a/b. Behaviorally, the deficit in glutamate release observed in the ventral hippocampus of PRS rats is causally related to reduced risk-taking behavior (Marrocco et al., 2012, 2014; Verhaeghe et al., 2022), and is positively correlated with poor social memory (Marrocco et al., 2014; Mairesse et al., 2015; Morley-Fletcher et al., 2018). Unlike the profound PRS-induced effects on ventral hippocampus deficit in glutamate release, PRS does not change in the ventral hippocampus of males the release activity of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the CNS (Marrocco et al., 2012, 2014; Morley-Fletcher et al., 2018). The unchanged GABA release in males has also been observed in other brain regions (Marrocco et al., 2012) involved in the regulation of learning and recognition memory (i.e. dorsal hippocampus), emotion and

cognition (prefrontal and perirhinal cortex) and decision-making behaviors such as striatum. However, the absence of effect of PRS on GABA release is not disregarded since there are no differences in ionotropic $GABA_A$ subunits expression in preweanling PRS rats in the hippocampus, despite an increased number of vocalizations in response to maternal separation (Laloux et al., 2012). On the other hand, by blocking metabotropic $GABA_B$ and mGlu2/3 subunit in the ventral hippocampus we could reverse the glutamate release deficit in the ventral hippocampus (Marrocco et al., 2012). Altogether, these findings indicate that PRS alters stress- and emotion-related cognitive functions *via* an imbalance between the excitatory and inhibitory neurotransmission in the hippocampal circuitries involving metabotropic receptors. Variations in the expression and/or insertion in the membrane of ionotropic glutamate receptors, which act as the sensors of the efficiency of the glutamate release such as AMPAR, should also be explored in the PRS programming of the glutamatergic synapse. AMPARs represent an excellent indicator of the functioning of the excitatory synapse and consist of four homologous pore-forming subunits (GluA1-4) that generally assemble into heterodimers, with GluA1/GluA2 and GluA2/A3 being the most present subunits in the hippocampus. GluA1 is trafficking-regulated during long-term potentiation, while GluA2/GluA3 are involved in the constitutive trafficking since they replace the preexisting GluA1 subunits in an activity-independent manner (Henley et al., 2011). Since constitutive trafficking becomes relevant to maintaining the synaptic strength in the absence of activity, GluA2/GluA3 activity represents a molecular mechanism for memory consolidation and emotional stress (Plant et al., 2006; Clem and Huganir, 2010; Liu et al., 2010). Interestingly, poor maternal care has been reported to regulate the expression and functioning of AMPA

receptors in the hippocampus (Bredy et al., 2004; Pickering et al., 2006), as well as of $GABA_A$-α1 subunit in different brain regions (Caldji et al., 2003). As PRS reduces maternal care (Gatta et al., 2018), in support of an involvement of AMPA receptor system in the programming induced by PRS, we previously showed a correction of PRS glutamate and behavioral phenotype by chronic treatment with a positive allosteric modulator of AMPARs (Morley-Fletcher et al., 2018). Brain excitation and inhibition levels shape the response to environmental stimulation of the underlying neural systems being therefore critical for the onset of cognitive and emotional skills acquisition during sensitive periods (Zacharopoulus et al., 2021). Thus, next to mGluRs and their modulation of the glutamate release machinery, changes in ionotropic AMPA/$GABA_A$ input balance during early life, may play a crucial role in the PRS programming. Most of the behavioral and molecular alterations induced by PRS are observed selectively in males, indicating that the early perinatal period represents a specific window of sensitivity, during which offspring are susceptible to the programming effects of PRS combined with sex differences. This also indicates that PRS induces an enhanced resilience to age-related diseases in females (Verhaeghe et al., 2022). Therefore, we explored in PRS rats of both sexes the synaptic expression of GluA2/GluA3 and $GABA_A$-α1 subunits, in order to evidence a sex-dependent PRS effect on excitatory/inhibitory balance and its involvement in emotional and cognitive behaviors. Also, by comparing cognitive performance of young adult and elderly rats in relation with GluA2/GluA3 subunits as well as presynaptic exocytotic glutamate machinery, we explored in both sexes the age-inducing effect of PRS.

11.2 MATERIALS AND METHODS

Animals

Thirty nulliparous female Sprague-Dawley rats, weighing approximately 250 g, were purchased from Charles River (France) and housed under standard conditions with a 12 h light/dark cycle (lights on 7am: lights off 7 pm). After group housing (five females/cage) for two weeks, each female was individually housed for one week with a sexually experienced male rat. Following that, a gain of at least 10 grams was considered as an index of pregnant status.

Stress procedure and maternal behavior

The stress procedure was performed on two breeding sets, using 30 females (15 CONTs and 15 stressed dams) according to our standard protocol (Maccari et al., 1995), as shown in figure 1. Briefly, from day 11 of gestation until delivery, pregnant females were subjected to restraint in a transparent plastic cylinder and exposed to bright light during three daily sessions of 45 min. Control pregnant females were left undisturbed in their home cages and were handled once per week. In order to assess the quality of gestational stress, maternal behavior (nursing behavior, grooming, licking, and carrying pups) was monitored for 24 h every day during the first 7 postpartum days with small infrared cameras placed in the animal cage rack where cages containing lactating females were placed. Because gestational stress induces a reduction of maternal behavior (<40% of maternal behavior in stressed mothers) (Gatta et al., 2018), we refer to the whole procedure as perinatal stress (PRS). After weaning, male and female offspring from the litter with a balanced sex ratio were used for the experiments. Animals were housed in groups of two or three and

maintained under similar environmental conditions during their entire life span; 3-5 month and 21–22-month-old rats were used in all experiments.

Experimental design

The experimental design is shown in figure 1. Two separate sets of male and female Cont and PRS rats were used for behavioral and biochemical analysis, adult (3-5 months) and aged (21-22 months). Behavioral tests were performed when the rats were 3 months old and 21 months old. One week after behavioral assessment, brain structures were collected, and biochemical analysis was carried in the hippocampus (ventral and dorsal) and the prefrontal cortex. All experiments followed the rules of European Communities Council Directive 86/609/EEC. The local Committee CEEA-75 (Comité d'Ethique en Experimentation Animale Nord-Pas de Calais, 75) approved the experimental procedures.

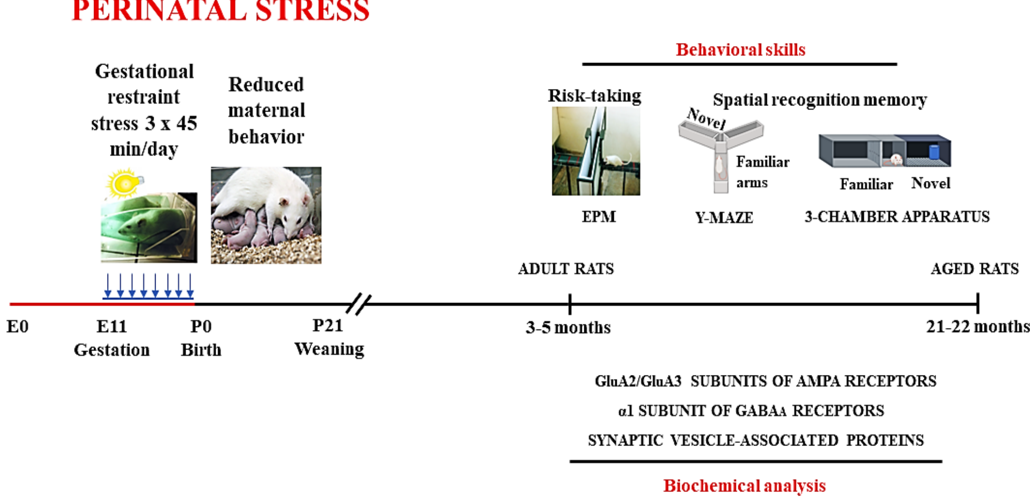


Figure 1. Experimental design and timeline Induction of perinatal stress (PRS) and maternal behavior analysis in the first postpartum week were followed by behavioral and biochemical measurements in adult (3-5 mo.) and aged (21–22 mo.) male and female progeny as indicated.

Behavioral studies

Spatial recognition memory

Spatial memory performance of adult and aged rats was performed in a behavioral task based on spontaneous recognition paradigm. For this purpose, two different apparatuses were used.

Y-maze

For both adult and aged rats we used a two-trial memory task in a Y-maze apparatus (adapted from Verhaeghe et al., 2022) made of gray plastic with three identical arms (50 cm) enclosed with 32 cm high side walls and illuminated by dim light (40 lux). Each arm was equipped with two infrared beams, one at each end of the arm. The maze floor was covered with rat odor-saturated sawdust, and the sawdust was mixed between each session to eliminate olfactory cues. Visual cues were placed in the testing room and kept constant during the behavioral testing sessions. The task consisted of two trials separated by a time interval. In the first trial (acquisition phase), one arm of the Y-maze was closed, and animals could visit the two other arms for 5 min. During the intertrial interval (IT= 6h), rats were returned in their home cages located in the animal room. During the second trial (retention phase), animals had free access to the three arms and were again allowed to explore the maze for 5 min. The time spent in the novel arm (previously closed in the first trial) was calculated as a percentage of the total time spent in all three arms during the first 3 min of the second trial. Time spent in the novel arm above chance (i.e., 33%) indicates spatial recognition.

Three-chamber apparatus

This test (adapted from Darnaudery et al., 2002) was used in a separate set of adult rats since the Y-maze did not reveal significant differences. The apparatus consisted of two equal compartments (30x30x10 cm) connected by an alley (30x10 cm large, 45 cm high) with two opposite openings, one per compartment, which could be closed by sliding doors. Boxes were illuminated by dim light and covered with opaque plates (30x70 cm large) that isolated apparatus from visual cues in the testing room. The floor of the apparatus was equipped with photoelectric beams to record time spent in each compartment. To carry out the test, we created two distinct spatial configurations of the apparatus, using square parallelepipeds (12x2 cm large, 45 cm high) made of grey Plexiglas. In the first of these configurations, referred to as the ''acquisition configuration'', both compartments were unchanged; in the second configuration, referred to as ''restitution configuration'', we placed square parallelepiped in one of two compartments (novel compartment). The task consisted of two trials separated by a time interval. In the first trial (acquisition phase), the rat was placed in the central alley and, 30s later, the sliding doors were opened, and the animal was free to explore the acquisition configuration for 10 minutes. During the inter-trial interval (ITI=4h), rats were housed in their home cages located in a room different from the test room. During the second trial, the animal was placed into the central alley and allowed to explore the restitution configuration for 15 min. Score of recognition during restitution trial was calculated as percentage of ratio of time spent (0-5 min) in the novel compartment *vs.* time recorded in the familiar compartment; a score above 100% indicates spatial recognition.

Risk-taking behavior in the elevated-plus maze test

Risk-taking behavior of PRS or control progeny was assessed in the elevated-plus maze test (EPM) (adapted from Morley-Fletcher et al., 2018). Briefly, the test was performed for 5 min early in the afternoon (between 1 and 4 pm) and began with the placement of the rat in the center of the maze with the head facing a closed arm. For aged animals, we used a custom-made EPM apparatus described by Vallée and Colleagues (1999), with closed and open arms of 20 × 20 cm (Vallée et al., 1999). The closed arms' luminosity was approximately 25 lx, and the luminosity of the open arms was approximately 50 lx. Behavior was recorded by a video camera and manually scored by a trained observer blind to the animals' condition (PRS and control) using a software package (Noldus, The Observer®). The time spent in the open and closed arms was measured, and the percentage of time spent in the open arms was calculated and analyzed as risk-taking behavior.

Western blot analysis

The hippocampus (ventral and dorsal) and prefrontal cortex of adult and aged rats were rapidly dissected and immediately stored at -80 °C. Glutamate-related proteins, synaptic vesicle-associated proteins and $GABA_A$ receptor subunit $\alpha1$ were assessed in synaptosomal fraction. To isolate synaptosomal fraction, tissue was manually homogenized with a potter in ten volumes of HEPES-buffered sucrose (0.32 M sucrose, 4 mM HEPES pH 7.4). All procedures were performed at 4 °C. Homogenates were centrifuged at 1000 × g for 10 min, and the resulting supernatants were centrifuged at 10,000 × g for 15 min. The pellet obtained from the second centrifugation was resuspended in ten volumes of HEPES-buffered sucrose

(Marrocco et al. 2012). This pellet contained the crude synaptosomal fraction. BCA assay was used to determine protein concentration. Synaptosomal lysates were resuspended in Laemmli reducing buffer, and 20 μg for synaptosomal fraction of each sample were loaded. One of the samples was used as an internal control and was loaded in each gel to ensure sample homogeneity between different gels and compare samples from different gels.

Proteins were first separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels according to their molecular weight and then transferred to nitrocellulose membranes (Bio-Rad). The transfer was performed at 4 °C in a buffer containing 35 mM Tris, 192 mM glycine, and 20% methanol. After transfer, blots were incubated in a blocking solution containing Tris-buffered saline and 5% (w/v) non-fat milk. All the following antibodies were first tested with control samples to determine the optimal conditions for use. To analyze several proteins per membrane, membranes were cut according to the molecular weight of the protein of interest. We used the following primary antibodies on synaptosomal fraction: rabbit polyclonal anti-synapsin Ia/b (1:4000; catalog. #sc-20780), rabbit polyclonal anti-synaptophysin (1:8000; catalog #sc-9116), rabbit polyclonal anti-syntaxin (1:4000, catalog #sc-13994), rabbit polyclonal anti-synapsin IIa (1:4000; catalog #sc-25538), all purchased from Santa Cruz Biotechnology; mouse monoclonal anti-rab3a (1:2000; catalog. #107111), mouse monoclonal anti-Munc-18 (1:2000; catalog #116011), mouse polyclonal anti-vesicle-associated membrane proteins (VAMP) (1:1500; catalog. #104111) which were purchased from Synaptic Systems; rabbit monoclonal anti-GluA2 (1/2000; catalog #ab206293), mouse monoclonal anti-GluA3 (1/800; catalog #MAB5416) purchased from Merck; rabbit polyclonal anti-$GABA_A$ receptor

α1 (1:1000. catalog #06-868, Sigma-Aldrich). To ensure that each lane was loaded with an equivalent amount of proteins, the blots were probed with a mouse monoclonal anti-b-actin (1:1500; catalog #A5316, Sigma). All primary antibodies were incubated overnight at 4 °C. Horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit antibodies (purchased from GE-Healthcare) were used at a dilution of 1:5000 and incubated for 1 h at room temperature. Bands were visualized with an enhanced chemiluminescence system (ECL enhancer Thermofisher). After immunoblotting, digitized images of bands immunoreactive for target antibodies and actin were acquired (FUSION®), and the area of immunoreactivity corresponding to each band was measured using ImageJ. A ratio of target to actin was then determined and data were expressed as normalized with respect to the control males of age-matched group.

Statistical analysis

Behavioral and biochemical data were expressed as the mean ± standard error of the mean (S.E.M.) and analyzed using a parametric analysis of variance (ANOVA) with Group (CONT *vs*. PRS) and Sex (male *vs.* female) as independent variables. Post-hoc comparisons were performed using Newman-Keuls or Fisher LSD. A p-value of <0.05 was considered statistically significant.

11.3 RESULTS

Sex-specific effect of PRS on risk-taking behavior, spatial recognition memory and balance AMPAR/GABA$_A$ in adult rats

Animals were assessed for risk-taking behavior in the EPM (Fig. 2A). As expected, we observed a clear sex-dependent effect with PRS reducing risk-taking behavior in males, while increasing it in females with respect to their corresponding control group (*PRS × sex interaction*, n=5-7 rats/group, $F_{(1,21)}$=11.780, p=0.002). We also evaluated spatial recognition memory in both adult and aged rats by using two different tests. First, we used the Y-maze test (Fig. 2B), and observed that, despite a low recognition score in control animals (33-34%), PRS had a trend to reduce the cognitive performance (*PRS effect*, n=9 rats/group, $F_{(1,32)}$=3.272, p=0.079) in adult males with respect to the control unstressed group, although not significantly (p=0.06). Therefore, for the assessment of spatial recognition memory in adults, we used a second test such as the three chamber-apparatus (Fig. 2C) to measure the recognition score (percentage of time spent in the novel compartment). Here, PRS reduced recognition score in males, while increasing it in females, with respect to their corresponding control group (*PRS x sex interaction*, n=6-7 rats/group, $F_{(1,22)}$= 15.1245, p= 0.0007). Also, control unstressed females displayed a reduced time spent in the novel compartment as compared to control males. We assessed GluA2 and GluA3 of AMPA glutamate receptors subunits in prefrontal cortex as well as dorsal and ventral hippocampus. In the prefrontal cortex (Fig. 2D), *PRS effect* failed to reach significance (n=10 rats/group, $F_{(1,36)}$= 3.798, p= 0.059). However, a separate analysis in females indicated a reduction in GluA2 protein levels only in the PRS female group (n=10 rats/group, $F_{(1,18)}$=5.987, p=0.02). Concerning GluA3 protein levels, we observed a *PRS effect* (n=9-10

rats/group, $F_{(1,33)}$=12.573, p=0.001) with reduced GluA3 subunit, in both sexes as compared to respective controls. In the dorsal hippocampus (Fig. 2E), we observed a *PRS x sex interaction* for the expression of GluA2 subunit (n=8-10 rats/group, $F_{(1,34)}$= 7.770, p=0.009) with decreased GluA2 levels in males with respect to controls but increased GluA2 in females relative to PRS males (Neuman-Keuls (NK): p=0.02 and p=0.03, respectively). PRS also modified GluA3 levels (*PRS effect,* n=7-10 rats/group, $F_{(1,32)}$=7.025, p=0.012) by reducing GluA3 in males (NK: p<0.05). In the ventral hippocampus (Fig. 2F), no effect of PRS was detected in GluA2 and GluA3 protein expression, while females, independently of the group, showed an increase of GluA3 subunit levels compared to males (*sex effect*, n=6-9 rats/group, $F_{(1,26)}$= 28.283, p= 0.000015). Overall, PRS induced a sex-dimorphic cognitive decline selectively in males, in association with decreased GluA2 and GluA3 levels in dorsal hippocampus. Concerning $GABA_A$-α1, in the prefrontal cortex (Fig. 2G), a *PRS x sex interaction* (n=8-10 rats/group, $F_{(1,33)}$=15.771, p=0.0003) indicated that subunit α1 of $GABA_A$ receptors was significantly reduced in PRS females with respect to control group (NK: p=0.002), while $GABA_A$-α1 was higher in control unstressed females compared to males (NK: p=0.003). In the dorsal hippocampus (Fig. 2H), females showed higher $GABA_A$-α1 protein levels compared to males (*sex effect*, n= 6-10 rats/group, $F_{(1,32)}$= 19.572, p= 0.0001). PRS increased $GABA_A$-α1 particularly in females (*PRS effect*, n= 6-10 rats/group, $F_{(1,32)}$= 7.006, p= 0.012). In the ventral hippocampus (Fig. 2I), females displayed higher levels of $GABA_A$-α1 protein (*sex effect*, n=8-10 rats/group, $F_{(1,32)}$=4.836, p=0.035), but no effect of PRS was detected . Thus, PRS females displayed more risk-taking behavior than males and presented an increased expression of $GABA_A$ receptor protein in the dorsal hippocampus.

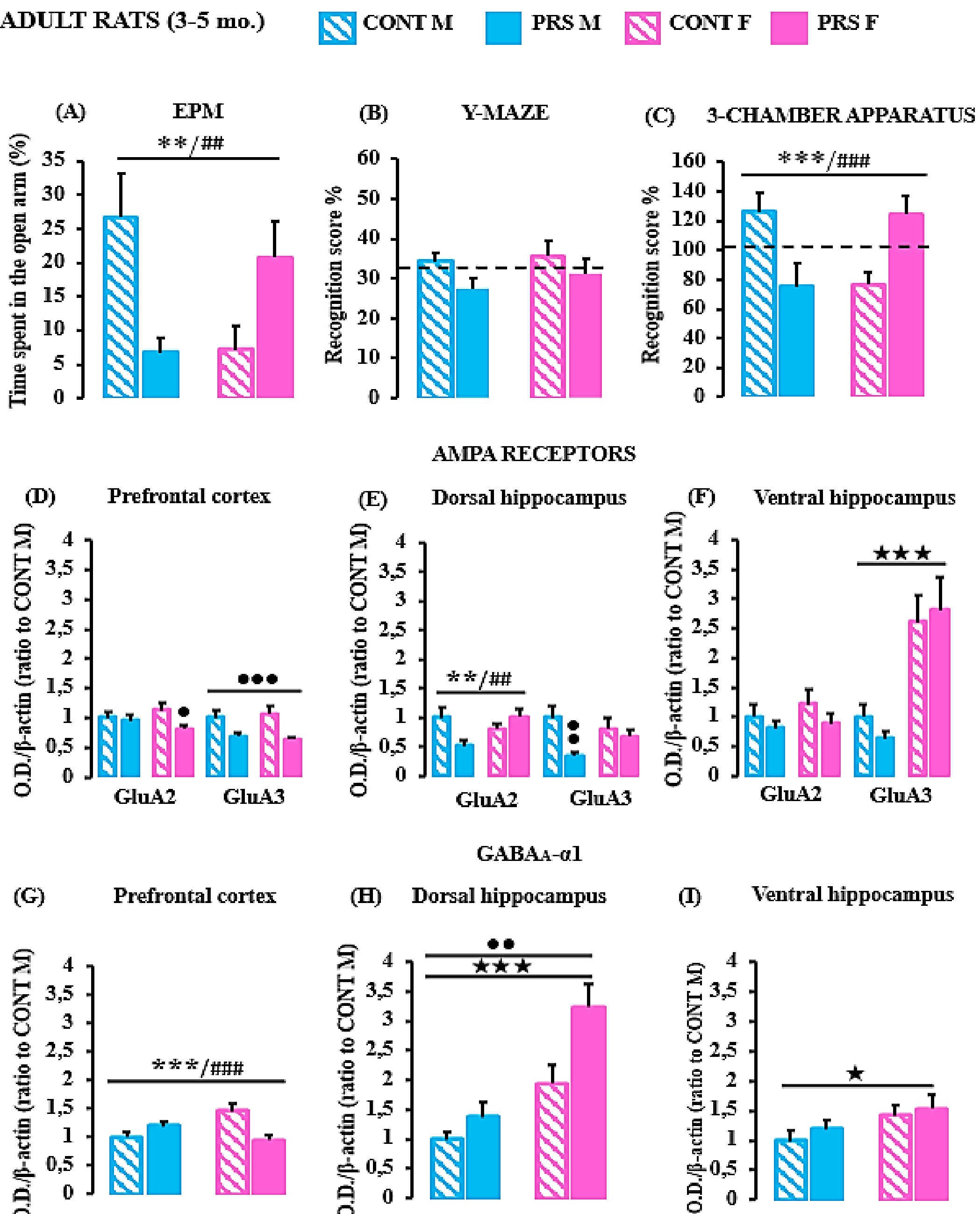


Figure 2. Sex-specific effect of PRS on risk-taking behavior, spatial recognition memory and AMPAR/GABAA balance in adult rats. Risk-taking behavior assessed in the elevated plus maze **(A)**, spatial recognition memory using the Y-maze **(B)**, and the 3-chamber apparatus (**C)** in adult PRS and control rats of both sexes. Values are expressed as means ± S.E.M. (n=5-7 rats/group). Immunoblots analysis of GluA2 and GluA3 subunits of AMPA receptors (**D-F**) and GABAA-α1 receptor subunit (**G-I**) in synaptosomal fractions obtained from prefrontal cortex, the dorsal hippocampus and the ventral hippocampus of adult male and female PRS and control unstressed rats**.** Values are expressed as means ± S.E.M. (n=6-10 rats/group), PRS *x* sex interaction: */#=p<0.05; **/##=p<0.01; ***/###=p<0.001. PRS effect: PRS *vs.* Control •=p<0.05; ••=p<0.01; •••=p<0.001. Sex effect: Females *vs.* Males ★ =p<0.05; ★★=p<0.01; ★★★=p<0.001.

Persistency in aged males of the PRS-induced sex-specific impairment of risk-taking behavior, spatial recognition memory and AMPAR/GABA$_A$ balance observed at adulthood

Animals were assessed for risk-taking behavior in the EPM (Fig. 3A). We observed the persistency of the sex-dimorphic profile observed in adults, with reduced risk-taking behavior in males, and increased levels in females with respect to their corresponding control group (*PRS × sex interaction*, n=6-10 rats/group, $F_{(1,26)}=13.127$, p=0.001). When we assessed aged rats for spatial recognition memory by using the Y-maze test (Fig. 3B), we observed a clear sex-dependent effect of PRS (*PRS x sex interaction*, n=6-7 rats/group, $F_{(1,23)}=7.800$, p=0.01), with PRS reducing the recognition score in males (NK: p=0.02) but not in PRS females, which displayed better recognition than males (NK: p=0.02). Concerning GluA2 subunit in the prefrontal cortex (Fig. 3C), a *PRS x sex interaction* (n=5-7 rats/group, $F_{(1,20)}= 4.603$, p=0.044) indicated that GluA2 protein levels were significantly lower in PRS males with respect to control group (NK: p=0.008), while GluA2 subunit of AMPA receptors was reduced in control unstressed females compared to males (NK: p=0.02). The same profile was observed for GluA3 (*PRS x sex interaction,* n=5-8 rats/group, $F_{(1,22)}=10.882$, p=0.003). In the dorsal hippocampus (Fig. 3D), a *PRS x sex interaction (*n=5-9 rats/group, $F_{(1,22)}= 4.652$, p= 0.042) indicated that GluA2 protein levels were significantly lower in PRS males with respect to control group (NK: p=0.006), while GluA2 subunit of AMPA receptors was reduced in control unstressed females compared to males (NK: p=0.006). The same profile was observed with respect to GluA3 subunit protein levels (*PRS x sex interaction,* n=8-10 rats/group, $F_{(1,21)}= 8.353$, p=0.008). In the ventral hippocampus (Fig. 3E), we did not observe any difference due to PRS or sex in the expression of GluA2

and GluA3 subunits. Thus, the effect of PRS on AMPAR was region- sex-specific and persisted until ageing. A *PRS x sex interaction* was also observed for $GABA_A$-α1 in the prefrontal cortex (Fig. 3F) with reduced levels of expression in PRS aged males and control unstressed females (*PRS x sex interaction*, n=3-6 rats/group, $F_{(1,16)}$= 4.352, p=0.05; NK p=0.035 and p=0.01 *vs.* the male control group, respectively). In the dorsal hippocampus (Fig. 3G), females displayed higher levels of $GABA_A$-α1 (*sex effect*, n=5-7 $F_{(1,20)}$=14.023, p=0.0012) but no effect of PRS was detected. As it is, no effects of PRS were identified for any brain region in aged females.

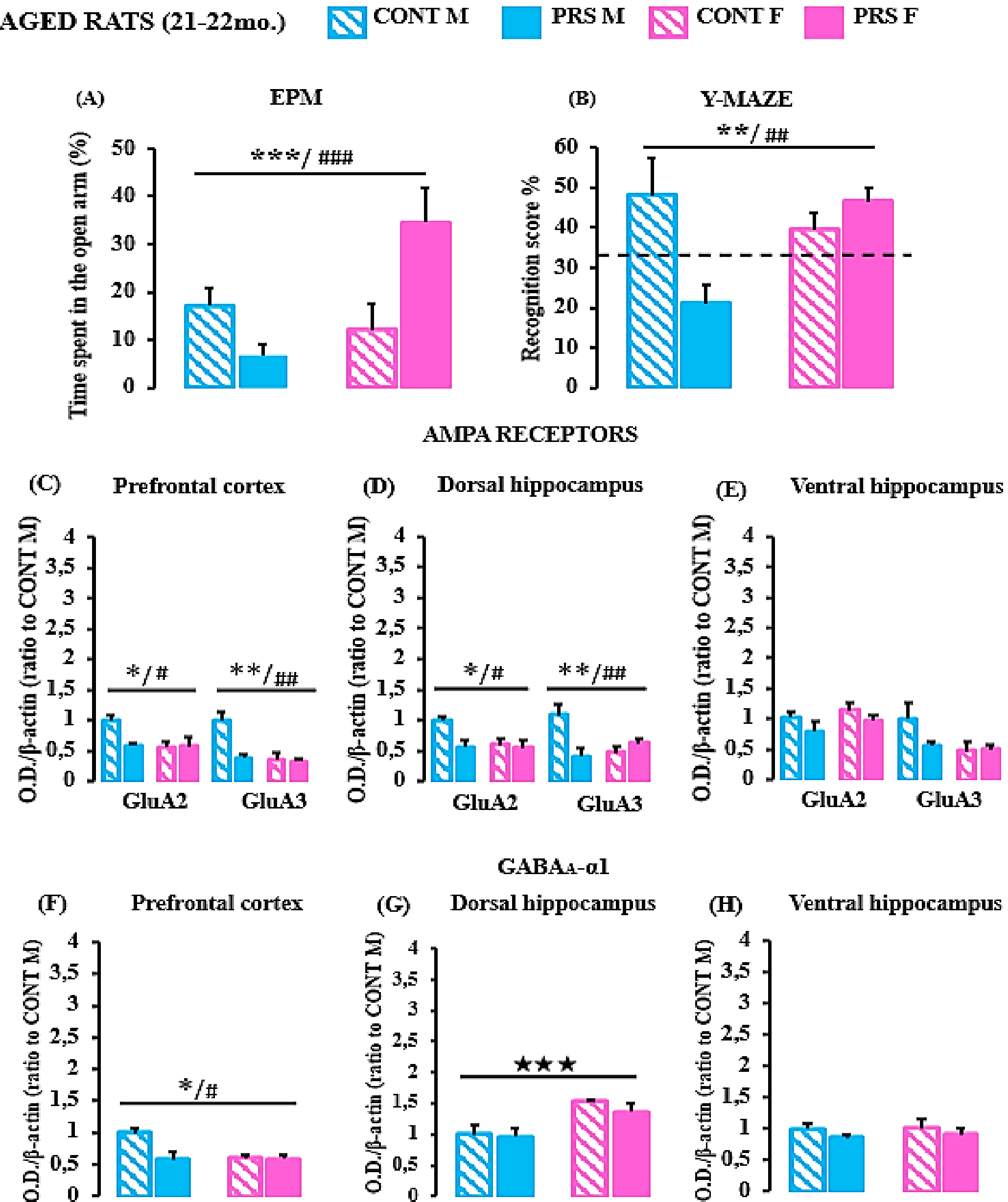
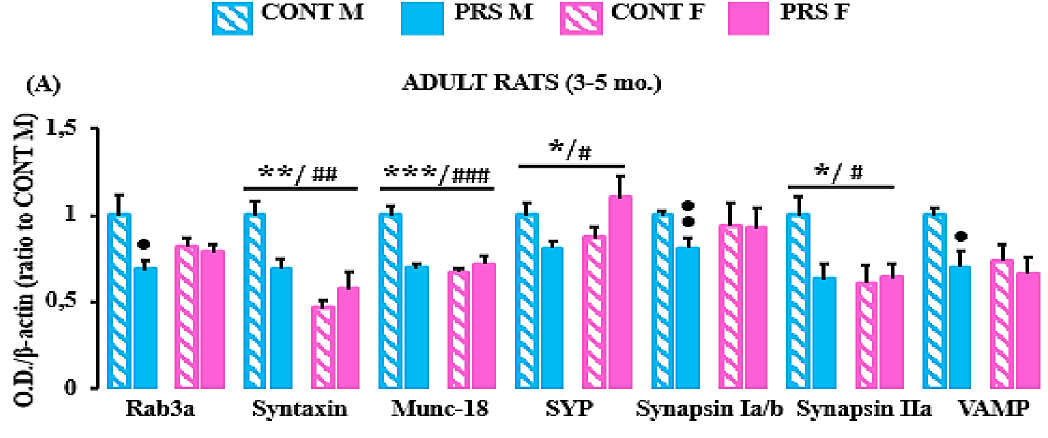


Figure 3. Persistency in aged males of the PRS-induced sex-specific impairment of spatial recognition memory and AMPAR/GABAA balance observed at adulthood. Risk-taking behavior assessed in the elevated-plus maze **(A)** and spatial recognition memory using the Y-maze **(B)** in aged PRS and control unstressed rats of both sexes. Values are expressed as means ± S.E.M. (n=6-10 rats/group). Immunoblots analysis of GluA2 and GluA3 subunits of AMPA receptors (**C-E**) and GABAA-α1 receptor subunit (**F-H**) in synaptosomal fractions obtained from prefrontal cortex, the dorsal hippocampus and the ventral hippocampus of aged PRS and control unstressed rats of both sexes. Values are expressed as means ± S.E.M. (n=2-9 rats/group). PRS *x* sex interaction: */#=p<0.05; **/##=p<0.01; ***/###=p<0.001. PRS effect: PRS *vs*. Control •= p<0.05; ••= p<0.01; •••= p<0.001. Sex effect: Females *vs*. Males ★ =p<0.05; ★★=p<0.01; ★★★=p<0.001.

PRS programs a lifelong sex-specific reduction of the presynaptic exocytotic machinery in the ventral hippocampus

We measured by western blot analysis synaptic vesicles-associated proteins expression levels in the ventral hippocampus of adult and aged rats. In adult rats (Fig 4A), we observed a *PRS x sex interaction* (n=6-7 rats/group), for protein levels of SynapsinIIa ($F_{(1,21)}$=4.494, p=0.046), Syntaxin ($F_{(1,21)}$=7.6541, p=0.011) and Munc-18 (n=6-7 rats/group, $F_{(1,21)}$=20.525, p=0.0002). Indeed, PRS males displayed reductions in protein levels with respect to control males for SynapsinIIa (NK: p=0.03), Syntaxin (NK: p=0.009) and Munc-18 (NK: p=0.0002). Moreover, these proteins were also reduced in control unstressed females with respect to control males (SynapsinIIa, NK: p=0.04; Syntaxin, NK: p= 0.0005; Munc-18, NK: p=0.0002). Levels of Synapsin Iab were not affected by PRS or sex when analysis was conducted on all groups, whereas a separate analysis in males indicated a marked reduction in PRS group (n=6-7 rats/group; $F_{(1,11)}$=9.550, p=0.010). Concerning Rab3a, *PRS* modified protein expression levels *(PRS effect,* n=6 rats/group; $F_{(1,20)}$=5.785, p=0.025) by reducing Rab3a in males (NK: p=0.026), with a trend for reduced levels in control unstressed females with respect to control males (*PRS x sex interaction*, $F_{(1,20)}$= 3.947, p= 0.06). PRS also reduced Synaptophisin (SYP) in males while increasing it in adult females compared to the corresponding control groups (n=6-7 rats/group, *PRS x sex interaction,* $F_{(1,21)}$=6.546, p=0.018). Finally, PRS reduced levels of VAMP (Synaptobrevin) with greater extent in males (*PRS effect*, n=6-7rats/group, $F_{(1,21)}$=5.468, p=0.029) and a trend for a *sex effect* ($F_{(1,21)}$=3.504, p=0.075) indicated a reduction of VAMP in control unstressed females with respect to males. In aged rats (Fig. 4B), we observed a *PRS x sex interaction* (n=6-7 rats/group) for protein levels of Syntaxin ($F_{(1,21)}$=6.774, p=0.016) and Rab3a $F_{(1,22)}$=4.770,

p=0.039) with reduced levels in male PRS rats (NK: p=0.004; Fisher LSD: p=0.02, respectively) and female controls (NK: p=0.004; Fisher LSD: p=0.03, respectively) with respect to control males. With respect to SYP, a main *sex effect* (n=6-7 rats/group, $F_{(1,23)}$=9.25, p=0.006) indicated a reduction of protein levels in females with respect to males in the control group (NK: p=0.010). A separate analysis in the male aged group, revealed a near to significance *PRS effect* for lower levels of SYP in PRS males (n=6-7 rats/group, $F_{(1,11)}$=4.590, p=0.0550). Thus, the PRS induced-reduction of presynaptic vesicles-associated proteins in the ventral hippocampus of males persisted up to ageing.



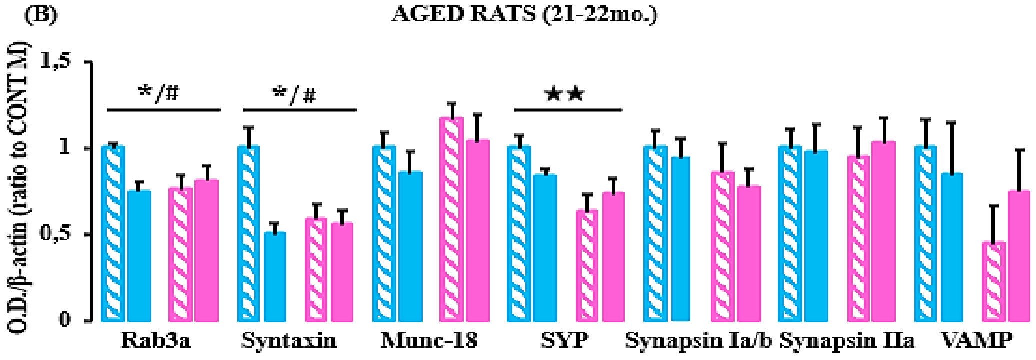


Figure 4. PRS induces in adult rats a sex-specific reduction of presynaptic vesicles-associated proteins in the ventral hippocampus, which persists in aged males. Immunoblots analysis of presynaptic vesicles-associated proteins in synaptosomal fractions obtained from the ventral hippocampus of adult **(A)** and aged **(B)** PRS and control unstressed rats of both sexes. Values are expressed as means ± S.E.M. (n=6-7 rats/group). PRS *x* sex interaction: */# =p<0.05; **/## =p<0.01; ***/### =p<0.001. PRS effect: PRS *vs.* Control

•=$p<0.05$; ••=$p<0.01$; ••• = $p<0.001$. Sex effect: Females *vs*. Males ★= $p<0.05$; ★★ = $p<0.01$; ★ ★ ★ =$p<0.001$.

11.4 DISCUSSION

PRS induced a sex-dimorphic pattern in emotional reactivity in the elevated-plus maze, with reduced open arm exploration (risk-taking behavior) in males while enhancing it in females. The same profile was observed in spatial recognition memory, with reduced recognition score in PRS males and higher recognition memory in PRS females. Furthermore, this dimorphic pattern persisted in aged rats. Thus, our findings indicate that PRS accelerates cognitive decline in males, since reduced recognition score was already present in adult males, and increases resilience in females, while control unstressed adult females, which also displayed reduced risk-taking behavior, presented a lower recognition score with respect to control males and PRS females. Interestingly, the cognitive performance in PRS animals mirrored the PRS sex-dimorphic emotional phenotype, which is repeatedly observed in adult (Zuena et al., 2008) as well as old animals (Veraeghe et al., 2022). Therefore, emotional reactivity, which is disrupted by PRS in a sex-dependent way, shapes the outcome in cognitive performance. Of note, in humans, social cognition such as interpersonal functioning is impaired in young adults displaying social anxiety during COVID-19 pandemic (Alvi et al., 2022). Moreover, in humans, cognitive flexibility and decision-making processes are reduced by anxiety. In animal models, exploratory/risk-taking behavior is reduced by stress (Park and Moghaddam, 2017). Remarkably, AMPA/GABA balance was in accordance with the sex-dimorphic changes induced by PRS on emotional/cognitive behaviors. Indeed, we observed in PRS males a clear reduction in the density of both GluA2 and GluA3 subunits in the dorsal

hippocampus and the prefrontal cortex of adult and elderly male PRS rats, compared with the unstressed controls. In PRS females, GluA2 was increased in the dorsal hippocampus, while both GluA2 and GluA3 subunits were reduced in the prefrontal cortex. Thus, AMPAR expression profile in these two brain regions is in total agreement with the behavioral phenotype in risk-taking and recognition memory induced by PRS with impaired performances in males. Interestingly, GluA2 displayed a sex/region-dependent profile, so that changes in dorsal hippocampus were compensated by an opposite profile in the prefrontal cortex. Indeed, GluA2, which is increased in females and reduced in males in the dorsal hippocampus, was reduced in females and did not change in males in the prefrontal cortex. Therefore, ionotropic AMPARs and in particular GluA2, are actively involved in the shaping of the emotional cognitive profile induced by PRS. On the other hand, no differences due to PRS were observed in the ventral hippocampus. This is not surprising in the ventral hippocampus where presynaptic mGlu2/3 and postsynaptic mGlu5 receptors as well as the excitatory machinery were mainly regulated respect to ionotropic glutamate receptors (Verhaeghe et al., 2022). Moreover, previous data in aged animals indicate that PRS does not alter the expression of ionotropic glutamate receptors in the ventral hippocampus, besides reduced levels of NMDA GluN1 receptor in aged males, while ionotropic receptors (both NMDA and AMPA) are reduced in the dorsal hippocampus of PRS aged males (Verhaeghe et al., 2022). So, PRS modifies ionotropic receptors in the dorsal hippocampus and mGluRs in the ventral hippocampus. The $\alpha 1$ subunit of the pentameric $GABA_A$ receptor was also studied in adult and aged rats of both sexes. PRS enhanced the expression of the $\alpha 1$ subunit of $GABA_A$ in the dorsal hippocampus of both sexes and

reduced it in the prefrontal cortex exclusively in females. In aged PRS subjects a reduction of $GABA_A$-α1 subunit protein levels in the prefrontal cortex was observed in the male gender. There were no changes in other brain structures, while PRS reduced $GABA_A$-α1 only in the prefrontal cortex of aged males. Of note, PRS had no effect in the ventral hippocampus for AMPAR and $GABA_A$-α1 subunits. Interestingly, in the ventral hippocampus all females, independently of PRS, displayed higher levels of GluA3 and $GABA_A$ subunits, evidencing a peculiar sex effect. As a whole, PRS males did not display notable changes in GABA in the ventral hippocampus, thus reinforcing previous evidence (Marrocco et al., 2012; Laloux et al., 2012). In PRS females, we observed a compensatory mechanisms of the levels AMPA/GABA receptors mediated by the dorsal hippocampus with respect to prefrontal cortex, which is in agreement with their behavioral resilience. It is widely accepted that $GABA_A$, NMDA and AMPA display a sequential participation in neuronal excitation during development in the hippocampus, with GABA acting as an excitatory transmitter in early postnatal stage, and glutamatergic synaptic transmission being first purely NMDA receptor-based and lacking functional AMPA receptors (Ben-Ari et al., 1997). Of note, the silencing of ionotropic receptors is able to alter $GABA_A$Rs synaptogenesis during development, while excitatory synaptic transmission is normal in hippocampal neurons devoid of $GABA_A$ (Duan et al., 2019). Therefore, excitatory/inhibitory balance seems to be mostly dependent of glutamatergic transmission. This specific functional hierarchy could explain the lack of effect of PRS on GABA neurotransmission in the ventral hippocampus of PRS males. In fact, because PRS males exhibit a hypo-glutamatergic synapse, one possible consequence would be a weakened

glutamate input on the configuration of GABA neurotransmission that remains unchanged. In support of this hypothesis, although the effect of PRS on glutamate/GABA depolarization-evoked release has not been investigated in females yet, we observed that PRS females display higher levels of both GluA2 and $GABA_A$ in the dorsal hippocampus. The expression profile in the AMPA/GABA balance observed in PRS adult females could also explain their increased stress resilience resulting in increased risk-taking behavior and better cognitive performance (Verhaeghe et al., 2022). Altogether, the data obtained indicate the involvement of excitatory/inhibitory imbalance as potential molecular mechanism in memory impairment, which is sex-dependent in the PRS rat. In the ventral hippocampus, the expression of the presynaptic vesicles-associated proteins was selectively reduced in PRS males, while no changes were observed in females. In particular, learning processes are modulated by synapsins (Kushner et al., 2005; Porton et al., 2010; Barbieri et al., 2018), which are involved in the clustering of synaptic vesicles to the reserve pool near the release sites, but also by synaptophysin, which acts as a regulator of the SNARE complex (Hinz et al., 2001; Li et al. 2017). Their reduction in males, but not PRS females, is thus in agreement with the reduced cognition score in PRS males. Of note, synapsin Ia/b is the only SNARE protein, which keeps being downregulated by PRS also in the dorsal hippocampus of adult males (Marrocco et al., 2012). Notably, we observed a similar scenario, with a selective sex-dimorphic effect, also in the ventral hippocampus of aged rats. In particular, PRS reduced the expression of Rab3a and syntaxin in aged males, whereas no alterations were found in the expression of any synaptic-vesicles proteins in PRS females. Therefore, the sexually dimorphic effects induced by PRS on SNARE proteins persisted throughout

ageing and was exclusively at the expense of proteins specifically deputed to glutamate release such Syntaxin and Rab3a. The results we obtained, highlight that PRS induces alterations that are strictly sex-dependent, consistently with previous data obtained in PRS rats at different ages (Reynaert et al., 2016; Morley-Fletcher et al., 2019; Verhaeghe et al., 2022). Indeed, PRS demasculinizes males that exhibit a profile similar to that of female unstressed rats. Measuring of sex hormones in plasma indicates decreased plasma testosterone and increased dihydrotestosterone levels in PRS males, while PRS reduced estradiol plasma levels in females. A reduction in testosterone levels represents a lifelong endocrine outcome of PRS, having been observed in fetal, adult and aged life (Ward and Weisz, 1984; Reynaert et al., 2016; Verhaeghe et al., 2022). To our knowledge, the few studies comparing the expression of AMPAR with respect to biological sex, suggest a modulation of AMPAR by estradiol which appears however region specific. For instance, estradiol is without effect on AMPAR binding density in the hippocampus and the dentate gyrus, but it reduces AMPAR binding density in the striatum, the frontal cortex and the nucleus accumbens (D'Souza et al., 2003). Moreover, expression of AMPARs GluA2/A3 in the hypothalamus is 2-fold higher in unstressed females with respect to males in gonadectomized animals after estradiol supplementation, with no changes in septum or amygdala (Diano et al., 1997). This estradiol-dependent modulation is also true for GABA, at least in the hippocampus, where several studies indicate a role for estradiol-dependent formation of new spines (Woolley and McEwen, 1992), which requires modification of GABAergic transmission in developing and adult hippocampus. Indeed, during development, estradiol would increase the impact of excitatory GABAergic transmission to hippocampal neurons,

while in the adult brain, estradiol would suppress GABAergic inhibitory transmission (Wójtowicz and Mozrzymas, 2010). As a consequence, increased excitability in CA1 is favored, and the formation of new dendritic spines is facilitated. Finally, levels of estradiol are higher in PRS aged males (Verhaeghe et al., 2022), which display reduced levels of $GABA_A$-α1 in the prefrontal cortex. Also, the activity of aromatase, the enzyme that converts testosterone to estradiol, is upregulated by PRS in males and downregulated in females in the dorsal hippocampus, but there is no effect of PRS on aromatase in the ventral hippocampus or prefrontal cortex (Verhaeghe et al., 2022). This supports a sex steroid-dependent region-specific regulation of AMPARs and $GABA_A$-α1 subunit, which is modulated by PRS. In conclusion, our findings strongly support the idea that early-life stress causes long-lasting effects on brain development in a sex-dependent way and provide the first evidence that ageing processes of cognition and AMPA/GABA transmission are selectively accelerated in males and that females are protected against PRS. Our findings may contribute to take into account a sex approach in pharmacological treatment strategies involving glutamate transmission, which would take into account individual variability programmed by early life stress.

GENERAL DISCUSSION AND CONCLUSIONS

Early adverse events impair the emotional, psychological, and social well-being of children and youth, causing long-term alterations in brain architecture programming and increasing vulnerability to stress-related disorders (Brown et al., 2009; Kessler et al., 2010; Friedman et al., 2015). Employing animal models of postweaning social isolation and perinatal stress, I investigated the role of glutamatergic neurotransmission in the manifestation of aberrant emotional, cognitive and social phenotypes induced by stress early in life. The main results of my research activity can be summarized in the following two points: (i) the absence of social stimuli during adolescence induces neurochemical and behavioral phenotype that mimics some behavioral features of a mouse model of autism spectrum disorders (Caruso et al., 2022); (ii) perinatal stress (PRS) induces long-term emotional, cognitive and biochemical programming alterations in a sex- and age-dependent way. Particularly, stress-induced alterations of cognitive performance, social interaction, and reactivity to novelty during the critical periods of life converge toward severe impairment of glutamate neurotransmission (i.e., metabotropic glutamate receptors of type II and AMPA glutamate receptors), underscoring its key role in determining and maintaining the proper developmental trajectory of individuals across the lifespan. On the one hand, these findings add to the growing literature suggesting that glutamate neurotransmission is deeply involved in the pathophysiological mechanisms of stress-related disorders; on the other hand, they add evidence to the complex picture of molecular actors involved in the manifestation of behavioral disruptions observed in stress-related disorders. Particularly, the identification and characterization of molecular pathways susceptible to stress-induced alterations in early life

could provide new insights into the developmental trajectories determining the manifestation of maladaptation programmed by early adverse events and underlying the development of psychopathology in adulthood. These findings could pave the way for the development of innovative pharmacological treatment strategies for neuropsychiatric disorders involving glutamatergic neurotransmission and taking into account sex- and age-dependent individual variability programmed by early life stress.

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REFERENCES

Acosta, G., Freidman, D. P., Grant, K. A., & Hemby, S. E. (2012). Alternative splicing of AMPA subunits in prefrontal cortical fields of cynomolgus monkeys following chronic ethanol self-administration. Frontiers in psychiatry, 2, 72. doi: 10.3389/fpsyt.2011.00072.

Adams, T., & Rosenkranz, J. A. (2016). Social Isolation During Postweaning Development Causes Hypoactivity of Neurons in the Medial Nucleus of the Male Rat Amygdala. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 41(7), 1929–1940. doi: 10.1038/npp.2015.364.

Agorastos, A., Pervanidou, P., Chrousos, G. P., & Baker, D. G. (2019). Developmental Trajectories of Early Life Stress and Trauma: A Narrative Review on Neurobiological Aspects Beyond Stress System Dysregulation. Frontiers in psychiatry, 10, 118. doi: 10.3389/fpsyt.2019.00118.

Ajayi, A. F., & Akhigbe, R. E. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertility research and practice, 6, 5. doi : 10.1186/s40738-020-00074-3.

Akinbo, O. I., McNeal, N., Hylin, M., Hite, N., Dagner, A., & Grippo, A. J. (2022). The Influence of Environmental Enrichment on Affective and Neural Consequences of Social Isolation Across Development. Affective science, 3(4), 713–733. doi: 10.1007/s42761-022-00131-8.

Al-Amin, M. M., Sultana, R., Sultana, S., Rahman, M. M., & Reza, H. M. (2016). Astaxanthin ameliorates prenatal LPS-exposed behavioral deficits and oxidative stress in adult offspring. BMC neuroscience, 17, 11. doi: 10.1186/s12868-016-0245-z.

Alvi, T., Kumar, D., & Tabak, B. A. (2022). Social anxiety and behavioral assessments of social cognition: A systematic review. Journal of affective disorders, 311, 17–30. doi: 10.1016/j.jad.2022.04.130.

Alyamani, R. A. S., & Murgatroyd, C. (2018). Epigenetic Programming by Early-Life Stress. Progress in molecular biology and translational science, 157, 133–150. doi: 10.1016/bs.pmbts.2018.01.004.

An, D., Chen, W., Yu, D. Q., Wang, S. W., Yu, W. Z., Xu, H., Wang, D. M., Zhao, D., Sun, Y. P., Wu, J. C., Tang, Y. Y., & Yin, S. M. (2017). Effects of social isolation, re-socialization and age on cognitive and aggressive

behaviors of Kunming mice and BALB/c mice. Animal science journal = Nihon chikusan Gakkaiho, 88(5), 798–806. doi: 10.1111/asj.12688.

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology, 36(3), 415–425. doi : 10.1016/j.psyneuen.2010.03.007.

Anand, K. S., & Dhikav, V. (2012). Hippocampus in health and disease: An overview. Annals of Indian Academy of Neurology, 15(4), 239–246. doi: 10.4103/0972-2327.104323.

Andersen, J. V., Markussen, K. H., Jakobsen, E., Schousboe, A., Waagepetersen, H. S., Rosenberg, P. A., & Aldana, B. I. (2021). Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology, 196, 108719. doi: 10.1016/j.neuropharm.2021.108719.

Angelucci, L., Patacchioli, F. R., Scaccianoce, S., Di Sciullo, A., Cardillo, A., & Maccari, S. (1985). A model for later-life effects of perinatal drug exposure: maternal hormone mediation. Neurobehavioral toxicology and teratology, 7(5), 511–517. PMID: 4080068.

Anggono, V., & Huganir, R. L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. Current opinion in neurobiology, 22(3), 461–469. doi: 10.1016/j.conb.2011.12.006.

Arnhold, M. M., Wotus, C., & Engeland, W. C. (2007). Differential regulation of parvocellular neuronal activity in the paraventricular nucleus of the hypothalamus following single vs. repeated episodes of water restriction-induced drinking. Experimental neurology, 206(1), 126–136. doi: 10.1016/j.expneurol.2007.04.010.

Atladóttir, H. O., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. Journal of autism and developmental disorders, 40(12), 1423–1430. doi: 10.1007/s10803-010-1006-y.

Banker, S. M., Gu, X., Schiller, D., & Foss-Feig, J. H. (2021). Hippocampal contributions to social and cognitive deficits in autism spectrum disorder. Trends in neurosciences, 44(10), 793–807. doi: 10.1016/j.tins.2021.08.005.

Bannerman, D. M., Sprengel, R., Sanderson, D. J., McHugh, S. B., Rawlins, J. N., Monyer, H., & Seeburg, P. H. (2014). Hippocampal synaptic plasticity,

spatial memory and anxiety. Nature reviews. Neuroscience, 15(3), 181–192. doi: 10.1038/nrn3677.

Barbazanges, A., Piazza, P. V., Le Moal, M., & Maccari, S. (1996). Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. The Journal of neuroscience : the official journal of the Society for Neuroscience, 16(12), 3943–3949. doi: 10.1523/JNEUROSCI.16-12-03943.1996.

Barbieri, R., Contestabile, A., Ciardo, M. G., Forte, N., Marte, A., Baldelli, P., Benfenati, F., & Onofri, F. (2018). Synapsin I and Synapsin II regulate neurogenesis in the dentate gyrus of adult mice. Oncotarget, 9(27), 18760–18774. doi: 10.18632/oncotarget.24655.

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., & Robinson, J. S. (1993). Fetal nutrition and cardiovascular disease in adult life. Lancet (London, England), 341(8850), 938–941. doi: 10.1016/0140-6736(93)91224-a.

Barker, D. J. (1995). Fetal origins of coronary heart disease. BMJ 311, 171–174. doi:10.1136/bmj.311.6998.171.

Barker, D. J., & Clark, P. M. (1997). Fetal undernutrition and disease in later life. Reviews of reproduction, 2(2), 105–112. doi : 10.1530/ror.0.0020105.

Baron-Cohen, S., Ring, H. A., Bullmore, E. T., Wheelwright, S., Ashwin, C., & Williams, S. C. (2000). The amygdala theory of autism. Neuroscience and biobehavioral reviews, 24(3), 355–364. doi: 10.1016/s0149-7634(00)00011-7.

Barros, V. G., Rodríguez, P., Martijena, I. D., Pérez, A., Molina, V. A., & Antonelli, M. C. (2006). Prenatal stress and early adoption effects on benzodiazepine receptors and anxiogenic behavior in the adult rat brain. Synapse (New York, N.Y.), 60(8), 609–618. doi: 10.1002/syn.20336.

Bartsch, T. (2012). The Hippocampus in Neurological Disease. The Clinical Neurobiology of the Hippocampus: An Integrative view. Oxford Scholarship Online. doi: 10.1093/acprof:oso/9780199592388.003.0011.

Beery, A. K., & Kaufer, D. (2015). Stress, social behavior, and resilience: insights from rodents. Neurobiology of stress, 1, 116–127. doi: 10.1016/j.ynstr.2014.10.004.

Begni, V., Sanson, A., Pfeiffer, N., Brandwein, C., Inta, D., Talbot, S. R., Riva, M. A., Gass, P., & Mallien, A. S. (2020). Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity. PloS one, 15(10), e0240439. doi: 10.1371/journal.pone.0240439.

Ben-Ari, Y., Khazipov, R., Leinekugel, X., Caillard, O., & Gaiarsa, J. L. (1997). GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. Trends in neurosciences, 20(11), 523–529. doi: 10.1016/s0166-2236(97)01147-8.

Benchenane, K., Tiesinga, P. H., & Battaglia, F. P. (2011). Oscillations in the prefrontal cortex: a gateway to memory and attention. Current opinion in neurobiology, 21(3), 475–485. doi: 10.1016/j.conb.2011.01.004.

Benno, R., Smirnova, Y., Vera, S., Liggett, A., & Schanz, N. (2009). Exaggerated responses to stress in the BTBR T+tf/J mouse: an unusual behavioral phenotype. Behavioural brain research, 197(2), 462–465. doi: 10.1016/j.bbr.2008.09.041.

Bicks, L. K., Koike, H., Akbarian, S., & Morishita, H. (2015). Prefrontal Cortex and Social Cognition in Mouse and Man. Frontiers in psychology, 6, 1805. doi: 10.3389/fpsyg.2015.01805.

Blanke, M. L., & VanDongen, A. M. J. (2009). Activation Mechanisms of the NMDA Receptor. In A. M. Van Dongen (Ed.), Biology of the NMDA Receptor. CRC Press/Taylor & Francis. PMID: 21204408.

Bockmühl, Y., Patchev, A. V., Madejska, A., Hoffmann, A., Sousa, J. C., Sousa, N., Holsboer, F., Almeida, O. F., & Spengler, D. (2015). Methylation at the CpG island shore region upregulates Nr3c1 promoter activity after early-life stress. Epigenetics, 10(3), 247–257. doi: 10.1080/15592294.2015.1017199.

Bodzęta, A., Scheefhals, N., & MacGillavry, H. D. (2021). Membrane trafficking and positioning of mGluRs at presynaptic and postsynaptic sites of excitatory synapses. Neuropharmacology, 200, 108799. doi: 10.1016/j.neuropharm.2021.108799.

Boero, G., Pisu, M. G., Biggio, F., Muredda, L., Carta, G., Banni, S., Paci, E., Follesa, P., Concas, A., Porcu, P., & Serra, M. (2018). Impaired glucocorticoid-mediated HPA axis negative feedback induced by juvenile social isolation in male rats. Neuropharmacology, 133, 242–253. doi: 10.1016/j.neuropharm.2018.01.045.

Bolivar, V. J., Walters, S. R., & Phoenix, J. L. (2007). Assessing autism-like behavior in mice: variations in social interactions among inbred strains. Behavioural Brain Research, 176(1), 21–26. doi: 10.1016/j.bbr.2006.09.007.

Bowman, R. E., MacLusky, N. J., Sarmiento, Y., Frankfurt, M., Gordon, M., & Luine, V. N. (2004). Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central

neurotransmitters. Endocrinology, 145(8), 3778–3787. doi: 10.1210/en.2003-1759.

Bozdagi, O., Sakurai, T., Papapetrou, D., Wang, X., Dickstein, D. L., Takahashi, N., Kajiwara, Y., Yang, M., Katz, A. M., Scattoni, M. L., Harris, M. J., Saxena, R., Silverman, J. L., Crawley, J. N., Zhou, Q., Hof, P. R., & Buxbaum, J. D. (2010). Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. Molecular autism, 1(1), 15. doi: 10.1186/2040-2392-1-15.

Bredy, T. W., Zhang, T. Y., Grant, R. J., Diorio, J., & Meaney, M. J. (2004). Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. The European journal of neuroscience, 20(5), 1355–1362. doi: 10.1111/j.1460-9568.2004.03599.x.

Brielmaier, J., Matteson, P. G., Silverman, J. L., Senerth, J. M., Kelly, S., Genestine, M., Millonig, J. H., DiCicco-Bloom, E., & Crawley, J. N. (2012). Autism-relevant social abnormalities and cognitive deficits in engrailed-2 knockout mice. PloS one, 7(7), e40914. doi : 10.1371/journal.pone.0040914.

Brown, D. W., Anda, R. F., Tiemeier, H., Felitti, V. J., Edwards, V. J., Croft, J. B., & Giles, W. H. (2009). Adverse childhood experiences and the risk of premature mortality. American journal of preventive medicine, 37(5), 389–396. doi: 10.1016/j.amepre.2009.06.021.

Bruno, V., Caraci, F., Copani, A., Matrisciano, F., Nicoletti, F., & Battaglia, G. (2017). The impact of metabotropic glutamate receptors into active neurodegenerative processes: A "dark side" in the development of new symptomatic treatments for neurologic and psychiatric disorders. Neuropharmacology, 115, 180–192. doi: 10.1016/j.neuropharm.2016.04.044.

Burger, P. M., Mehl, E., Cameron, P. L., Maycox, P. R., Baumert, M., Lottspeich, F., De Camilli, P., & Jahn, R. (1989). Synaptic vesicles immunoisolated from rat cerebral cortex contain high levels of glutamate. Neuron, 3(6), 715–720. doi : 10.1016/0896-6273(89)90240-7.

Burket, J. A., Herndon, A. L., Winebarger, E. E., Jacome, L. F., & Deutsch, S. I. (2011). Complex effects of mGluR5 antagonism on sociability and stereotypic behaviors in mice: possible implications for the pharmacotherapy of autism spectrum disorders. Brain research bulletin, 86(3-4), 152–158. doi: 10.1016/j.brainresbull.2011.08.001.

Byrnes, K. R., Loane, D. J., & Faden, A. I. (2009). Metabotropic glutamate receptors as targets for multipotential treatment of neurological

disorders. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics, 6(1), 94–107. doi: 10.1016/j.nurt.2008.10.038.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proceedings of the National Academy of Sciences of the United States of America, 95(9), 5335–5340. doi: 10.1073/pnas.95.9.5335.

Caldji, C., Diorio, J., & Meaney, M. J. (2003). Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 28(11), 1950–1959. doi: 10.1038/sj.npp.1300237.

Careaga, M., Schwartzer, J., & Ashwood, P. (2015). Inflammatory profiles in the BTBR mouse: how relevant are they to autism spectrum disorders?. Brain, behavior, and immunity, 43, 11–16. doi: 10.1016/j.bbi.2014.06.006.

Caruso, A., Nicoletti, F., Gaetano, A., & Scaccianoce, S. (2019). Risk Factors for Alzheimer's Disease: Focus on Stress. Frontiers in pharmacology, 10, 976. doi: 10.3389/fphar.2019.00976.

Caruso, A., Ricceri, L., Caruso, A., Nicoletti, F., Gaetano, A., & Scaccianoce, S. (2022a). Postweaning social isolation and autism-like phenotype: A biochemical and behavioral comparative analysis. Behavioural brain research, 428, 113891. doi: 10.1016/j.bbr.2022.113891.

Caruso, A., Gaetano, A., & Scaccianoce, S. (2022b). Corticotropin-Releasing Hormone: Biology and Therapeutic Opportunities. Biology, 11(12), 1785. doi: 10.3390/biology11121785.

Caudal, D., Godsil, B. P., Mailliet, F., Bergerot, D., & Jay, T. M. (2010). Acute stress induces contrasting changes in AMPA receptor subunit phosphorylation within the prefrontal cortex, amygdala and hippocampus. PloS one, 5(12), e15282. doi: 10.1371/journal.pone.0015282.

César-Razquin, A., Snijder, B., Frappier-Brinton, T., Isserlin, R., Gyimesi, G., Bai, X., Reithmeier, R. A., Hepworth, D., Hediger, M. A., Edwards, A. M., & Superti-Furga, G. (2015). A Call for Systematic Research on Solute Carriers. Cell, 162(3), 478–487. doi: 10.1016/j.cell.2015.07.022.

Chang, C. H., Hsiao, Y. H., Chen, Y. W., Yu, Y. J., & Gean, P. W. (2015). Social isolation-induced increase in NMDA receptors in the hippocampus

exacerbates emotional dysregulation in mice. Hippocampus, 25(4), 474–485. doi : 10.1002/hipo.22384.

Chang, C. H., Su, C. L., & Gean, P. W. (2018). Mechanism underlying NMDA blockade-induced inhibition of aggression in post-weaning socially isolated mice. Neuropharmacology, 143, 95–105. doi: 10.1016/j.neuropharm.2018.09.019.

Chater, T. E., & Goda, Y. (2014). The role of AMPA receptors in postsynaptic mechanisms of synaptic plasticity. Frontiers in cellular neuroscience, 8, 401. doi: 10.3389/fncel.2014.00401.

Chater, T. E., & Goda, Y. (2022). The Shaping of AMPA Receptor Surface Distribution by Neuronal Activity. Frontiers in synaptic neuroscience, 14, 833782. doi : 10.3389/fnsyn.2022.833782.

Chen, W., Mahadomrongkul, V., Berger, U. V., Bassan, M., DeSilva, T., Tanaka, K., Irwin, N., Aoki, C., & Rosenberg, P. A. (2004). The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 24(5), 1136–1148. doi: 10.1523/JNEUROSCI.1586-03.2004.

Chen, Y., Baram, T (2016). Toward Understanding How Early-Life Stress Reprograms Cognitive and Emotional Brain Networks. Neuropsychopharmacol 41, 197–206. doi : 10.1038/npp.2015.181.

Chen, T. J., & Kukley, M. (2020). Glutamate receptors and glutamatergic signalling in the peripheral nerves. Neural regeneration research, 15(3), 438–447. doi: 10.4103/1673-5374.266047.

Cherubini, E., Gaiarsa, J. L., & Ben-Ari, Y. (1991). GABA: an excitatory transmitter in early postnatal life. Trends in neurosciences, 14(12), 515–519. doi : 10.1016/0166-2236(91)90003-d.

Chittajallu, R., Vignes, M., Dev, K. K., Barnes, J. M., Collingridge, G. L., & Henley, J. M. (1996). Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. Nature, 379(6560), 78–81. doi: 10.1038/379078a0.

Choi, C. H., Schoenfeld, B. P., Bell, A. J., Hinchey, P., Kollaros, M., Gertner, M. J., Woo, N. H., Tranfaglia, M. R., Bear, M. F., Zukin, R. S., McDonald, T. V., Jongens, T. A., & McBride, S. M. (2011). Pharmacological reversal of synaptic plasticity deficits in the mouse model of fragile X syndrome by group II mGluR antagonist or lithium treatment. Brain research, 1380, 106–119. doi: 10.1016/j.brainres.2010.11.032.

Cioni, G., & Sgandurra, G. (2013). Normal psychomotor development. Handbook of clinical neurology, 111, 3–15. doi: 10.1016/B978-0-444-52891-9.00001-4.

Clem, R. L., & Huganir, R. L. (2010). Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science (New York, N.Y.), 330(6007), 1108–1112. doi: 10.1126/science.1195298.

Colgin L. L. (2011). Oscillations and hippocampal-prefrontal synchrony. Curr. Opin. Neurobiol. 21, 467–474. doi: 10.1016/j.conb.2011.04.006.

Contractor, A., Mulle, C., & Swanson, G. T. (2011). Kainate receptors coming of age: milestones of two decades of research. Trends in neurosciences, 34(3), 154–163. doi: 10.1016/j.tins.2010.12.002.

Costa Cruz, P. H., & Kawahara, Y. (2021). RNA Editing in Neurological and Neurodegenerative Disorders. Methods in molecular biology (Clifton, N.J.), 2181, 309–330. doi: 10.1007/978-1-0716-0787-9_18.

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacology, biochemistry, and behavior, 86(2), 189–199. doi: 10.1016/j.pbb.2006.12.001.

Crupi, R., Impellizzeri, D., & Cuzzocrea, S. (2019). Role of Metabotropic Glutamate Receptors in Neurological Disorders. Frontiers in molecular neuroscience, 12, 20. doi: 10.3389/fnmol.2019.00020.

D'Souza, D. N., Harlan, R. E., & Garcia, M. M. (2003). Modulation of glutamate receptor expression by gonadal steroid hormones in the rat striatum. Brain research bulletin, 59(4), 289–292. doi: 10.1016/s0361-9230(02)00885-7.

Daimon, C. M., Jasien, J. M., Wood, W. H., 3rd, Zhang, Y., Becker, K. G., Silverman, J. L., Crawley, J. N., Martin, B., & Maudsley, S. (2015). Hippocampal Transcriptomic and Proteomic Alterations in the BTBR Mouse Model of Autism Spectrum Disorder. Frontiers in physiology, 6, 324. doi: 10.3389/fphys.2015.00324.

Dalley, J. W., Theobald, D. E., Pereira, E. A. C., Li, P. M. M. C., & Robbins, T. W. (2002). Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. Psychopharmacology, 164(3), 329–340. doi: 10.1007/s00213-002-1215-y.

Dana, K., Finik, J., Koenig, S., Motter, J., Zhang, W., Linaris, M., Brumberg, J. C., & Nomura, Y. (2019). Prenatal Exposure to Famine and Risk for Development of Psychopathology in Adulthood: A Meta-Analysis. Journal of psychiatry and psychiatric disorders, 3(5), 227–240. doi: 10.26502/jppd.2572-519X0077.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. Physiology & behavior, 106(1), 29–39. doi: 10.1016/j.physbeh.2011.08.019.

Dang, Y. H., Liu, P., Ma, R., Chu, Z., Liu, Y. P., Wang, J. B., Ma, X. C., & Gao, C. G. (2015). HINT1 is involved in the behavioral abnormalities induced by social isolation rearing. Neuroscience letters, 607, 40–45. doi: 10.1016/j.neulet.2015.08.026.

Darnaudéry, M., Pallarès, M., Piazza, P. V., Le Moal, M., & Mayo, W. (2002). The neurosteroid pregnenolone sulfate infused into the medial septum nucleus increases hippocampal acetylcholine and spatial memory in rats. Brain research, 951(2), 237–242. doi: 10.1016/s0006-8993(02)03166-9.

Darnaudéry, M., & Maccari, S. (2008). Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain research reviews, 57(2), 571–585. doi : 10.1016/j.brainresrev.2007.11.004.

Day-Wilson, K. M., Jones, D. N. C., Southam, E., Cilia, J., & Totterdell, S. (2006). Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle. Neuroscience, 141(3), 1113–1121. doi : 10.1016/j.neuroscience.2006.04.048.

de Angelis, L., & File, S. E. (1979). Acute and chronic effects of three benzodiazepines in the social interaction anxiety test in mice. Psychopharmacology, 64(2), 127–129. doi : 10.1007/BF00496051.

de Kloet, E. R., & Reul, J. M. (1987). Feedback action and tonic influence of corticosteroids on brain function: A concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology, 12(2), 83–105. doi: 10.1016/0306-4530(87)90040-0.

de Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. Endocrine reviews, 19(3), 269–301. doi: 10.1210/edrv.19.3.0331.

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. Nature reviews. Neuroscience, 6(6), 463–475. doi: 10.1038/nrn1683.

de Kloet, E. R., Karst, H., & Joëls, M. (2008). Corticosteroid hormones in the central stress response: quick-and-slow. Frontiers in neuroendocrinology, 29(2), 268–272. doi : 10.1016/j.yfrne.2007.10.002.

de Kloet, E. R., Otte, C., Kumsta, R., Kok, L., Hillegers, M. H., Hasselmann, H., Kliegel, D., & Joëls, M. (2016). Stress and Depression: a Crucial Role of the Mineralocorticoid Receptor. Journal of neuroendocrinology, 28(8), 10.1111/jne.12379. doi : 10.1111/jne.12379.

de Kloet, E. R., Meijer, O. C., de Nicola, A. F., de Rijk, R. H., & Joëls, M. (2018). Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. Frontiers in neuroendocrinology, 49, 124–145. doi: 10.1016/j.yfrne.2018.02.003.

de Kloet, E. R., de Kloet, S. F., de Kloet, C. S., & de Kloet, A. D. (2019). Top-down and bottom-up control of stress-coping. Journal of neuroendocrinology, 31(3), e12675. doi: 10.1111/jne.12675.

de Vasconcellos-Bittencourt, A. P., Vendite, D. A., Nassif, M., Crema, L. M., Frozza, R., Thomazi, A. P., Nieto, F. B., Wofchuk, S., Salbego, C., da Rocha, E. R., & Dalmaz, C. (2011). Chronic stress and lithium treatments alter hippocampal glutamate uptake and release in the rat and potentiate necrotic cellular death after oxygen and glucose deprivation. Neurochemical research, 36(5), 793–800. doi: 10.1007/s11064-011-0404-7.

Dehorter, N., & Del Pino, I. (2020). Shifting Developmental Trajectories During Critical Periods of Brain Formation. Frontiers in cellular neuroscience, 14, 283. doi: 10.3389/fncel.2020.00283.

Deminière, J. M., Piazza, P. V., Guegan, G., Abrous, N., Maccari, S., Le Moal, M., & Simon, H. (1992). Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. Brain research, 586(1), 135–139. doi: 10.1016/0006-8993(92)91383-p.

DeMorrow S. (2018). Role of the Hypothalamic-Pituitary-Adrenal Axis in Health and Disease. International journal of molecular sciences, 19(4), 986. doi: 10.3390/ijms19040986.

Diano, S., Naftolin, F., & Horvath, T. L. (1997). Gonadal steroids target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: a morphological and biochemical study. Endocrinology, 138(2), 778–789. doi: 10.1210/endo.138.2.4937.

Diering, G. H., & Huganir, R. L. (2018). The AMPA Receptor Code of Synaptic Plasticity. Neuron, 100(2), 314–329. doi: 10.1016/j.neuron.2018.10.018.

Dong, X. X., Wang, Y., & Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta pharmacologica Sinica, 30(4), 379–387. doi: 10.1038/aps.2009.24.

Doumazane, E., Scholler, P., Zwier, J. M., Trinquet, E., Rondard, P., & Pin, J. P. (2011). A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 25(1), 66–77. doi: 10.1096/fj.10-163147.

Du, X., Li, J., Li, M., Yang, X., Qi, Z., Xu, B., Liu, W., Xu, Z., & Deng, Y. (2020). Research progress on the role of type I vesicular glutamate transporter (VGLUT1) in nervous system diseases. Cell & bioscience, 10, 26. doi: 10.1186/s13578-020-00393-4.

Duan, J., Pandey, S., Li, T., Castellano, D., Gu, X., Li, J., Tian, Q., & Lu, W. (2019). Genetic Deletion of $GABA_A$ Receptors Reveals Distinct Requirements of Neurotransmitter Receptors for GABAergic and Glutamatergic Synapse Development. Frontiers in cellular neuroscience, 13, 217. doi: 10.3389/fncel.2019.00217.

Einon, D. F., & Morgan, M. J. (1977). A critical period for social isolation in the rat. Developmental psychobiology, 10(2), 123–132. doi: 10.1002/dev.420100205.

Eissa, N., Al-Houqani, M., Sadeq, A., Ojha, S. K., Sasse, A., & Sadek, B. (2018). Current Enlightenment About Etiology and Pharmacological Treatment of Autism Spectrum Disorder. Frontiers in neuroscience, 12, 304. doi: 10.3389/fnins.2018.00304.

Ellegood, J., & Crawley, J. N. (2015). Behavioral and Neuroanatomical Phenotypes in Mouse Models of Autism. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics, 12(3), 521–533. doi: 10.1007/s13311-015-0360-z.

Eriksen, J., Li, F., & Edwards, R. H. (2020). The mechanism and regulation of vesicular glutamate transport: Coordination with the synaptic vesicle cycle. Biochimica et biophysica acta. Biomembranes, 1862(12), 183259. doi: 10.1016/j.bbamem.2020.183259.

Erzurumlu, R. S., & Gaspar, P. (2012). Development and critical period plasticity of the barrel cortex. The European journal of neuroscience, 35(10), 1540–1553. doi: 10.1111/j.1460-9568.2012.08075.x.

Estes, M. L., & McAllister, A. K. (2016). Maternal immune activation: Implications for neuropsychiatric disorders. Science (New York, N.Y.), 353(6301), 772–777. doi: 10.1126/science.aag3194.

Evans, A. J., Gurung, S., Henley, J. M., Nakamura, Y., & Wilkinson, K. A. (2019). Exciting Times: New Advances Towards Understanding the Regulation and Roles of Kainate Receptors. Neurochemical research, 44(3), 572–584. doi : 10.1007/s11064-017-2450-2.

Fanselow M. S., Dong H.-W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65, 7–19. doi: 10.1016/j.neuron.2009.11.031.

Farbstein, D., Hollander, N., Peled, O., Apter, A., Fennig, S., Haberman, Y., Gitman, H., Yaniv, I., Shkalim, V., Pick, C. G., & Benaroya-Milshtein, N. (2021). Social isolation in mice: behavior, immunity, and tumor growth. Stress, 24(2), 229–238. doi: 10.1080/10253890.2020.1777976.

Faridar, A., Jones-Davis, D., Rider, E., Li, J., Gobius, I., Morcom, L., Richards, L. J., Sen, S., & Sherr, E. H. (2014). Mapk/Erk activation in an animal model of social deficits shows a possible link to autism. Molecular autism, 5, 57. doi: 10.1186/2040-2392-5-57.

Featherstone D. E. (2010). Intercellular glutamate signaling in the nervous system and beyond. ACS chemical neuroscience, 1(1), 4–12. doi: 10.1021/cn900006n.

Ferdman, N., Murmu, R. P., Bock, J., Braun, K., & Leshem, M. (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. Behavioural brain research, 180(2), 174–182. doi: 10.1016/j.bbr.2007.03.011.

Ferraguti, F., & Shigemoto, R. (2006). Metabotropic glutamate receptors. Cell and tissue research, 326(2), 483–504. doi: 10.1007/s00441-006-0266-5.

Finsterwald, C., & Alberini, C. M. (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies. Neurobiology of learning and memory, 112, 17–29. doi: 10.1016/j.nlm.2013.09.017.

Fitzgerald, E., Hor, K., & Drake, A. J. (2020). Maternal influences on fetal brain development: The role of nutrition, infection and stress, and the potential for intergenerational consequences. Early human development, 150, 105190. doi: 10.1016/j.earlhumdev.2020.105190.

Fone, K. C. F., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neuroscience and Biobehavioral Reviews, 32(6), 1087–1102. doi: 10.1016/j.neubiorev.2008.03.003.

Franklin, T. B., Saab, B. J., & Mansuy, I. M. (2012). Neural mechanisms of stress resilience and vulnerability. Neuron, 75(5), 747–761. doi: 10.1016/j.neuron.2012.08.016.

Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., & Nicoll, R. A. (2001). Kainate receptors depress excitatory synaptic transmission at CA3-->CA1 synapses in the hippocampus via a direct presynaptic action. The Journal of neuroscience : the official journal of the Society for Neuroscience, 21(9), 2958–2966. doi: 10.1523/JNEUROSCI.21-09-02958.2001.

Friedman, E. M., Karlamangla, A. S., Gruenewald, T. L., Koretz, B., & Seeman, T. E. (2015). Early life adversity and adult biological risk profiles. Psychosomatic medicine, 77(2), 176–185. doi: 10.1097/PSY.0000000000000147.

Frodl, T., Reinhold, E., Koutsouleris, N., Reiser, M., & Meisenzahl, E. M. (2010). Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. Journal of psychiatric research, 44(13), 799–807. doi: 10.1016/j.jpsychires.2010.01.006.

Frye, C. A., & Llaneza, D. C. (2010). Corticosteroid and neurosteroid dysregulation in an animal model of autism, BTBR mice. Physiology & behavior, 100(3), 264–267. doi: 10.1016/j.physbeh.2010.03.005.

Fujiwara, H., Han, Y., Ebihara, K., Awale, S., Araki, R., Yabe, T., & Matsumoto, K. (2017). Daily administration of yokukansan and keishito prevents social isolation-induced behavioral abnormalities and down-regulation of phosphorylation of neuroplasticity-related signaling molecules in mice. BMC complementary and alternative medicine, 17(1), 195. doi: 10.1186/s12906-017-1710-7.

Fung, L. K., & Hardan, A. Y. (2015). Developing Medications Targeting Glutamatergic Dysfunction in Autism: Progress to Date. CNS drugs, 29(6), 453–463. doi: 10.1007/s40263-015-0252-0.

Gamallo, A., Villanua, A., Trancho, G., & Fraile, A. (1986). Stress adaptation and adrenal activity in isolated and crowded rats. Physiology & behavior, 36(2), 217–221. doi: 10.1016/0031-9384(86)90006-5.

Gambardella, P., Greco, A. M., Sticchi, R., Bellotti, R., & Di Renzo, G. (1994). Individual housing modulates daily rhythms of hypothalamic catecholaminergic system and circulating hormones in adult male rats. Chronobiology international, 11(4), 213–221. doi: 10.3109/07420529409067790.

Gamo, N. J., & Arnsten, A. F. (2011). Molecular modulation of prefrontal cortex: rational development of treatments for psychiatric disorders. Behavioral neuroscience, 125(3), 282–296. doi: 10.1037/a0023165.

Gan, Q., Salussolia, C. L., & Wollmuth, L. P. (2015). Assembly of AMPA receptors: mechanisms and regulation. The Journal of physiology, 593(1), 39–48. doi: 10.1113/jphysiol.2014.273755.

Gangi, D. N., Messinger, D. S., Martin, E. R., & Cuccaro, M. L. (2016). Dopaminergic variants in siblings at high risk for autism: Associations with initiating joint attention. Autism research : official journal of the International Society for Autism Research, 9(11), 1142–1150. doi: 10.1002/aur.1623.

Garin, C. M., Nadkarni, N. A., Pépin, J., Flament, J., & Dhenain, M. (2022). Whole brain mapping of glutamate distribution in adult and old primates at 11.7T. NeuroImage, 251, 118984. doi: 10.1016/j.neuroimage.2022.118984.

Gasbarri, A., & Pompili, A. (2014). 4 - Involvement of Glutamate in Learning and Memory, Editor(s): Alfredo Meneses, Identification of Neural Markers Accompanying Memory, Elsevier,2014, Pages 63-77, ISBN 9780124081390. doi: 10.1016/B978-0-12-408139-0.00004-3.

Gatta, E., Mairesse, J., Deruyter, L., Marrocco, J., Van Camp, G., Bouwalerh, H., Lo Guidice, J. M., Morley-Fletcher, S., Nicoletti, F., & Maccari, S. (2018). Reduced maternal behavior caused by gestational stress is predictive of life span changes in risk-taking behavior and gene expression due to altering of the stress/anti-stress balance. Neurotoxicology, 66, 138–149. doi: 10.1016/j.neuro.2018.04.005.

Gentsch, C., Lichtsteiner, M., & Feer, H. (1981). Locomotor activity, defecation score and corticosterone levels during an openfield exposure: a comparison among individually and group-housed rats, and genetically selected rat lines. Physiology & behavior, 27(1), 183–186. doi: 10.1016/0031-9384(81)90320-6.

Ghit, A., Assal, D., Al-Shami, A. S., & Hussein, D. E. E. (2021). GABA$_A$ receptors: structure, function, pharmacology, and related disorders. Journal, genetic engineering & biotechnology, 19(1), 123. doi: 10.1186/s43141-021-00224-0.

Glasgow, N. G., Siegler Retchless, B., & Johnson, J. W. (2015). Molecular bases of NMDA receptor subtype-dependent properties. The Journal of physiology, 593(1), 83–95. doi : 10.1113/jphysiol.2014.273763.

Głombik, K., Stachowicz, A., Ślusarczyk, J., Trojan, E., Budziszewska, B., Suski, M., Kubera, M., Lasoń, W., Wędzony, K., Olszanecki, R., & Basta-Kaim, A. (2015). Maternal stress predicts altered biogenesis and the profile of mitochondrial proteins in the frontal cortex and hippocampus of adult offspring rats. Psychoneuroendocrinology, 60, 151–162. doi: 10.1016/j.psyneuen.2015.06.015.

Golubeva, A. V., Crampton, S., Desbonnet, L., Edge, D., O'Sullivan, O., Lomasney, K. W., Zhdanov, A. V., Crispie, F., Moloney, R. D., Borre, Y. E., Cotter, P. D., Hyland, N. P., O'Halloran, K. D., Dinan, T. G., O'Keeffe, G. W., & Cryan, J. F. (2015). Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. Psychoneuroendocrinology, 60, 58–74. doi: 10.1016/j.psyneuen.2015.06.002.

Goodell, D. J., Ahern, M. A., Baynard, J., Wall, V. L., & Bland, S. T. (2017). A novel escapable social interaction test reveals that social behavior and mPFC activation during an escapable social encounter are altered by post-weaning social isolation and are dependent on the aggressiveness of the stimulus rat. Behavioural brain research, 317, 1–15. doi: 10.1016/j.bbr.2016.09.025.

Gould, G. G., Burke, T. F., Osorio, M. D., Smolik, C. M., Zhang, W. Q., Onaivi, E. S., Gu, T. T., DeSilva, M. N., & Hensler, J. G. (2014). Enhanced novelty-induced corticosterone spike and upregulated serotonin 5-HT1A and cannabinoid CB1 receptors in adolescent BTBR mice. Psychoneuroendocrinology, 39, 158–169. doi: 10.1016/j.psyneuen.2013.09.003).

Gould, J. M., Smith, P. J., Airey, C. J., Mort, E. J., Airey, L. E., Warricker, F. D. M., Pearson-Farr, J. E., Weston, E. C., Gould, P. J. W., Semmence, O. G., Restall, K. L., Watts, J. A., McHugh, P. C., Smith, S. J., Dewing, J. M., Fleming, T. P., & Willaime-Morawek, S. (2018). Mouse maternal protein restriction during preimplantation alone permanently alters brain neuron proportion and adult short-term memory. Proceedings of the National

Academy of Sciences of the United States of America, 115(31), E7398–E7407. doi: 10.1073/pnas.1721876115.

Granata, L., Valentine, A., Hirsch, J. L., Honeycutt, J., & Brenhouse, H. (2021). Trajectories of Mother-Infant Communication: An Experiential Measure of the Impacts of Early Life Adversity. Frontiers in human neuroscience, 15, 632702. doi: 10.3389/fnhum.2021.632702.

Gray, J. D., Kogan, J. F., Marrocco, J., & McEwen, B. S. (2017). Genomic and epigenomic mechanisms of glucocorticoids in the brain. Nature Reviews. Endocrinology, 13(11), 661–673. doi: 10.1038/nrendo.2017.97.

Greger, I. H., Watson, J. F., & Cull-Candy, S. G. (2017). Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. Neuron, 94(4), 713–730. doi : 10.1016/j.neuron.2017.04.009.

Gué, M., Bravard, A., Meunier, J., Veyrier, R., Gaillet, S., Recasens, M., & Maurice, T. (2004). Sex differences in learning deficits induced by prenatal stress in juvenile rats. Behavioural brain research, 150(1-2), 149–157. doi: 10.1016/S0166-4328(03)00250-X.

Guo, C., & Ma, Y. Y. (2021). Calcium Permeable-AMPA Receptors and Excitotoxicity in Neurological Disorders. Frontiers in neural circuits, 15, 711564. doi : 10.3389/fncir.2021.711564.

Guskov, A., Jensen, S., Faustino, I., Marrink, S. J., & Slotboom, D. J. (2016). Coupled binding mechanism of three sodium ions and aspartate in the glutamate transporter homologue Glt_{Tk}. Nature communications, 7, 13420. doi : 10.1038/ncomms13420.

Haapanen, M. J., Perälä, M. M., Salonen, M. K., Kajantie, E., Simonen, M., Pohjolainen, P., Pesonen, A. K., Räikkönen, K., Eriksson, J. G., & von Bonsdorff, M. B. (2018). Early life stress and frailty in old age: the Helsinki birth cohort study. BMC geriatrics, 18(1), 179. doi: 10.1186/s12877-018-0873-5.

Hall, F. S., Ghaed, S., Pert, A., & Xing, G. (2002). The effects of isolation rearing on glutamate receptor NMDAR1A mRNA expression determined by in situ hybridization in Fawn hooded and Wistar rats. Pharmacology, biochemistry, and behavior, 73(1), 185–191. doi. 10.1016/s0091-3057(02)00796-7.

Haller J. (2018). The role of central and medial amygdala in normal and abnormal aggression: A review of classical approaches. Neuroscience and biobehavioral reviews, 85, 34–43. doi: 10.1016/j.neubiorev.2017.09.017.

Hanley J. G. (2018). The Regulation of AMPA Receptor Endocytosis by Dynamic Protein-Protein Interactions. Frontiers in cellular neuroscience, 12, 362. doi : 10.3389/fncel.2018.00362.

Hansen, K. B., Yi, F., Perszyk, R. E., Furukawa, H., Wollmuth, L. P., Gibb, A. J., & Traynelis, S. F. (2018). Structure, function, and allosteric modulation of NMDA receptors. The Journal of general physiology, 150(8), 1081–1105. doi: 10.1085/jgp.201812032.

Hao, L. Y., Hao, X. Q., Li, S. H., & Li, X. H. (2010). Prenatal exposure to lipopolysaccharide results in cognitive deficits in age-increasing offspring rats. Neuroscience, 166(3), 763–770. doi: 10.1016/j.neuroscience.2010.01.006.

Haratizadeh, S., Parvan, M., Mohammadi, S., Shabani, M., & Nozari, M. (2021). An overview of modeling and behavioral assessment of autism in the rodent. International Journal of Developmental Neuroscience, 81(3), 221–228. doi: 10.1002/jdn.10096.

Hatch, A. M., Wiberg, G. S., Zawidzka, Z., Cann, M., Airth, J. M., & Grice, H. C. (1965). Isolation syndrome in the rat. Toxicology and applied pharmacology, 7(5), 737–745. doi: 10.1016/0041-008x(65)90132-8.

Hawkins R. A. (2009). The blood-brain barrier and glutamate. The American journal of clinical nutrition, 90(3), 867S–874S. doi: 10.3945/ajcn.2009.27462BB.

Heaney, C. F., & Kinney, J. W. (2016). Role of GABA(B) receptors in learning and memory and neurological disorders. Neuroscience and biobehavioral reviews, 63, 1–28. doi: 10.1016/j.neubiorev.2016.01.007.

Hellemans, K. G., Benge, L. C., & Olmstead, M. C. (2004). Adolescent enrichment partially reverses the social isolation syndrome. Brain research. Developmental brain research, 150(2), 103–115. doi: 10.1016/j.devbrainres.2004.03.003.

Henley, J. M., Barker, E. A., & Glebov, O. O. (2011). Routes, destinations and delays: recent advances in AMPA receptor trafficking. Trends in neurosciences, 34(5), 258–268. doi: 10.1016/j.tins.2011.02.004.

Henley, J. M., & Wilkinson, K. A. (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. Dialogues in clinical neuroscience, 15(1), 11–27. doi: 10.31887/DCNS.2013.15.1/jhenley.

Henry, C., Kabbaj, M., Simon, H., Le Moal, M., & Maccari, S. (1994). Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young

and adult rats. Journal of neuroendocrinology, 6(3), 341–345. doi: 10.1111/j.1365-2826.1994.tb00591.x.

Herman, J. P., McKlveen, J. M., Solomon, M. B., Carvalho-Netto, E., & Myers, B. (2012). Neural regulation of the stress response: glucocorticoid feedback mechanisms. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas, 45(4), 292–298. doi: 10.1590/s0100-879x2012007500041.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. Comprehensive Physiology, 6(2), 603–621. doi: 10.1002/cphy.c150015.

Hermes, G., Li, N., Duman, C., & Duman, R. (2011). Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. Physiology & behavior, 104(2), 354–359. doi: 10.1016/j.physbeh.2010.12.019.

Hertz L. (2013). The Glutamate-Glutamine (GABA) Cycle: Importance of Late Postnatal Development and Potential Reciprocal Interactions between Biosynthesis and Degradation. Frontiers in endocrinology, 4, 59. doi: 10.3389/fendo.2013.00059.

Hill, M. N., Eiland, L., Lee, T. T. Y., Hillard, C. J., & McEwen, B. S. (2019). Early life stress alters the developmental trajectory of corticolimbic endocannabinoid signaling in male rats. Neuropharmacology, 146, 154–162. doi: 10.1016/j.neuropharm.2018.11.036.

Hinz, B., Becher, A., Mitter, D., Schulze, K., Heinemann, U., Draguhn, A., & Ahnert-Hilger, G. (2001). Activity-dependent changes of the presynaptic synaptophysin-synaptobrevin complex in adult rat brain. European journal of cell biology, 80(10), 615–619. doi: 10.1078/0171-9335-00196.

Hoffmann, L. C., Schütte, S. R., Koch, M., & Schwabe, K. (2009). Effect of "enriched environment" during development on adult rat behavior and response to the dopamine receptor agonist apomorphine. Neuroscience, 158(4), 1589–1598. doi: 10.1016/j.neuroscience.2008.11.035.

Holt-Lunstad, J. (2018). Why Social Relationships Are Important for Physical Health: A Systems Approach to Understanding and Modifying Risk and Protection. Annual Review of Psychology, 69, 437–458. doi: 10.1146/annurev-psych-122216-011902.

Horder, J., Petrinovic, M. M., Mendez, M. A., Bruns, A., Takumi, T., Spooren, W., Barker, G. J., Künnecke, B., & Murphy, D. G. (2018). Glutamate and GABA in autism spectrum disorder-a translational magnetic resonance spectroscopy study in man and rodent models. Translational psychiatry, 8(1), 106. doi: 10.1038/s41398-018-0155-1.

Hosseini, E., Farid Habibi, M., Babri, S., Mohaddes, G., Abkhezr, H., & Heydari, H. (2022). Maternal stress induced anxiety-like behavior exacerbated by electromagnetic fields radiation in female rats offspring. PloS one, 17(8), e0273206. doi: 10.1371/journal.pone.0273206.

Hunt, D. L., & Castillo, P. E. (2012). Synaptic plasticity of NMDA receptors: mechanisms and functional implications. Current opinion in neurobiology, 22(3), 496–508. doi: 10.1016/j.conb.2012.01.007.

Iadarola, N. D., Niciu, M. J., Richards, E. M., Vande Voort, J. L., Ballard, E. D., Lundin, N. B., Nugent, A. C., Machado-Vieira, R., & Zarate, C. A., Jr (2015). Ketamine and other N-methyl-D-aspartate receptor antagonists in the treatment of depression: a perspective review. Therapeutic advances in chronic disease, 6(3), 97–114. doi: 10.1177/2040622315579059.

Ibi, D., Takuma, K., Koike, H., Mizoguchi, H., Tsuritani, K., Kuwahara, Y., Kamei, H., Nagai, T., Yoneda, Y., Nabeshima, T., & Yamada, K. (2008). Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. Journal of neurochemistry, 105(3), 921–932. doi: 10.1111/j.1471-4159.2007.05207.x.

Ieraci, A., Mallei, A., & Popoli, M. (2016). Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. Neural plasticity, 2016, 6212983. doi: 10.1155/2016/6212983.

Ikawa, D., Makinodan, M., Iwata, K., Ohgidani, M., Kato, T. A., Yamashita, Y., Yamamuro, K., Kimoto, S., Toritsuka, M., Yamauchi, T., Fukami, S. I., Yoshino, H., Okumura, K., Tanaka, T., Wanaka, A., Owada, Y., Tsujii, M., Sugiyama, T., Tsuchiya, K., Mori, N., … Kishimoto, T. (2017). Microglia-derived neuregulin expression in psychiatric disorders. Brain, behavior, and immunity, 61, 375–385. doi: 10.1016/j.bbi.2017.01.003.

Iturra-Mena, A. M., Arriagada-Solimano, M., Luttecke-Anders, A., & Dagnino-Subiabre, A. (2018). Effects of prenatal stress on anxiety- and depressive-like behaviours are sex-specific in prepubertal rats. Journal of neuroendocrinology, 30(7), e12609. doi: 10.1111/jne.12609.

Ivy, A. S., Rex, C. S., Chen, Y., Dubé, C., Maras, P. M., Grigoriadis, D. E., Gall, C. M., Lynch, G., & Baram, T. Z. (2010). Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience, 30(39), 13005–13015. doi: 10.1523/JNEUROSCI.1784-10.2010.

Jash, S., & Sharma, S. (2022). Pathogenic Infections during Pregnancy and the Consequences for Fetal Brain Development. Pathogens (Basel, Switzerland), 11(2), 193. doi: 10.3390/pathogens11020193.

Jiang, X., Ma, H., Wang, Y., & Liu, Y. (2013). Early life factors and type 2 diabetes mellitus. Journal of diabetes research, 2013, 485082. doi: 10.1155/2013/485082.

Jin, X. T., & Smith, Y. (2011). Localization and functions of kainate receptors in the basal ganglia. Advances in experimental medicine and biology, 717, 27–37. doi: 10.1007/978-1-4419-9557-5_3.

Joëls M. (2006). Corticosteroid effects in the brain: U-shape it. Trends in pharmacological sciences, 27(5), 244–250. doi: 10.1016/j.tips.2006.03.007.

Joëls M. (2018). Corticosteroids and the brain. The Journal of endocrinology, 238(3), R121–R130. doi: 10.1530/JOE-18-0226.

Jones-Davis, D. M., Yang, M., Rider, E., Osbun, N. C., da Gente, G. J., Li, J., Katz, A. M., Weber, M. D., Sen, S., Crawley, J., & Sherr, E. H. (2013). Quantitative trait loci for interhemispheric commissure development and social behaviors in the BTBR T^{+} tf/J mouse model of autism. PloS one, 8(4), e61829. doi: 10.1371/journal.pone.0061829.

Jun, C., Choi, Y., Lim, S. M., Bae, S., Hong, Y. S., Kim, J. E., & Lyoo, I. K. (2014). Disturbance of the glutamatergic system in mood disorders. Experimental neurobiology, 23(1), 28–35. doi: 10.5607/en.2014.23.1.28.

Kadriu, B., Musazzi, L., Henter, I. D., Graves, M., Popoli, M., & Zarate, C. A., Jr (2019). Glutamatergic Neurotransmission: Pathway to Developing Novel Rapid-Acting Antidepressant Treatments. The international journal of neuropsychopharmacology, 22(2), 119–135. doi: 10.1093/ijnp/pyy094.

Kallarackal, A. J., Kvarta, M. D., Cammarata, E., Jaberi, L., Cai, X., Bailey, A. M., & Thompson, S. M. (2013). Chronic stress induces a selective decrease in AMPA receptor-mediated synaptic excitation at hippocampal temporoammonic-CA1 synapses. The Journal of neuroscience : the official

journal of the Society for Neuroscience, 33(40), 15669–15674. doi: 10.1523/JNEUROSCI.2588-13.2013.

Kalivas P. W. (2009). The glutamate homeostasis hypothesis of addiction. Nature reviews. Neuroscience, 10(8), 561–572. doi : 10.1038/nrn2515.

Karki, P., Lee, E., & Aschner, M. (2013). Manganese neurotoxicity: a focus on glutamate transporters. Annals of occupational and environmental medicine, 25(1), 4. doi: 10.1186/2052-4374-25-4.

Karst, H., & Joëls, M. (2005). Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells. Journal of neurophysiology, 94(5), 3479–3486. doi: 10.1152/jn.00143.2005.

Katz, L. C., & Shatz, C. J. (1996). Synaptic activity and the construction of cortical circuits. Science (New York, N.Y.), 274(5290), 1133–1138. doi: 10.1126/science.274.5290.1133.

Keesom, S. M., Finton, C. J., Sell, G. L., & Hurley, L. M. (2017). Early-Life Social Isolation Influences Mouse Ultrasonic Vocalizations during Male-Male Social Encounters. PloS one, 12(1), e0169705. doi: 10.1371/journal.pone.0169705.

Kessler, R. C., McLaughlin, K. A., Green, J. G., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., Aguilar-Gaxiola, S., Alhamzawi, A. O., Alonso, J., Angermeyer, M., Benjet, C., Bromet, E., Chatterji, S., de Girolamo, G., Demyttenaere, K., Fayyad, J., Florescu, S., Gal, G., Gureje, O., Haro, J. M., … Williams, D. R. (2010). Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. The British journal of psychiatry, 197(5), 378–385. doi: 10.1192/bjp.bp.110.080499.

Kim, D. G., Gonzales, E. L., Kim, S., Kim, Y., Adil, K. J., Jeon, S. J., Cho, K. S., Kwon, K. J., & Shin, C. Y. (2019). Social Interaction Test in Home Cage as a Novel and Ethological Measure of Social Behavior in Mice. Experimental neurobiology, 28(2), 247–260 doi: 10.5607/en.2019.28.2.247.

Kim, G. S., Lee, H., & Jeong, Y. (2021). Altered dorsal functional connectivity after post-weaning social isolation and resocialization in mice. NeuroImage, 245, 118740. doi: 10.1016/j.neuroimage.2021.118740.

Kirsten, T. B., Chaves-Kirsten, G. P., Chaible, L. M., Silva, A. C., Martins, D. O., Britto, L. R., Dagli, M. L., Torrão, A. S., Palermo-Neto, J., & Bernardi, M. M. (2012). Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to

lipopolysaccharide. Journal of neuroscience research, 90(10), 1903–1912. doi: 10.1002/jnr.23089.

Kitchener, P., Di Blasi, F., Borrelli, E., & Piazza, P. V. (2004). Differences between brain structures in nuclear translocation and DNA binding of the glucocorticoid receptor during stress and the circadian cycle. The European journal of neuroscience, 19(7), 1837–1846. doi: 10.1111/j.1460-9568.2004.03267.x.

Knudsen E. I. (2004). Sensitive periods in the development of the brain and behavior. Journal of cognitive neuroscience, 16(8), 1412–1425. doi: 10.1162/0898929042304796.

Koch, M. D., & Arnold, W. J. (1972). Effects of early social deprivation on emotionality in rats. Journal of comparative and physiological psychology, 78(3), 391–399. doi: 10.1037/h0032380.

Koehl, M., Darnaudéry, M., Dulluc, J., Van Reeth, O., Le Moal, M., & Maccari, S. (1999). Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. Journal of neurobiology, 40(3), 302–315. PMID: 10440731.

Koehl, M., & Abrous, D. N. (2011). A new chapter in the field of memory: adult hippocampal neurogenesis. The European journal of neuroscience, 33(6), 1101–1114. doi: 10.1111/j.1460-9568.2011.07609.x.

Koenigs, M., & Grafman, J. (2009). The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. Behavioural brain research, 201(2), 239–243. doi: 10.1016/j.bbr.2009.03.004.

Koike, H., Ibi, D., Mizoguchi, H., Nagai, T., Nitta, A., Takuma, K., Nabeshima, T., Yoneda, Y., & Yamada, K. (2009). Behavioral abnormality and pharmacologic response in social isolation-reared mice. Behavioural brain research, 202(1), 114–121. doi: 10.1016/j.bbr.2009.03.028.

Kokare, D. M., Dandekar, M. P., Singru, P. S., Gupta, G. L., & Subhedar, N. K. (2010). Involvement of α-MSH in the social isolation induced anxiety- and depression-like behaviors in rat. Neuropharmacology, 58(7), 1009–1018. doi: 10.1016/j.neuropharm.2010.01.006.

Köles, L., Kató, E., Hanuska, A., Zádori, Z. S., Al-Khrasani, M., Zelles, T., Rubini, P., & Illes, P. (2016). Modulation of excitatory neurotransmission by neuronal/glial signalling molecules: interplay between purinergic and

glutamatergic systems. Purinergic signalling, 12(1), 1–24. doi: 10.1007/s11302-015-9480-5.

Kong, L., Chen, K., Tang, Y., Wu, F., Driesen, N., Womer, F., Fan, G., Ren, L., Jiang, W., Cao, Y., Blumberg, H. P., Xu, K., & Wang, F. (2013). Functional connectivity between the amygdala and prefrontal cortex in medication-naive individuals with major depressive disorder. Journal of psychiatry & neuroscience : JPN, 38(6), 417–422. doi: 10.1503/jpn.120117.

Koning, A. C. A. M., Buurstede, J. C., van Weert, L. T. C. M., & Meijer, O. C. (2019). Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective. Journal of the Endocrine Society, 3(10), 1917–1930. doi: 10.1210/js.2019-00158.

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents. Methods in molecular biology (Clifton, N.J.), 1916, 69–74. doi: 10.1007/978-1-4939-8994-2_4.

Krogsgaard-Larsen, P., Honoré, T., Hansen, J. J., Curtis, D. R., & Lodge, D. (1980). New class of glutamate agonist structurally related to ibotenic acid. Nature, 284(5751), 64–66. doi : 10.1038/284064a0.

Kuhn, H. G., Toda, T., & Gage, F. H. (2018). Adult Hippocampal Neurogenesis: A Coming-of-Age Story. The Journal of neuroscience : the official journal of the Society for Neuroscience, 38(49), 10401–10410. doi: 10.1523/JNEUROSCI.2144-18.2018.

Kumar, A., & Foster, T. C. (2019). Alteration in NMDA Receptor Mediated Glutamatergic Neurotransmission in the Hippocampus During Senescence. Neurochemical research, 44(1), 38–48. doi: 10.1007/s11064-018-2634-4.

Kushner, S. A., Elgersma, Y., Murphy, G. G., Jaarsma, D., van Woerden, G. M., Hojjati, M. R., Cui, Y., LeBoutillier, J. C., Marrone, D. F., Choi, E. S., De Zeeuw, C. I., Petit, T. L., Pozzo-Miller, L., & Silva, A. J. (2005). Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(42), 9721–9734. doi: 10.1523/JNEUROSCI.2836-05.2005.

Lacey, R. E., Kumari, M., & Bartley, M. (2014). Social isolation in childhood and adult inflammation: evidence from the National Child Development Study. Psychoneuroendocrinology, 50, 85–94. doi: 10.1016/j.psyneuen.2014.08.007.

Laloux, C., Mairesse, J., Van Camp, G., Giovine, A., Branchi, I., Bouret, S., Morley-Fletcher, S., Bergonzelli, G., Malagodi, M., Gradini, R., Nicoletti, F., Darnaudéry, M., & Maccari, S. (2012). Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. Psychoneuroendocrinology, 37(10), 1646–1658. doi: 10.1016/j.psyneuen.2012.02.010.

Lapiz, M. D., Fulford, A., Muchimapura, S., Mason, R., Parker, T., & Marsden, C. A. (2003). Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neuroscience and behavioral physiology, 33(1), 13–29. doi: 10.1023/a:1021171129766.

Larsen, B., & Luna, B. (2018). Adolescence as a neurobiological critical period for the development of higher-order cognition. Neuroscience and biobehavioral reviews, 94, 179–195. doi:10.1016/j.neubiorev.2018.09.005.

Lauri, S. E., Ryazantseva, M., Orav, E., Vesikansa, A., & Taira, T. (2021). Kainate receptors in the developing neuronal networks. Neuropharmacology, 195, 108585. doi : 10.1016/j.neuropharm.2021.108585.

Lee, C. H., Lü, W., Michel, J. C., Goehring, A., Du, J., Song, X., & Gouaux, E. (2014). NMDA receptor structures reveal subunit arrangement and pore architecture. Nature, 511(7508), 191–197. doi: 10.1038/nature13548.

Lee, B. K., Magnusson, C., Gardner, R. M., Blomström, Å., Newschaffer, C. J., Burstyn, I., Karlsson, H., & Dalman, C. (2015). Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain, behavior, and immunity, 44, 100–105. doi: 10.1016/j.bbi.2014.09.001.

Lee, A. R., Kim, J. H., Cho, E., Kim, M., & Park, M. (2017). Dorsal and Ventral Hippocampus Differentiate in Functional Pathways and Differentially Associate with Neurological Disease-Related Genes during Postnatal Development. Frontiers in molecular neuroscience, 10, 331. doi: 10.3389/fnmol.2017.00331.

Lehre, K. P., Levy, L. M., Ottersen, O. P., Storm-Mathisen, J., & Danbolt, N. C. (1995). Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. The Journal of neuroscience : the official journal of the Society for Neuroscience, 15(3 Pt 1), 1835–1853. doi: 10.1523/JNEUROSCI.15-03-01835.1995.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America, 97(20), 11032–11037. doi: 10.1073/pnas.97.20.11032.

Levelt, C. N., & Hübener, M. (2012). Critical-period plasticity in the visual cortex. Annual review of neuroscience, 35, 309–330. doi: 10.1146/annurev-neuro-061010-113813.

Levitt, P., & Veenstra-VanderWeele, J. (2015). Neurodevelopment and the origins of brain disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 40(1), 1–3. doi: 10.1038/npp.2014.237.

Levitz, J., Habrian, C., Bharill, S., Fu, Z., Vafabakhsh, R., & Isacoff, E. Y. (2016). Mechanism of Assembly and Cooperativity of Homomeric and Heteromeric Metabotropic Glutamate Receptors. Neuron, 92(1), 143–159. doi: 10.1016/j.neuron.2016.08.036.

Li, K., & Xu, E. (2008). The role and the mechanism of gamma-aminobutyric acid during central nervous system development. Neuroscience bulletin, 24(3), 195–200. doi: 10.1007/s12264-008-0109-3.

Li, J., Olsen, J., Vestergaard, M., Obel, C., Baker, J. L., & Sørensen, T. I. (2010). Prenatal stress exposure related to maternal bereavement and risk of childhood overweight. PloS one, 5(7), e11896. doi: 10.1371/journal.pone.0011896.

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Li, X. Y., Aghajanian, G., & Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biological psychiatry, 69(8), 754–761. doi: 10.1016/j.biopsych.2010.12.015.

Li, M., Long, C., & Yang, L. (2015). Hippocampal-prefrontal circuit and disrupted functional connectivity in psychiatric and neurodegenerative disorders. BioMed research international, 2015, 810548. doi: 10.1155/2015/810548.

Li, L. J., Hu, R., Lujan, B., Chen, J., Zhang, J. J., Nakano, Y., Cui, T. Y., Liao, M. X., Chen, J. C., Man, H. Y., Feng, H., & Wan, Q. (2016). Glycine Potentiates AMPA Receptor Function through Metabotropic Activation of GluN2A-Containing NMDA Receptors. Frontiers in molecular neuroscience, 9, 102. doi : 10.3389/fnmol.2016.00102.

Li, X., Wang, C., Wang, W., Yue, C., & Tang, Y. (2017). Neonatal exposure to BDE 209 impaired learning and memory, decreased expression of hippocampal core SNAREs and synaptophysin in adult rats. Neurotoxicology, 59, 40–48. doi: 10.1016/j.neuro.2017.01.004.

Li, Y., Shen, M., Stockton, M. E., & Zhao, X. (2019). Hippocampal deficits in neurodevelopmental disorders. Neurobiology of learning and memory, 165, 106945. doi: 10.1016/j.nlm.2018.10.001.

Li, M., Fu, X., Xie, W., Guo, W., Li, B., Cui, R., & Yang, W. (2020). Effect of Early Life Stress on the Epigenetic Profiles in Depression. Frontiers in cell and developmental biology, 8, 867. doi: 10.3389/fcell.2020.00867.

Lin, C. H., Huang, Y. J., Lin, C. J., Lane, H. Y., & Tsai, G. E. (2014). NMDA neurotransmission dysfunction in mild cognitive impairment and Alzheimer's disease. Current pharmaceutical design, 20(32), 5169–5179. doi: 10.2174/1381612819666140110115603.

Liu, Y., Formisano, L., Savtchouk, I., Takayasu, Y., Szabó, G., Zukin, R. S., & Liu, S. J. (2010). A single fear-inducing stimulus induces a transcription-dependent switch in synaptic AMPAR phenotype. Nature neuroscience, 13(2), 223–231. doi : 10.1038/nn.2474.

Liu, X., Wu, R., Tai, F., Ma, L., Wei, B., Yang, X., Zhang, X., & Jia, R. (2013). Effects of group housing on stress induced emotional and neuroendocrine alterations. Brain research, 1502, 71–80. doi: 10.1016/j.brainres.2013.01.044.

Liu, Z. W., Yu, Y., Lu, C., Jiang, N., Wang, X. P., Xiao, S. Y., & Liu, X. M. (2019). Postweaning Isolation Rearing Alters the Adult Social, Sexual Preference and Mating Behaviors of Male CD-1 Mice. Frontiers in behavioral neuroscience, 13, 21. doi: 10.3389/fnbeh.2019.00021.

Liu, M. Y., Wei, L. L., Zhu, X. H., Ding, H. C., Liu, X. H., Li, H., Li, Y. Y., Han, Z., Li, L. D., Du, Z. W., Zhou, Y. P., Zhang, J., Meng, F., Tang, Y. L., Liu, X., Wang, C., & Zhou, Q. G. (2023). Prenatal stress modulates HPA axis homeostasis of offspring through dentate TERT independently of glucocorticoids receptor. Molecular psychiatry, 28(3), 1383–1395. doi : 10.1038/s41380-022-01898-9.

Lonart, G., & Südhof, T. C. (2000). Assembly of SNARE core complexes prior to neurotransmitter release sets the readily releasable pool of synaptic vesicles. The Journal of biological chemistry, 275(36), 27703–27707. PMID: 10970903.

Lowy, M. T., Gault, L., & Yamamoto, B. K. (1993). Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the

hippocampus. Journal of neurochemistry, 61(5), 1957–1960. doi: 10.1111/j.1471-4159.1993.tb09839.x.

Lowy, M. T., Wittenberg, L., & Yamamoto, B. K. (1995). Effect of acute stress on hippocampal glutamate levels and spectrin proteolysis in young and aged rats. Journal of neurochemistry, 65(1), 268–274. doi: 10.1046/j.1471-4159.1995.65010268.x.

Lu, L., Bao, G., Chen, H., Xia, P., Fan, X., Zhang, J., Pei, G., & Ma, L. (2003). Modification of hippocampal neurogenesis and neuroplasticity by social environments. Experimental neurology, 183(2), 600–609. doi: 10.1016/s0014-4886(03)00248-6.

Lu, W., Shi, Y., Jackson, A. C., Bjorgan, K., During, M. J., Sprengel, R., Seeburg, P. H., & Nicoll, R. A. (2009). Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron, 62(2), 254–268. doi: 10.1016/j.neuron.2009.02.027.

Lukasz, B., O'Sullivan, N. C., Loscher, J. S., Pickering, M., Regan, C. M., & Murphy, K. J. (2013). Peripubertal viral-like challenge and social isolation mediate overlapping but distinct effects on behaviour and brain interferon regulatory factor 7 expression in the adult Wistar rat. Brain, behavior, and immunity, 27(1), 71–79. doi: 10.1016/j.bbi.2012.09.011.

Lukkes, J. L., Watt, M. J., Lowry, C. A., & Forster, G. L. (2009). Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. Frontiers in behavioral neuroscience, 3, 18. doi: 10.3389/neuro.08.018.2009.

Luna, B., Marek, S., Larsen, B., Tervo-Clemmens, B., & Chahal, R. (2015). An integrative model of the maturation of cognitive control. Annual review of neuroscience, 38, 151–170. doi: 10.1146/annurev-neuro-071714-034054.

Lüscher, C., & Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harbor perspectives in biology, 4(6), a005710. doi: 10.1101/cshperspect.a005710.

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., & Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. The Journal of neuroscience : the official journal of the Society for Neuroscience, 15(1 Pt 1), 110–116. doi: 10.1523/JNEUROSCI.15-01-00110.1995.

Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A. R., Cinque, C., & Van Reeth, O. (2003). Prenatal stress and long-term consequences:

implications of glucocorticoid hormones. Neuroscience and biobehavioral reviews, 27(1-2), 119–127. doi: 10.1016/s0149-7634(03)00014-9.

Maccari, S., & Morley-Fletcher, S. (2007). Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology, 32 Suppl 1, S10–S15. doi. 10.1016/j.psyneuen.2007.06.005.

Maccari, S., Krugers, H. J., Morley-Fletcher, S., Szyf, M., & Brunton, P. J. (2014). The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. Journal of neuroendocrinology, 26(10), 707–723. doi: 10.1111/jne.12175.

Maccari, S., Polese, D., Reynaert, M. L., Amici, T., Morley-Fletcher, S., and Fagioli, F. (2017). Early-life experiences and the development of adult diseases with a focus on mental illness: The Human Birth Theory. Neuroscience 342, 232–251. doi: 10.1016/j.neuroscience.2016.05.042.

Madalena, K. M., & Lerch, J. K. (2017). The Effect of Glucocorticoid and Glucocorticoid Receptor Interactions on Brain, Spinal Cord, and Glial Cell Plasticity. Neural plasticity, 2017, 8640970. doi: 10.1155/2017/8640970.

Mahler, S. V., Hensley-Simon, M., Tahsili-Fahadan, P., LaLumiere, R. T., Thomas, C., Fallon, R. V., Kalivas, P. W., & Aston-Jones, G. (2014). Modafinil attenuates reinstatement of cocaine seeking: role for cystine-glutamate exchange and metabotropic glutamate receptors. Addiction biology, 19(1), 49–60. doi : 10.1111/j.1369-1600.2012.00506.x.

Mairesse, J., Gatta, E., Reynaert, M. L., Marrocco, J., Morley-Fletcher, S., Soichot, M., Deruyter, L., Camp, G. V., Bouwalerh, H., Fagioli, F., Pittaluga, A., Allorge, D., Nicoletti, F., & Maccari, S. (2015). Activation of presynaptic oxytocin receptors enhances glutamate release in the ventral hippocampus of prenatally restraint stressed rats. Psychoneuroendocrinology, 62, 36–46. doi: 10.1016/j.psyneuen.2015.07.005.

Makinodan, M., Rosen, K. M., Ito, S., & Corfas, G. (2012). A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science (New York, N.Y.), 337(6100), 1357–1360. doi: 10.1126/science.1220845.

Makinodan, M., Ikawa, D., Yamamuro, K., Yamashita, Y., Toritsuka, M., Kimoto, S., Yamauchi, T., Okumura, K., Komori, T., Fukami, S. I., Yoshino, H., Kanba, S., Wanaka, A., & Kishimoto, T. (2017). Effects of the mode of re-socialization after juvenile social isolation on medial prefrontal cortex

myelination and function. Scientific reports, 7(1), 5481. doi: 10.1038/s41598-017-05632-2.

Maksymetz, J., Moran, S. P., & Conn, P. J. (2017). Targeting metabotropic glutamate receptors for novel treatments of schizophrenia. Molecular brain, 10(1), 15. doi: 10.1186/s13041-017-0293-z.

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain, behavior, and immunity, 26(4), 607–616. doi : 10.1016/j.bbi.2012.01.011.

Man H. Y. (2011). GluA2-lacking, calcium-permeable AMPA receptors--inducers of plasticity?. Current opinion in neurobiology, 21(2), 291–298. doi : 10.1016/j.conb.2011.01.001.

Mango, D., Caruso, A., Saidi, A., Nisticò, R., & Scaccianoce, S. (2019). The positive allosteric modulator at mGlu2 receptors, LY487379, reverses the effects of chronic stress-induced behavioral maladaptation and synaptic dysfunction in the adulthood. Synapse, 73(9), e22101. doi: 10.1002/syn.22101.

Mannaioni, G., Marino, M. J., Valenti, O., Traynelis, S. F., & Conn, P. J. (2001). Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. The Journal of neuroscience : the official journal of the Society for Neuroscience, 21(16), 5925–5934. doi: 10.1523/JNEUROSCI.21-16-05925.2001.

Mantsch J. R. (2022). Corticotropin releasing factor and drug seeking in substance use disorders: Preclinical evidence and translational limitations. Addiction neuroscience, 4, 100038. doi: 10.1016/j.addicn.2022.100038.

Marmiroli, P., & Cavaletti, G. (2012). The glutamatergic neurotransmission in the central nervous system. Current medicinal chemistry, 19(9), 1269–1276. doi: 10.2174/092986712799462711.

Marrocco, J., Mairesse, J., Ngomba, R. T., Silletti, V., Van Camp, G., Bouwalerh, H., Summa, M., Pittaluga, A., Nicoletti, F., Maccari, S., & Morley-Fletcher, S. (2012). Anxiety-like behavior of prenatally stressed rats is associated with a selective reduction of glutamate release in the ventral hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience, 32(48), 17143–17154. doi: 10.1523/JNEUROSCI.1040-12.2012.

Marrocco, J., Reynaert, M. L., Gatta, E., Gabriel, C., Mocaër, E., Di Prisco, S., Merega, E., Pittaluga, A., Nicoletti, F., Maccari, S., Morley-Fletcher, S., & Mairesse, J. (2014). The effects of antidepressant treatment in prenatally stressed rats support the glutamatergic hypothesis of stress-related disorders. The Journal of neuroscience : the official journal of the Society for Neuroscience, 34(6), 2015–2024. doi: 10.1523/JNEUROSCI.4131-13.2014.

Marrocco, J., Verhaeghe, R., Bucci, D., Di Menna, L., Traficante, A., Bouwalerh, H., Van Camp, G., Ghiglieri, V., Picconi, B., Calabresi, P., Ravasi, L., Cisani, F., Bagheri, F., Pittaluga, A., Bruno, V., Battaglia, G., Morley-Fletcher, S., Nicoletti, F., & Maccari, S. (2020). Maternal stress programs accelerated aging of the basal ganglia motor system in offspring. Neurobiology of stress, 13, 100265. doi: 10.1016/j.ynstr.2020.100265.

Martin, S., Henley, J. M., Holman, D., Zhou, M., Wiegert, O., van Spronsen, M., Joëls, M., Hoogenraad, C. C., & Krugers, H. J. (2009). Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. PloS one, 4(3), e4714. doi: 10.1371/journal.pone.0004714.

Martineau, M., Guzman, R. E., Fahlke, C., & Klingauf, J. (2017). VGLUT1 functions as a glutamate/proton exchanger with chloride channel activity in hippocampal glutamatergic synapses. Nature communications, 8(1), 2279. doi : 10.1038/s41467-017-02367-6.

Masini, E., Loi, E., Vega-Benedetti, A. F., Carta, M., Doneddu, G., Fadda, R., & Zavattari, P. (2020). An Overview of the Main Genetic, Epigenetic and Environmental Factors Involved in Autism Spectrum Disorder Focusing on Synaptic Activity. International journal of molecular sciences, 21(21), 8290. doi: 10.3390/ijms21218290.

Masneuf, S., Lowery-Gionta, E., Colacicco, G., Pleil, K. E., Li, C., Crowley, N., Flynn, S., Holmes, A., & Kash, T. (2014). Glutamatergic mechanisms associated with stress-induced amygdala excitability and anxiety-related behavior. Neuropharmacology, 85, 190–197. doi: 10.1016/j.neuropharm.2014.04.015.

Massie, A., Boillée, S., Hewett, S., Knackstedt, L., & Lewerenz, J. (2015). Main path and byways: non-vesicular glutamate release by system xc(-) as an important modifier of glutamatergic neurotransmission. Journal of neurochemistry, 135(6), 1062–1079. doi : 10.1111/jnc.13348.

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., & Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. Nature, 349(6312), 760–765. doi: 10.1038/349760a0.

Matsumoto, K., Fujiwara, H., Araki, R., & Yabe, T. (2019). Post-weaning social isolation of mice: A putative animal model of developmental disorders. Journal of pharmacological sciences, 141(3), 111–118. doi: 10.1016/j.jphs.2019.10.002.

Matz, J., Gilyan, A., Kolar, A., McCarvill, T., & Krueger, S. R. (2010). Rapid structural alterations of the active zone lead to sustained changes in neurotransmitter release. Proceedings of the National Academy of Sciences of the United States of America, 107(19), 8836–8841. doi: 10.1073/pnas.0906087107.

McEwen B. S. (2004). Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Annals of the New York Academy of Sciences, 1032, 1–7. doi: 10.1196/annals.1314.001.

McEwen B. S. (2005). Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism: clinical and experimental, 54(5 Suppl 1), 20–23. doi: 10.1016/j.metabol.2005.01.008.

McEwen B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological reviews, 87(3), 873–904. Doi: 10.1152/physrev.00041.2006.

McEwen B. S. (2008). Understanding the potency of stressful early life experiences on brain and body function. Metabolism: clinical and experimental, 57 Suppl 2(Suppl 2), S11–S15. doi: 10.1016/j.metabol.2008.07.006.

McEwen B. S. (2012). The ever-changing brain: cellular and molecular mechanisms for the effects of stressful experiences. Developmental neurobiology, 72(6), 878–890. doi :10.1002/dneu.20968.

McEwen, B. S., Gray, J., & Nasca, C. (2015a). Recognizing Resilience: Learning from the Effects of Stress on the Brain. Neurobiology of Stress, 1, 1–11. doi: 10.1016/j.ynstr.2014.09.001.

McEwen, B. S., Bowles, N. P., Gray, J. D., Hill, M. N., Hunter, R. G., Karatsoreos, I. N., & Nasca, C. (2015b). Mechanisms of stress in the brain. Nature neuroscience, 18(10), 1353–1363. doi: 10.1038/nn.4086.

McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 41(1), 3–23. doi: 10.1038/npp.2015.171.

McEwen B. S. (2017). Allostasis and the Epigenetics of Brain and Body Health Over the Life Course: The Brain on Stress. JAMA psychiatry, 74(6), 551–552. doi: 10.1001/jamapsychiatry.2017.0270.

McFarlane, H. G., Kusek, G. K., Yang, M., Phoenix, J. L., Bolivar, V. J., & Crawley, J. N. (2008). Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes, Brain, and Behavior, 7(2), 152–163. doi: 10.1111/j.1601-183X.2007.00330.x.

Meijer, O. C., Buurstede, J. C., & Schaaf, M. J. M. (2019). Corticosteroid Receptors in the Brain: Transcriptional Mechanisms for Specificity and Context-Dependent Effects. Cellular and molecular neurobiology, 39(4), 539–549. doi: 10.1007/s10571-018-0625-2.

Melendez, R. I., Gregory, M. L., Bardo, M. T., & Kalivas, P. W. (2004). Impoverished rearing environment alters metabotropic glutamate receptor expression and function in the prefrontal cortex. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology, 29(11), 1980–1987. doi: 10.1038/sj.npp.1300507.

Mercier, F., Kwon, Y. C., & Douet, V. (2012). Hippocampus/amygdala alterations, loss of heparan sulfates, fractones and ventricle wall reduction in adult BTBR T+ tf/J mice, animal model for autism. Neuroscience letters, 506(2), 208–213. doi : 10.1016/j.neulet.2011.11.007.

Meyza, K. Z., & Blanchard, D. C. (2017). The BTBR mouse model of idiopathic autism - Current view on mechanisms. Neuroscience and biobehavioral reviews, 76(Pt A), 99–110. doi: 10.1016/j.neubiorev.2016.12.037.

Mifsud, K. R., & Reul, J. M. H. M. (2018). Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain. Stress (Amsterdam, Netherlands), 21(5), 389–402. doi:10.1080/10253890.2018.1456526.

Milstein, A. D., & Nicoll, R. A. (2009). TARP modulation of synaptic AMPA receptor trafficking and gating depends on multiple intracellular domains. Proceedings of the National Academy of Sciences of the United States of America, 106(27), 11348–11351. doi: 10.1073/pnas.0905570106.

Moghaddam B. (1993). Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. Journal of neurochemistry, 60(5), 1650–1657. doi: 10.1111/j.1471-4159.1993.tb13387.x.

Moghaddam B. (2002). Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biological psychiatry, 51(10), 775–787. doi: 10.1016/s0006-3223(01)01362-2.

Monroy, E., Hernández-Torres, E., & Flores, G. (2010). Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. Journal of chemical neuroanatomy, 40(2), 93–101. doi: 10.1016/j.jchemneu.2010.05.005.

Moore K. E. (1968). Studies with chronically isolated rats: tissue levels and urinary excretion of catecholamines and plasma levels of corticosterone. Canadian journal of physiology and pharmacology, 46(4), 553–558. doi: 10.1139/y68-080.

Moretto, E., Murru, L., Martano, G., Sassone, J., & Passafaro, M. (2018). Glutamatergic synapses in neurodevelopmental disorders. Progress in neuro-psychopharmacology & biological psychiatry, 84(Pt B), 328–342. doi: 10.1016/j.pnpbp.2017.09.014.

Morinan, A., & Leonard, B. E. (1980). Some anatomical and physiological correlates of social isolation in the young rat. Physiology & behavior, 24(3), 637–640. doi: 10.1016/0031-9384(80)90265-6gen.

Morley-Fletcher, S., Darnaudery, M., Koehl, M., Casolini, P., Van Reeth, O., & Maccari, S. (2003). Prenatal stress in rats predicts immobility behavior in the forced swim test. Effects of a chronic treatment with tianeptine. Brain research, 989(2), 246–251. doi: 10.1016/s0006-8993(03)03293-1.

Morley-Fletcher, S., Mairesse, J., Soumier, A., Banasr, M., Fagioli, F., Gabriel, C., Mocaer, E., Daszuta, A., McEwen, B., Nicoletti, F., & Maccari, S. (2011). Chronic agomelatine treatment corrects behavioral, cellular, and biochemical abnormalities induced by prenatal stress in rats. Psychopharmacology, 217(3), 301–313. doi: 10.1007/s00213-011-2280-x.

Morley-Fletcher, S., Zuena, A. R., Mairesse, J., Gatta, E., Van Camp, G., Bouwalerh, H., Riozzi, B., Battaglia, G., Pittaluga, A., Olivero, G., Mocaer, E., Bretin, S., Nicoletti, F., & Maccari, S. (2018). The reduction in glutamate release is predictive of cognitive and emotional alterations that are corrected by the positive modulator of AMPA receptors S 47445 in perinatal stressed rats. Neuropharmacology, 135, 284–296. doi : 10.1016/j.neuropharm.2018.03.018.

Morley-Fletcher, S., Mairesse, J., Van Camp, G., Reynaert, M. L., Gatta, E., Marrocco, J., Bouwalerh, H., Nicoletti, F., & Maccari, S. (2019). Perinatal

Stress Programs Sex Differences in the Behavioral and Molecular Chronobiological Profile of Rats Maintained Under a 12-h Light-Dark Cycle. Frontiers in molecular neuroscience, 12, 89. doi: 10.3389/fnmol.2019.00089.

Morrison, J. H., & Hof, P. R. (1997). Life and death of neurons in the aging brain. Science, 278(5337), 412–419. doi: 10.1126/science.278.5337.412.

Morrison, J. H., & Baxter, M. G. (2012). The ageing cortical synapse: hallmarks and implications for cognitive decline. Nature reviews. Neuroscience, 13(4), 240–250. doi: 10.1038/nrn3200.

Moussawi, K., Riegel, A., Nair, S., & Kalivas, P. W. (2011). Extracellular glutamate: functional compartments operate in different concentration ranges. Frontiers in systems neuroscience, 5, 94. doi: 10.3389/fnsys.2011.00094.

Moy, S. S., & Nadler, J. J. (2008). Advances in behavioral genetics: mouse models of autism. Molecular Psychiatry, 13(1), 4–26. doi: 10.1038/sj.mp.4002082.

Muller, C. L., Anacker, A. M. J., & Veenstra-VanderWeele, J. (2016). The serotonin system in autism spectrum disorder: From biomarker to animal models. Neuroscience, 321, 24–41. doi : 10.1016/j.neuroscience.2015.11.010.

Mumtaz, F., Khan, M. I., Zubair, M., & Dehpour, A. R. (2018). Neurobiology and consequences of social isolation stress in animal model-A comprehensive review. Biomedicine & pharmacotherapy, 105, 1205–1222. doi: 10.1016/j.biopha.2018.05.086.

Musazzi, L., Milanese, M., Farisello, P., Zappettini, S., Tardito, D., Barbiero, V. S., Bonifacino, T., Mallei, A., Baldelli, P., Racagni, G., Raiteri, M., Benfenati, F., Bonanno, G., & Popoli, M. (2010). Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. PloS one, 5(1), e8566. doi: 10.1371/journal.pone.0008566.

Näätänen, R., Kujala, T., Kreegipuu, K., Carlson, S., Escera, C., Baldeweg, T., & Ponton, C. (2011). The mismatch negativity: an index of cognitive decline in neuropsychiatric and neurological diseases and in ageing. Brain: a journal of neurology, 134(Pt 12), 3435–3453. doi: 10.1093/brain/awr064.

Nasca, C., Xenos, D., Barone, Y., Caruso, A., Scaccianoce, S., Matrisciano, F., Battaglia, G., Mathé, A. A., Pittaluga, A., Lionetto, L., Simmaco, M., & Nicoletti, F. (2013). L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. Proceedings of the

National Academy of Sciences of the United States of America, 110(12), 4804–4809. doi: 10.1073/pnas.1216100110.

Nasca, C., Bigio, B., Zelli, D., Nicoletti, F., & McEwen, B. S. (2015a). Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. Molecular psychiatry, 20(6), 755–763. doi: 10.1038/mp.2014.96.

Nasca, C., Zelli, D., Bigio, B., Piccinin, S., Scaccianoce, S., Nisticò, R., & McEwen, B. S. (2015b). Stress dynamically regulates behavior and glutamatergic gene expression in hippocampus by opening a window of epigenetic plasticity. Proceedings of the National Academy of Sciences of the United States of America, 112(48), 14960–14965. doi: 10.1073/pnas.1516016112.

Nasca, C., Bigio, B., Zelli, D., de Angelis, P., Lau, T., Okamoto, M., Soya, H., Ni, J., Brichta, L., Greengard, P., Neve, R. L., Lee, F. S., & McEwen, B. S. (2017). Role of the Astroglial Glutamate Exchanger xCT in Ventral Hippocampus in Resilience to Stress. Neuron, 96(2), 402–413.e5. doi: 10.1016/j.neuron.2017.09.020.

Nasir, M., Trujillo, D., Levine, J., Dwyer, J. B., Rupp, Z. W., & Bloch, M. H. (2020). Glutamate Systems in DSM-5 Anxiety Disorders: Their Role and a Review of Glutamate and GABA Psychopharmacology. Frontiers in psychiatry, 11, 548505. doi: 10.3389/fpsyt.2020.548505.

Nelson, C. A., 3rd, & Gabard-Durnam, L. J. (2020). Early Adversity and Critical Periods: Neurodevelopmental Consequences of Violating the Expectable Environment. Trends in neurosciences, 43(3), 133–143. doi: 10.1016/j.tins.2020.01.002.

Nemeroff C. B. (2004). Neurobiological consequences of childhood trauma. The Journal of clinical psychiatry, 65 Suppl 1, 18–28. PMID: 14728093.

Nicoletti, F., Meek, J. L., Iadarola, M. J., Chuang, D. M., Roth, B. L., & Costa, E. (1986a). Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. Journal of neurochemistry, 46(1), 40–46. doi: 10.1111/j.1471-4159.1986.tb12922.x.

Nicoletti, F., Wroblewski, J. T., Novelli, A., Alho, H., Guidotti, A., & Costa, E. (1986b). The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. The Journal of neuroscience : the official journal of

the Society for Neuroscience, 6(7), 1905–1911. doi: 10.1523/JNEUROSCI.06-07-01905.1986.

Nicoletti, F., Bockaert, J., Collingridge, G. L., Conn, P. J., Ferraguti, F., Schoepp, D. D., Wroblewski, J. T., & Pin, J. P. (2011). Metabotropic glutamate receptors: from the workbench to the bedside. Neuropharmacology, 60(7-8), 1017–1041. doi: 10.1016/j.neuropharm.2010.10.022.

Nicoletti, F., Bruno, V., Ngomba, R. T., Gradini, R., & Battaglia, G. (2015). Metabotropic glutamate receptors as drug targets: what's new? Current opinion in pharmacology, 20, 89–94. doi: 10.1016/j.coph.2014.12.002.

Nicoletti, F., Orlando, R., Di Menna, L., Cannella, M., Notartomaso, S., Mascio, G., Iacovelli, L., Matrisciano, F., Fazio, F., Caraci, F., Copani, A., Battaglia, G., & Bruno, V. (2019). Targeting mGlu Receptors for Optimization of Antipsychotic Activity and Disease-Modifying Effect in Schizophrenia. Frontiers in psychiatry, 10, 49. doi: 10.3389/fpsyt.2019.00049.

Niescier, R. F., & Lin, Y. C. (2021). The Potential Role of AMPA Receptor Trafficking in Autism and Other Neurodevelopmental Conditions. Neuroscience, 479, 180–191. doi : 10.1016/j.neuroscience.2021.09.013.

Niswender, C. M., & Conn, P. J. (2010). Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual review of pharmacology and toxicology, 50, 295–322. doi: 10.1146/annurev.pharmtox.011008.145533.

Ohgi, Y., Futamura, T., & Hashimoto, K. (2015). Glutamate Signaling in Synaptogenesis and NMDA Receptors as Potential Therapeutic Targets for Psychiatric Disorders. Current molecular medicine, 15(3), 206–221. doi: 10.2174/1566524015666150330143008.

Okada, R., Fujiwara, H., Mizuki, D., Araki, R., Yabe, T., & Matsumoto, K. (2015). Involvement of dopaminergic and cholinergic systems in social isolation-induced deficits in social affiliation and conditional fear memory in mice. Neuroscience, 299, 134–145. doi: 10.1016/j.neuroscience.2015.04.064.

Olivenza, R., Moro, M. A., Lizasoain, I., Lorenzo, P., Fernández, A. P., Rodrigo, J., Boscá, L., & Leza, J. C. (2000). Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. Journal of neurochemistry, 74(2), 785–791. doi: 10.1046/j.1471-4159.2000.740785.x.

Owens, D. F., & Kriegstein, A. R. (2002). Is there more to GABA than synaptic inhibition?. Nature reviews. Neuroscience, 3(9), 715–727. doi : 10.1038/nrn919.

Pajarillo, E., Rizor, A., Lee, J., Aschner, M., & Lee, E. (2019). The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: Potential targets for neurotherapeutics. Neuropharmacology, 161, 107559. doi : 10.1016/j.neuropharm.2019.03.002.

Pal M. M. (2021). Glutamate: The Master Neurotransmitter and Its Implications in Chronic Stress and Mood Disorders. Frontiers in human neuroscience, 15, 722323. doi: 10.3389/fnhum.2021.722323.

Palacios-García, I., Lara-Vásquez, A., Montiel, J. F., Díaz-Véliz, G. F., Sepúlveda, H., Utreras, E., Montecino, M., González-Billault, C., & Aboitiz, F. (2015). Prenatal stress down-regulates Reelin expression by methylation of its promoter and induces adult behavioral impairments in rats. PloS one, 10(2), e0117680. doi: 10.1371/journal.pone.0117680.

Pampliega, O., Domercq, M., Villoslada, P., Sepulcre, J., Rodríguez-Antigüedad, A., & Matute, C. (2008). Association of an EAAT2 polymorphism with higher glutamate concentration in relapsing multiple sclerosis. Journal of neuroimmunology, 195(1-2), 194–198. doi : 10.1016/j.jneuroim.2008.01.011.

Paoletti, P., Bellone, C., & Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nature reviews. Neuroscience, 14(6), 383–400. doi : 10.1038/nrn3504.

Park, J., & Moghaddam, B. (2017). Impact of anxiety on prefrontal cortex encoding of cognitive flexibility. Neuroscience, 345, 193–202. doi: 10.1016/j.neuroscience.2016.06.013.

Parkin, G. M., Udawela, M., Gibbons, A., & Dean, B. (2018). Glutamate transporters, EAAT1 and EAAT2, are potentially important in the pathophysiology and treatment of schizophrenia and affective disorders. World journal of psychiatry, 8(2), 51–63. doi: 10.5498/wjp.v8.i2.51.

Patel, N., Crider, A., Pandya, C. D., Ahmed, A. O., & Pillai, A. (2016). Altered mRNA Levels of Glucocorticoid Receptor, Mineralocorticoid Receptor, and Co-Chaperones (FKBP5 and PTGES3) in the Middle Frontal

Gyrus of Autism Spectrum Disorder Subjects. Molecular neurobiology, 53(4), 2090–2099. doi: 10.1007/s12035-015-9178-2.

Pavlides, C., Watanabe, Y., Magariños, A. M., & McEwen, B. S. (1995). Opposing roles of type I and type II adrenal steroid receptors in hippocampal long-term potentiation. Neuroscience, 68(2), 387–394. doi: 10.1016/0306-4522(95)00151-8.

Pei, W., Huang, Z., Wang, C., Han, Y., Park, J. S., & Niu, L. (2009). Flip and flop: a molecular determinant for AMPA receptor channel opening. Biochemistry, 48(17), 3767–3777. doi: 10.1021/bi8015907.

Peng, S., Zhang, Y., Zhang, J., Wang, H., & Ren, B. (2011). Glutamate receptors and signal transduction in learning and memory. Molecular biology reports, 38(1), 453–460. doi: 10.1007/s11033-010-0128-9.

Pereda-Pérez, I., Popović, N., Otalora, B. B., Popović, M., Madrid, J. A., Rol, M. A., & Venero, C. (2013). Long-term social isolation in the adulthood results in CA1 shrinkage and cognitive impairment. Neurobiology of learning and memory, 106, 31–39. doi: 10.1016/j.nlm.2013.07.004.

Perego, C., Vanoni, C., Bossi, M., Massari, S., Basudev, H., Longhi, R., & Pietrini, G. (2000). The GLT-1 and GLAST glutamate transporters are expressed on morphologically distinct astrocytes and regulated by neuronal activity in primary hippocampal cocultures. Journal of neurochemistry, 75(3), 1076–1084. doi : 10.1046/j.1471-4159.2000.0751076.x.

Peterlik, D., Flor, P. J., & Uschold-Schmidt, N. (2016). The Emerging Role of Metabotropic Glutamate Receptors in the Pathophysiology of Chronic Stress-Related Disorders. Current neuropharmacology, 14(5), 514–539. doi: 10.2174/1570159x13666150515234920.

Petrovic, M. M., Viana da Silva, S., Clement, J. P., Vyklicky, L., Mulle, C., González-González, I. M., & Henley, J. M. (2017). Metabotropic action of postsynaptic kainate receptors triggers hippocampal long-term potentiation. Nature neuroscience, 20(4), 529–539. doi: 10.1038/nn.4505.

Petty F. (1995). GABA and mood disorders: a brief review and hypothesis. Journal of affective disorders, 34(4), 275–281. doi: 10.1016/0165-0327(95)00025-i.

Pickering, C., Gustafsson, L., Cebere, A., Nylander, I., & Liljequist, S. (2006). Repeated maternal separation of male Wistar rats alters glutamate receptor expression in the hippocampus but not the prefrontal cortex. Brain research, 1099(1), 101–108. doi: 10.1016/j.brainres.2006.04.136.

Pinky, N. F., Wilkie, C. M., Barnes, J. R., & Parsons, M. P. (2018). Region- and Activity-Dependent Regulation of Extracellular Glutamate. The Journal of neuroscience : the official journal of the Society for Neuroscience, 38(23), 5351–5366. doi: 10.1523/JNEUROSCI.3213-17.2018.

Plant, K., Pelkey, K. A., Bortolotto, Z. A., Morita, D., Terashima, A., McBain, C. J., Collingridge, G. L., & Isaac, J. T. (2006). Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nature neuroscience, 9(5), 602–604. doi: 10.1038/nn1678.

Popoli, M., Yan, Z., McEwen, B. S., and Sanacora, G. (2011). The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. Nat. Rev. Neurosci. 13, 22–37. doi:10.1038/nrn3138.

Porton, B., Rodriguiz, R. M., Phillips, L. E., Gilbert, J. W., 4th, Feng, J., Greengard, P., Kao, H. T., & Wetsel, W. C. (2010). Mice lacking synapsin III show abnormalities in explicit memory and conditioned fear. Genes, brain, and behavior, 9(3), 257–268. doi: 10.1111/j.1601-183X.2009.00555.x.

Provenzano, G., Corradi, Z., Monsorno, K., Fedrizzi, T., Ricceri, L., Scattoni, M. L., & Bozzi, Y. (2016). Comparative Gene Expression Analysis of Two Mouse Models of Autism: Transcriptome Profiling of the BTBR and En2 (-/-) Hippocampus. Frontiers in neuroscience, 10, 396. doi: 10.3389/fnins.2016.00396.

Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Glutamate. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10807/

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Brose, N., & Ehrenreich, H. (2009). Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes, brain, and behavior, 8(4), 416–425. doi : 10.1111/j.1601-183X.2009.00487.x.

Rasetti, R., Mattay, V. S., Wiedholz, L. M., Kolachana, B. S., Hariri, A. R., Callicott, J. H., Meyer-Lindenberg, A., & Weinberger, D. R. (2009). Evidence that altered amygdala activity in schizophrenia is related to clinical state and not genetic risk. The American journal of psychiatry, 166(2), 216–225. doi: 10.1176/appi.ajp.2008.08020261.

Reimers, J. M., Milovanovic, M., & Wolf, M. E. (2011). Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. Brain research, 1367, 223–233. doi: 10.1016/j.brainres.2010.10.016.

Reinwald, J. R., Becker, R., Mallien, A. S., Falfan-Melgoza, C., Sack, M., Clemm von Hohenberg, C., Braun, U., Cosa Linan, A., Gass, N., Vasilescu, A. N., Tollens, F., Lebhardt, P., Pfeiffer, N., Inta, D., Meyer-Lindenberg, A., Gass, P., Sartorius, A., & Weber-Fahr, W. (2018). Neural Mechanisms of Early-Life Social Stress as a Developmental Risk Factor for Severe Psychiatric Disorders. Biological psychiatry, 84(2), 116–128. doi: 10.1016/j.biopsych.2017.12.010.

Reissner, K. J., Gipson, C. D., Tran, P. K., Knackstedt, L. A., Scofield, M. D., & Kalivas, P. W. (2015). Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. Addiction biology, 20(2), 316–323. doi : 10.1111/adb.12127.

Reul, J. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology, 117(6), 2505–2511. doi: 10.1210/endo-117-6-2505.

Reynaert, M. L., Marrocco, J., Mairesse, J., Lionetto, L., Simmaco, M., Deruyter, L., Allorge, D., Moles, A., Pittaluga, A., Maccari, S., Morley-Fletcher, S., Van Camp, G., & Nicoletti, F. (2016). Hedonic sensitivity to natural rewards is affected by prenatal stress in a sex-dependent manner. Addiction biology, 21(6), 1072–1085. doi: 10.1111/adb.12270.

Ribeiro, F. M., Vieira, L. B., Pires, R. G., Olmo, R. P., & Ferguson, S. S. (2017). Metabotropic glutamate receptors and neurodegenerative diseases. Pharmacological research, 115, 179–191. doi: 10.1016/j.phrs.2016.11.013.

Rivera, D. S., Lindsay, C. B., Oliva, C. A., Codocedo, J. F., Bozinovic, F., & Inestrosa, N. C. (2020a). Effects of long-lasting social isolation and re-socialization on cognitive performance and brain activity: a longitudinal study in Octodon degus. Scientific reports, 10(1), 18315. doi: 10.1038/s41598-020-75026-4.

Rivera, D. S., Lindsay, C. B., Oliva, C. A., Bozinovic, F., & Inestrosa, N. C. (2020b). "Live together, die alone": The effect of re-socialization on behavioural performance and social-affective brain-related proteins after a long-term chronic social isolation stress. Neurobiology of stress, 14, 100289. doi: 10.1016/j.ynstr.2020.100289.

Rodríguez-Moreno, A., & Lerma, J. (1998). Kainate receptor modulation of GABA release involves a metabotropic function. Neuron, 20(6), 1211–1218. doi: 10.1016/s0896-6273(00)80501-2.

Rojas D. C. (2014). The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. Journal of neural transmission (Vienna, Austria : 1996), 121(8), 891–905. doi: 10.1007/s00702-014-1216-0.

Roshan-Milani, S., Seyyedabadi, B., Saboory, E., Parsamanesh, N., & Mehranfard, N. (2021). Prenatal stress and increased susceptibility to anxiety-like behaviors: role of neuroinflammation and balance between GABAergic and glutamatergic transmission. Stress, 24(5), 481–495. doi: 10.1080/10253890.2021.1942828.

Ros-Simó, C., & Valverde, O. (2012). Early-life social experiences in mice affect emotional behaviour and hypothalamic-pituitary-adrenal axis function. Pharmacology, biochemistry, and behavior, 102(3), 434–441. doi: 10.1016/j.pbb.2012.06.001.

Rothstein, J. D., Martin, L., Levey, A. I., Dykes-Hoberg, M., Jin, L., Wu, D., Nash, N., & Kuncl, R. W. (1994). Localization of neuronal and glial glutamate transporters. Neuron, 13(3), 713–725. doi: 10.1016/0896-6273(94)90038-8.

Rotschafer, S. E., Trujillo, M. S., Dansie, L. E., Ethell, I. M., & Razak, K. A. (2012). Minocycline treatment reverses ultrasonic vocalization production deficit in a mouse model of Fragile X Syndrome. Brain research, 1439, 7–14. doi : 10.1016/j.brainres.2011.12.041.

Roullet, F. I., Wöhr, M., & Crawley, J. N. (2011). Female urine-induced male mice ultrasonic vocalizations, but not scent-marking, is modulated by social experience. Behavioural Brain Research, 216(1), 19–28. doi: 10.1016/j.bbr.2010.06.004.

Ruan, Y., Miyagi, A., Wang, X., Chami, M., Boudker, O., & Scheuring, S. (2017). Direct visualization of glutamate transporter elevator mechanism by high-speed AFM. Proceedings of the National Academy of Sciences of the United States of America, 114(7), 1584–1588. doi: 10.1073/pnas.1616413114.

Rudolph, U., & Knoflach, F. (2011). Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. Nature reviews. Drug discovery, 10(9), 685–697. doi: 10.1038/nrd3502.

Rugh, R. (1990) The Mouse: Its Reproduction and Development. Oxford University Press, Oxford UK.

Sahakian, B.J., Robbins, T.W. & Iversen, S.D (1977). The effects of isolation rearing on exploration in the rat. Animal Learning & Behavior 5, 193–198. doi: 10.3758/BF03214077.

Salari, N., Rasoulpoor, S., Rasoulpoor, S., Shohaimi, S., Jafarpour, S., Abdoli, N., Khaledi-Paveh, B., & Mohammadi, M. (2022). The global prevalence of autism spectrum disorder: a comprehensive systematic review and meta-analysis. Italian journal of pediatrics, 48(1), 112. doi: 10.1186/s13052-022-01310-w.

Sánchez, M. M., Aguado, F., Sánchez-Toscano, F., & Saphier, D. (1998). Neuroendocrine and immunocytochemical demonstrations of decreased hypothalamo-pituitary-adrenal axis responsiveness to restraint stress after long-term social isolation. Endocrinology, 139(2), 579–587. doi: 10.1210/endo.139.2.5720.

Sanz-Clemente, A., Nicoll, R. A., & Roche, K. W. (2013). Diversity in NMDA receptor composition: many regulators, many consequences. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry, 19(1), 62–75. doi: 10.1177/1073858411435129.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine reviews, 21(1), 55–89. doi: 10.1210/edrv.21.1.0389.

Scaccianoce, S., Del Bianco, P., Paolone, G., Caprioli, D., Modafferi, A. M., Nencini, P., & Badiani, A. (2006). Social isolation selectively reduces hippocampal brain-derived neurotrophic factor without altering plasma corticosterone. Behavioural brain research, 168(2), 323–325. doi: 10.1016/j.bbr.2005.04.024.

Scattoni, M. L., Gandhy, S. U., Ricceri, L., & Crawley, J. N. (2008). Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. PloS one, 3(8), e3067. doi: 10.1371/journal.pone.0003067.

Scattoni, M. L., Ricceri, L., & Crawley, J. N. (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. Genes, brain, and behavior, 10(1), 44–56. doi: 10.1111/j.1601-183X.2010.00623.x.

Scattoni, M. L., Martire, A., Cartocci, G., Ferrante, A., & Ricceri, L. (2013). Reduced social interaction, behavioural flexibility and BDNF signalling in the BTBR T+ tf/J strain, a mouse model of autism. Behavioural brain research, 251, 35–40. doi: 10.1016/j.bbr.2012.12.028.

Schipper, L., van Heijningen, S., Karapetsas, G., van der Beek, E. M., & van Dijk, G. (2020). Individual housing of male C57BL/6J mice after weaning

impairs growth and predisposes for obesity. PloS one, 15(5), e0225488. doi: 10.1371/journal.pone.0225488.

Schmeisser, M. J., Ey, E., Wegener, S., Bockmann, J., Stempel, A. V., Kuebler, A., Janssen, A. L., Udvardi, P. T., Shiban, E., Spilker, C., Balschun, D., Skryabin, B. V., Dieck, S.t, Smalla, K. H., Montag, D., Leblond, C. S., Faure, P., Torquet, N., Le Sourd, A. M., Toro, R., … Boeckers, T. M. (2012). Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature, 486(7402), 256–260. doi : 10.1038/nature11015.

Schoepp D. D. (2001). Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. The Journal of pharmacology and experimental therapeutics, 299(1), 12–20. PMID: 11561058.

Schrijver, N. C., Bahr, N. I., Weiss, I. C., & Würbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacology, biochemistry, and behavior, 73(1), 209–224. doi: 10.1016/s0091-3057(02)00790-6.

Schulz, K. M., Pearson, J. N., Neeley, E. W., Berger, R., Leonard, S., Adams, C. E., & Stevens, K. E. (2011). Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. Physiology & behavior, 104(2), 340–347. doi: 10.1016/j.physbeh.2011.02.021.

Seckl J. R. (2008). Glucocorticoids, developmental 'programming' and the risk of affective dysfunction. Progress in brain research, 167, 17–34. doi: 10.1016/S0079-6123(07)67002-2.

Seese, R. R., Maske, A. R., Lynch, G., & Gall, C. M. (2014). Long-term memory deficits are associated with elevated synaptic ERK1/2 activation and reversed by mGluR5 antagonism in an animal model of autism. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 39(7), 1664–1673. doi: 10.1038/npp.2014.13.

Seffer, D., Rippberger, H., Schwarting, R. K., & Wöhr, M. (2015). Pro-social 50-kHz ultrasonic communication in rats: post-weaning but not post-adolescent social isolation leads to social impairments-phenotypic rescue by re-socialization. Frontiers in behavioral neuroscience, 9, 102. doi: 10.3389/fnbeh.2015.00102.

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. Journal of visualized experiments : JoVE, (96), e52434. doi: 10.3791/52434.

Sengpiel F. (2007). The critical period. Current biology : CB, 17(17), R742–R743. doi: 10.1016/j.cub.2007.06.017.

Senst, L., Baimoukhametova, D., Sterley, T. L., & Bains, J. S. (2016). Sexually dimorphic neuronal responses to social isolation. eLife, 5, e18726. doi: 10.7554/eLife.18726.

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. (2021). The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions. Frontiers in behavioral neuroscience, 14, 601939. doi: 10.3389/fnbeh.2020.601939.

Shimizu, K., Kurosawa, N., & Seki, K. (2016). The role of the AMPA receptor and 5-HT(3) receptor on aggressive behavior and depressive-like symptoms in chronic social isolation-reared mice. Physiology & behavior, 153, 70–83. doi: 10.1016/j.physbeh.2015.10.026.

Shpyleva, S., Ivanovsky, S., de Conti, A., Melnyk, S., Tryndyak, V., Beland, F. A., James, S. J., & Pogribny, I. P. (2014). Cerebellar oxidative DNA damage and altered DNA methylation in the BTBR T+tf/J mouse model of autism and similarities with human post mortem cerebellum. PloS one, 9(11), e113712. doi : 10.1371/journal.pone.0113712.

Sigurdsson, T., & Duvarci, S. (2016). Hippocampal-Prefrontal Interactions in Cognition, Behavior and Psychiatric Disease. Frontiers in systems neuroscience, 9, 190. doi: 10.3389/fnsys.2015.00190.

Silk, J. B., Beehner, J. C., Bergman, T. J., Crockford, C., Engh, A. L., Moscovice, L. R., Wittig, R. M., Seyfarth, R. M., & Cheney, D. L. (2010). Strong and consistent social bonds enhance the longevity of female baboons. Current biology : CB, 20(15), 1359–1361. doi: 10.1016/j.cub.2010.05.067.

Silverman, J. L., Tolu, S. S., Barkan, C. L., & Crawley, J. N. (2010a). Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 35(4), 976–989. doi: 10.1038/npp.2009.201.

Silverman, J. L., Yang, M., Turner, S. M., Katz, A. M., Bell, D. B., Koenig, J. I., & Crawley, J. N. (2010b). Low stress reactivity and neuroendocrine

factors in the BTBR T+tf/J mouse model of autism. Neuroscience, 171(4), 1197–1208. doi: 10.1016/j.neuroscience.2010.09.059.

Silverman, J. L., Smith, D. G., Rizzo, S. J., Karras, M. N., Turner, S. M., Tolu, S. S., Bryce, D. K., Smith, D. L., Fonseca, K., Ring, R. H., & Crawley, J. N. (2012). Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. Science translational medicine, 4(131), 131ra51. doi.org/10.1126/scitranslmed.3003501.

Sladeczek, F., Pin, J. P., Récasens, M., Bockaert, J., & Weiss, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. Nature, 317(6039), 717–719. doi: 10.1038/317717a0.

Smith, S. E., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007). Maternal immune activation alters fetal brain development through interleukin-6. The Journal of neuroscience : the official journal of the Society for Neuroscience, 27(40), 10695–10702. doi: 10.1523/JNEUROSCI.2178-07.2007.

Smith, K. E., & Pollak, S. D. (2020). Early life stress and development: potential mechanisms for adverse outcomes. Journal of neurodevelopmental disorders, 12(1), 34. doi: 10.1186/s11689-020-09337-y.

Stein, A. D., Obrutu, O. E., Behere, R. V., & Yajnik, C. S. (2019). Developmental undernutrition, offspring obesity and type 2 diabetes. Diabetologia, 62(10), 1773–1778. doi: 10.1007/s00125-019-4930-1.

Stephens, M. A., & Wand, G. (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. Alcohol research : current reviews, 34(4), 468–483. PMID: 23584113.

Sugiyama, H., Ito, I., & Hirono, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature, 325(6104), 531–533. doi: 10.1038/325531a0.

Suglia, S. F., Campo, R. A., Brown, A. G. M., Stoney, C., Boyce, C. A., Appleton, A. A., Bleil, M. E., Boynton-Jarrett, R., Dube, S. R., Dunn, E. C., Ellis, B. J., Fagundes, C. P., Heard-Garris, N. J., Jaffee, S. R., Johnson, S. B., Mujahid, M. S., Slopen, N., Su, S., & Watamura, S. E. (2020). Social Determinants of Cardiovascular Health: Early Life Adversity as a Contributor to Disparities in Cardiovascular Diseases. The Journal of pediatrics, 219, 267–273. doi: 10.1016/j.jpeds.2019.12.063.

Tanaka, K., Osako, Y., Takahashi, K., Hidaka, C., Tomita, K., & Yuri, K. (2019). Effects of post-weaning social isolation on social behaviors and oxytocinergic activity in male and female rats. Heliyon, 5(5), e01646. doi: 10.1016/j.heliyon.2019.e01646.

Taylor, S. E., Welch, W. T., Kim, H. S., & Sherman, D. K. (2007). Cultural differences in the impact of social support on psychological and biological stress responses. Psychological science, 18(9), 831–837. doi: 10.1111/j.1467-9280.2007.01987.x.

Thibado, J. K., Tano, J. Y., Lee, J., Salas-Estrada, L., Provasi, D., Strauss, A., Marcelo Lamim Ribeiro, J., Xiang, G., Broichhagen, J., Filizola, M., Lohse, M. J., & Levitz, J. (2021). Differences in interactions between transmembrane domains tune the activation of metabotropic glutamate receptors. eLife, 10, e67027. doi: 10.7554/eLife.67027.

Toth, I., & Neumann, I. D. (2013). Animal models of social avoidance and social fear. Cell and tissue research, 354(1), 107–118. doi: 10.1007/s00441-013-1636-4.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: structure, regulation, and function. Pharmacological reviews, 62(3), 405–496. doi: 10.1124/pr.109.002451.

Tulogdi, A., Tóth, M., Barsvári, B., Biró, L., Mikics, E., & Haller, J. (2014). Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats. Developmental psychobiology, 56(1), 49–57. doi: 10.1002/dev.21090.

Turnock-Jones, J. J., Jennings, C. A., Robbins, M. J., Cluderay, J. E., Cilia, J., Reid, J. L., Taylor, A., Jones, D. N., Emson, P. C., & Southam, E. (2009). Increased expression of the NR2A NMDA receptor subunit in the prefrontal cortex of rats reared in isolation. Synapse, 63(10), 836–846. doi: 10.1002/syn.20665.

Uzunova, G., Pallanti, S., & Hollander, E. (2016). Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics. The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry, 17(3), 174–186. doi: 10.3109/15622975.2015.1085597.

Vallée, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low

anxiety in adult offspring: correlation with stress-induced corticosterone secretion. The Journal of neuroscience : the official journal of the Society for Neuroscience, 17(7), 2626–2636. doi: 10.1523/JNEUROSCI.17-07-02626.1997.

Vallée, M., MacCari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999). Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. The European journal of neuroscience, 11(8), 2906–2916. doi: 10.1046/j.1460-9568.1999.00705.x.

Valzelli L. (1973). The "isolation syndrome" in mice. Psychopharmacologia, 31(4), 305–320. doi. 10.1007/BF00421275.

Van den Hove, D. L., Blanco, C. E., Aendekerk, B., Desbonnet, L., Bruschettini, M., Steinbusch, H. P., Prickaerts, J., & Steinbusch, H. W. (2005). Prenatal restraint stress and long-term affective consequences. Developmental neuroscience, 27(5), 313–320. doi: 10.1159/000086711.

Van den Hove, D. L., Leibold, N. K., Strackx, E., Martinez-Claros, M., Lesch, K. P., Steinbusch, H. W., Schruers, K. R., & Prickaerts, J. (2014). Prenatal stress and subsequent exposure to chronic mild stress in rats; interdependent effects on emotional behavior and the serotonergic system. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology, 24(4), 595–607. doi: 10.1016/j.euroneuro.2013.09.006.

van Kerkhof, L. W., Damsteegt, R., Trezza, V., Voorn, P., & Vanderschuren, L. J. (2013). Social play behavior in adolescent rats is mediated by functional activity in medial prefrontal cortex and striatum. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 38(10), 1899–1909. doi: 10.1038/npp.2013.83.

Van Pett, K., Viau, V., Bittencourt, J. C., Chan, R. K., Li, H. Y., Arias, C., Prins, G. S., Perrin, M., Vale, W., & Sawchenko, P. E. (2000). Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. The Journal of comparative neurology, 428(2), 191–212. doi: 10.1002/1096-9861(20001211)428:2<191::aid-cne1>3.0.co;2-u.

Van Waes, V., Enache, M., Dutriez, I., Lesage, J., Morley-Fletcher, S., Vinner, E., Lhermitte, M., Vieau, D., Maccari, S., & Darnaudéry, M. (2006). Hypo-response of the hypothalamic-pituitary-adrenocortical axis after an ethanol challenge in prenatally stressed adolescent male rats. The European journal of neuroscience, 24(4), 1193–1200. doi: 10.1111/j.1460-9568.2006.04973.x.

VanTieghem, M. R., & Tottenham, N. (2018). Neurobiological Programming of Early Life Stress: Functional Development of Amygdala-Prefrontal Circuitry and Vulnerability for Stress-Related Psychopathology. Current topics in behavioral neurosciences, 38, 117–136. doi: 10.1007/7854_2016_42.

Vekovischeva, O. Y., Aitta-Aho, T., Echenko, O., Kankaanpää, A., Seppälä, T., Honkanen, A., Sprengel, R., & Korpi, E. R. (2004). Reduced aggression in AMPA-type glutamate receptor GluR-A subunit-deficient mice. Genes, brain, and behavior, 3(5), 253–265. doi: 10.1111/j.1601-1848.2004.00075.x.

Venero, C., & Borrell, J. (1999). Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: a microdialysis study in freely moving rats. The European journal of neuroscience, 11(7), 2465–2473. doi: 10.1046/j.1460-9568.1999.00668.x.

Verhaeghe, R., Gao, V., Morley-Fletcher, S., Bouwalerh, H., Van Camp, G., Cisani, F., Nicoletti, F., & Maccari, S. (2022). Maternal stress programs a demasculinization of glutamatergic transmission in stress-related brain regions of aged rats. GeroScience, 44(2), 1047–1069. doi: 10.1007/s11357-021-00375-5.

Viveros, M. P., Hernández, R., Martínez, I., & González, P. (1988). Effects of social isolation and crowding upon adrenocortical reactivity and behavior in the rat. Revista espanola de fisiologia, 44(3), 315–321. PMID: 3231885.

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 22(15), 6810–6818. doi: 10.1523/JNEUROSCI.22-15-06810.2002.

Wadhwa, P. D., Buss, C., Entringer, S., & Swanson, J. M. (2009). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Seminars in reproductive medicine, 27(5), 358–368. doi : 10.1055/s-0029-1237424.

Wang, J. Q., Arora, A., Yang, L., Parelkar, N. K., Zhang, G., Liu, X., Choe, E. S., & Mao, L. (2005). Phosphorylation of AMPA receptors: mechanisms and synaptic plasticity. Molecular neurobiology, 32(3), 237–249. doi: 10.1385/MN:32:3:237.

Wang, Z., Edwards, J. G., Riley, N., Provance, D. W., Jr, Karcher, R., Li, X. D., Davison, I. G., Ikebe, M., Mercer, J. A., Kauer, J. A., & Ehlers, M. D. (2008). Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. Cell, 135(3), 535–548. doi: 10.1016/j.cell.2008.09.057.

Wang, D. D., & Kriegstein, A. R. (2009). Defining the role of GABA in cortical development. The Journal of physiology, 587(Pt 9), 1873–1879. doi: 10.1113/jphysiol.2008.167635.

Wang, X. D., Rammes, G., Kraev, I., Wolf, M., Liebl, C., Scharf, S. H., Rice, C. J., Wurst, W., Holsboer, F., Deussing, J. M., Baram, T. Z., Stewart, M. G., Müller, M. B., & Schmidt, M. V. (2011). Forebrain CRF_1 modulates early-life stress-programmed cognitive deficits. The Journal of neuroscience : the official journal of the Society for Neuroscience, 31(38), 13625–13634. doi: 10.1523/JNEUROSCI.2259-11.2011.

Wang, H., & Zhuo, M. (2012). Group I metabotropic glutamate receptor-mediated gene transcription and implications for synaptic plasticity and diseases. Frontiers in pharmacology, 3, 189. doi: 10.3389/fphar.2012.00189.

Ward, I. L., & Weisz, J. (1984). Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology, 114(5), 1635–1644. doi: 10.1210/endo-114-5-1635.

Waters, R. C., & Gould, E. (2022). Early Life Adversity and Neuropsychiatric Disease: Differential Outcomes and Translational Relevance of Rodent Models. Frontiers in systems neuroscience, 16, 860847. doi: 10.3389/fnsys.2022.860847.

Watkins, J. C., & Evans, R. H. (1981). Excitatory amino acid transmitters. Annual review of pharmacology and toxicology, 21, 165–204. doi: 10.1146/annurev.pa.21.040181.001121.

Wei, H., Ding, C., Jin, G., Yin, H., Liu, J., & Hu, F. (2015). Abnormal glutamate release in aged BTBR mouse model of autism. International journal of clinical and experimental pathology, 8(9), 10689–10697. PMID: 26617779.

Wei, H., Ma, Y., Liu, J., Ding, C., Hu, F., & Yu, L. (2016a). Proteomic analysis of cortical brain tissue from the BTBR mouse model of autism: Evidence for changes in STOP and myelin-related proteins. Neuroscience, 312, 26–34. doi: 10.1016/j.neuroscience.2015.11.003.

Wei, H., Ma, Y., Ding, C., Jin, G., Liu, J., Chang, Q., Hu, F., & Yu, L. (2016b). Reduced Glutamate Release in Adult BTBR Mouse Model of Autism Spectrum Disorder. Neurochemical research, 41(11), 3129–3137. doi: 10.1007/s11064-016-2035-5.

Weinstock, M., Matlina, E., Maor, G. I., Rosen, H., & McEwen, B. S. (1992). Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary

adrenal system in the female rat. Brain research, 595(2), 195–200. doi: 10.1016/0006-8993(92)91049-k.

Weinstock M. (2001). Effects of maternal stress on development and behaviour in rat offspring. Stress (Amsterdam, Netherlands), 4(3), 157–167. doi: 10.3109/10253890109035015.

Weinstock M. (2008). The long-term behavioural consequences of prenatal stress. Neuroscience and biobehavioral reviews, 32(6), 1073–1086. doi: 10.1016/j.neubiorev.2008.03.002.

Weinstock M. (2011). Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. Stress, 14(6), 604–613. doi: 10.3109/10253890.2011.588294.

Weinstock M. (2016). Prenatal stressors in rodents: Effects on behavior. Neurobiology of stress, 6, 3–13. doi: 10.1016/j.ynstr.2016.08.004.

Weiss, I. C., Pryce, C. R., Jongen-Rêlo, A. L., Nanz-Bahr, N. I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behavioural brain research, 152(2), 279–295. doi: 10.1016/j.bbr.2003.10.015.

Wenthold, R. J., Petralia, R. S., Blahos J, I. I., & Niedzielski, A. S. (1996). Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 16(6), 1982–1989. doi: 10.1523/JNEUROSCI.16-06-01982.1996.

Wiesel, T. N., & Hubel, D.H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. Journal of neurophysiology, 26, 1003–1017. doi: 10.1152/jn.1963.26.6.1003.

Willard, S. S., & Koochekpour, S. (2013). Glutamate, glutamate receptors, and downstream signaling pathways. International journal of biological sciences, 9(9), 948–959. doi: 10.7150/ijbs.6426.

Wilson, J. M., Khabazian, I., Pow, D. V., Craig, U. K., & Shaw, C. A. (2003). Decrease in glial glutamate transporter variants and excitatory amino acid receptor down-regulation in a murine model of ALS-PDC. Neuromolecular medicine, 3(2), 105–118. doi : 10.1385/NMM:3:2:105.

Wilson, C. A., & Koenig, J. I. (2014). Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology, 24(5), 759–773. doi: 10.1016/j.euroneuro.2013.11.008.

Witkin, J. M., Pandey, K. P., & Smith, J. L. (2022). Clinical investigations of compounds targeting metabotropic glutamate receptors. Pharmacology, biochemistry, and behavior, 219, 173446. doi : 10.1016/j.pbb.2022.173446.

Wöhr, M., Roullet, F. I., & Crawley, J. N. (2011). Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. Genes, Brain, and Behavior, 10(1), 35–43. doi: 10.1111/j.1601-183X.2010.00582.x.

Wójtowicz, T., & Mozrzymas, J. W. (2010). Estradiol and GABAergic transmission in the hippocampus. Vitamins and hormones, 82, 279–300. doi: 10.1016/S0083-6729(10)82015-1.

Wongwitdecha, N., & Marsden, C. A. (1996). Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behavioural brain research, 75(1-2), 27–32. doi: 10.1016/0166-4328(96)00181-7.

Woolley, C. S., & McEwen, B. S. (1992). Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. The Journal of neuroscience : the official journal of the Society for Neuroscience, 12(7), 2549–2554. doi: 10.1523/JNEUROSCI.12-07-02549.1992.

Wu, J., Song, T. B., Li, Y. J., He, K. S., Ge, L., & Wang, L. R. (2007). Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats. Brain research, 1141, 205–213. doi: 10.1016/j.brainres.2007.01.024.

Wu, C., & Sun, D. (2015). GABA receptors in brain development, function, and injury. Metabolic brain disease, 30(2), 367–379. doi : 10.1007/s11011-014-9560-1.

Xu, P., Chen, A., Li, Y., Xing, X., & Lu, H. (2019). Medial prefrontal cortex in neurological diseases. Physiological genomics, 51(9), 432–442. doi: 10.1152/physiolgenomics.00006.2019.

Yadav, R., Dravid, S. M., Yuan, H., & Traynelis, S. F. (2016). AMPA receptors: Molecular biology and pharmacology. In The Curated Reference Collection in Neuroscience and Biobehavioral Psychology (pp. 311-318). Elsevier Science Ltd. doi: 10.1016/B978-0-12-809324-5.02325-7.

Yaka, R., Salomon, S., Matzner, H., and Weinstock, M. (2007). Effect of varied gestational stress on acquisition of spatial memory, hippocampal LTP and synaptic proteins in juvenile male rats. Behav. Brain Res. 179, 126–132. doi:10.1016/j.bbr.2007.01.018.

Yang, M., Bozdagi, O., Scattoni, M. L., Wöhr, M., Roullet, F. I., Katz, A. M., Abrams, D. N., Kalikhman, D., Simon, H., Woldeyohannes, L., Zhang, J. Y., Harris, M. J., Saxena, R., Silverman, J. L., Buxbaum, J. D., & Crawley, J. N. (2012). Reduced excitatory neurotransmission and mild autism-relevant phenotypes in adolescent Shank3 null mutant mice. The Journal of neuroscience : the official journal of the Society for Neuroscience, 32(19), 6525–6541. doi: 10.1523/JNEUROSCI.6107-11.2012.

Yang, H., Huh, S. O., & Hong, J. S. (2015). Enhancement of Short-Term Memory by Methyl-6-(Phenylethynyl)-Pyridine in the BTBR T+tf/J Mouse Model of Autism Spectrum Disorder. Endocrinology and metabolism (Seoul, Korea), 30(1), 98–104. doi: 10.3803/EnM.2015.30.1.98.

Yavas, E., Gonzalez, S., & Fanselow, M. S. (2019). Interactions between the hippocampus, prefrontal cortex, and amygdala support complex learning and memory. F1000Research, 8, F1000 Faculty Rev-1292. doi: 10.12688/f1000research.19317.1.

Yeh, C. M., Huang, C. C., & Hsu, K. S. (2012). Prenatal stress alters hippocampal synaptic plasticity in young rat offspring through preventing the proteolytic conversion of pro-brain-derived neurotrophic factor (BDNF) to mature BDNF. The Journal of physiology, 590(4), 991–1010. doi: 10.1113/jphysiol.2011.222042.

Young, A.B., & Chu, D.C.M. (1990). Distribution of GABAA and GABAB receptors in mammalian brain: Potential targets for drug development. Drug Development Research, 21, 161-167. doi: 10.1002/ddr.430210303.

Yuen, E. Y., Wei, J., Liu, W., Zhong, P., Li, X., & Yan, Z. (2012). Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. Neuron, 73(5), 962–977. doi: 10.1016/j.neuron.2011.12.033.

Zacharopoulos, G., Sella, F., Cohen Kadosh, K., Hartwright, C., Emir, U., & Cohen Kadosh, R. (2021). Predicting learning and achievement using GABA and glutamate concentrations in human development. PLoS biology, 19(7), e3001325. doi: 10.1371/journal.pbio.3001325.

Zhang, X. H., Jia, N., Zhao, X. Y., Tang, G. K., Guan, L. X., Wang, D., Sun, H. L., Li, H., & Zhu, Z. L. (2013). Involvement of pGluR1, EAAT2 and EAAT3 in offspring depression induced by prenatal stress. Neuroscience, 250, 333–341. doi : 10.1016/j.neuroscience.2013.04.031.

Zhang, X., Ge, T. T., Yin, G., Cui, R., Zhao, G., & Yang, W. (2018). Stress-Induced Functional Alterations in Amygdala: Implications for

Neuropsychiatric Diseases. Frontiers in neuroscience, 12, 367. doi: 10.3389/fnins.2018.00367.

Zhang, L., Huang, C. C., Dai, Y., Luo, Q., Ji, Y., Wang, K., Deng, S., Yu, J., Xu, M., Du, X., Tang, Y., Shen, C., Feng, J., Sahakian, B. J., Lin, C. P., & Li, F. (2020). Symptom improvement in children with autism spectrum disorder following bumetanide administration is associated with decreased GABA/glutamate ratios. Translational psychiatry, 10(1), 9. doi: 10.1038/s41398-020-0692-2.

Zhao, X., Sun, L., Jia, H., Meng, Q., Wu, S., Li, N., & He, S. (2009). Isolation rearing induces social and emotional function abnormalities and alters glutamate and neurodevelopment-related gene expression in rats. Progress in neuro-psychopharmacology & biological psychiatry, 33(7), 1173–1177. doi: 10.1016/j.pnpbp.2009.06.016.

Zhou, Q., & Sheng, M. (2013). NMDA receptors in nervous system diseases. Neuropharmacology, 74, 69–75. doi : 10.1016/j.neuropharm.2013.03.030.

Zhou, Y., & Danbolt, N. C. (2014). Glutamate as a neurotransmitter in the healthy brain. Journal of neural transmission, 121(8), 799–817. doi: 10.1007/s00702-014-1180-8.

Zhu, J. J., Esteban, J. A., Hayashi, Y., & Malinow, R. (2000). Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nature neuroscience, 3(11), 1098–1106. doi: 10.1038/80614.

Zhu, Y., Carvey, P. M., & Ling, Z. (2007). Altered glutathione homeostasis in animals prenatally exposed to lipopolysaccharide. Neurochemistry international, 50(4), 671–680. doi: 10.1016/j.neuint.2006.12.013.

Zohar, I., & Weinstock, M. (2011). Differential effect of prenatal stress on the expression of corticotrophin-releasing hormone and its receptors in the hypothalamus and amygdala in male and female rats. Journal of neuroendocrinology, 23(4), 320–328. doi: 10.1111/j.1365-2826.2011.02117.x.

Zohar, I., Dosoretz-Abittan, L., Shoham, S., & Weinstock, M. (2015). Sex dependent reduction by prenatal stress of the expression of 5HT1A receptors in the prefrontal cortex and CRF type 2 receptors in the raphe nucleus in rats: reversal by citalopram. Psychopharmacology, 232(9), 1643–1653. doi: 10.1007/s00213-014-3803-z.

Zoicas, I., & Kornhuber, J. (2019). The Role of the N-Methyl-D-Aspartate Receptors in Social Behavior in Rodents. International journal of molecular sciences, 20(22), 5599. doi: 10.3390/ijms20225599.

Zuena, A. R., Mairesse, J., Casolini, P., Cinque, C., Alemà, G. S., Morley-Fletcher, S., Chiodi, V., Spagnoli, L. G., Gradini, R., Catalani, A., Nicoletti, F., & Maccari, S. (2008). Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PloS one, 3(5), e2170. doi: 10.1371/journal.pone.0002170.