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Cell-Specific Effects of the Mineralocorticoid Receptor

The effects of mineralocorticoid receptor deletion in glutamatergic or GABAergic neurons on emotional and cognitive functions

Huanqing Yang

Munich, Germany

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First examiner: PD. Dr. Mathias Schmidt

Second examiner: Prof. Dr. Anton Sirota

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List of Abbreviations

5-HT: 5-Hydroxytryptamin;serotonin;

11β-HSD: 11β-hydroxysteroid dehydrogenase;

11β-HSD2: 11β-hydroxysteroid dehydrogenases types 2;

Aβ: β-amyloid;

aCSF artificial cerebrospinal fluid;

ACTH: adrenocorticotropic hormone;

ADHD: Attention-Deficit/Hyperactivity Disorder;

AF-1: activation functional domain 1;

AF-2: activation functional domain 2;

ASD: Autism Spectrum Disorder;

BLA: basolateral amygdala;

BP: bipolar disorder;

BPD: borderline personality disorder;

CA1: cornu Ammonis 1;

CA3: cornu Ammonis 3;

CAV1: caveolin-1;

CNS: central nervous system;

CORT: corticosterone;

CRH: corticotropin-releasing hormone;

CSDS: chronic social defeat stress;

DAXX: death domain-associated protein;

DBD: DNA binding domain;

DG: dentate gyrus;

DRR1: down-regulated in renal cell carcinoma 1;

EGFR: epidermal growth factor receptor;

ELL: 11-19 lysine-rich leukemia;

ERK: extracellular signal-regulated kinase;

EPM: elevated plus maze;

FAF1: Fas-associated factor 1;

FC: Fear conditioning;

fEPSPs: field excitatory postsynaptic potentials;

FLASH: FLICE-associated huge protein;

GABA: γ-aminobutyric acid;

GC: glucocorticoid;

GR: glucocorticoid receptor;

hMR: human MR;

HPA: hypothalamic-pituitary-adrenal;

HRE: hormone response element;

hsp90: heat shock protein 90;

IGF1R: insulin-like growth factor1 receptor;

ISH: In situ hybridization;

LBD: ligand-binding domain;

LTP: long-term potentiation;

MC2R: melanocortin-2 receptor;

mEPSCs: miniature excitatory postsynaptic currents;

MCI: mild cognitive impairment;

mPFC: medial prefrontal cortex;

MWM: Morris water maze;

NCoR: nuclear receptor corepressor;

NAc: nucleus accumbens;

NOR: novel object recognition;

NTS: nucleus tractus solitarius;

MR: mineralocorticoid receptor;

NTD: N-terminal domain;

OF: open field;

PDGFR: platelet-derived growth factor receptor;

PFA: paraformaldehyde;

PGC-1: PPAR-γ coactivator 1;

PI3K: phosphoinositide-3-kinase;

PIAS1: protein inhibitor of activated STAT1;

PKB/Akt: protein kinase B/Akt;

PKC: protein kinases C;

PKD: protein kinases D;

PTMs post-translational modifications;

PTSD: post-traumatic stress disorder;

PVN: paraventricular nucleus;

SEM: standard error of the mean;

SHR spontaneously hypertensive rat;

SMRT: silencing mediator of retinoic acid and thyroid hormone receptor;

SNPs: single-nucleotide polymorphism;

SOR: spatial object recognition;

SRCs: steroid receptor coactivators;

SSRIs: selective serotonin reuptake inhibitors;

TGFβ: transforming growth factor-β;

WHO: World Health Organization.

Abstract

Stress is a normal response to situational pressures or demands. Exposure to stress activates the hypothalamic-pituitary-adrenal (HPA) axis and leads to the release of corticosteroids, which act in the brain via two distinct receptors: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). MR plays a key role in cognitive and emotional function, making it a key player in the body's response to stress. This thesis aims to investigate the effects of MR on emotional behavior and cognitive function in specific cell types (glutamatergic neurons and GABAergic neurons). Therefore, I first performed baseline behavioral testing in mice lacking MR in glutamatergic neurons as well as in GABAergic neurons. Lack of MR in glutamatergic neurons results in an anxiety-like phenotype, whereas lack of MR in GABAergic neurons appears to affect cognitive function. Following, further cognitive behavioral experiments were carried out on mice lacking MR in GABAergic neurons, and it was found that mice lacking MR in GABAergic neurons performed better in cognitive tasks under stressful conditions. Then, electrophysiological experiments were used to observe whether the neuronal functions of glutamatergic neurons and GABAergic neurons lacking MR were changed. Morphological changes of glutamatergic neurons lacking MR were observed by Golgi staining. Moreover, I conducted behavioral tests on these two strains of mice after chronic social stress, not only on male mice, but also on female mice, in order to observe the emotional behavior and cognitive behavior of mice of different sexes after experiencing chronic stress. Furthermore, in single-cell RNA sequencing data we identified cell-type specific downstream MR target genes, with Fam107a affected in MR-deficient glutamatergic neurons, and Npy being affected in MR-deficient GABAergic neurons. Normalizing Fam107a expression in mice lacking MR in glutamatergic neurons through viral manipulations was able to partially rescue the genotype-induced phenotype. Given the central role of MR in cognitive and emotional functioning, and its importance as a target for promoting resilience, future research should investigate how MR modulation can be used to alleviate disturbances in emotion and behavior, as well as cognitive impairment, in patients with stress-related psychiatric disorders.

1 Introduction

1.1 Stress and psychiatric disorders

1.1.1 Stress-related psychiatric disorders

Mental health is of utmost importance for individual well-being and success in life. In recent years, with the rapid development of society, mental disorders have become increasingly common worldwide. Mental health disorders pose a significant public health concern, and mental health disorders have become a major public health problem. According to the World Health Organization (WHO), nearly 1 billion people are affected by one or more mental illnesses, which equals nearly one-eighth of the world's population (United Nations, 2022); World Health Organization, 2022b). In addition to the physical impact on the individual, mental health conditions can also have significant social implications, including reduced productivity, increased healthcare costs, diminished quality of life, and added burdens on family members and caregivers. Among them, one of the most prominent social impacts is their economic burden on society. According to the Global Burden of Disease, Injury, and Risk Factor Study, the global cost of lost productivity due to mental health disorders will reach a staggering $16.3 trillion by 2030. Mental disorders therefore stand among the top ten health challenges faced worldwide (GBD 2019 Mental Disorders Collaborators, 2022).

There are numerous causes of psychiatric disorders, including genetic factors, environmental influences, and lifestyle choices. Among them, stress plays an important role in some psychiatric disorders. With the development of society, stress-related psychiatric disorders pose a significant and increasingly serious public health issue worldwide. After the COVID-19 pandemic, there has been a significant increase in the number of mental disorders, with anxiety and depression seeing the largest increases (World Health Organization, 2022a). A meta-analysis showed that the prevalence of mental disorders (depression, anxiety, post-traumatic stress disorder, bipolar disorder, and schizophrenia) was significantly higher in areas of war and conflict, accounting for 22.1% of the interviewed population (Charlson et al., 2019).

In summary, stress-related psychiatric disorders have significant social consequences and affect an individual's ability to function effectively in personal and professional life. Therefore, studying the pathogenesis of stress-related psychiatric disorders is crucial for enhancing our understanding of these complex conditions. Through research on the mechanisms underlying

stress-related psychiatric disorders, we can gain deeper insights into the potential biological, psychological, and social factors involved in disease development. This research can help identify risk factors and early warning signs, improve diagnostic accuracy, provide information for the development of more effective treatment strategies, and offer potential targets for the development of new pharmacological and non-pharmacological interventions, ultimately leading to better symptom relief.

1.1.2 Stress and resilience

According to the preceding text, we know that stress is the culprit of many mental illnesses, but it is also an unavoidable aspect of human existence. Whether it's daily work troubles, interpersonal conflicts, financial pressure, or traumatic events, humans universally experience stress. However, stress is not entirely negative. As a physical and emotional response to perceived threats or demands, it is a fundamental mechanism that helps us cope with challenging situations. Some short-term or moderate levels of stress can improve attention and alertness, motivate individuals to take action, enhance performance, and effectively complete tasks (El Zein et al., 2015; Marin et al., 2010; Tang et al., 2007). Acute stress also can temporarily boost the immune system, aiding our survival in threatening situations (Dhabhar, 2018). Conversely, excessive and prolonged stress can lead to a range of adverse physical and mental health outcomes, including psychiatric disorders (Yaribeygi et al., 2017). As a matter of fact, it is not the stress itself that determines the outcome, but rather how we cope with and manage these sources of stress.

Individuals exhibit significant differences in their ability to cope and recover when faced with adversity. Resilience refers to the ability to adapt, recover, and maintain psychological well-being in the face of stressors. It reflects an individual's capacity to effectively cope with and bounce back from adversity (H. Liu et al., 2018). The relationship between stress and resilience is complex and multifaceted. On a neurobiological level, stress experiences activate intricate interactions among physiological, cognitive, and emotional responses. The stress response is often referred to as the "fight or flight" response, involving activation of the autonomic nervous system and the release of stress hormones like cortisol (Russell & Lightman, 2019). Resilience involves the functioning of brain regions involved in emotion regulation, cognitive control, and reward processing (Kalisch et al., 2015). Understanding the underlying mechanisms of stress and resilience is crucial for promoting mental health, preventing mental disorders, and fostering overall well-being throughout the lifespan.

1.1.3 Hypothalamic-pituitary-adrenal axis and corticosteroid receptors

The relationship between stress, resilience, and mental illness is complex and involves many different kinds of biological processes. One key factor is the stress response system, which includes the hypothalamic-pituitary-adrenal (HPA) axis and stress hormones such as CORT (cortisol in humans or corticosterone in rodents). The HPA axis is an essential part of the neuroendocrine system and is involved in the control of stress responses that stimulate the production and release of adrenal cortisol (Herman et al., 2016). When the body encounters a stressor (both physiological and psychological), the paraventricular nucleus of the hypothalamus synthesizes and secretes corticotropin-releasing hormone (CRH), which in turn acts on the anterior pituitary gland to stimulate the release of adrenocorticotropic hormone (ACTH). ACTH promotes the synthesis and secretion of glucocorticoid (GC) hormones by binding to the melanocortin-2 receptor (MC2R) in the adrenal cortex (Fink, 2016; Gupta, 2019; Rao & Androulakis, 2019). These stress hormones bind to corticosteroid receptors in peripheral tissues as well as in different brain regions, including the hippocampus, prefrontal cortex, and amygdala. Activation of corticosteroid receptors influences the stress response and regulates subsequent behavior and physiological adaptations, including effects on immune function, cardiovascular health, and the central nervous system.

The actions of cortisol and corticosterone are mediated through binding to specific corticosteroid receptors. Glucocorticoid genomic signaling involves the binding of glucocorticoids to two nuclear receptors: type I is the mineralocorticoid receptor (MR), and type II is the glucocorticoid receptor (GR), both of which are members of the nuclear receptor superfamily (Antoni, 2019; Rao & Androulakis, 2019; Reul & de Kloet, 1985; Yiallouris et al., 2019). These receptors are primarily located in the cytoplasm of target cells, where they act as ligand-activated transcription factors, regulating the expression of specific genes in response to stress hormones. MRs have high affinity for cortisol and corticosterone and are typically occupied under basal, non-stress conditions. They are primarily involved in regulating salt and water balance, among other physiological processes. On the other hand, GRs have a lower affinity for cortisol and corticosterone and are activated during periods of stress, playing a central role in the stress response. While the actions of corticosteroid receptors are crucial for an appropriate stress response, dysregulation or prolonged activation of these receptors can have adverse effects on health (Joëls, 2018). Chronic stress and prolonged exposure to high levels of cortisol or corticosterone can lead to a range of negative consequences, including immune suppression, metabolic disturbances, cardiovascular complications, as well as mental

health disorders such as depression and anxiety (Dziurkowska & Wesolowski, 2021; Seiler et al., 2020).

There has been a lot of research on GR and stress-related psychiatric disorders (De Nicola et al., 2020; Finsterwald & Alberini, 2014; Spijker & van Rossum, 2009). The activation of GRs leads to changes in gene expression, influencing the synthesis of proteins involved in energy metabolism, immune regulation, and stress adaptation. Additionally, GRs can regulate neuronal excitability and synaptic plasticity, impacting cognitive function and emotional regulation. The contribution of MRs, on the other hand, has not received as much attention. However, in recent years, studies have confirmed that by conveying hormone signals and stimulating the expression of target genes, MRs regulate a number of physiological and pathological responses relevant to brain function and disease. MRs have a vital role in cognition, emotion, and behavior, in addition to regulating water and salt balance (Chen et al., 2016; Paul et al., 2022; Wingenfeld & Otte, 2019).

Considering that MR may be a potential therapeutic target for psychiatric disorders, this thesis focuses on MRs to investigate their relationship with emotions, cognition, and behavior.

1.2 MR biological background[1]

1.2.1MR structure

1.2.1.1 Gene structure

The human MR (hMR) NR3C2 gene is located at q31.1-q31.2 region of chromosome 4, consists of 450 bases, and encodes a 984 amino acid protein of 107 KDa (Arriza et al., 1987; Fan et al., 1989; Morrison et al., 1990). There are 10 exons in the hMR gene; the first 2 exons (1α and 1β) are not translated, and the remaining 8 exons encode the entire MR protein, thereby resulting in the generation of two possible transcripts, hMRα and hMRβ (Zennaro et al., 1995). Different from humans, the MR gene in rats is located on chromosome 19q11, has 3 MR splice variants: αMR, βMR, and γMR, and encodes 981 amino acids (Kwak et al., 1993; Viengchareun et al., 2007). Further, some studies have found the splice variant MR+4 of MR in various human and rat tissues (Wickert et al., 1998). In mice, the MR gene has a similar structure of that in rats and

[1] The sections 1.2, 1.3 and 1.4 are taken from Yang et al.(2023).

encodes 978 amino acids (Viengchareun et al., 2007). The MR gene is evolutionarily conserved in most mammals (Rivers et al., 2009). Patel and colleagues (1989) found that in the coding region and the 3' untranslated region, there is an 88% open reading frame nucleic acid homology between rat and human kidney MR.

1.2.1.2 Protein structure

As a member of the nuclear receptor superfamily, MR shares a common protein structure with GR (Koning et al., 2019), which is composed of three independent domains. These three main functional domains are the N-terminal domain (NTD), the DNA binding domain (DBD), and the C-terminal ligand-binding domain (LBD) (Couette et al., 1996). The NTD is located at the NH2-terminal and plays a key role in regulating the specificity of MR action. It is highly conserved, with at least 50% homology between different species (Pascual-Le Tallec & Lombès, 2005; Viengchareun et al., 2007). MR DBD, which located in the middle of the protein, exhibits a considerable degree of similarity (roughly 94%) to GR DBD, and has a role in binding specific target DNA sequences and hormone response elements (HRE) (Hudson et al., 2014; Viengchareun et al., 2007). Finally, the LBD is located at the COOH-terminal and is responsible for binding to specific hormone ligands, and mediating the translocation of MR from the cytoplasm to the nucleus, and its homology between species is as high as 80%-97% (K. Fischer et al., 2010; Fuller et al., 2012; Grossmann et al., 2021; Mangelsdorf et al., 1995; Sheppard, 2002; Viengchareun et al., 2007; Zennaro et al., 1995).

1.2.2 MR ligands

Steroid hormone receptors are ligand-activated and switch from an inactive state to an active state by binding to their corresponding hormones (Torchia et al., 1998). Since MR LBD shares high homology with GR LBD, MR has two main endogenous ligands: aldosterone and cortisol in humans, or CORT in rodents (Baker et al., 2013). MR has a high affinity for cortisol (Kd = 0.5 nM), 10-fold higher than GR (Kd = 5 nM) (Meijer et al., 2018). The circulating concentration of cortisol in the blood is about 100-1000 times that of aldosterone (Syed & Qureshi, 2012). Even if only 5%-10% of cortisol is actively free, cortisol levels remain much higher than aldosterone in plasma (Cizza & Rother, 2012; Mifsud & Reul, 2018). Consequently, MR will be entirely occupied by cortisol except for when the circadian cycle of cortisol release is at its lowest point. Aldosterone has the same affinity for binding MR as cortisol, and since aldosterone dissociates from MR more slowly than cortisol, the aldosterone-MR complex is more stable and potent.

As a member of the nuclear receptor superfamily, MR is essential for the control of sodium and potassium transport in epithelial cells, especially in the kidney and colon (Fuller & Young, 2005; Viengchareun et al., 2007). MR is also widely distributed in non-epithelial tissues both humans and animals. For example, MR is expressed not only in cardiac myocytes, but also in endothelial and smooth muscle cells of the vascular system (Christy et al., 2003). In the central nervous system, the hippocampus has the highest MR abundance. While the dominant ligands of brain MR are cortisol/CORT, MR binds to aldosterone in selective brain regions, i.e. in the brainstem, to regulate the physiology and behavior associated with salt balance (Fuller & Verity, 1990). In the reproductive system, MR is expressed in the granulosa cells of the ovary (Fru et al., 2006). Progesterone, androgens and their derivatives can also bind to MR and exhibit partial activation or antagonism(Quinkler et al., 2004). Therefore, progesterone is considered by some as another physiological ligand for MR. In humans and rodents, progesterone antagonizes MR activation via aldosterone, and in sharks and chickens, progesterone activates MR directly (Baker & Katsu, 2020). It appears that MR acts as a "type I corticosteroid receptor" in these species (Gustavson et al., 2008). The existing mineralocorticoid antagonists, such as spironolactone and eplerenone, have been used clinically as antihypertensive and cardiovascular protection drugs (Lainscak et al., 2015). Drospirenone as a new progestin also has certain MR antagonist activity (Motivala & Pitt, 2007). Taken together, this shows that MR ligands are diverse (see Table 1)(Fuller et al., 2012; Paul et al., 2022; Reul et al., 2000) and additional mechanisms are required to regulate their selectivity.

Table 1. The ligands of mineralocorticoid receptor

	Source	Type	Name
Agonist	Endogenous	Steroidal	Aldosterone
			Cortisol
	Synthetic	Steroidal	18-Oxocortisol
			Fludrocortisone
Antagonist	Endogenous	Steroidal	Progesterone
	Synthetic	Steroidal	Spironolactone
			7α-thiomethylspironolactone (TMS)
			6β-hydroxy-7α-thiomethylspironolactone (HTMS)
			Canrenone
			prorenone
			Potassium canrenoate
			Mexrenone
			Eplerenone
		Non-steroidal	Apararenone
			Esaxerenone
			Finerenone

1.2.3 Cellular mechanisms of MR

In the absence of ligands, MR mostly remains in the cytoplasm, and is inactivated by binding to heat shock protein 90 (hsp90) and other chaperone proteins (Lombès et al., 1994; Robertson et al., 1993). When the ligand enters the cell and binds to the MR, specific activation domains control the activity of MR functions. One is an autonomous activation function 1 (AF-1) located at the N-terminus, and the other is transcriptional activation function 2 (AF-2), which is located at the C-terminus (Yokota et al., 2007). The ligand-receptor complex undergoes a conformational change, dissociating from hsp90 , and the MR is converted into a DBD-active form, thereby initiating a nuclear translocation signal (Weikum et al., 2018; J. Yang & Young, 2009). After MR enters the nucleus, it binds to the HRE on the target gene via DBD, and

mediates transcriptional activation or repression under the combined action of transcriptional co-regulators (see Figure 1) (Fuller & Young, 2005). When aldosterone engages with MR, the interaction between N-terminus and C-terminus is enhanced and the internal structure of MR changes, resulting in the recruitment of particular synergistic transcription factors and the promotion of the matching aldosterone effects. This interaction between NTD and LBD is aldosterone-dependent, as cortisol and MR have no such impact when combined (Fuller, 2015).

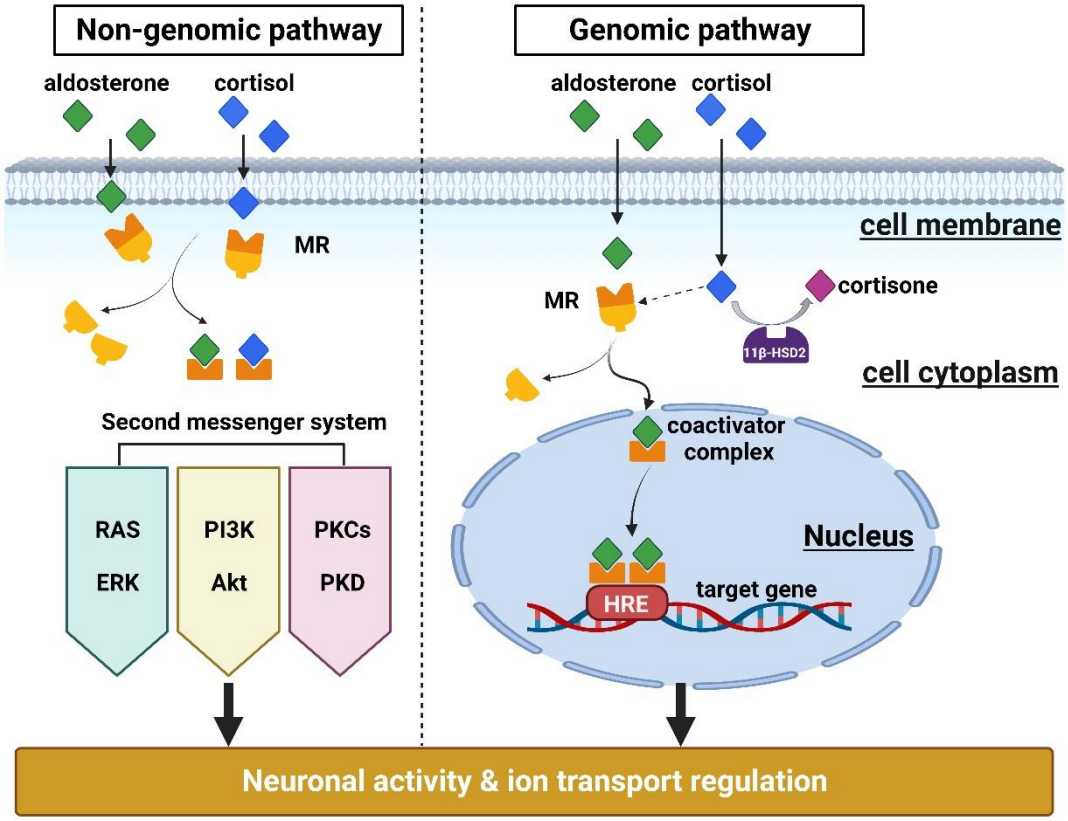


Figure 1: Schematic representation of intracellular genomic and non-genomic signaling pathways following MR ligand binding. Rapid MR signaling via non-genomic pathways activates downstream receptor kinases and second messenger systems, ultimately leading to alterations in neuronal activity. Conversely, for the genomic pathway, ligand-activated MR dissociates from its chaperone protein complex, translocates to the nucleus and regulates gene transcription of HRE-containing target cells. In 11ß-HSD2 containing cells the main natural ligand of MR is aldosterone, as the high-affinity ligand cortisol (or corticosterone) will be converted. In cells not containing 11ß-HSD2, as for example many neurons, the main ligand for MR is cortisol or corticosterone. MR: mineralocorticoid receptor; HRE: hormone response element; RAS: renin-angiotensin system; ERK: extracellular signal-regulated kinase; PI3K: Phosphatidylinositide 3-kinases; Akt: protein kinase B; PKC: protein kinases C; PKD: protein kinases D. (Illustration is created with Biorender.com).

In addition, some researchers have proposed a fast, non-genomic action of membrane-associated MR, distinct from the classical intracellular function of MR as transcription factor (Groeneweg et al., 2012; Wehling, 2005). This non-genomic effect manifests rapidly, and also

disappears quickly, indicating that its action is exerted at the level of the cell membrane (Keller-Wood & Dallman, 1984). For instance, within the first hour of administration, cortisol exerts numerous effects on cognition and mood, and these early effects can only be explained by early non-genomic effects, also highlighting the significance of MR in the initial stages of the stress response (Joëls et al., 2012).

Although it has been found that other steroids, such as estrogen, may exert rapid non-genomic effects by binding to signal transduction protein-associated membrane receptors (Arnal et al., 2017; Schumacher, 1990), MR, unlike other steroid receptors, lacks palmitoyl ionization sites and cannot be inserted into the membrane (Nicolaides et al., 2017). Nevertheless, some researchers suggested that MR can communicate with the cell membrane through the scaffold proteins striatin and caveolin-1 (CAV1) (Ashton et al., 2015; Coutinho et al., 2014). Other studies have found that MR exploits many intracellular signaling cascades to more rapidly alter cellular function through transactivation of receptor kinases. Examples include epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and insulin-like growth factor1 receptor (IGF1R), as well as some second messenger systems like extracellular signal-regulated kinase (ERK) cascade, phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt) pathway, and protein kinases C (PKC) and D (PKD) (see Figure 1) (Nagata et al., 2019; Ong & Young, 2017; Parker et al., 2018; Ruhs et al., 2017). MRs that mediate non-genomic effects are thought to be identical to MRs involved in genomic signaling, with the exception that they may be translocated to the membrane rather than the nucleus, and rapid effects of aldosterone can be induced by aldosterone-BSA in cells without nuclear MRs (Karst et al., 2022). Additional evidence for MR-mediated rapid and non-genomic effects were reported for MR ligands fused to BSA, thereby prohibiting the intracellular diffusion of the ligands. For example, a study using Aldosterone-BSA found that this extracellular MR ligand activates the PKCα pathway, induces MR phosphorylation, and triggers cross-talk between the nongenomic and genomic responses in renal collecting duct cells (Le Moëllic et al., 2004). While there is substantial evidence of rapid MR-mediated effects in peripheral tissues (Hermidorff et al., 2017), the situation in the brain and specifically in 11β-HSD type 2 (11β-HSD2) negative cells is still largely unclear. Visual proof of MR located and acting at the membrane level is difficult, therefore the membrane receptor hypothesis so far lacks direct evidence. Integrating the nongenomic and genomic activities of MR is an intriguing topic that needs more research.

1.2.4 MR selectivity

As mentioned above, many substances have similar affinities to MR, and MR signals can be modulated at various levels. The following will describe how MR selects the corresponding ligand and produces a specific response.

1.2.4.1 Selectivity at the pre-receptor level

The tissue distribution, expression, and coordination aspects of MR influence the mineralocorticoid actions it mediates. Specifically, the modulation of local MR ligand concentrations through specific enzymes is modulating MR function at the pre-receptor level (Penning et al., 2008). Important enzymes in this regard are the 11β-hydroxysteroid dehydrogenases (11β-HSD) types 1 and 2, which regulate intracellular glucocorticoid levels. In humans, cortisol is converted to cortisone by 11β-HSD2, which is a derivative with very low affinity for MR and cannot bind or activate MR, hence allowing tissue-specific MR regulation (Funder, 1991; Odermatt & Atanasov, 2009). Similarly in rodents, corticosterone is converted to 11-deoxycorticosterone, and neither product of the conversion can bind to or activate MR (Odermatt & Atanasov, 2009). Aldosterone can activate MR in aldosterone target cells due to 11β-HSD2's inactivation of cortisol and corticosterone (Gomez-Sanchez & Gomez-Sanchez, 2014). Therefore, the entry of aldosterone and the absence of cortisol action on MR are governed by the activity of 11β-HSD2 (Edwards et al., 1988; Funder et al., 1988). However, since 11β-HSD2 is not expressed in many non-epithelial tissues, such as heart, nervous system or adipocytes, MR selectivity may be controlled by mechanisms at the post-receptor level (Faresse, 2014; Gomez-Sanchez & Gomez-Sanchez, 2014; Penning, 2003). Further, there are few neurons in the brain that can co-express 11β-HSD2 and MR, and are aldosterone-sensitive. This kind of neurons have been found to be located mainly in the nucleus tractus solitarius (NTS) and to be efferent to the nucleus accumbens (NAc) via a multisynaptic pathway relayed from the dorsolateral pons and paraventricular thalamic nucleus (Gasparini et al., 2019; Geerling et al., 2006; Shekhtman et al., 2007). However, 11β-HSD2 mRNA is certainly not sufficient to explain MR selectivity, which is further modulated at the post-receptor level.

1.2.4.2 Selectivity at the post-receptor level

MR requires the presence of transcriptional co-factors in order to bind to ligands and exert its physiological effects, and their binding is affected by multiple post-translational modifications (PTMs), including phosphorylation, glycosylation, ubiquitination, methylation, acetylation,

sumoylation, oxidative stress, etc. (Gadasheva et al., 2021). Among the large number of transcriptional co-factors that have been found, their functions and cellular expression are in various forms—some of which play a role in transcriptional activation, while others affect transcriptional repression (McKenna et al., 1999; Torchia et al., 1998; J. Yang & Young, 2009). Coactivators and corepressors are involved in chromatin remodeling (Z. Liu et al., 1999). As a transcriptional MR coactivators, both 11-19 lysine-rich leukemia (ELL) and FLICE-associated huge protein (FLASH), can interact with AF-1 (Yang & Young, 2009); PPAR-γ coactivator 1 (PGC-1), on the other hand, acts by interacting with AF-2 (Knutti et al., 2000), and Ubiquitin conjugating enzyme 9 (Ubc9) interacts with the NTD of MR and thus exerts physiological effects (Yokota et al., 2007). Another example is the protein tesmin, which can coactivate aldosterone- and deoxycorticosterone-induced MR transactivation, but not cortisol-induced MR-mediated transactivation (Fuller, 2015). There are proteins that function as coactivators for numerous transcription factors, but the function of p160 family members or steroid receptor coactivators (SRCs) appears to be restricted to the nuclear receptor family (Meijer et al., 2000). As a corepressor, the nuclear receptor corepressor (NCoR) and thyroid hormone receptor (SMRT) interact with LBD (Wang et al., 2004), and death domain-associated protein (DAXX) and protein inhibitor of activated STAT1 (PIAS1) interact with NTD Role (J. Yang & Young, 2009). Fas-associated factor 1 (FAF1) has the ability to suppress aldosterone-activated MR transactivation after interacting with the sumoylated MR, and aldosterone increases the activation of MR target genes when endogenous FAF1 in cells was silenced (Wang et al., 2019). Different ligands in conjunction with MR can recruit distinct transcription coordinating factors, and these transcription-coordinating factors decide distinct outcomes (Faresse, 2014; Gadasheva et al., 2021; Weber et al., 2008).

1.2.5 Expression and distribution of MR

MR is expressed in many tissues throughout the body. MR expression in the periphery is predominant in epithelial tissues such as distal parts of the nephron, liver, distal colon, airway, sweat glands, inner ear, etc. (Gorini et al., 2019). In epithelial tissues, where cortisol has a low affinity for MR, the conversion of cortisol to corticosterone can be accomplished by 11β-HSD2, thus ensuring that aldosterone, the main physiological ligand in epithelial tissues, can bind to MR to exert its proper physiological effects (Edwards et al., 1988). In addition, many non-epithelial tissues such as cardiovascular, skin, placenta, ovary and testis, adipose tissue and the brain have been identified as expressing MR (Cole & Young, 2017). In contrast to epithelial tissues, the expression of 11β-HSD2 is almost absent or relatively low in many of these tissues.

Thus, glucocorticoids become the main ligands for MR in most non-epithelial tissues (Marzolla et al., 2014). Differences in the tissue distribution of MR and GR were also related to receptor selectivity, and MR and GR are expressed at varying amounts on various cell types. In the immune system, for instance, GR is substantially more expressed than MR. In the central nervous system, however, many brain areas express CORT-preferred MR, including the prefrontal cortex, hippocampus, lateral septal thalamic nucleus, hypothalamic nucleus, and medial and central amygdala (Reul & de Kloet, 1985). In the majority of cases MR is co-expressed with GR, for example in the hippocampus (Gomez-Sanchez, 2014; Sarabdjitsingh et al., 2012). A single-cell sequencing study in the human temporal lobe cortex showed that in most inhibitory neurons, the expression levels of MR and GR were not high, and only two kinds of GABAergic neurons show higher expression of MR than of GR. The opposite was found to be true in glutamatergic neurons, where GR is generally expressed at a higher level than MR (Koning et al., 2019). Although in the brain, MR is commonly regarded as expressed mainly in neurons, recent single-cell mRNA expression analyses reveal that it is also expressed in astrocytes (Viho et al., 2022) and microglia (Bast et al., 2018; Odermatt & Kratschmar, 2012). Little attention has been given to potential sex differences in MR expression and distribution so far, although an early study studies found that in the hippocampus, MR mRNA was significantly higher expressed in females than in males (Watzka et al., 2000). As differential phenotypes were also observed between male and female forebrain-specific MR knockout mice (Ter Horst et al., 2012), a more detailed study of MR-related sex differences is highly warranted.

1.3 MR in the brain

There are many studies on the relationship between MR and the renal and cardiovascular systems, which are summarized elsewhere (Nakamura et al., 2022; Ravid & Laffin, 2022; van der Heijden et al., 2022). However, MR does not only play a physiological role in the aforementioned organ systems, but also in the brain despite being only widely distributed in the brain's limbic system. Below we discuss the main functions of MR in the brain (see Figure 2).

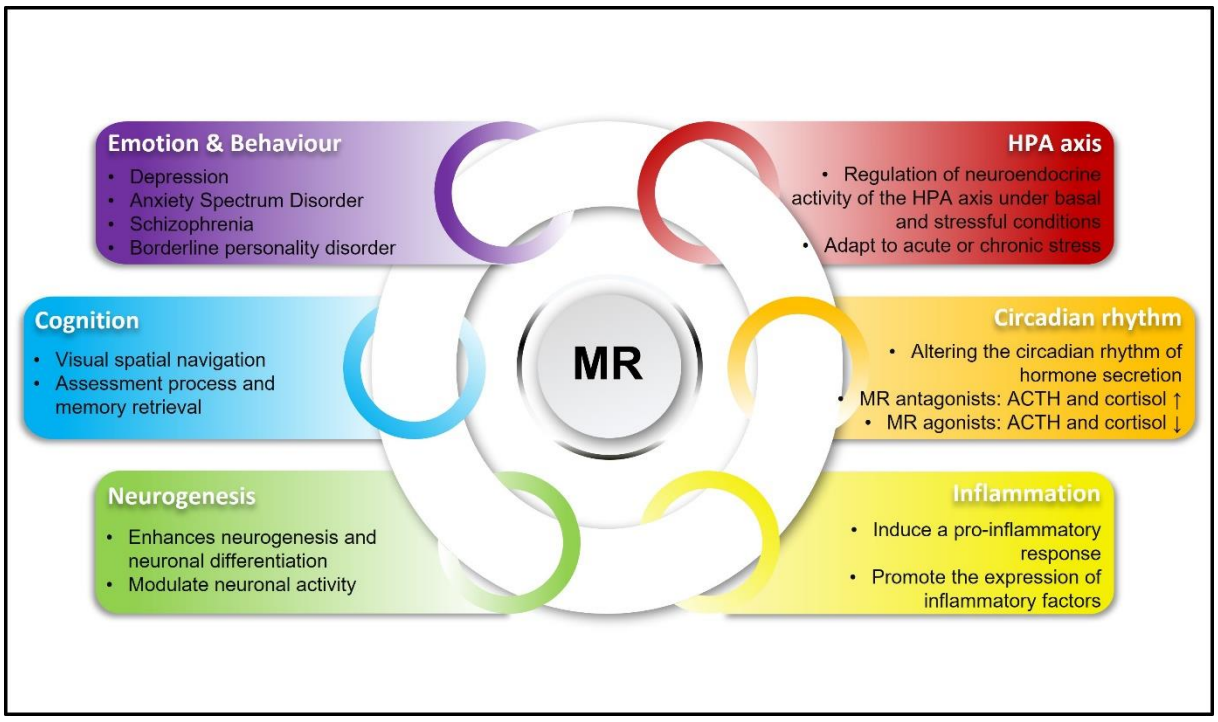


Figure 2: Overview of MR functions in the brain. In the brain, MR regulates the HPA axis activity and circadian rhythm, modulates neurogenesis, participates in neuroinflammation and affects mood, behavior, and cognition.

1.3.1 HPA axis and circadian rhythm

In the brain, MR is mainly expressed in neurons in the limbic system and prefrontal cortex (Kloet et al., 1998), both of which are known to regulate HPA axis activity. Here, MR is thought to function through γ-aminobutyric acid (GABA)-ergic neuron-mediated tonic inhibition projections to the medial parvicellular neurons of the PVN, which control ACTH secretion through CRH and AVP (Goncharova, 2020; Herman et al., 2016). It is well known that MR can modulate the neuroendocrine activity of the HPA axis, both under basal conditions and in stressful environments (Berardelli et al., 2013; Chen et al., 2016; Murck et al., 2014). Moreover, different MR variants have different effects on the activity of the basal HPA axis. MR (hydrochloride 2C/G) G-carriers exhibit higher basal cortisol levels than C-carriers (Kuningas et al., 2007; Muhtz et al., 2011). For the MR GA haplotype, the inhibitory effect of dexamethasone on CAR was significantly higher in female carriers than in male carriers (van Leeuwen et al., 2010), highlighting the variant-specific and sex-specific functions of MR. Using forebrain-specific MR deletion in animal studies, it was found that forebrain MR may modulate the HPA axis by inhibiting the secretion of corticosterone. Both basal corticosterone secretion and stress-induced corticosterone secretion were altered in transgenic mice, and corticosterone levels were significantly increased under stress inhibition (ter Horst, van der Mark, et al., 2012).

Under stress conditions, administering metyrapone to rats was discovered to enhance the binding of MR and GR to GRE present in the genes targeted by GC, and the transcriptional activity of these genes varied depending on the gene. But metyrapone did not completely result in the elimination of the effects of stress on plasma CORT, MR, and GR binding to HRE (Kennedy et al., 2020).

HPA axis activity follows a distinct circadian rhythm. CORT secretion is usually high at the beginning of the activity phase (e.g., morning in humans and evening in rodents) and declines to reach a trough at the start of the rest phase. GCs exhibit strong time dependence, with almost all physiological effects accompanied by similar rhythms (De Nobrega et al., 2020; Gaffey et al., 2016; Hood & Amir, 2017; Oster et al., 2017). MR plays a unique role in regulating the HPA axis, and it cooperates with GR to control the basal activity of the HPA axis. Administration of MR antagonists, whether in the morning or in the afternoon, increases ACTH and cortisol secretion during circadian quiescence, altering circadian-driven HPA activity (Berardelli et al., 2010; Oitzl et al., 1995; van Haarst et al., 1997). In addition, it has been shown that systemic treatment of the MR antagonist potassium canrenoate during the nadir phase of the circadian rhythm increases cortisol secretion (Grottoli et al., 2002). Following administration of the MR agonist fludrocortisone (0.5 mg), nocturnal HPA axis activity was significantly suppressed and cortisol and ACTH were sharply reduced (Buckley et al., 2007). However, limbic MR deficiency has no effect on basal circadian HPA axis activity in the cornu Ammonis 1 (CA1) region (Berger et al., 2006a).

1.3.2 Neurogenesis

In adult mammals, new neurons continue to be generated in the granule cells of the hippocampal dentate gyrus (DG) (Cameron et al., 1993; Kempermann et al., 2015) and the subventricular zone (SVZ) (Doetsch et al., 1999; Ponti et al., 2013). Recently, there is also some new evidence supporting the idea that selected neurogenesis may also occur in other brain regions, such as the hypothalamus, striatum, substantia nigra, cortex, and amygdala (Jurkowski et al., 2020). However, the role of the MR in relation to neurogenesis has so far only been studied in the hippocampus. Specifically, endogenous glucocorticoids are a known regulator of neurogenesis, and a prominent involvement of MR in neurogenesis and modulation of neuronal activity in the hippocampus has been demonstrated (Brown, 2008; Marver et al., 1974). Several studies have revealed increased proliferation following MR activation (Anacker et al., 2013; Fischer et al., 2002). Anacker and colleagues (2013) discovered that activation of MR

promoted proliferation after exposure to low concentrations of cortisol in hippocampal progenitor cells, and that MR mediated proliferation of astrocytes. In contrast, high concentrations of cortisol, which result in the additional activation of GR, lead to reduced hippocampal progenitor cell proliferation and neuronal differentiation, as well as inhibited MR-induced increases in astrogliosis. Mechanistically, low concentrations of cortisol were shown to enhance Notch/Hes signaling, while high concentrations of cortisol inhibit transforming growth factor-β-SMAD2/3 (TGFβ-SMAD2/3) signaling, both of which contribute to the proliferation of neural progenitor cells. In a prenatal stress model in rats, short-term stress and mild prenatal stress can enhance hippocampal neurogenesis and neuronal differentiation, which can improve the learning ability of adult offspring (A. Fujioka et al., 2006; T. Fujioka et al., 2001). Interestingly, only MR was demonstrated to be involved in this process. In this case, MR in fetal hippocampal neurons may be activated by corticosterone, while GR is only activated by high concentrations of corticosterone under long-term and severe stress (T. Fujioka et al., 1999).

Conversely, MR activation has been shown to block adrenalectomy-induced cell proliferation (Rodriguez et al., 1998). Montaron et al. (2003) showed similar results, where low doses of MR agonist (20 mg/L aldosterone) prevented adrenalectomy-induced cell death, while high doses of MR agonist (30 mg/L aldosterone) prevented adrenalectomy-induced cell proliferation. These findings contradict those of previously mentioned studies, but the mechanisms of adrenalectomy and MR activation are not fully understood, and differences in these results may be due to a variety of reasons.

Similar to what was observed after adrenalectomy, Gass et al. (2000) found reduced neurogenesis and amount of dentate granule cells in MR knockout mice, likely reflecting elevated corticosterone levels. Interestingly, a similar effect was not found in GR knockout mice, underlining an important role of the MR after adrenalectomy. A similar situation was observed in rats, with increased apoptosis in the dentate gyrus following administration of the GR agonist dexamethasone, possibly due to decreased MR activity and plasma corticosterone levels (Hassan et al., 1996; Sousa et al., 1999). Taken together, MR activation and the balance of MR and GR seem to be crucial to regulate hippocampal neurogenesis.

1.3.3 Neuroinflammation: Focus on microglia

A characteristic hallmark of chronic stress is the induction of pro-inflammatory mechanisms (Hayashi & Cortopassi, 2015; Miller & Sadeh, 2014). In numerous rodent experiments,

researchers have found that microglia alter morphology, phagocytic activity, and synaptic plasticity, and are increased in sensitivity after chronic stress (Bisht et al., 2018). As microglia are the main immune cells of the central nervous system (CNS), their activation can lead to inflammation and neuronal damage following chronic stress. Glucocorticoids are important regulators of inflammation, and thus, MR and GR play important roles in the regulation of inflammation (Bay-Richter et al., 2012; Ozacmak et al., 2014). In microglia, MR and GR are co-expressed in the presence of 11β-HSD1 (Odermatt & Kratschmar, 2012). In regulating neuroinflammation and NF-κB activity in microglia, MR controls the NF-κB pathway and inflammatory mediators in microglia by coordinating with GR. 11β-HSD1 can locally regulate the balance mediated by MR and GR, and only low (20 nM) and moderate (50 nM) concentrations of corticosterone can promote the expression of interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) in a MR-dependent manner (Chantong et al., 2012). After aldosterone treatment, LPS-induced interleukin-1β (IL-1β) and IL-6 mRNA expression was found to be increased in the PFC and CSF of rats (Bay-Richter et al., 2012). In the spontaneously hypertensive rat (SHR) model, lesion-induced brain tissue damage triggers a pro-inflammatory feedforward cascade driven by MR activation in response to endogenous corticosterone. In hippocampus, hyperactive MR and enhanced MR/GR co-localization promote inflammatory effects. Further, in spontaneously hypertensive rats, hyperactivation of MR is associated with a pro-inflammatory environment, resulting from an activated state of microglia (Brocca et al., 2019). CORT-activated MRs were also shown to mediate inflammatory and fibrotic responses in the presence of the 11β-HSD2 inhibitor carbenoxolone and salt (Wilson et al., 2009). Given these findings of MR in the brain's inflammatory response, the role of MR in the regulation of CNS inflammation needs further, in-depth study.

1.4 MR and psychiatric disorders

There are many types of psychiatric disorders, but in general, they mainly manifest as disturbances in thinking and cognition, emotional regulation impairment, or behavioral abnormalities. In the following section, we will review the relationship between MR and psychiatric disorders in two parts: abnormal emotions and behaviors, and cognitive impairment.

1.4.1 Abnormal emotions and behaviors

Depression and Anxiety Spectrum Disorders are the major disorders of emotional and behavioral impairment in contemporary society. In the regulation of stress responses, MR and GR control adaptation to environmental demands in a complementary manner. Disruption of

the MR/GR balance may lead to hyperactivity of the HPA axis, and this is one of the hypotheses for the etiology of depression (Qi et al., 2013). Hyperactivity of the HPA axis and elevated GC affect brain serotonin and stress adaptation-dependent responses, possibly leading to depression (Haleem & Gul, 2020; Keller et al., 2017). Persistent effects of stress on the HPA axis early in growth and development may lead to increased susceptibility to depression in adulthood (Mello et al., 2003; Heim, 2000; Shea et al., 2005). In adolescence, elevated cortisol often precedes the onset of depression and may be a risk factor of this disease (Zajkowska et al., 2022). Other researchers have discovered that in comparison to healthy individuals, patients experiencing depression have lower concentrations of morning cortisol and a disrupted daily rhythm of aldosterone release, resulting in an increased level of aldosterone at night (Izakova et al., 2020). Another study reported that compared with healthy controls, basal cortisol levels were elevated in depressed patients throughout the circadian cycle (Belvederi Murri et al., 2014). Irregular work schedules and chronic stress can lead to disruption of circadian rhythms and increase the risk of mental illness (den Boon & Sarabdjitsingh, 2017). In line with these observations, MR mRNA expression in hippocampus, prefrontal cortex, and cingulate gyrus is significantly reduced in depressed patients (Klok, Alt, et al., 2011; Medina et al., 2013; Patel et al., 2000). There is accumulating genetic and functional evidence for a direct contribution of MR to mood disorders, which is further amplified by sex differences. For example, the MR CA haplotype is associated with female susceptibility to depression, and this effect has also been linked to childhood abuse (Endedijk et al., 2020; Vinkers et al., 2015). Further, MR haplotype 2 genetic variants are associated with individual differences in antidepressant ability in women, as evidenced by more feelings of optimist and fewer feelings of hopelessness (Klok, Giltay, et al., 2011).

In addition, there are interesting associations of MR with 5-Hydroxytryptamin (serotonin, 5-HT), a monoamine neurotransmitter involved in the regulation of mood, learning and memory (Šalamon Arčan et al., 2022). The 5-HT1A receptor is abundant in the hippocampus, where it colocalizes with MR (H. Meltzer, 1989; H. Y. Meltzer, 1990). 5-HT release and transport are inhibited by MR and GR, and 5-HT1A receptor-mediated hyperpolarization is reduced following MR downregulation (Joëls, 2001). Furthermore, it was shown that use of antidepressants can increase MR expression (DeRijk et al., 2008). During antidepressant treatment, adjunctive use of MR agonist fludrocortisone can reduce the latency of SSRIs therapeutic effect and improve efficacy (Otte et al., 2010).

Enhanced negative feedback of the HPA axis was found not only in patients with depression, but also in patients with post-traumatic stress disorder (PTSD) (Young et al., 1994). Individuals with no diagnosis but elevated scores on the Anxiety Scale, along with those diagnosed with anxiety disorders and depression, exhibit heightened sensitivity of the HPA axis to stress and greater susceptibility to post-traumatic stress disorder (Ancelin et al., 2017; Faye et al., 2018; Juruena et al., 2018; Lupien et al., 2018; Quevedo et al., 2019). After receiving the mineralocorticoid receptor antagonist spironolactone, patients with PTSD exhibit heightened cortisol responses and increased cortisol production in response to CRH activation (Kellner et al., 2002). Other investigators have sought to explore changes in HPA response and its relationship to MR by blocking cortisol synthesis with metyrapone, but several studies have found inconsistent results. One study reported no significant differential HPA response between PTSD patients and controls (Kellner et al., 2004), while in another study, PTSD patients have been shown to have a decreased ACTH response to metyrapone (Neylan et al., 2003). Yet another study found increased ACTH and cortisol values in PTSD patients and no group differences in salt corticosteroid receptor-mediated feedback inhibition (Otte et al., 2006). In PTSD patients, abnormally low or down-regulated MR in hippocampus may result in considerable tissue loss (Zhe et al., 2008). However, the role of MR in anxiety behavior remains controversial and a recent study has found that MR may contribute differentially to depressive or anxiety symptoms. After adrenalectomy, the patient's aldosterone level returned to normal, and the depression scale showed relief of depressive symptoms, but the anxiety scale scores did not change significantly; with MR antagonists, the patient had high levels of aldosterone, and anxiety scales showed improvement in anxiety symptoms, but there were no significant changes in depression scale scores (Murck et al., 2021). There are also findings suggesting that patients with increased aldosterone are more prone to comorbidities of anxiety disorder (Sonino et al., 2011). Thus, further research is needed to clarify the role of MR in anxiety (Kellner & Wiedemann, 2008).

In addition to depression and anxiety spectrum disorders, there are also other psychiatric disorders with emotional-behavioral disturbances that were associated with MR expression or function. For example, The NR3C2-4 region is highly methylated in female schizophrenic patients (Qing et al., 2020). Decreased MR mRNA expression was found in postmortem prefrontal cortex of bipolar disorder (BP) and schizophrenia patients (Xing et al., 2004). Moreover, treatment with the MR antagonist spironolactone partially improved anxiety symptoms in patients with bipolar disorder (Juruena et al., 2009) and has been proposed as a

potential therapeutic agent for improving negative symptoms, such as lack of motivation, anhedonia, apathy, social withdrawal (Zandifar et al., 2022). Furthermore, MR stimulation has been shown to improve affective empathy in individuals. Wingenfeld et al. (2014) found that fludrocortisone-stimulated MR enhances emotional empathy in women with borderline personality disorder (BPD).

In rodent animal models, the HPA axis was also more sensitive to stress exposure in animals that exhibited anxiety- and depression-like behaviors (Faye et al., 2018; Fox et al., 2018; Goncharova & Oganyan, 2018; Oh et al., 2018). Hippocampal MR was shown to affect anxiety-like behavior in rats (Smythe et al., 1997). For example animals lacking forebrain MR exhibit increased anxiety-like behaviors (Brinks et al., 2009) whereas MR overexpression in the forebrain can reduce anxiety behavior (Lai et al., 2007; Mitra et al., 2009). In a social isolation model in rats, MR expression was found to be down-regulated in the hippocampus, which may lead to reduced resilience to acute stress (Boero et al., 2018). A similar result was found in another study, where acute restraint stress of less than 2 hours reduced MR heteronuclear RNA in hippocampus, but not MR mRNA (Herman & Watson, 1995). However, it has also been shown that acute stress (forced swimming and novelty stress) caused an increase in MR expression levels in the rat hippocampus, followed by an increased inhibitory tonus mediated by MR on HPA axis activity (Gesing et al., 2001). Importantly, while most studies focused exclusively on males, there are also several reports showing that MR effects are sex-specific. For example, chronic severe stress increases MR expression in the hippocampus of female adult rats, but not males. However, when rats were exposed to chronic unpredictable stress when they were young, females showed MR downregulation, but males showed MR upregulation as they matured. This difference may be related to the length of time between rats being subjected to stress stimuli and being sacrificed (Karandrea et al., 2000; Kitraki et al., 2004). Chronic corticosterone treatment in mice resulted in decreased hippocampal and hypothalamic MR expression and depressive-like behaviors, but co-administration of the MR antagonist spironolactone prevented the mice from developing depression-like behaviors (Wu et al., 2013). The aldosterone system has been observed to have a correlation with depressive symptoms, and prolonged administration of the mineralocorticoid aldosterone may result in augmented depression- and anxiety-like behaviors; however, the underlying mechanism remains to be elucidated (Bay-Richter et al., 2012; Hlavacova et al., 2012; Hlavacova & Jezova, 2008). In addition, researchers found that non-genomic effects of MR may modulate anxiety-like behaviors, as anxiety-like behaviors occur 30 minutes after MR antagonist administration (Chen

et al., 2019; Groeneweg et al., 2012).

1.4.2 Cognitive impairment

Glutamate is essential for the creation and maintenance of synapses, learning and memory, and cellular metabolism. Glucocorticoids, and both acute and chronic stress stimulate glutamate release in the brain (Popoli et al., 2011). In response to stress, CA1 hippocampal excitability is increased, resulting in MR activation via a quick and transitory non-genomic action and an increase in presynaptic membrane glutamate release (Karst et al., 2005a; Olijslagers et al., 2008). With the assistance of limbic system-related membrane proteins (Qiu et al., 2010), MR can facilitate information acquisition and retrieval of stored information, enabling humans to govern the evaluation of novel settings and pick appropriate behavioral response strategies. However, the increase in excitability and the release of glutamate are inhibited by the activated GR (de Kloet et al., 2009; Myers et al., 2014; Oitzl & de Kloet, 1992). Several investigations have demonstrated that non-contextual delivery of stress or use of the MR antagonist spironolactone prior to contextual fear conditioning can lessen contextual fear by interfering with memory formation via non-genomic effects (Sajadi et al., 2006; Zhou et al., 2010, 2011). Other studies have indicated that the MR antagonist spironolactone produces impairments in long-term potentiation (LTP), selective attention, and working memory function under stress (Avital et al., 2006; Cornelisse et al., 2011). Stimulating MR with flucortisone induces more emotional risk-behavior responses in young, healthy participants rather than conservative rational strategies (Deuter et al., 2017).

An important aspect of MR function, especially in the context of cognition, is its effect on the neuronal network level. MR has been reported to modulate neurotransmitter release from presynaptic terminals (Gulyaeva, 2021), but so far it is largely unclear how MR function at this level is regulated by neuronal activity. There are few studies suggesting that stress-induced changes in learning and memory may involve upregulation of connections between the amygdala and the hippocampus and striatum, which are sensitive to MR blockade (Schwabe et al., 2013). Further, following stress exposure MR-dependent amygdala activity is delayed, suggesting a time-dependent effect of stress on neural activity and memory processes (Vogel et al., 2017). While GR activation is believed to involve information acquirement and memory consolidation, the process of MR activation involves the utilisation of contextual information retrieval (Joëls, 2008; Joëls et al., 2012; Lupien & McEwen, 1997; Roozendaal, McReynolds, et al., 2009; Zhou et al., 2011). However, further studies are needed to elucidate the role of MR

especially on the non-genomic level to affect neurotransmission and consequently brain circuit activity.

MR has been demonstrated in many clinical studies to affect cognitive function in patients with mental illness. In mild cognitive impairment (MCI) patients, cognition is impaired possibly because of the reduced MR/GR ratio due to hippocampal atrophy (Kline & Mega, 2020). The MR antagonist spironolactone ameliorated β-amyloid (Aβ) induced cognitive impairment in Alzheimer's disease and reduced expression of Aβ (Chen et al., 2020). The situation is more complex with MR agonist treatment, where depending on context and setting both improvements and impairments of memory performance were reported. Among depressed subjects, only older patients had impaired verbal learning and visuospatial memory following treatment with the MR agonist fludrocortisone (Otte, Wingenfeld, Kuehl, Richter, et al., 2015). Conversely, fludrocortisone significantly improved memory and executive function in young depressed patients (Otte, Wingenfeld, Kuehl, Kaczmarczyk, et al., 2015). PTSD patients and BPD patients improved working memory after fludrocortisone treatment (Wingenfeld & Wolf, 2015). However, another study has found that in women with BPD, their verbal learning and visuospatial memory were impaired compared with controls (Wingenfeld & Otte, 2019). Based on these studies, MR agonist therapy seems to have different effects on different types of patients. Finally, there are also genetic studies showing that under stress induction, carriers of the MR gene Val variant have deficits in reward learning, exhibiting an impaired ability to modulate behavior (Bogdan et al., 2010). MR variation in the hippocampus and medial temporal region is associated with encoding and retrieval of long-term memory, and some NR3C2 single-nucleotide polymorphism (SNPs) can cause changes in verbal memory performance (Keller et al., 2017). Together, these data underline the important role of MR in cognitive function, but the directionality of the effect is still controversial and likely context-dependent.

The important role of MR in cognition has been corroborated using animal studies. Knockout of MR in the mouse forebrain leads to impaired learning, deficits in working memory (Berger et al., 2006a), and improved spatial memory following increased neuronal MR expression (Lai et al., 2007; Mitra et al., 2009; Rozeboom et al., 2007). Further, rats injected with spironolactone have impaired spatial memory and altered behavioral strategies (Oitzl & de Kloet, 1992; Yau et al., 1999). Interestingly, other studies have shown the opposite result: chronic administration of MR antagonist spironolactone to Nrg1-tg transgenic mice partially improved working

memory, and improved the manifestations of what are considered human schizophrenia-positive symptoms (Wehr et al., 2017). Similar to studies in humans the corticosterone synthesis blocker metyrapone was also used to differentiate between MR and GR-mediated effects on cognition. Using a higher dose of metyrapone (50 mg/kg) to inhibit corticosterone synthesis in male Sprague-Dawley rats, the latency of animals looking for an escape platform in MWM was significantly longer than that in the control group (Roozendaal et al., 1996). The high dose of metyrapone also dramatically decreased the percentage of fear-induced immobility. This may be due to a sharp drop in corticosterone levels that reduced MR occupancy, resulting in impaired information acquisition performance (Oitzl & de Kloet, 1992; Roozendaal et al., 1996). However, more recent studies also question the utility of metyrapone due to apparent off-target effects on MR and GR activity (Kennedy et al., 2020). The different outcomes of genetic versus pharmacological MR manipulation might be due to developmental effects of MR deletion, time-dependence of MR deactivation or region- and cell type-specificity of the effects. For example, McEown and Treit's study (2011) showed that in the three brain regions, dorsal hippocampus, ventral hippocampus and medial prefrontal cortex (mPFC), only after the ventral hippocampus was injected with MR antagonists, would unconditioned fear of rats be reduced. The effect of MR on cognitive function was also shown to be related to sex. Following chronic stress, increased MR expression was found only in the hippocampal cornu Ammonis 3 (CA3) region of female, but not male rats, paralleling their spatial memory improvement (Kitraki et al., 2004). Forebrain MR-deficient female mice were unable to distinguish between cued and contextual fears as accurately as male mice in a fear conditioning memory test (ter Horst, van der Mark, et al., 2012). Taken together, the animal data on MR function on cognition support the human data, showing that MR modulation has a direct effect on cognitive processes that are dependent on the specific context, including brain region, the cell type, the external condition and sex.

1.5 Sex differences in psychiatric disorders

As is well known, sex differences play a significant role in the development, function, and susceptibility to various physiological and psychological processes in the brain. Many psychiatric disorders exhibit sex differences in terms of incidence, symptom presentation, and treatment (Gobinath et al., 2017; Riecher-Rössler, 2017). In terms of incidence, men have a higher proportion in neurodevelopmental psychiatric disorders such as Autism Spectrum Disorder (ASD) and Attention-Deficit/Hyperactivity Disorder (ADHD), which are more prevalent in men and exhibit different clinical presentations between men and women (Arnett et al., 2015;

May et al., 2019; Napolitano et al., 2022; Werling & Geschwind, 2013). On the other hand, womend tend to be more susceptible to mental disorders that manifest in adulthood (Bao & Swaab, 2011). For instance, women are more prone to stress-related mental disorders such as depression and anxiety, with rates nearly double those of men (Duman et al., 2019; Riecher-Rössler, 2017; Terlizzi & Villarroel, 2020). Regarding symptom presentation, men suffering from schizophrenia are more likely to exhibit prominent negative symptoms and neurocognitive deficits, while women tend to show more emotional symptoms (Leger & Neill, 2016; Li et al., 2022; Mendrek & Mancini-Marïe, 2016). In terms of treatment, female patients with depression show better response to selective serotonin reuptake inhibitors (SSRIs) compared to male patients (Sramek et al., 2016). Pre-menopausal women have a better response to traditional antipsychotic drugs and benzodiazepines compared to men of the same age group, and they often require relatively lower doses (Yonkers et al., 1992). This may be attributed to sex differences in pharmacokinetic characteristics such as drug metabolism or clearance rates (Franconi & Campesi, 2014; LeGates et al., 2019). Moreover, different sexes also exhibit varying sensitivity to the side effects of psychotropic medications (Marazziti et al., 2013).

Sex differences in behavior have long been recognized in various species, including mice. Especially after experiencing stress, the sex of mice leads to different behavioral patterns, affecting multiple domains including social behavior, emotional responses, learning and memory, and endocrine responses to stress. It has been found that female mice exposed to social defeat stress exhibit a reduction in social interaction, whereas male social responses remain largely unaffected (Trainor et al., 2011). Oxytocin increases social interaction in males but not females exposed to social defeat stress (Bangasser & Cuarenta, 2021). In terms of oxytocin neuron activation, male mice display a rapid and short-lived response following social defeat, whereas in females, the reactivity of oxytocin neurons lasts longer and is further heightened after repeated social defeat (Duque-Wilckens et al., 2020).

The stressed mice also show significant sexual differences in emotional behavior and cognition after stress. Most studies suggest that after experiencing early life stress in mice, the anxiety level of adult males increases (Tsuda & Ogawa, 2012; Veenema et al., 2007), and significantly increase depression-like behavior in adolescent males (He et al., 2020). However, female mice do not seem to be significantly affected by early life stress (Bailoo et al., 2014; Bondar et al., 2018; Kundakovic et al., 2013). In unfamiliar environments, female mice did not exhibit the pronounced anxiety-like behavior oobserved in male mice (ter Horst, de Kloet, et al., 2012).

Additionally, female rodents display fewer anxiety-like behaviors during the proestrus and estrus phases (ter Horst, de Kloet, et al., 2012). Regarding spatial learning and memory, most studies indicate that male rodents tend to exhibit faster learning and better performance than females in various maze tasks (Bowman et al., 2006; Jonasson, 2005; Rizk-Jackson et al., 2006). However, it should be noted that chronic stress appears to impair cognitive function more in males than in females (Luine et al., 2017). Furthermore, traumatic stress leads to increased aggression in male mice (Nelson & Trainor, 2007), but it also preserves nonaggressive social interactions (Nordman et al., 2020). However, some studies suggest that this increase in aggression occurs specifically in socially isolated mice (Chang & Gean, 2019; Matsumoto et al., 2005; Toth et al., 2011).

Due to these sex differences, it is necessary to adopt different treatment approaches for different sexes in the process of treating mental illnesses.

1.6 Aims of the thesis

Although it has been found that MR is closely associated with various stress-related psychiatric disorders and has an impact on the brain's emotions, behaviors, and cognition, its specific role in certain brain regions or cell types remains poorly understood. In order to gain a better understanding of the role of MR in glutamatergic and GABAergic neurons, and to explore whether MR could serve as a potential biomarker or therapeutic target for stress-related psychiatric disorders, the following questions are proposed:

1. What is the contribution of the MR in glutamatergic or GABAergic neurons to emotion, cognition, and behavior?

2. Is the function of MR in glutamatergic or GABAergic neurons sex-dependent?

3. Is the response elicited by acute and chronic stress mediated by MRs in glutamatergic or GABAergic neurons?

4. Does the MR in glutamatergic or GABAergic neurons contribute to changes in neuronal structure, function and activity?

5. Are there cell type-specific down-stream genetic targets of MR signaling in glutamatergic or GABAergic neurons?

2 MATERIALS AND METHODS

2.1 Animals

2.1.1Generation of $MR^{lox/lox}$-Nex-Cre mouse line

The creation of MR-floxed mice was previously described (Berger et al., 2006b). To conditionally knock out MR in forebrain glutamatergic neurons, $MR^{lox/lox}$ mice were crossed with Nex-Cre animals (Goebbels et al., 2006). The transcription factor with a helix-loop-helix structure Nex (also known as NeuroD6/Math2) is a marker of neuronal precursors in the embryonic cortex. It is active in glutamatergic neurons in the neocortex, hippocampus, amygdala, and olfactory bulb of the adult mouse brain, and it is highly expressed in dorsally differentiated neurons of the telencephalon. Cre activity begins during development, starting from embryonic day 11.5. The resulting offspring with a deletion of MR in glutamatergic forebrain neurons ($MR^{lox/lox}$-Nex-Cre, referred to as MR^{Nex}) and their control $MR^{lox/lox}$ littermates (referred to as Ctrl) were used.

2.1.2 Generation of $MR^{lox/lox}$-Dlx-Cre mouse line

Similar to the generation of MR^{Nex}, We crossed $MR^{lox/lox}$ mice with Dlx-Cre animals to obtain conditional MR mutant mice in GABAergic cells (Monory et al., 2006). The Dlx5 and Dlx6 genes are involved in the development and maturation of the brain, involving many aspects such as neuron differentiation, migration, maturation and protrusion formation. They greatly influence the growth of GABAergic neurons. Cre activity begins during development, starting at embryonic day E10. Finally, we had offspring with deletion of MR in GABA neurons ($MR^{lox/lox}$-Dlx-Cre, referred to as MR^{Dlx}) and their control $MR^{lox/lox}$ littermates (referred to as Ctrl).

2.1.3 Genotyping

Genotyping was performed by PCR using the following primers: MR-flox-7, 5´- CTG-GAG-ATC-TGA-ACT-CCA-GGC-T-3´; MR-flox-10, 5´-TAG-AAA-CAC-TTC-GTA-AAG-TAG-AGC-T-3´ and MR-flox-8, 5´-CCT-AGA-GTT-CCT-GAG-CTG-CTG-A-3´. Standard PCR conditions resulted in a 285-bp wild-type and a 335-bp floxed PCR product. In Nex-Cre and Dlx5/6-Cre mice, the presence of Cre was evaluated using the primers CRE-F 5′-GAT-CGC-TGC-CAG-GAT-ATA-CG-3′, CRE-R 5′-AAT-CGC-CATCTT- CCA-GCA-G-3′, Thy-F 5´-TCT-GAG-TGG-CAA-AGG-ACC-TTA-GG-3´ and Thy-R 5´- CCA-CTG-GTG-AGG-TTG-AGG-3´, resulting in a Cre-specific PCR product of 574 bp and a control PCR product of Thy1 of 372 bp.

All mouse lines used were on a mixed 129S2/Sv×C57BL/6 genetic background. All animals used in single experiments were littermates. Experimenters were always blind to genotype.

2.1.4 Standard housing conditions

All studies were conducted in conformity with the European Communities' Council Directive 2010/63/EU, as well as the Guidelines for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany. The mice used in the experiments were all ranged in age from 8 to 20 weeks and both sexes were included. Unless otherwise specified, during the experiment mice were housed individually in cages (IVC; 30 cm × 16 cm × 16 cm; 501 cm^2) with adequate bedding and nesting material and wooden tunnels for environmental enrichment for 1 week prior to behavioral testing or hormonal assessment. Animals were kept in a standard laboratory environment with *ad libitum* food (Altromin 1318, Altromin GmbH, Germany) and water, a central airflow system (Tecniplast, IVC Green Line-GM500), and maintained in a 12:12 hours light-dark cycle at a constant temperature of 23±2 °C and 55% humidity. Semi-randomly chosen experimental groups were assigned to the animals, and data analysis was performed blinded to group assignment. Behavioral testing was performed during the light period between 8:00 am and 1:00 pm.

2.2 Experimental design for MR^{Nex} mice

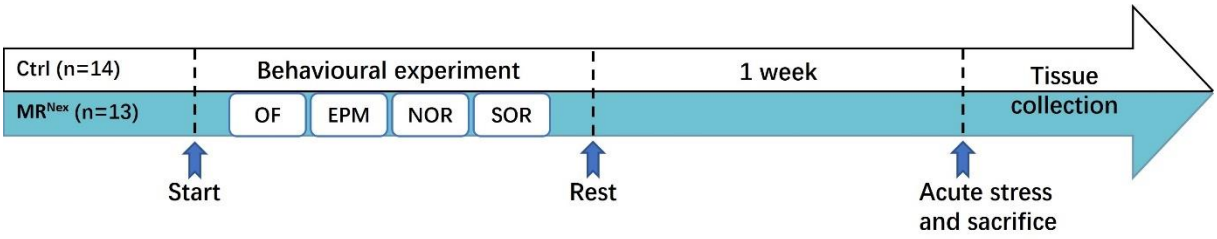
2.2.1 Experiment 1

To understand in detail the phenotype of the transgenic mice lacking glutamatergic MR in the forebrain, behavioral experiments were conducted on the mice under baseline conditions. Two cohorts of animals were used, and all animals were aged between 12 and 20 weeks.

Only male mice were used in the first cohort. All the behavioral experiments were carried out on both Ctrl group (n=14) and MR^{Nex} group (n=13) under the baseline conditions, without any stressful stimulation. Experiments were carried out in the following order: open field (OF) test, elevated plus maze (EPM) test, novel object recognition (NOR) test, and spatial object recognition (SOR) test to test the anxiety and cognition of these two strains of mice. A week following the behavioral tests, all the mice were exposed to an acute restraint stress, and blood was collected following the onset of the restraint stress. Finally, animals from all groups were deeply anesthetized and decapitated under basal conditions, and tissue (brain, blood, adrenals) was collected for further analyses (see Figure 3A).

In the second cohort, 13 Ctrl mice and 11 MR^{Nex} mice were used. First, the home cage locomotion test to assess home cage behavior (n=8 per group) was performed. After that the Morris water maze (MWM) to further investigate the learning and memory of MR^{Nex} mice was applied (see Figure 3B).

A. Cohort 1



B. Cohort 2

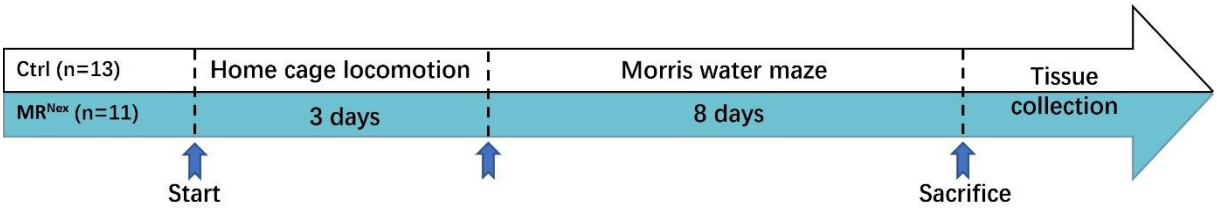


Figure 3. Timeline of MR^{Nex} mice baseline experiments.

2.2.2 Experiment 2

2.2.2.1 Chronic social defeat stress on male mice

The commonly used chronic social defeat stress (CSDS) paradigm was employed to further investigate whether there are significant changes in the performance of the mice after stress, including physiological indicators as well as emotional behavior, could be observed after stress. A cohort of 44 male mice (25 MR^{Nex}, 19 Ctrl) were tested to examine their anxiety-related behavior and cognition. All of their age ranged from 12 to 20 weeks. The KO and WT animals were divided into two groups: the non-stressed group and the stress group. For the non-stressed group, they were single housed without any stressor. However, for the stress group, a 21-day CSDS paradigm was utilised, the mice in stress group were attacked by a different CD1 mouse every day (see 1.4.2). After the attack, the stressed mice were housed in the same cage with CD1 but separated by a transparent panel. On days 15 to 18 of CSDS, the behavioral tests

in the order of OF, EPM, NOR, SOR were carried out, collecting the data for body weight and fur status during the experiments. At last, the mice were sacrificed and the brains, adrenals, and blood were collected for the future experiment (see Figure 4).

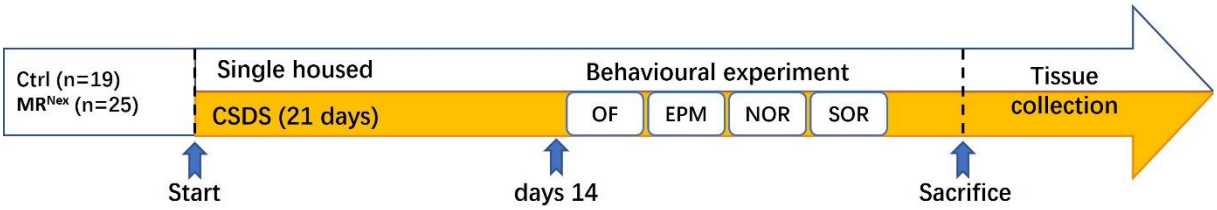


Figure 4. Timeline of male MR^{Nex} mice CSDS experiment.

2.2.2.2 CSDS on female mice

It is well known that there are significant differences in the responses of males and females to stress exposure. To study the performance of neuroendocrine and behavioral responses of female mice under baseline and after stress, a CSDS experiment was conducted on female mice as well. A cohort of 42 female mice (23 MR^{Nex}, 19 Ctrl) were tested to examine their anxiety-related behavior and cognition. All of their age ranged from 12 to 20 weeks. Similar to the male mice, the female mice were also divided into two groups: the non-stressed group and the stress group. In the non-stressed group, the mice were single-housed without any additional stressors. In the stress group, a 21-day CSDS paradigm was applied, in which the mice were attacked daily by a distinct CD1 mouse. After attack, the mice were housed in a same cage with CD1 but separate by a transparent panel. The behavioral tests were carried on after 2 weeks CSDS, collecting the data for body weight and fur status during the experiments. At last, the mice were sacrificed and the brains, adrenals, blood were collected for the future experiment (see Figure 5).

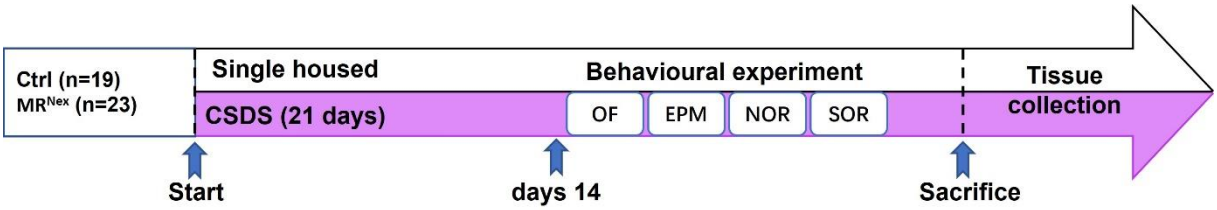


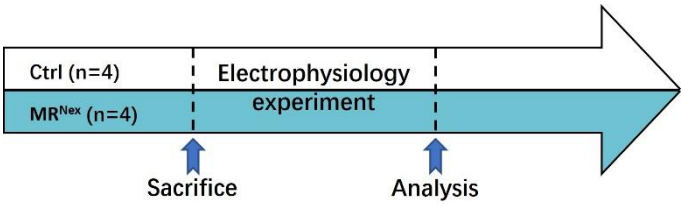
Figure 5. Timeline of female MR^{Nex} mice CSDS experiment

2.2.3 Experiment 3

2.2.3.1 Function of glutamatergic neurons

Electrophysiology experiments were designed to gain insight into the role of lacking MR in the function of glutamatergic neurons and how it may be involved in learning and memory processes. 2 cohorts of animals were used in this study, 4 Ctrl mice and 4 MR^{Nex} mice were in each cohort. All of the mice utilised in this study ranged in age from 8 to 12 weeks (see Figure 7).

A. Cohort 1



B. Cohort 2

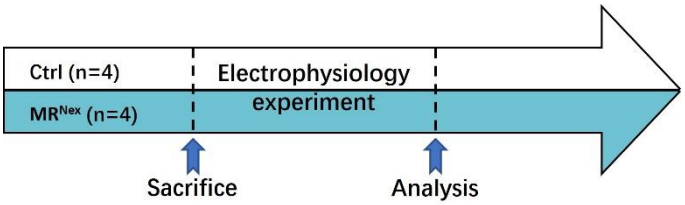


Figure 6. Timeline of electrophysiology experiment

2.2.3.2 Neuron structure detection

To study the structure of neurons in the ventral hippocampus, 4 Ctrl mice and 4 MR^{Nex} mice were used to conduct Golgi staining. All the adult mice in this study which no more than 24 weeks (see Figure 8).

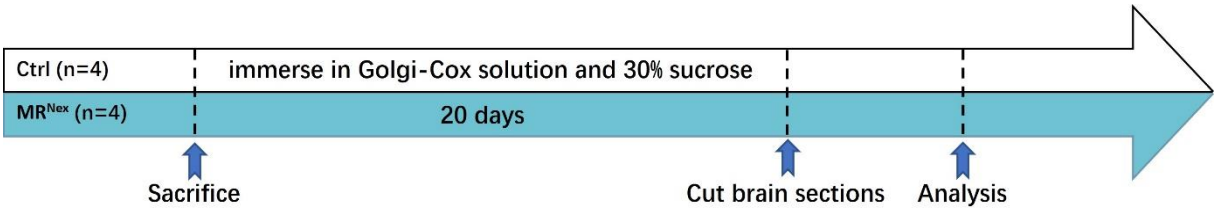


Figure 7. Timeline of Golgi staining experiment

2.2.4 Experiment 4

To verify that MR-mediated downregulation of Fam107a contributes to increased anxiety-related behaviors in male mice, stereotaxic surgery was performed on male mice. A total of 18 MR^{Nex} and 18 Ctrl rodents between 8 and 12 weeks of age were selected. They were divided into two groups, with one group receiving a control virus injection (AAV1/2-CAG-Fam107a-IRES-eGFP-WPRE-bGHp(A)) and the other receiving a Fam107a overexpressing virus injection (AAV1/2-CAG-IRES-eGFP-WPRE-bGHp(A)). Four weeks after virus injection, behavioral experiments were conducted using OF and EPM to assess anxiety-like behavior, and NOR test and SOR test to assess cognitive ability. Finally, mice were sacrificed, their brains, adrenals, and blood were collected for RNAScope, immunostaining, and CORT level measurement (see Figure 8).

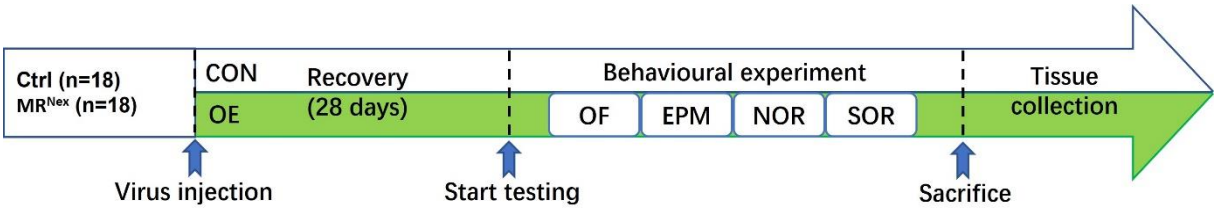


Figure 8. Timeline of MR^{Nex} mice Fam107a AAV virus injection experiment

2.2.5 Experiment 5

To confirm previous results due to specific rescue of Fam107a in glutamatergic neurons, Cre-positive mice acquired infection with a DIO-cre-dependent Fam107a overexpression virus, specifically targeting the CA3 glutamatergic cell population. In addition to use 22 male MR^{Nex} mice in stereotactic surgery, 24 Nex-cre positive mice were used as the control group. All of them were aged between 8 and 12 weeks. The animals were divided into two groups: one was injected with the control virus (AAV1/2-CMV-DIO-eGFP), the other one was injected with the DIO vectors for Cre-inducible expression of Fam107a (AAV1/2-CMV-DIO-FAM107A_310122). The behavioral experiments were conducted four weeks later. Using OF and EPM to test their anxiety-like behavior, using NOR test and SOR test to test their cognition. In the end, the mice were sacrificed, and the brains, adrenals, and blood were collected for RNAScope analysis and CORT level measurement (see Figure 9).

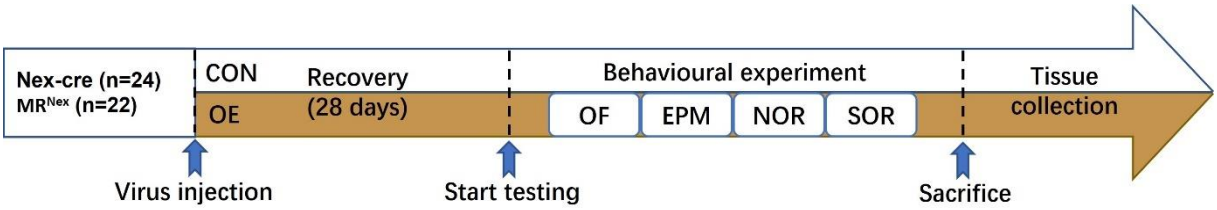


Figure 9. Timeline of MR^{Nex} mice DIO-Fam107a AAV virus injection experiment

2.3 Experimental design for MR^{Dlx} mice

2.3.1 Experiment 1

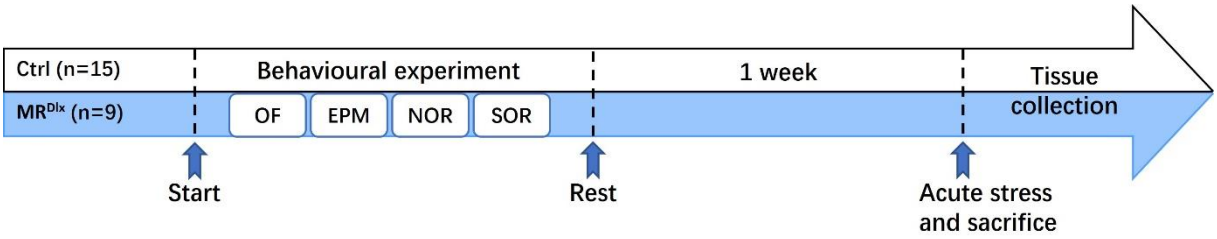
To understand in detail the phenotype of the transgenic mice after the lacking MR in the forebrain, some behavioral experiments were performed on the mice under the baseline. Two cohorts of animals were used in the experiments, and all animals were aged between 12 and 20 weeks.

In the first cohort, all the animals are male mice, 15 in Ctrl group and 9 in MR^{Dlx} group. All the behavioral experiments were carried out under the baseline conditions, without any stressful stimulation. Experiments were carried out in the order of OF, EPM, NOR and SOR to test the anxiety and cognition of these two strains of mice. A week following the behavioral tests, all the mice were exposed to an acute restraint stress, and blood was collected following the onset of the restraint stress. Finally, animals from all groups were deeply anesthetized and decapitated under basal conditions, and tissue (brain, blood, adrenals) was collected for further analyses (see Figure 10A).

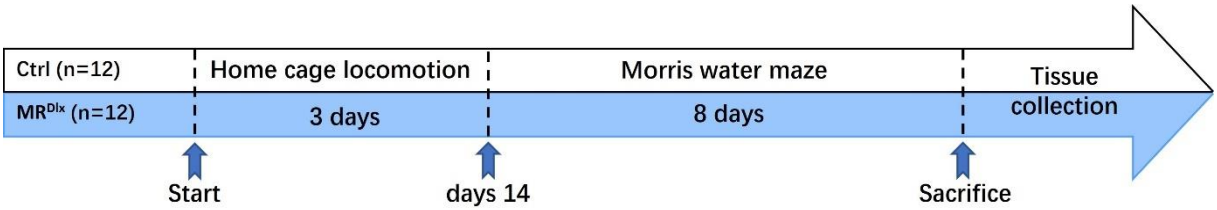
In the second cohort, a total of 12 Ctrl mice and 12 MR^{Dlx} mice were used. The behavior of the mice in the home cage was initially assessed by using the home cage locomotion test (8 control, 8 MR^{Dlx}). After that, MWM test was conducted to further investigate the learning and memory of MR^{Dlx} mice (see Figure 10B).

In the third cohort, there were 14 Ctrl mice and 14 MR^{Dlx} mice. Fear conditioning (FC) test was conducted on these mice to further investigate the learning and memory of MR^{Dlx} mice after stress (see Figure 10C).

A. Cohort 1



B. Cohort 2



C. Cohort 3

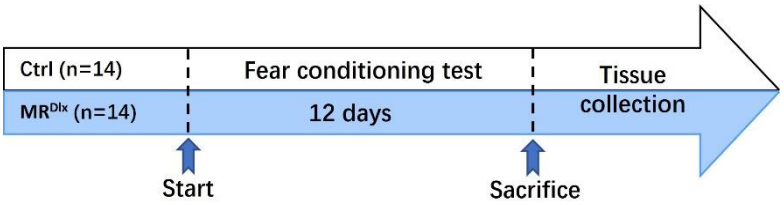


Figure 10. Timeline of MR^{Dlx} mice baseline experiment

2.3.2 Experiment 2

2.3.2.1 CSDS on male mice

To further investigate whether there are significant changes in the performance of mice after stress, including physiological indicators as well as emotional behavior, the CSDS paradigm was used. A cohort of 44 male mice (22 MR^{Dlx}, 22 Ctrl) were tested to examine their anxiety-related behavior and cognition. All of their age ranged from 12 to 20 weeks. The Ctrl and MR^{Dlx} animals were divided into two groups: the non-stressed group and the stress group. For the non-stressed group, they were single housed. A 21-day chronic social defeat stress paradigm was used for the stress group, the mice in stress group were attacked by a different CD1 mouse every day. After the attack, the mice were housed in the same cage with CD1 but separated by a transparent panel. The behavioral tests in the order of OF, EPM, NOR and SOR were carried

out after 2 weeks of CSDS, collecting the data for body weight and fur status during the experiments. At last, the mice were sacrificed, and the brains, adrenals, and blood were collected for the future experiment (see Figure 11).

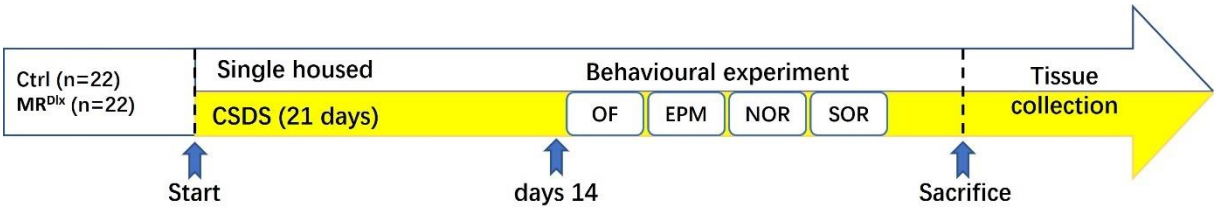


Figure 11. Timeline of male MR^{Dlx} mice CSDS experiment

2.3.2.2 CSDS on female mice

It is well known that there are significant differences in the responses of males and females to stress exposure. To study the performance of neuroendocrine and behavioral responses of female mice after stress, CSDS experiment was conducted on female mice as well. A cohort of 40 female mice (19 MR^{Dlx}, 21 Ctrl) were tested to examine their anxiety-related behavior and cognition. All of their age ranged from 12 to 20 weeks. The Ctrl and MR^{Dlx} mice were divided into two groups: the non-stressed group and the stress group. For the non-stressed group, they were housed. The 21-day chronic social defeat stress paradigm was also used in the stress group, the mice in stress group were attacked by a different CD1 mouse every day. After attack, the mice were housed in a same cage with CD1 but separate by a transparent panel. the behavioral tests were carried on after 2 weeks CSDS, collect the data for body weight and fur status during the experiments. At last, the mice were sacrificed and the brains, adrenals, blood were collected for the future experiment (see Figure 12).

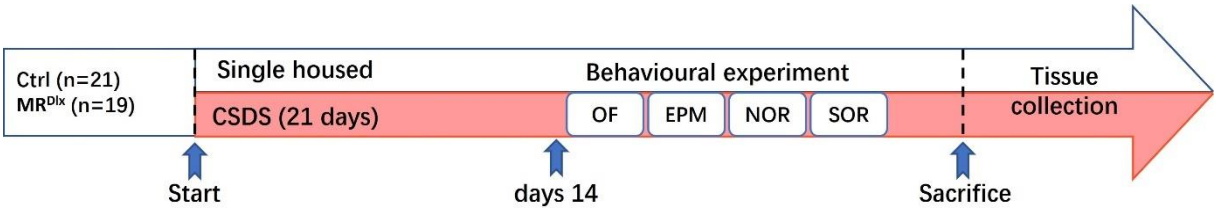


Figure 12. Timeline of female MR^{Dlx} mice CSDS experiment

2.3.3 Experiment 3

Electrophysiology experiments were designed to gain insight into the role of lacking MR in the

function of glutamatergic neurons and how it may be involved in learning and memory processes. A total of 6 Ctrl mice and 6 MR^{Dlx} animals were used in this study. All the mice utilised in this study ranged in age from 8 to 12 weeks (see Figure 13).

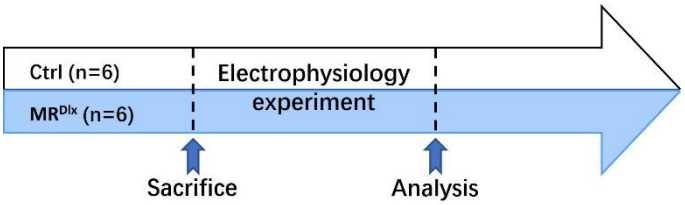


Figure 13. Timeline of MR^{Dlx} mice electrophysiology experiment

2.4 Stress paradigms

2.4.1 Acute stress paradigm

The restraint stress paradigm was utilised for acute stress (Paré & Glavin, 1986). Mice are restrained into 50 ml falcon tubes with a hole in the top and the lid respectively, which allow the mice to breathe properly and move their tails (see Figure 15). The acute restraint stress lasted for 30 min. At the end of 30 min, tail blood was collected from mice (Fluttert et al., 2000), and blood samples were collected in 1.5 ml EDTA-coated microcentrifuge tubes (Kabe Labortechnik, Germany) for CORT level testing. The mice were then put back in their cages, allowed to regain calm for 60 min, and the tail blood was collected again at 90 min of the experiment to test another CORT level. Blood samples for basal CORT levels were collected in the morning of the last experimental day without any stimulation.

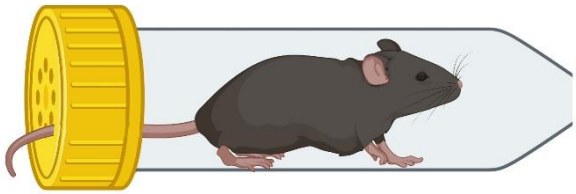


Figure 14. Acute restraint stress paradigm

2.4.2 Chronic social defeat stress paradigm

The CSDS paradigm is commonly used to induce anxiety- and depression-related endophenotypes in mice and was performed as previously described (van Doeselaar et al., 2021;

Wagner et al., 2011). Before the experiment began, the more aggressive male CD1 mice were selected for use in the CSDS experiment. In order to lessen the predictability of stressors and limit the effects of habituation, the 21-days CSDS experiment was carried out between 12:00 pm and 16:00 pm every day, with slightly different experimental time each day. The CD1 mice remained in their cages and dominated during the experiment period.

For the male mice, the MR^{Nex} mice and Ctrl mice in the CSDS group will be directly placed in different CD1 mice's cages (30 cm x 16 cm) every day, and the attack process will last no more than 5 minutes. The animals will be separated if there is an aggressive fight during the attack period to avoid serious injury to the animals (see Figure 16). For the female mice, in order to provoke a CD1 resident attack, first, they were covered with urine which was collected urine from C57Bl/6n male mice previously and kept at room temperature. A brush was used to apply the urine to several parts of the mice body (head, back, tail and especially at the vaginal orifice). Later placed them in different CD1 mice's cages to start the CSDS as described before. If male CD1 mice showed interest in females or exhibited mating-like behavior, they were immediately separated. After the defeat, the mice in the CSDS group spent the following 24 hours in the same cage with the CD1 mice that attacked them, but they were separated by a transparent panel. During this time, they were able to sense each other's presence through smell and sight but were not able to make physical contact. Animals in the control group were kept alone in their own cages for 21 days.

During the experiment, the mice in the control group and the CSDS group were weighed every week, and the skin and hair condition of the CSDS group mice was scored daily. According to prior research (Mineur et al., 2003), the condition of the fur was scored on a 4-point scale, with 1 representing a clean and well-groomed fur and 4 representing a shaggy, unkempt fur, including severe wound marks. Intermediate fur status is indicated by grades of 2 and 3, respectively. Animals with severe injuries were excluded.

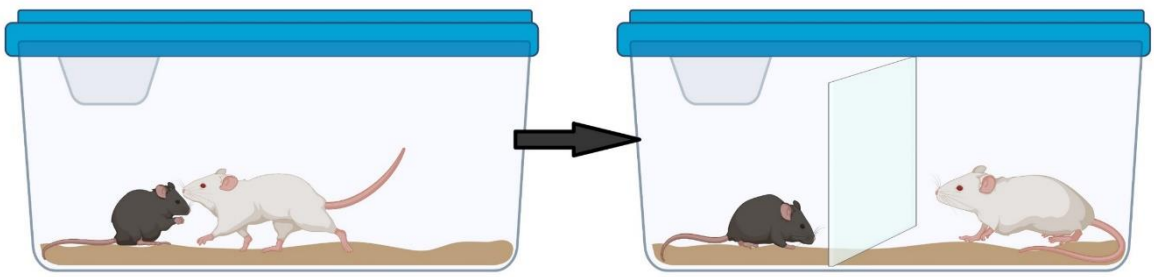


Figure 15. Chronic social defeat stress paradigm

2.5 Assessment of home cage behavior

Home cage locomotion test was conducted on animals in order to evaluate their basal cage activities. Eight mice per group were individually placed in cages equipped with an infrared mouse motion detector/data logger (Mouse-E-Motion; Infra-E-Motion, Hamburg, Germany). Measurements were performed for 72 consecutive hours during a light cycle and a dark cycle, during which time mice had free access to food and water (see Figure 17). The data indicate the total number of seconds in which any movement occurred. The data recording device was configured to detect movement in seconds of mice put in cages, and these numbers were gathered throughout a 30-min period.

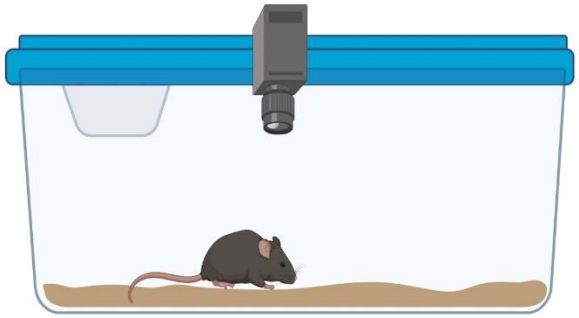


Figure 16. The home cage locomotion test

2.6 Behavioral Experiments

All behavioral tests were performed in a dedicated animal facility, adjacent to the animal housing room. To prevent any potential behavioral alterations due to circadian rhythmic changes in corticosterone levels, tests took place between 7:00 am and 1:00 pm. The tests include OF test, EPM test, NOR test, SOR test, MWM test and FC test. Among which, OF and EMP are mostly used to assess the anxiety behavior of mice, while NOR, SOR, MWM, and FC are primarily used to assess the cognitive function of mice. Using the automated video tracking system Any-maze (Any-maze 6.18; Stoelting Co) to record, track, and assess the tests. If a manual assessment is required, it is performed by skilled workers who are blinded to the experimental conditions.

2.6.1 OF Test

The OF test is commonly utilised in studies of exploratory behavior and anxiety-like behaviors. This experiment also reflects the mice's natural tendency to explore and gather information about their environment. Mice have a tendency to spend more time exploring in corners than in the centre of an open field when they experience high levels of anxiety or fear. As previously described (Schmidt et al., 2007; Sterlemann et al., 2008), the open field used for the test is made of gray polyvinyl chloride plastic material with a size of 50 cm x 50 cm x 50 cm. The test lasted for a total of 15 minutes in low light conditions (about 15 lux). During the test, the mice were placed in a certain corner of the open field, and allowed to explore the arena freely (see Figure 18). The results were evaluated using Any-maze software, which divided the total period into three 5-min segments, to analyse the distance the mice moved in the central area, the corners, and the entire open field, and time the mice spent in those areas.

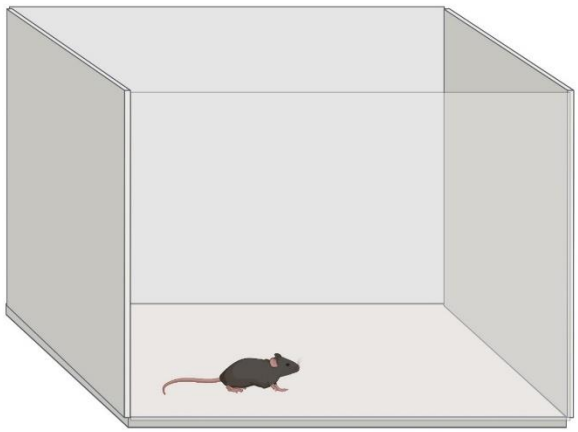


Figure 17. The OF test

2.6.2 EPM Test

The EPM test is a behavioral test used to assess anxiety-like behavior. The amount of time animals spend in open arms versus closed arms is used as an indicator of anxiety-like behavior, the preference for the open arms is seen as a sign of less anxious behavior. As previously described (Schmidt et al., 2007; Sterlemann et al., 2008), the elevated maze apparatus used in the test was made of grey polyvinyl chloride plastic material with two opposing open arms (30 cm × 5 cm × 0.5 cm) and two opposing closed arms (30 cm × 5 cm × 15 cm), connected into a cross shape by a middle platform (5 cm × 5 cm) with a height of 50 cm. The test was

conducted under low light settings (approximately 10 lux with the closed arm and 20 lux with the open arm) for a total of 10 minutes. During the test, the mice were placed on the middle platform area facing the closed arm, and allowed to explore the arms freely (see Figure 19). The results were evaluated using Any-maze software, analysing the percentage of the distance the mice moved in the open arm, the percentage of time spent in the open arm, and the number of times the mice entered the open arm. Any mice that fell from the open arms during the test were not included in the analysis.

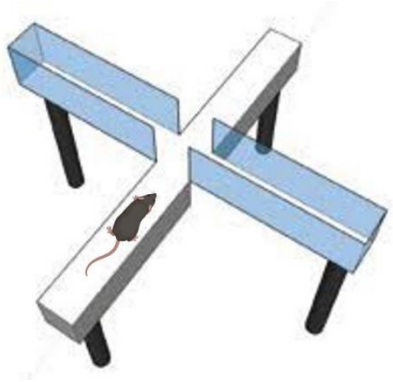


Figure 18. The EPM test

2.6.3 NOR Test

Compared with familiar objects, rodents naturally tend to spend more time exploring unfamiliar one (Ennaceur, 2010). The NOR test is commonly used in research studies utilising rodent models, enabling researchers to assess recognition memory and learning. As shown in the figure, the objects used in the experiment are divided into two types, A and B, each of which is built by 13 Lego® bricks. Variable in shape and colour yet created to attain a consistent volume. To eliminate the mice's unfamiliarity, the experimental arena uses a large open home cage (specific size), with bedding on the bottom of the cage. At the beginning of the experiment, the mice were given a 15-minute familiarization period, and two same objects (make sure the objects had no odour) were placed 5 cm from the rear of the arena, and the mice were then placed in the arena away from the objects and allowed to explore freely. Following the familiarisation period, the mice were returned to their home cages for 20 minutes before a 5-minute testing period. One of the two identical objects was replaced by a different object in the arena, the mouse was placed in the same position of the arena as before. Once the mouse touched the object with its nose, front paws, or whiskers, or if it obviously smelled that the object was in proximity, any approach to the object was considered an interaction.

Before experimenting with the next mouse, the arena must be cleaned with paper towels to remove any traces of mouse urine and faeces, and the odour must be eliminated with 75% alcohol (see Figure 20). The results were evaluated using Any-maze software, analysing the ratio of time, and number of times the mice explored objects in new and old objects, respectively.

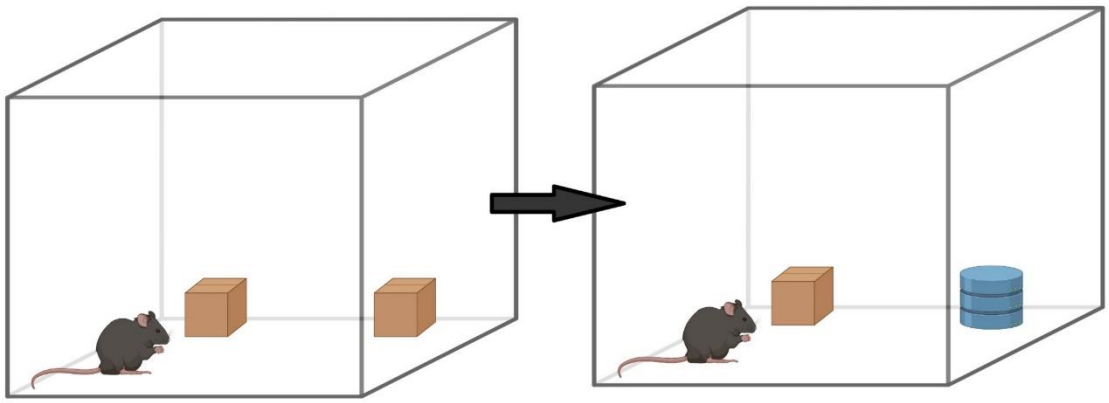


Figure 19. The NOR text

2.6.4 SOR Test

Rodents are not only interested in novel objects and are particularly interested in objects that are placed in novel locations (Ennaceur, 2010). The SOR test can be used to assess spatial abilities, visual memory, and cognitive functioning on rodents. As shown in the figure, the objects used in the experiment were all built from 13 LEGO® bricks to ensure uniform shape and size. To eliminate the mice's unfamiliarity, the experimental arena uses a large open home cage (specific size), with bedding on the bottom of the cage. At the beginning of the experiment, the mice were given a 15-minute familiarization period, and two objects (make sure the objects had no odour) were placed 5 cm from the rear of the arena, and the mice were then placed in the arena away from the objects and allowed to explore freely. Following the familiarisation period, the mice were returned to their home cages for 20 minutes before a 5-minute testing period. In preparation for the test, one of the objects was relocated to the front of the arena, and the mouse was placed in the same position of the arena as before. Once the mouse touched the object with its nose, front paws, or whiskers, or if it obviously smelled that the object was in proximity, any approach to the object was considered an interaction. Before experimenting with the next mouse, the arena must be cleaned with paper towels to remove

any traces of mouse urine and faeces, and the odour must be eliminated with 75% alcohol (see Figure 21). The results were evaluated using Any-maze software, analysing the ratio of time, and number of times the mice explored objects in new and old locations, respectively.

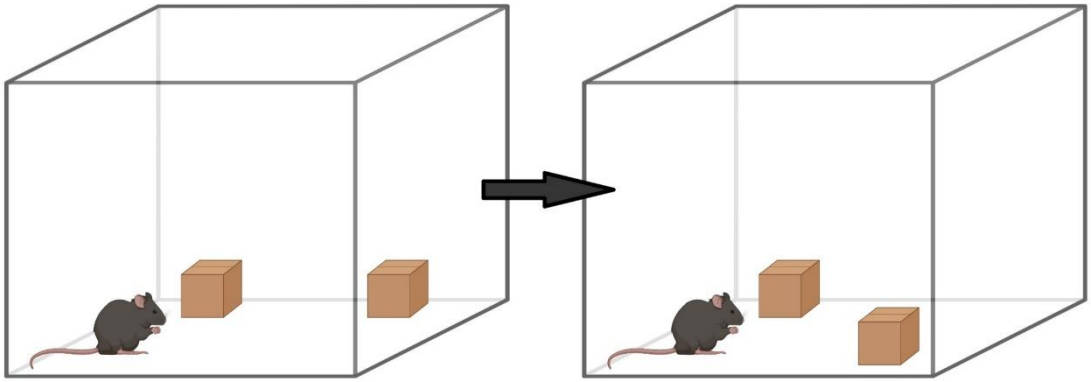


Figure 20. The SOR text

2.6.5 MWM

The MWM test is a cognitive task designed to evaluate spatial learning and memory in mice. In our experiment, the MWM test was carried out in a white circular pool with a diameter of 150 cm and a depth of 41 cm. The pool (W 309 cm × L 357 cm × H 283 cm) was placed in the centre of the room on a shelf which is 110 cm above the ground. There are four types of posters (stripes, rectangles, triangles and circles with different black and white patterns) put on the surrounding walls as visual cues in the room to guide the mice. The pool was filled to a depth of 33 cm, and the water temperature was maintained at 22-23°C throughout the test by adding warm water. The computer for video tracking is located in the southwest (SW) corner and the water pipe is in the northeast (NE) corner, neither of which is visible to the animals in the tank. The room is illuminated with indirect light and the light on the water surface reaches 11.5 lux, with two spotlights facing the wall and highlighting the distinctive landmarks. The escape platform is cylinder made of 8 cm × 8 cm transparent acrylic plexiglass, which is placed in the centre of the NE quadrant, 35 cm away from the wall, and submerged 1 cm below the water surface (see Figure 22).

First, the mice were allowed to adapt to the pool and water for one day, after which they underwent a 5-day training period to find the platform. Each animal was performed 4 trials per

day. The starting points for each of the four trials were randomly distributed along the SW area of the tank's perimeter and were each used for a different starting position. After lowering the mouse into the water facing the wall, the experimenter quickly sat in the SW corner while using Any-maze (v5.26; Stoelting, Dublin, Ireland) to start recording the trial. If the mouse found the platform within 90 seconds, it was allowed to stay on the platform for 10 seconds. The mouse could only swim for a maximum of 90 seconds before being taken from the pool. If the mouse did not find the platform within 90 seconds, it was guided onto the platform without touching it and then redirected for 10 seconds to a distal visual signal before being taken from the pool. Mice were taken out of the water pool and put back into their individual cages, and a heat lamp was set up safely away for 10 min to make sure the mice were completely dry. In order to reduce the variability in results caused by timing considerations, inter-trial intervals were 15 minutes per day, and tests were conducted at around the same time each day. On day 6, mice were subjected to a 60 seconds probe trial. The platform was removed from the pool at this time, and the mice were allowed to swim freely for 60 seconds.

The experiments were evaluated by Any-maze software, such as the training period, the average escape latency of 4 trials per day (for mice that did not find the platform within 90 seconds, the labelling escape latency was 90 seconds); the probe period, the mice's swim time, path length for platform quadrants, and their percentage of total swim time, total path length.

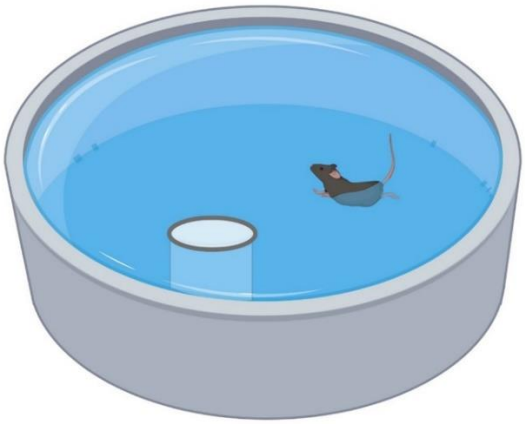


Figure 21. The MWM test

2.6.6 FC test

The FC test is primarily focused on studying fear learning and memory. It is also a kind of stress

test for mice. Equipment from Ugo Basile, Italy, was utilised, following the protocol adapted from Dias and Ressle (2014).

On the first day, the mice were put in a square chamber, and sprayed 80% alcohol on the wall of the chamber, so that the mice can link the environment and the smell. After one-minute habituation, the mice received five pairings of conditioned and unconditioned stimuli with a 5-minute inter-trial interval. The conditioned stimulus consisted of a 29.5 s, 9 kHz, 80 dB tone, while the unconditioned stimulus involved a 500 ms, 0.6 mA foot shock (see Figure 23). The percentage of time spent freezing to the tones was measured by Any-maze 4.20 (Stoelting) software.

24 hours later, contextual fear conditioning was carried out the next day. The mice were placed in the same environment as the previous day for 5 minutes, characterized by the same chamber shape and smell, but without any stimulation. Using Any-maze 4.20 software to measure the freezing time of the mice during these 5 minutes.

Subsequently, auditory cued fear conditioning was conducted on the third day. The animals were placed in a completely different environment, a triangular chamber sprayed with 1% acetic acid. The mice were still given 1 minute of adaptation time. Following the adaptation period, the mice were exposed to the same tone as on the first day (9 kHz; 80 dB; 30s) for a total of 10 repetitions, with an inter-trial interval of 1.5 minutes. However, no foot shock was administered after the tone. Measure the percentage of time frozen in response to the tone using Any-maze 4.20 software.

Figure 22. The FC test

2.7 Electrophysiology

Electrophysiology experiments were done by collaborators, and the specific methods are referred to previous study (Chang et al., 2022; Dine et al., 2015).

2.7.1 Electrophysiology on MR^{Nex} mice

MR^{Nex} mice aged 8-12 weeks were used for this experiment and their brains were obtained by decapitation immediately after isoflurane anesthesia. Brains were sectioned using a vibratome (HM650V, Thermo Scientific) in ice-cold carbogen gas (95% O_2/5% CO_2) saturated solution containing 87mM NaCl, 2.5mM KCl, 7mM $MgCl_2$, 0.5mM $CaCl_2$, 25mM $NaHCO_3$, 1.25mM NaH_2PO_4, 75mM sucrose and 10mM glucose, 350μm thick horizontal slices containing the ventral hippocampus were obtained. The slices were initially incubated in carbonized saline (containing 125mM NaCl, 2.5mM KCl, 1mM $MgCl_2$, 2mM $CaCl_2$, 25mM $NaHCO_3$, 1.25mMM NaH_2PO_4 and 10mM glucose) at 34°C for 30 minutes and then transferred to room temperature (23-25°C) for at least 30 minutes further incubated. All measurements were performed at room temperature.

For long-term potentiation (LTP) and paired-pulse facilitation experiments, slices were superfused with carbogenated physiological saline (4-5 ml/min flow rate). The field excitatory postsynaptic potentials (fEPSPs) at the CA3-CA1 synapses were evoked by delivering square pulse electrical stimulation (50 μs pulse width) through a bipolar tungsten electrode (50 μm tip diameter, ~0.5 MΩ nominal impedance) to the Schaffer collateral pathway. The fEPSPs were recorded using a glass microelectrode (filled with physiological saline, ~1 MΩ open-tip resistance) placed in the CA1 stratum radiatum. An fEPSP with a magnitude of around 50% of the amplitude of the population spike occurrence was elicited by adjusting the voltage stimulus intensity. The information was digitised at 5 kHz after being low-pass filtered at 1 kHz. Before and after inducing LTP, the neuronal tissue was stimulated with pulses every 15 s. High-frequency stimulation (HFS, 100 Hz for 1 s) generated long-term potentiation. In order to get the paired-pulse ratio, divide the slope of fEPSP2 by that of fEPSP1.

In the whole-cell patch clamp experiment, infrared videomicroscopy (Zeiss, Oberkochen, Germany) was used to identify individual CA1 pyramidal neurons. Subsequently, somatic whole-cell voltage clamp recordings were performed on these cells using an EPC 10 amplifier (HEKA) with the following specific parameters: holding potential of -70 mV, a seal resistance greater than 1 GΩ, a series resistance less than 20 MΩ, 10 mV liquid junction potential

correction, 3 kHz low-pass filter, and 15 kHz sampling rate. To ensure accurate recordings, carbogenated physiological saline solution was used at a flow rate of 2-3 ml/min, supplemented with 50 μM APV, 100 μM picrotoxin, and 1 μM TTX. The tip resistance of the patch pipette was 3-5 MΩ, and the intracellular solution contained: 125mM $CsCH_3SO_3$, 8mM NaCl, 10mM HEPES, 0.5mM EGTA, 4mM Mg-ATP, 0.3mM Na-GTP, and 20mM Na_2-phosphocreatine. 10 min after break-in to the cell, miniature excitatory postsynaptic currents (mEPSCs) mediated by AMPA receptors were recorded for 5 minutes. Finally, the data were analysed offline using Mini Analysis software (Synaptosoft).

2.7.2 Electrophysiology on MR^{Dlx} mice

After administering isoflurane to mice and promptly decapitating them, the brains were swiftly extracted from the cranial cavity. Using a vibratome, 350 m-thick coronal slices of the dorsal hippocampus were obtained in an ice-cold carbogen gas (95% O_2/5% CO_2)-saturated solution containing 87mM NaCl, 2.5mM KCl, 7mM $MgCl_2$, 0.5mM $CaCl_2$, 25mM $NaHCO_3$, 1.25mM NaH_2PO_4, 75mM sucrose and 10mM glucose, and 75 mM sucrose. Brain slices were then incubated at 34°C for 30 minutes in carbogenated physiological saline (containing 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose), followed by an incubation at room temperature (23-25°C) for at least 1 hour. At ambient temperature, all electrophysiological measurements were performed. The Ctrl and MR^{Dlx} mice were equally divided into two groups, one of which was the CORT group, and the Brain slices were stored in carbonated saline containing 1 uM CORT solution for 1 hour (Sigma-Aldrich Corticosterone, product nr. 27840, dissolved in 0.01 % EtOH; Merck KGaA, Darmstadt, DE); in vehicle group, brain slices were pre-incubated with carbogen saline vehicle solution containing 0.01% EtOH. Slices were rinsed for 30 minutes in pure carbogenated physiological saline after pre-incubation with CORT or vehicle solution. In the recording chamber, slices were superfused with carbogenated physiological saline (4-5 ml/min flow rate). fEPSPs at CA3 - CA1 synapses were elicited by square-pulse electrical stimuli (50us pulse width) delivered to the Schaffer collateral-commissural pathway via a bipolar tungsten electrode (50um pole diameter, 0.5 M nominal impedance). In the CA1 stratum radiatum, glass microelectrodes (filled with physiological saline, 1 Mopen-tip resistance) were used to record fEPSPs. The stimulation intensity was adjusted so as to generate a fEPSP that was 50% of the amplitude of a population spike. The recording data were 1 kHz low-pass filtered and 5 kHz digitalized. Before and after

the induction of LTP, which was induced by high-frequency stimulation (100 Hz for 1 s), a single stimulation pulse was administered to the neural tissue every 15 s.

2.8 Golgi-Cox staining and analysis of dendrites and spines

Mice were anesthetized with isoflurane and decapitated, and immediately extracted brains and immersed them in Golgi-Cox solution (Glaser & Van der Loos, 1981) for 14 days, followed by immersion in 30% sucrose solution for 5 days at room temperature in the dark. 120 μm thick serial coronal sections were cut on a Microm HM 650V vibratome (Thermo Scientific, Walldorf, Germany), mounted on Superfrost plus slides (Thermo Scientific).

Pyramidal neurons from the CA3 ventral hippocampus have been selected for structural analysis (6-8 neurons per area per animal). Neurons were traced at 40X and dendritic spines at 100X by using Neurolucida software (MicroBrightField Bioscience, Williston, VT). Sholl analysis was used to calculate the total dendrite length, and NeuroExplorer software (MicroBrightField) was used to count the connections at concentric circles (20 μm apart).

2.9 Stereotaxic Surgery

For virus injection, mice were anesthetized by inhalational isoflurane (Floren, Abbott), then placed in a stereotaxic frame (Kopf Instruments) and given 2% (v/v) O2, and maintained body temperature with a heating pad. Before surgery, mice were given i.p. injection of Meloxicam (Metacam®, Boehringer Ingelheim, Ingelheim am Rhein, Germany) 0.5 mg/kg per body weight. Inject AAV virus bilaterally using a 33-gauge injection needle with a 5 μl micro-syringes (Hamilton®, Bonaduz, GR, Switzerland). Utilize an automated microinjection pump (World Precision Instruments) to inject 300 nl of virus at a rate of 100 nl/min. To determine the injection coordinates, the Franklin and Paxinos mouse brain atlas have been used. The targeted coordinates, relative to bregma, were as follows: 0.8 mm posterior, 1.2 mm lateral, 4.3 mm ventral. Within 3 days after surgery, Metacam (1 mg/kg body weight) was mixed into the drinking water of the mice and administered as a systemic analgesic, and the body weight of the mice was monitored daily. After completion of the behavioral experiments, successful viral expression was verified by RNAScope.

2.10 Tissue collection and processing

At the end of the experiment, for the mice that will be used for the subsequent immunofluorescence experiment, performed perfusion after anesthetizing them with

isoflurane. First, perfused with 0.1 M PBS via the heart, and then the body is fixed with 4% (v/v) paraformaldehyde (PFA) perfusion. The brains were quickly removed and preserved in 4% (v/v) PFA at 4°C for 24 hours. Later, the brains were transferred to a 30% sucrose solution at 4°C. After the brains had completely settled to the bottom of the solution, they were kept at 4°C until they could be processed.

The rest of the animals were killed by decapitation under isoflurane anesthesia. The basal trunk blood was collected, the adrenal glands were removed and weighed, and brains were quickly frozen and stored at 80°C before being sectioned.

2.11 CORT measurements

All blood samples are collected and stored on ice, then centrifuged at 8000 rpm for 15 minutes at 4°C. Afterwards, 10 µl of plasma is transferred to newly labelled microcentrifuge tubes and stored in a -20°C freezer. During the thesis study, two methods were used to measure CORT level.

One was following the manufacturer's instructions, the CORT Double Antibody 125I Radioimmunoassay Kit (MP Biomedicals Inc., Eschwege, Germany; sensitivity 12.5 ng/ml) was used to measure corticosterone levels (ng/ml). The radioactivity was measured with a gamma counter (Packard Cobra II Auto Gamma, PerkinElmer). Finally, the CORT level was obtained by standard curve.

The other one was following the manufacturer's instructions, by using an Enzyme-linked Immunosorbent Assay (ELISA) kit (RE52211, TECAN, IBL Hamburg, Germany) to determine CORT level in plasma. Plasma from unstressed mice was diluted 1:3 with standard A (0 nmol/L, RE52217, TECAN, IBL Hamburg, Germany), plasma from stressed mice was diluted 1:10. For the assay 20 µl from the diluted sample mixture were used. The Standard Range was 5 – 240 nmol/L. The Analytical Sensitivity (Limit of Detection) is 1.680 nmol/L; Cross-Reactivity of other substances tested < 0.1 – 7.4 %; Intra-Assay <3.3 – 7.7 %; Inter-Assay <5 - 10.8 %. The Assay was measured with Photometer (EPOCH Microplate Spectrophotometer BioTek Instrumente), at a wavelength of 450 nm and at reference wavelength of 625 nm. Finally, the CORT level was obtained by standard curve with background subtraction (450nm-625nm).

2.12 In situ hybridization (ISH)

The frozen brain samples are sliced into 20 m thick coronal pieces using a cryostat at -20°C. After the sections have been dried, they are placed to Super Frost Plus slides and kept at -20°C until needed. Use a plasmid harbouring MR that has been linearized as a template to generate antisense cRNA probes. These probes are subsequently 35S-UTP-labeled according to the protocol described in prior literature (Schmidt et al., 2007). Start by acetylating tissue slices in a solution of 0.25 percent acetic anhydride in 0.1 millimolar triethanolamine hydrochloride. Following acetylation, brain pieces should be dehydrated in an ethanol gradient. After the sections have been produced, cRNA probes containing about 1.5 x 106 cpm of the 35S labelled riboprobe are administered in a volume of 100 l of hybridization buffer. The slices should be incubated at 55 degrees Celsius for one night with a coverslip to facilitate hybridization. The following day, after washing the sections in 2x SSC (standard saline citrate), apply 20 mg/l of RNAse A after removing the coverslips. The sections are first washed in a succession of room temperature SSC solutions, then immersed in 0.1x SSC at 65°C for 1 hour, and then dehydrated using progressively higher concentrations of ethanol. Allow the slides to air dry before mounting them on Kodak Biomax MR films. Autoradiographs should be digitised after film development. Measure optical density for expression analysis using the NIH ImageJ software. For each animal, separate readings were taken in representative areas of the left and right hippocampus, and the mean optical density was calculated. Make sure that measurements are taken with the noise from the background removed out. Fairly representative results can be obtained by exposing selected slides to 4°C in Kodak NTB2 emulsion (Eastman Kodak Co., Rochester, NY). Exposure period should be modified according on the typical degree of MR expression. Create the slides and examine the MR mRNA expression under a light microscope with a darkfield condenser.

2.13 RNAScope analysis and cell counting

Take out the brain previously stored in the -80°C freezer. Frozen brains were sectioned in the cryotome at -20°C with a coronal plane to a thickness of 20 µm. The cutted brain sections were attached to Super Frost Plus slides and stored at -20°C. The RNA Scope Fluorescent Multiplex Kit (Item 320850, Advanced Cell Diagnostics, Newark, CA, USA) was used for mRNA staining. mm-Fam107a-C1, mm-Nr3c2-C2, and mm-scl17a6-C3 probes were used for staining. The staining procedure was performed according to the manufacturer's instructions. First, slides with brain sections attached were fixed in 4% paraformaldehyde at 4°C for 15 min.

Subsequently, brain sections were successively dehydrated in ethanol concentrations ranging from 50% to 75% to 100%. After that, tissue pieces were incubated with Proteinase IV for 30 min at room temperature. Next, mm-Fam107a-C1, mm-Nr3c2-C2, and mm-scl17a6-C3 were mixed in a 50:1:1 ratio and hybridized with the sections at 40°C for 2 h, following four separate hybridizations with amplification reagents 1-4. Finally, after a quick re-staining of the sections with DAPI, the slides were covered and stored at 4°C until image acquisition. All images were acquired using a Zeiss inverted laser scanning confocal microscope and Zen software, using a 40× objective (n=3 animals per marker and condition). All images were collected with the same laser power, detector gain, and amplifier offset settings for each unique marker. Each image was acquired in a 1.0 µm z-stack. The mRNA in the cells was quantified and analysed using ImageJ and QuPath 0.3.2 software.

2.14 Immunofluorescence

The perfused and fixed brains were removed and 40 µm thick sections were prepared using a vibrating microtome (Microm HM 650 V, Thermo Fisher Scientific). After blocking, the sections were incubated with primary antibody (Rabbit anti-Fam107a (ab185459), Abcam, 1:1000) at 4°C for overnight and protected from light. The next day, sections are washed and incubated with the appropriate fluorescence-coupled secondary antibody (Goat anti-Rabbit IgG Alexa Fluor 488; Invitrogen, Thermo Fisher Scientific, 1:500) for 2 hours at room temperature and protected from light, then washed with PBS. Sections were mounted using Fluoromount-G mounting medium (SouthernBiotech) and then stored at -20°C for image acquisition. Images were captured using a Zeiss inverted laser scanning confocal microscope and Zen software. Z-stacks of pictures of the region of interest were generated for confocal imaging in increments of 1.0 m.

2.15 Single-cell RNA sequencing

Single-cell RNA sequencing was conducted by collaborators as described previously (Lopez et al., 2021). Male CD1 animals were trained for aggression and randomly assigned to acute social defeat stress or control conditions. Mice undergoing 5 minutes of acute social defeat were housed together with resident aggressor CD1 mice, and 5 hours later, the brains of stressed and control mice were collected after perfusion with PBS and placed in ice-cold oxygenated artificial cerebrospinal fluid (aCSF). aCSF is oxygenated using a 5% CO2 in O2 mixture. Cut 1000 µm thick sections using a VT1200/S Leica vibrating microtome. The ventral hippocampus (−2.46 mm Bregma to −3.52 mm Bregma) was manually extracted under the guidance of a

stereomicroscope (M205C, Leica). Dissociate the tissue with a papain dissociation system (Worthington) for 35 min at 37 °C in a shaking water bath. Cells were filtered through a 30 μm filter (Partec) and placed in cold aCSF before loading onto a 10X Genomics Chromium Chip. The 10X Genomics Single-Cell v3.0 kit (10x Genomics) was used. Perform reverse transcription and library preparation. Library concentration and fragment length were determined by qPCR using KAPA Library Quant (Kapa Biosystems) and Bioanalyzer High Sensitivity DNA Kit (Agilent), respectively. Libraries were pooled and single-pass sequenced on the Illumina NovaSeq 6000 System. Raw data were preprocessed using CellRanger (v 3.0.2) with default parameters. Data analysis was performed using SCANPY (1.7.1) and the AnnData data structure (0.7.4). The filtering threshold is determined independently for each sample. SCRUBLET (0.2.1) was utilised to detect and remove potential doublets, and the data were normalized using the pool-based normalization tool SCRAN (1.18.5). and apply Louvain clustering with a resolution of 0.5. The obtained clusters were then passed to SCRAN for normalization, taking into account the absence of significant batch effects. Finally, the data were batch corrected using SCANPY's ComBat implementation, which considered the knockout key as a batch variable, since samples with the same knockout key were sequenced together without significant batch effects. The final UMAP visualization of the data was generated via SCANPY using a kNN graph with 15 neighbors. Use the same graph to cluster the data using a Python implementation of Louvain's algorithm. Start with a resolution of 0.5. Marker genes for each cluster were detected using SCANPY 's rank_genes_groups function with default parameters (Welch's t-test). Clusters were annotated using literature-based marker genes. Subclustering is performed for finer annotation and similar subclusters are merged. Neural cell clusters in our data were enhanced by incorporating spatial information using literature-based markers for anatomical location in the hippocampus. Marker identification for pyramidal cells involved Neurod6 genes. Subsequently, expression patterns of Fibcd1 and Spock1 were analysed to specifically characterize pyramidal cells in CA1 and CA3 neurons. Trajectory inference for oligodendrocyte-associated cell types using CellRank (1.2.0) and PalantirKernel. Use Diffusion Pseudotime (sc.tl.dpt) on a kNN plot for a subset of data (k=50) to obtain pseudotime. DIFFXPY was used to perform differential expression tests. A pairwise test was performed for each cluster between control cells and stressed cells. Tests were performed using the Wald test and negative binomial GLM. The formula is as follows: $Y_{ij} \sim 1 + C_j + S_j$, where Cj is the condition label (Ctrl or Stress) and Sj is the sample covariate for the cell. For downstream visualization and analysis, genes with absolute Log2FC < 0.1 and mean expression < 0.1 were filtered out from the control and stress data.

2.16 Statistics analysis

The IBM SPSS Statistics 22 (IBM SPSS Statistics, IBM) software was used to analyse the data, and GraphPad Prism 8.0 was used to create the graphics (GraphPad Software). An independent Student's t-test was used to compare two groups. If the data are not normally distributed, use the nonparametric Mann–Whitney U test. Data from more than two groups were analysed with a Two-way Repeated Measures ANOVA model followed by Tukey post hoc analysis to determine statistical significance between groups. If behavioral data are not normally distributed, normalize the data using a log transformation before analysis. Post hoc independent samples t-tests were carried out if significant main or interaction effects were discovered. The ANOVA significance levels were set at $p<0.05$ for main effects and $p<0.1$ for interactions. The significance threshold was set at $p<0.05$ for each post hoc test. Values outside two standard deviations were considered outliers and excluded from the analysis. Data are visualized as mean ± standard error of the mean (SEM).

3 Results:

3.1 Validation of Cre-loxP-dependent MR knockout in forebrain glutamatergic neurons and its effect on anxiety-related behavior and cognition at baseline levels

We obtained mice with conditional knockout of MR in pyramidal neurons by mating female $MR^{lox/lox}$ mice with male Nex-cre mice. ISH was used to verify whether MR was successfully knocked out in the hippocampus (Figure 24A). We detected a significant reduction of MR in the CA1, CA2, CA3 and DG regions of the hippocampus. But due to the cre is not expressed in DG in this mouse line, the deletion of MR in DG was not expected, the continuing significant decrease could be explained by a secondary process. Following the successful knockout of MR, we continued our studies to look at the structural, functional, and molecular effects that would result from the absence of MR in glutamatergic pyramidal neurons.

As shown in Figure 24B, we performed a variety of behavioral experiments with male mice to observe the baseline of anxiety-related behavior and cognition for the MR^{Nex} mice. The experimental mice were in the age range from 12 to 20 weeks. After the behavioral tests, we gave the mice a week of rest to bring them back to a stress-free state, and then conducted acute restraint stress. The results revealed that CORT levels of both MR^{Nex} mice and Ctrl mice significantly increased following restraint (Figure 24C), and that CORT levels of both groups significantly dropped after being returned to their cages for an hour ($F_{(2, 73)}=426.6$, $p=0.000$), without an effect of the genotype ($F_{(1, 73)}=0.009$, $p=0.926$).

In baseline behavioral experiments, we used OF test and EPM test to detect the anxiety-like behavior of mice, and used the NOR test and SOR test to detect the cognitive function of mice (Figure 24B). In OF, we found that MR^{Nex} mice preferred to stay at the corners of the arena more than Ctrl mice did. Even though there is no difference in the total distance of MR^{Nex} mice and Ctrl mice in the entire OF arena ($t_{(25)}=-0.967$, $p=0.343$), MR^{Nex} mice spent significantly reduced time ($t_{(25)}=3.396$, $p=0.002$) and travelled significantly less distance ($t_{(25)}=4.325$, $p=0.000$) in the central area compared to the Ctrl group (Figure 24D). The anxiogenic phenotype of MR^{Nex} mice was confirmed in the EPM. Comparing MR^{Nex} mice to Ctrl mice, the time spent in the open arms ($t_{(23)}=2.107$, $p=0.046$), the distance in the open arms ($t_{(23)}=2.2232$, $p=0.036$), and the ratio entering the open arms ($t_{(23)}=2.689$, $p=0.013$) were all significantly decreased (Figure 24E). On the cognitive level, neither the NOR test nor the SOR test revealed

any statistically significant differences between MR^{Nex} mice and Ctrl mice regarding either the duration ($t_{(19)}=1.896$, $p=0.073$; $t_{(22)}=-0.856$, $p=0.401$) or frequency ($t_{(20)}=0.001$, $p=0.999$; $t_{(22)}=-1.341$, $p=0.193$) of touching novel objects, nor in their total time spent exploring objects ($t_{(20)}=1.327$, $p=0.199$; $t_{(21)}=-0.733$, $p=0.472$; Figure 24F, 24G, respectively). Thus, MR in glutamatergic forebrain neurons is regulating anxiety, but not cognition in male mice.

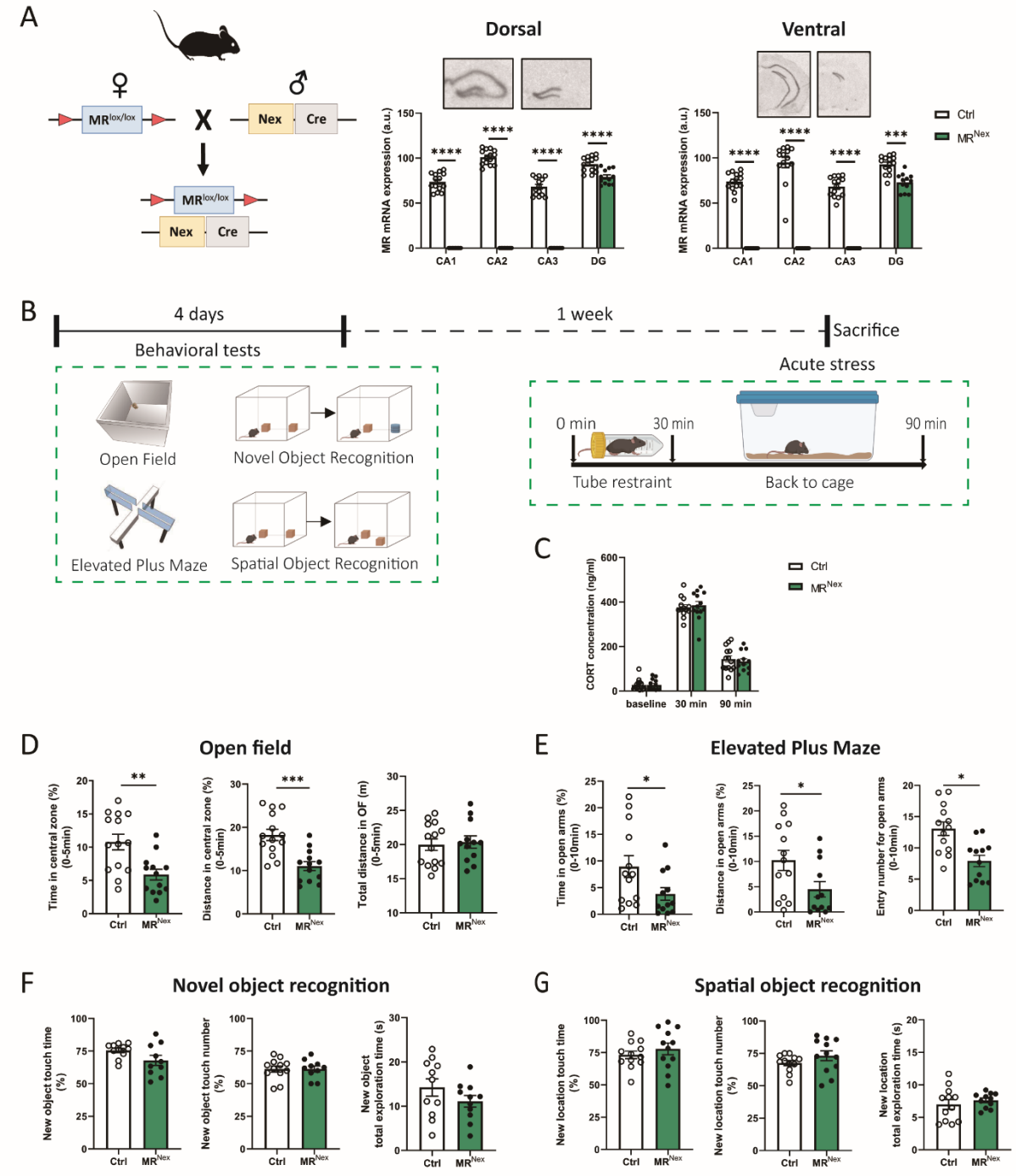


Figure 23 Breeding and validation of MR^{Dlx} mice, baseline behavioral experimental measurements.

(A) By breeding $MR^{lox/lox}$ mice with Nex-Cre mice, MR mRNA levels in glutamatergic forebrain neurons were significantly reduced. **(B)** Behavioral experiments were conducted on 12-20 week old male MR^{Nex} and Ctrl mice. After one week of behavioral experiments, these mice were exposed to acute stress paradigms. **(C)** CORT levels were significantly increased in both groups of mice following acute restraint. In OF test **(D)** and EPM test **(E)**, lack of MR in glutamatergic neurons reveals an anxiety-like phenotype. The cognitive function of mice in the NOR test **(F)** or SOR test **(G)** was not significantly affected by the loss of MR in glutamatergic neurons. ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$.*

In a separate cohort of mice, we assessed home cage locomotion and stressful learning in the MWM test (Figure 25A). For home cage locomotion there were no discernible differences between Ctrl and MR^{Nex} mice ($F_{(1, 108)}=1.780$, $p=0.1849$), which both displayed a clear circadian rhythm, with a substantial increase in activity at night compared to daytime ($F_{(11, 108)}=12.38$, $p=0.000$; Figure 25B). For the MWM test, the average time it takes for two groups of mice to find the platform decreases during the five days of training ($F_{(4, 100)}=7.910$, $p=0.000$), indicating that their capacity for learning is unhampered (Figure 25 C). Nevertheless, there was no noticeable distinction between MR^{Nex} mice and Ctrl mice. In the probe test of the formal experiment, both two groups of mice spent much more time than in the other quadrants ($F_{(3, 84)}=11.75$, $p=0.000$), but there was no discernible difference between these two groups ($F_{(1, 84)}=0.002$, $p=0.963$; Figure 25D).

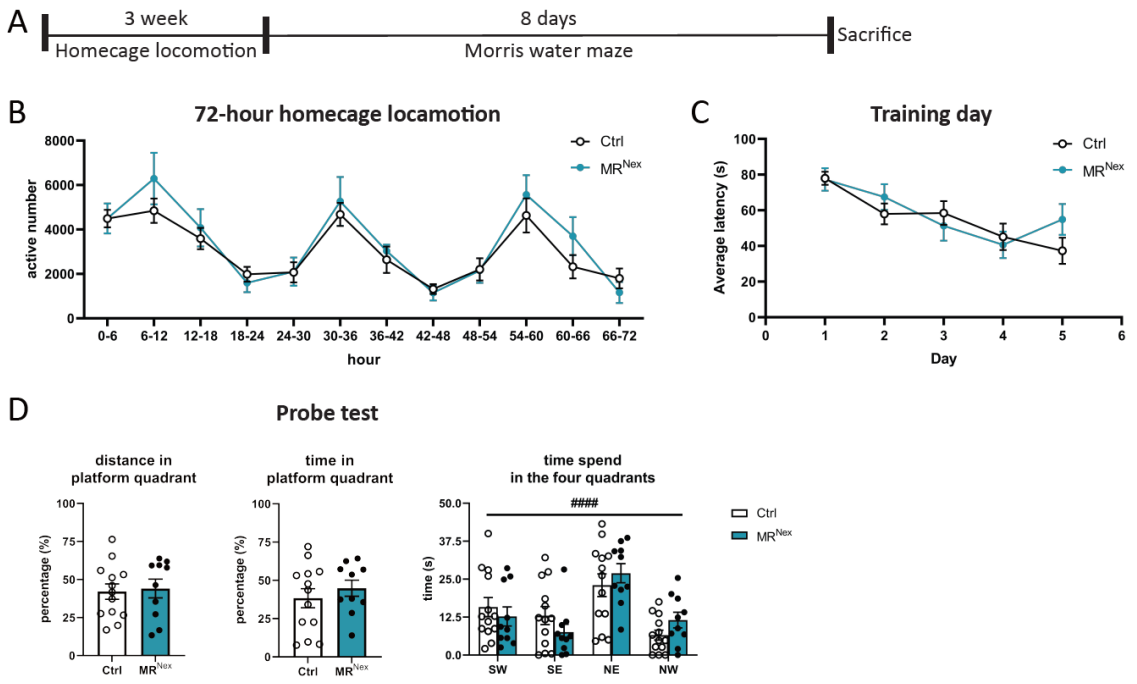


Figure 24 Experimental design and results for assessing home cage activity levels and spatial learning. (A) Experimental design. **(B)** Both MR^{Nex} mice and Ctrl mice had obvious circadian rhythms in

the activities of the home cage, but there was no significant difference between the two groups. **(C)** and **(D)** Loss of MR in glutamatergic neurons does not affect spatial learning in mice. ** $p < 0.05$, **$p < 0.01$, *** $p < 0.001$*.

Taken together, the data clearly indicate that mice lacking the MR in glutamatergic pyramidal neurons exhibit an increased anxiety-like behavior, while their learning and memory ability is unaffected.

3.2 Anxiety-related behavior and cognition changes in MR^{Nex} mice after chronic social defeat stress.

3.2.1 CSDS has mostly genotype-independent effects in male MR^{Nex} mice.

In addition to studying the phenotype of MR^{Nex} mice in basal conditions, we also investigated whether emotional behavior and cognitive function are altered in MR^{Nex} mice under stressful conditions. Therefore, mice underwent the chronic social defeat stress for 21 days, with behavioral testing on days 15 to 18 (Figure 25A). After the experiments, we measured the CORT level, there were no significant differences between genotypes ($F_{(1,37)}=0.110$, $p= 0.742$), between stress treatments ($F_{(1,37)}=3.843$, $p= 0.057$; Figure 25B).

We replicated the anxiogenic effect of glutamatergic MR deletion under non-stressed conditions in the OF, with a significant genotype effect for time ($F_{(1,36)}=4.948$, $p=0.030$) and distance ($F_{(1,37)}=5.273$, $p= 0.027$) in the central area. CSDS also resulted in an increase in anxiety for both Ctrl and MR^{Nex} mice, independent of genotype (centre time: $F_{(1,36)}=10.52$, $p=0.003$; centre distance: $F_{(1,37)}=7.227$, $p=0.011$). Also, the CSDS group moved in the entire box less than the non-stressed group overall ($F_{(1,39)}=13.36$, $p=0.001$; Figure 25C).

In EPM, the results were similar to those of OF. The percentage of times the CSDS group entered the open arm ($F_{(1,38)}=5.209$, $p=0.028$), as well as the time spent in the open arm ($F_{(1,38)}=5.663$, $p=0.022$) were significantly less than the non-stressed group. The MR^{Nex} mice spent less time ($F_{(1,38)}=6.268$, $p=0.017$) and the distance of the activity ($F_{(1,38)}=5.598$, $p=0.023$) than the Ctrl mice in the open arm, with no significant genotype x stress interaction ($F_{(1,38)}=0.3896$, $p=0.536$; $F_{(1,38)}=0.037$, $p=0.848$; $F_{(1,38)}=0.094$, $p=0.761$; Figure 25D).

In NOR, regardless of whether the mice received CSDS, there were no significant differences between MR^{Nex} animals and Ctrl mice (stress: percentage of time to explore the new object: $F_{(1, 38)}=2.141$, $p=0.152$; frequency of exploring new object: $F_{(1, 35)}=0.174$, $p=0.679$; total time spent

exploring new object: $F_{(1, 39)}=0.119, p=0.732;$ genotype: percentage of time to explore the new object: $F_{(1, 38)}=0.867, p=0.358$; frequency of exploring new object: $F_{(1, 35)}=0.499, p=0.484$; total time spent exploring new object: $F_{(1, 39)}=2.180, p=0.148$). And there is no interaction between genotype and stress (percentage of time to explore the new position: $F_{(1, 38)}=0.742, p=0.394$; frequency of exploring new position: $F_{(1, 35)}=1.22, p=0.277$; total time spent exploring new position: $F_{(1, 39)}=0.037, p=0.848$; Figure 25E). In SOR, we observed that while there was no obvious difference in the frequency of exploring the object in a new place (stress: $F_{(1, 37)}=0.347$, $p=0.559$; genotype: $F(1, 37)=2.010, p=0.165$; interaction: $F(1, 37)=1.86, p=0.181$) or the total amount of time spent exploring objects (stress: $F_{(1, 38)}=0.198, p=0.659$; genotype: $F_{(1, 38)}=2.151$, $p=0.151$; interaction: $F_{(1, 38)}=0.009, p=0.9245$), the percentage of MR^{Nex} mice touching the object in the new location was higher than that of Ctrl mice following CSDS, there is an interaction (stress: $F(1, 37)=0.331, p=0.568$; genotype: $F_{(1, 37)}=3.953, p=0.054$; interaction: $F_{(1,37)}=5.590, p=0.023;$ Figure 25F).

Taken together, the data demonstrate that mice indeed exhibit increased anxiety in response to chronic stress, although CSDS mostly has genotype-independent effects in male MR^{Nex} mice compared to controls.

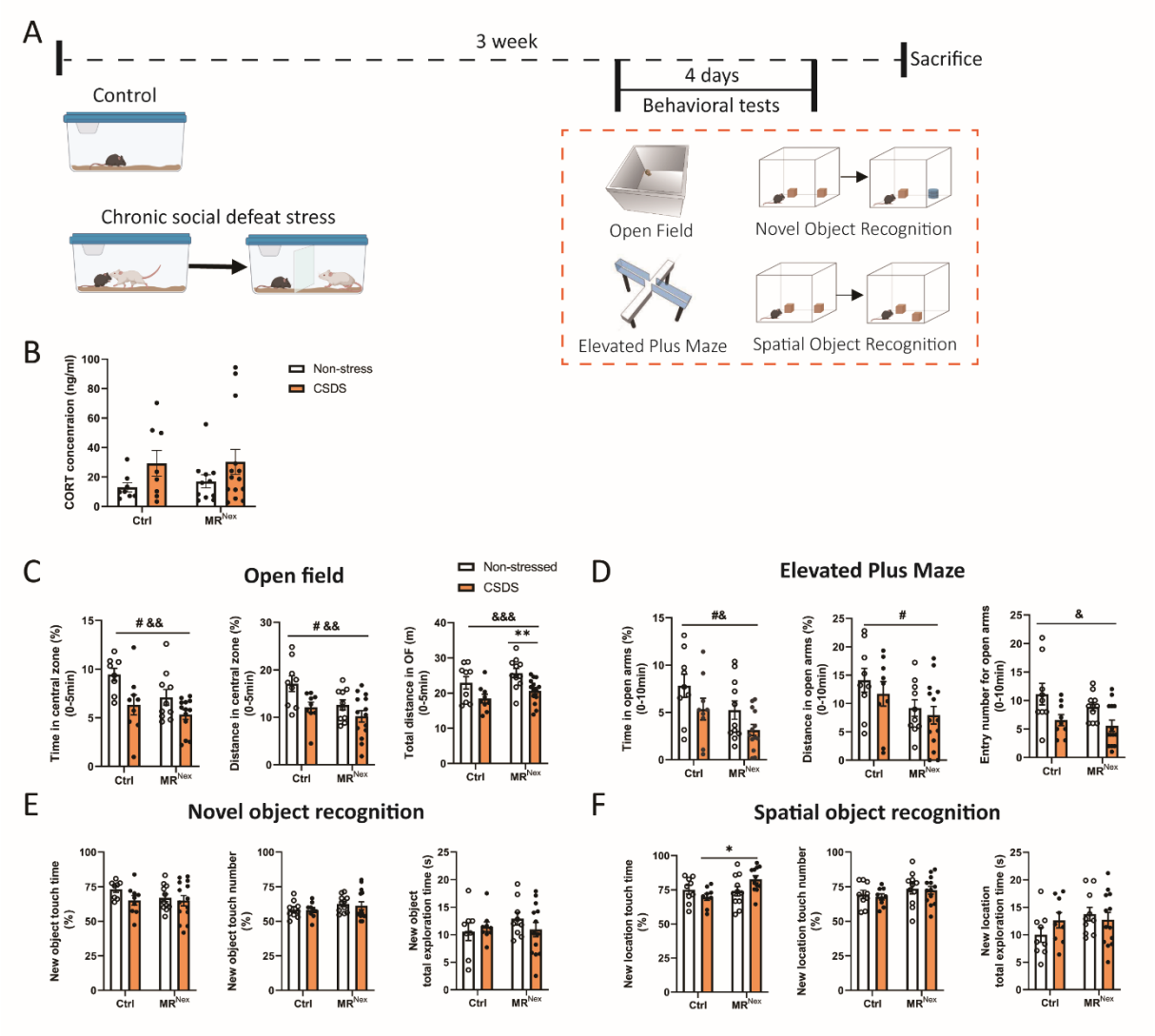


Figure 25 Behavioral studies on male mice under chronic social defeat stress. (A) The duration of the CSDS trial was 21 days, behavioral tests were carried out simultaneously during the third week of CSDS. **(B)** The CORT levels of male MR^{Nex} mice and Ctrl mice did not differ significantly between the groups. In the OF test **(C)**, after CSDS, both MR^{Nex} mice and Ctrl mice exhibited decreased activity duration and distance in the centre of the open field arena, with no significant difference between the two groups. In the EPM test **(D)**, after CSDS, both MR^{Nex} mice and Ctrl mice exhibited decreased activity duration and distance in the open arm, with no significant difference between the two groups. In the NOR test **(E)** and SOR test **(F)**, the changes in the two groups after CSDS are not significant. *$*p < 0.05$, $**p < 0.01$, $***p < 0.001$*. **#** means there's a difference between different genotypes. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. **&** means there's a difference between different treatments. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

3.2.2 The anxious phenotype in male MR^{Nex} mice is sex-specific.

Animals respond differently to stress depending on their sex. Previous research has

demonstrated that unlike male mice, female mice do not exhibit the same obvious neuroendocrine alterations in response to persistent stress (Dalla et al., 2011; van Doeselaar et al., 2021). Therefore, we also studied female MR^{Nex} animals in addition to male mice.

MR^{Nex} and Ctrl females were exposed to the female 21-day CSDS paradigm and behavioral testing was carried out on experimental days 15 to 18 (Figure 26A). After the experiments, we measured the CORT level, there were no significant differences between genotypes ($F_{(1,35)}=0.485$, $p= 0.491$), between stress treatments ($F_{(1,35)}=0.013$, $p= 0.909$; Figure 26B).

In OF test (Figure 26C), there was no discernible difference between the MR^{Nex} mice and Ctrl mice whether they experienced CSDS or not (percentage of time in central zone: $F_{(1, 37)}=0.259$, $p=0.613$; percentage of distance in central zone: $F_{(1, 37)}=0.053$, $p=0.819$; total distance: $F_{(1, 38)}=0.313$, $p=0.579$). However, compared to non-stressed animals, animals that had experienced CSDS displayed increased anxiety, they had a shorter staying time in the central area of the open field arena ($F_{(1,37)}=4.412$, $p=0.042$), had less movement in the whole open field arena ($F_{(1,37)}=8.993$, $p=0.048$). In EPM test, there was no discernible difference between the MR^{Nex} mice and Ctrl mice whether they experienced CSDS or not (percentage of time in open arms: $F_{(1, 36)}=0.628$, $p=0.433$; percentage of distance in open arms: $F_{(1, 36)}=1.143$, $p=0.292$; number of entries into open arms: $F_{(1, 36)}=3.088$, $p=0.087$). But the mice which experienced CSDS spent less time ($F_{(1,36)}=9.141$, $p=0.005$) and move less ($F_{(1,36)}=17.680$, $p=0.000$) in the open arm (Figure 26D). In both NOR and SOR, although the percentage of time and exposure to novel objects was higher than 50%, neither between the non-stressed group and the CSDS group, nor between the MR^{Nex} animals and the Ctrl mice showed any statistical differences. In NOR test, the percentage of time to explore the new object did not affected by stress ($F_{(1, 36)}=0.308$, $p=0.582$) and genotype ($F_{(1, 36)}=0.339$, $p=0.564$); the frequency of exploring new object did not affected by stress ($F_{(1, 36)}=2.290$, $p=0.139$) and genotype ($F_{(1, 36)}=1.742$, $p=0.195$); total time spent exploring new object did not affected by stress ($F_{(1, 36)}=0.058$, $p=0.811$) and genotype ($F_{(1, 36)}=1.017$, $p=0.320$; Figure 26E). In SOR test, the percentage of time to explore the new object did not affected by stress ($F_{(1, 37)}=0.453$, $p=0.505$) and genotype ($F_{(1, 37)}=2.231$, $p=0.144$); the frequency of exploring new object did not affected by stress ($F_{(1, 34)}=1.873$, $p=0.180$) and genotype ($F_{(1, 34)}=2.828$, $p=0.102$); total time spent exploring new object did not affected by stress ($F_{(1, 37)}=0.604$, $p=0.442$) and genotype ($F_{(1, 37)}=1.656$, $p=0.206$; Figure 26F).

The above experimental results are in line with other previous study findings. In contrast to

male mice, female mice did not exhibit the same evident alterations in emotional behavior and cognitive performance after CSDS.

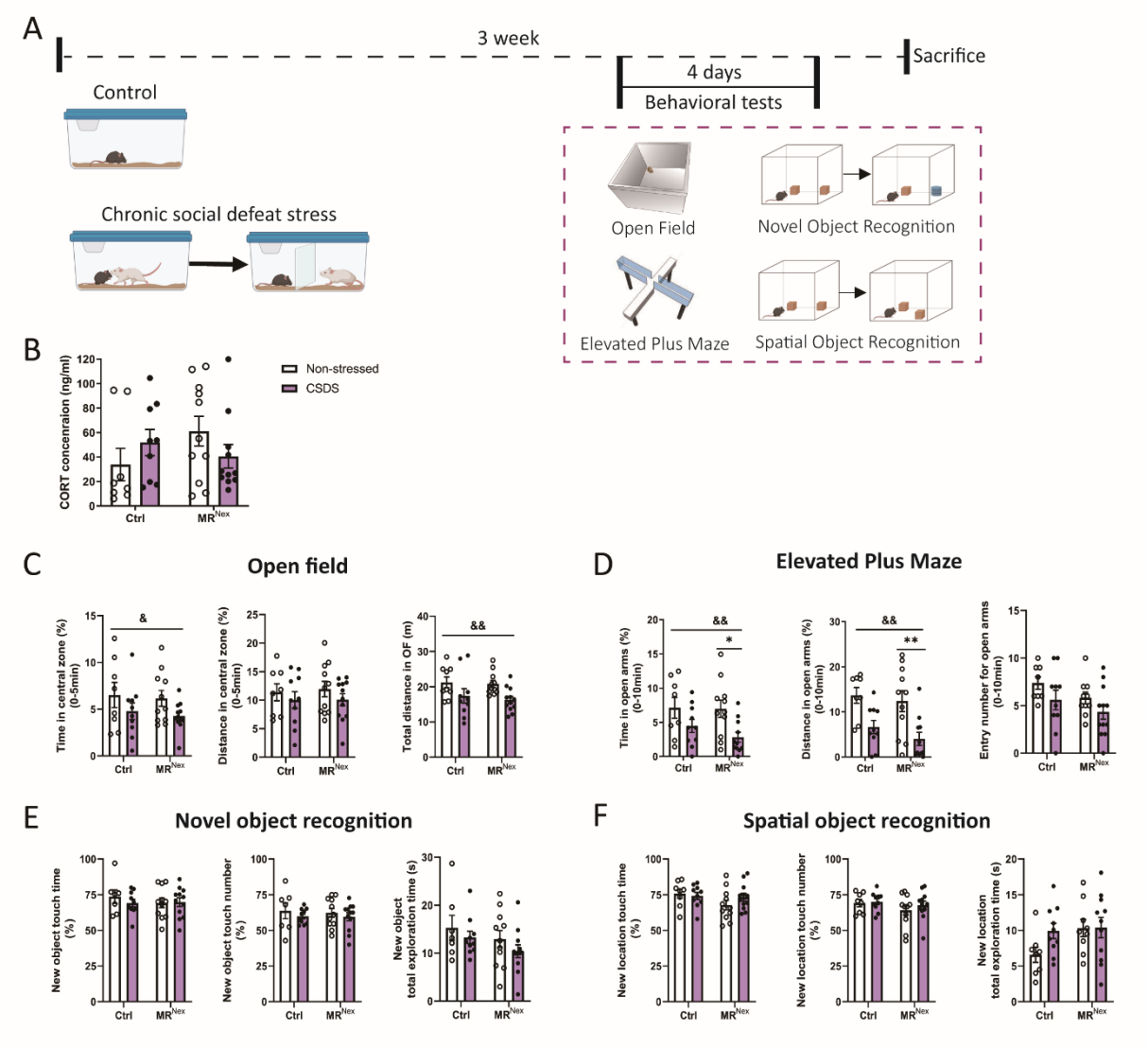


Figure 26 Behavioral studies on female mice under chronic social defeat stress. (A) The duration of the CSDS trial was 21 days, behavioral tests were carried out simultaneously during the third week of CSDS. **(B)** Their CORT levels of female mice did not differ significantly between the groups. **(C)** After CSDS, both MR^{Nex} mice and Ctrl mice exhibited decreased activity duration and distance in the centre of the open field arena, with no significant difference between the two groups. **(D)** After CSDS, both MR^{Nex} mice and Ctrl mice exhibited decreased activity duration and distance in the open arm, with no significant difference between the two groups. **(E)** and **(F)** In NOR test and SOR test, the changes in the two groups after CSDS are not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **#** means there's a difference between different genotypes. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. **&** means there's a difference between different treatments. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

3.3 Function and structure

3.3.1 Mice lacking MR in glutamatergic pyramidal neurons may show alterations in hippocampal glutamate neurotransmission

To further investigate whether lacking MR affects synaptic transmission, we performed evoked field potential recordings on mice, to measure LTP, paired-pulse facilitation, and excitatory postsynaptic potential in the CA1 area of the hippocampus (Figure 27A). After using high-frequency stimulation of the Schaffer collateral projections of CA3 to CA1, we didn't detect a significant difference in LTP between MR^{Nex} mice and Ctrl mice (Figure 27B). This may be the reason why cognitive function was not significantly impaired in MR^{Nex} mice in the previous behavioral experiments. In the paired-pulse ratio results, the ratio measured in MR^{Nex} mice was higher than that of Ctrl mice ($F_{(1,90)}=31.91$, $p=0.000$; Figure 27C), indicating a lower release probability of presynaptic vesicles in MR^{Nex} mice. To investigate this further, a separate cohort of mice was subjected to whole-cell patch clamp recordings, where, MR^{Nex} mice showed a lower frequency ($t_{(53)}=5.194$, $p=0.000$) of glutamate neurotransmitter release from presynaptic vesicles and a lower amplitude ($t_{(53)}=4.221$, $p=0.000$) in the post-synaptic AMPA receptors (Figure 27D). Based on the findings from the whole-cell patch clamp recordings, it is evident that MR inactivation in glutamatergic pyramidal neurons has a significant impact on glutamatergic signaling in the hippocampus. The lower frequency and amplitude observed in the recordings suggest that mice lacking MR in glutamate neurons may have altered presynaptic release of glutamate and post-synaptic response, possibly reflecting a decrease in the levels of post-synaptic AMPA receptors in CA1. However, additional experiments and controls are necessary to confirm these findings and fully elucidate the underlying mechanisms.

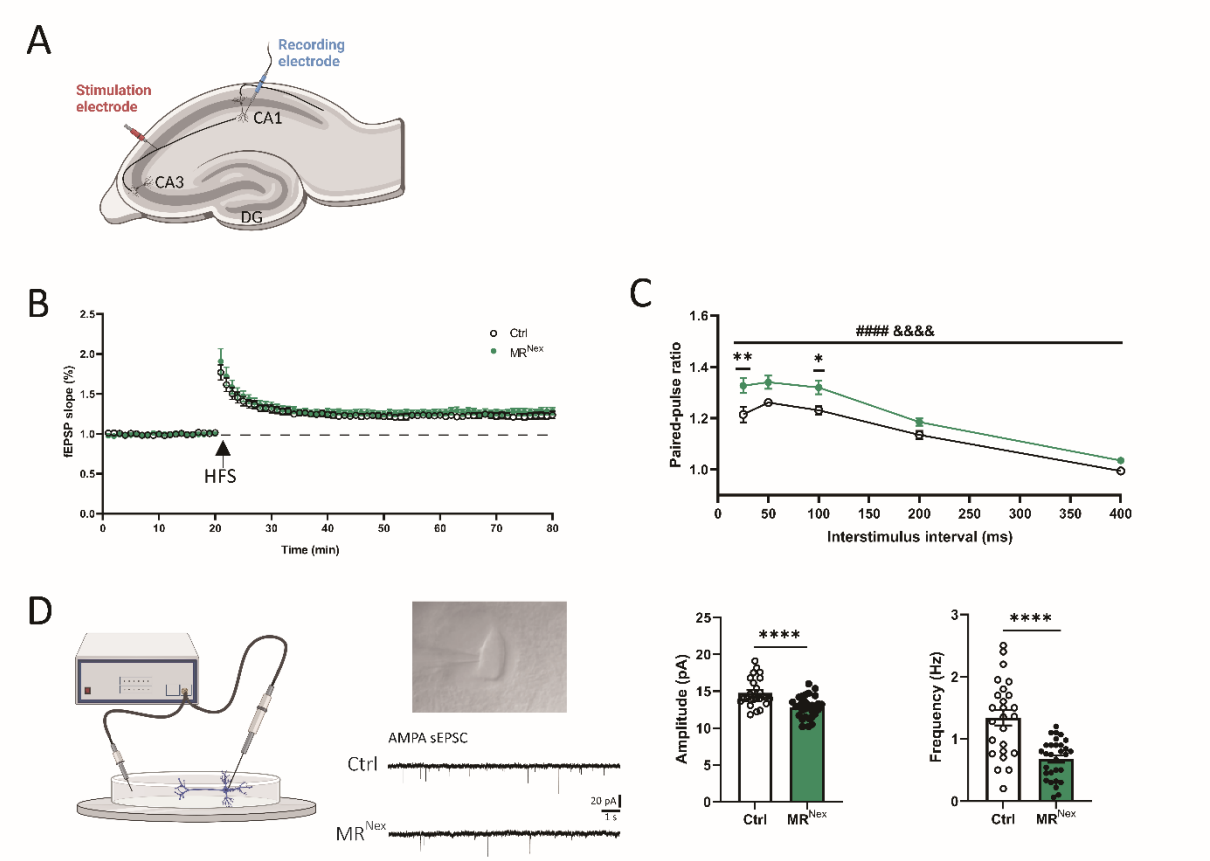


Figure 27 The function changes in MR^{Nex} mice. (A) and **(B)** There's no significant difference between MR^{Nex} mice and Ctrl mice in LTP. **(C)** The PPR of MR^{Nex} mice was higher than that of Ctrl mice. **(D)** A reduced frequency of glutamate neurotransmitter release from presynaptic vesicles and a lower amplitude at the post-synaptic AMPA receptors were seen in mice lacking MR during whole-cell patch clamp tests.

3.3.2 Deletion of MR in glutamatergic pyramidal neurons leads to structural changes in the hippocampus

To assess whether deleting MR in glutamatergic pyramidal neurons has an effect on neuronal morphology, we used Golgi staining (Figure 28A). As shown in Figure 28F, deletion of MR in glutamatergic neurons significantly reduced the dendritic length as well as the number of spines in CA3 compared to Ctrl mice time ($t_{(48)}=3.432$, $p=0.001$; $t_{(48)}=3.103$, $p=0.003$; $t_{(127)}=7.036$, $P<0.001$; Figure 28B). Sholl analysis of the number of dendritic interactions further revealed that MR loss had an effect on the complexity of dendritic branching, mainly at 50 µm to 100 µm away from the soma (50 µm: $p=0.041$, 60 µm: $p=0.003$, 70 µm: $p= 0.002$, 80 µm: $p=0.003$, 90 µm: $p= 0.011$, 100 µm: $p=0.017$; Figure 28C). This suggests that MR deletion mainly affects the mid segment of dendritic branches. Moreover, the deletion of MR in glutamatergic neurons significantly reduced the dendritic length compared with the control group ($t_{(60)}=2.174$, $p=0.034$; Figure 28C).

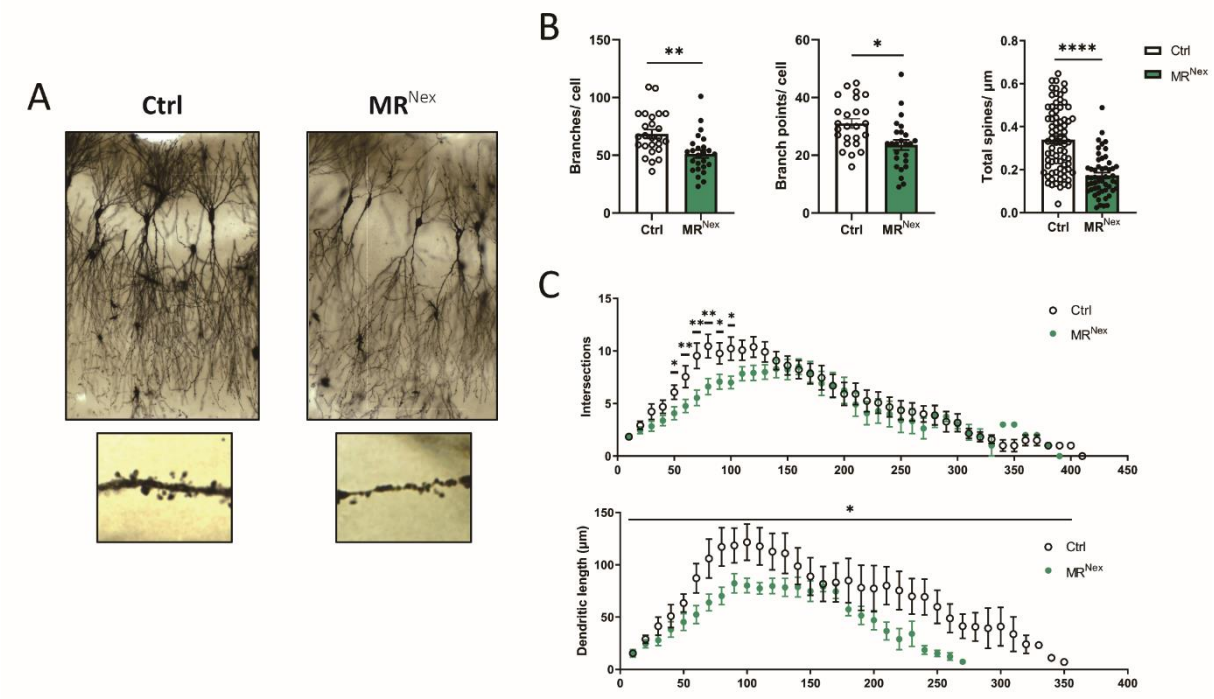


Figure 28 The structure changes in MR^{Nex} mice. (A) Deleting MR of glutamatergic pyramidal neurons affects morphology. **(B)** MR deletion in glutamatergic neurons significantly reduced the dendritic length and the number of spines in CA3 compared to Ctrl mice. **(C)** Deleting MR in glutamatergic neurons significantly reduced the dendritic length.

3.4 Single-cell RNA sequencing and viral expression

3.4.1 Differential genes

We chose five mice from each group (non-stressed group and stress group) for molecular characterization in order to get single-cell sequencing data. Ventral hippocampus from these mice were used in scRNA-seq experiments. The different clusters from the hippocampus are further subdivided into cell types such as neurons, oligodendrocytes, astrocytes, microglia, endothelial cells, ependymal cells, elongate cells, and vascular cells, and so on. As shown in the figure, we found that the CA3 Glut2 cluster had the greatest number of differential genes between the MR^{Nex} mice and the Ctrl group in the baseline group (Figure 29A). In the stress group, the CA3 Glut2 cluster also had a considerably higher number of differential genes in MR^{Nex} mice (Figure 29A). Therefore, we decided to focus our observations on the CA3 Glut2 cluster.

In the baseline group, there were 1872 differential genes in the CA3 Glut2 cluster, and in the stress group, there were 1173 differentially expressed genes in the CA3 Glut2 cluster. These

genes were merged and selected based on significant differences between Ctrl and MR^{Nex} mice at the baseline or between the non-stressed and stress groups within each genotype. Furthermore, genes were chosen if their fold change>|0.5|. This approach leads to the identification of 78 differentially expressed genes, as shown in the volcano diagram (Figure 29B). Among the strongly upregulated genes, we identified Nr3c1 (GR), which is known to be involved in the regulation of various physiological processes, most notably, stress response. Among the downregulated genes, we confirmed Nr3c2 (MR) and also Fkbp5, a gene previously described to be strongly regulated via MR in the hippocampus (Hartmann et al., 2021). Intriguingly, we also identified Fam107a as one of the most strongly downregulated genes in the CA3 Glut2 cluster in MR^{Nex} mice. We and others had previously shown that Fam107a, also known as down-regulated in renal cell carcinoma 1 (DRR1), is a stress-responsive actin bundling factor that is involved in synaptic plasticity and stress resilience. We therefore selected this gene for further functional analyses (Carbajosa et al., 2018; Schmidt et al., 2011). Through the validation of RNAScope, we can find that consistent with the single-cell RNA sequencing data, the expression of Fam107a gene is indeed decreased in MR^{Nex} mice ($t_{(92)}=9.274$, $p=0.000$; Figure 29C).

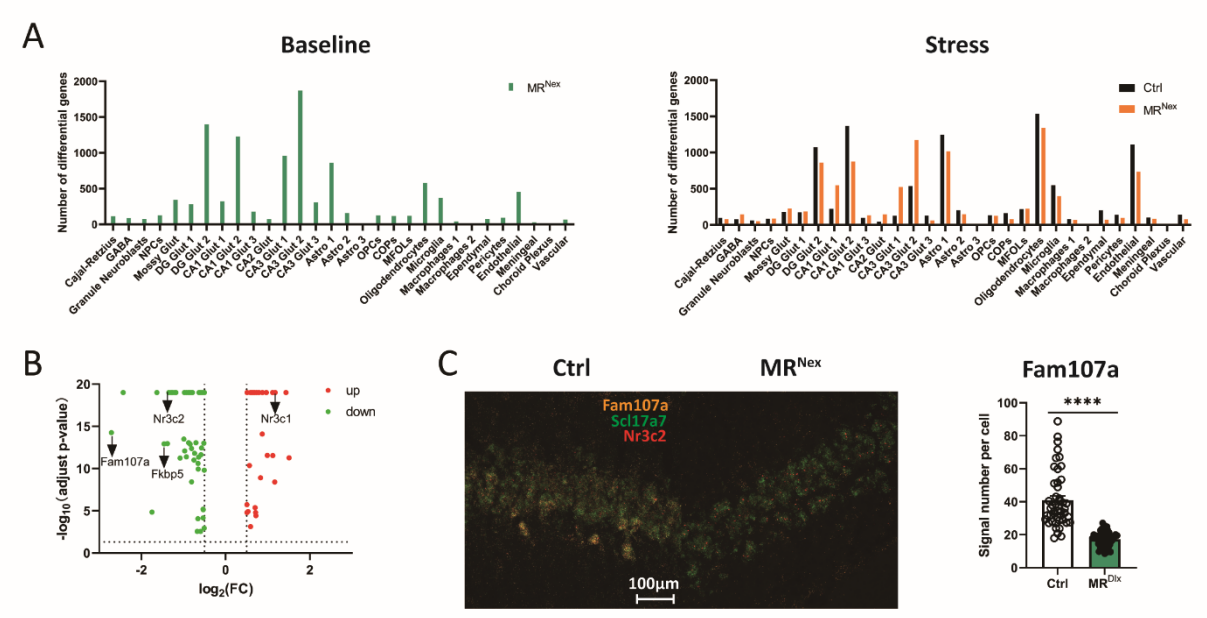


Figure 29 Single-cell RNA sequencing data of MR^{Nex} animals. (A) Histogram showing the number of differential genes in the baseline of MR^{Nex} animals. **(B)** Histogram showing the number of differential genes in MR^{Nex} animals during stress induction. **(C)** Volcano plot showing the Log2FC pattern of differential genes. Fam107a was selected for further manipulation.

3.4.2 Overexpression of Fam107a partially rescues the anxiety phenotype of MR^{Nex} mice.

To test our hypothesis that the MR-mediated downregulation of Fam107a contributes to the male-specific increase in anxiety-related behavior, we virally overexpressed Fam107a in the ventral CA3 region of the hippocampus of MR^{Nex} and Ctrl mice (Figure 30A). Successful viral expression was confirmed via immunofluorescence (Figure 30A). We then performed behavioral tests on the mice four weeks after virus injection.

In OF (Figure 30B), we discovered that the mice injected with the virus exhibited uneasiness and spent less time in the centre of the arena ($F_{(1,29)}=13.79$, $p=0.001$) and travelled less in the centre of the arena ($F_{(1,31)}=22.13$, $p=0.000$) than the mice receiving the empty virus. Since the mice in the MR^{Nex} group did not vary significantly, we found a significant interaction effect between genotype and overexpression of Fam107a on anxiety-like behavior ($F_{(1,31)}=6.466$, $p=0.016$). In EPM (Figure 30C), regardless of whether the mice were in the MR^{Nex} group or the Ctrl group, the number of times the mice entered the open arm ($F_{(1,32)}=7.778$, $p=0.009$), the duration of their stay ($F_{(1,31)}=27.53$, $p=0.000$), and the movement distance ($F_{(1,31)}=15.99$, $p=0.000$) all reduced following Fam107a overexpression. But we didn't see an interaction between genotype and overexpression of Fam107a. It demonstrates how the anxiety phenotype of MR^{Nex} mice may be partially rescued by Fam107a overexpression. But in both NOR and SOR experiments, we found no significant difference. In NOR (Figure 30D), overexpression of Fam107a did not affect their performance (percentage of time to explore the new object: $F_{(1,31)}=0.076$, $p=0.784$; frequency of exploring new object: $F_{(1,31)}=0.883$, $p=0.355$; total time spent exploring new object: $F_{(1,30)}=2.530$, $p=0.122$), also genotype did not show any significant effects (percentage of time to explore the new object: $F_{(1,31)}=0.181$, $p=0.673$; frequency of exploring new object: $F_{(1,31)}=0.005$, $p=0.944$; total time spent exploring new object: $F_{(1,30)}=0.271$, $p=0.606$). Similar in SOR (Figure 30E), overexpression of Fam107a did not affect their performance (percentage of time to explore the new position: $F_{(1,32)}=0.476$, $p=0.495$; frequency of exploring new position: $F_{(1,32)}=0.488$, $p=0.489$; total time spent exploring new position: $F_{(1,29)}=1.831$, $p=0.186$), also genotype did not show any significant effects (percentage of time to explore the new position: $F_{(1,32)}=0.246$, $p=0.623$; frequency of exploring new position: $F_{(1,32)}=1.915$, $p=0.176$; total time spent exploring new position: $F_{(1,29)}=0.325$, $p=0.573$).

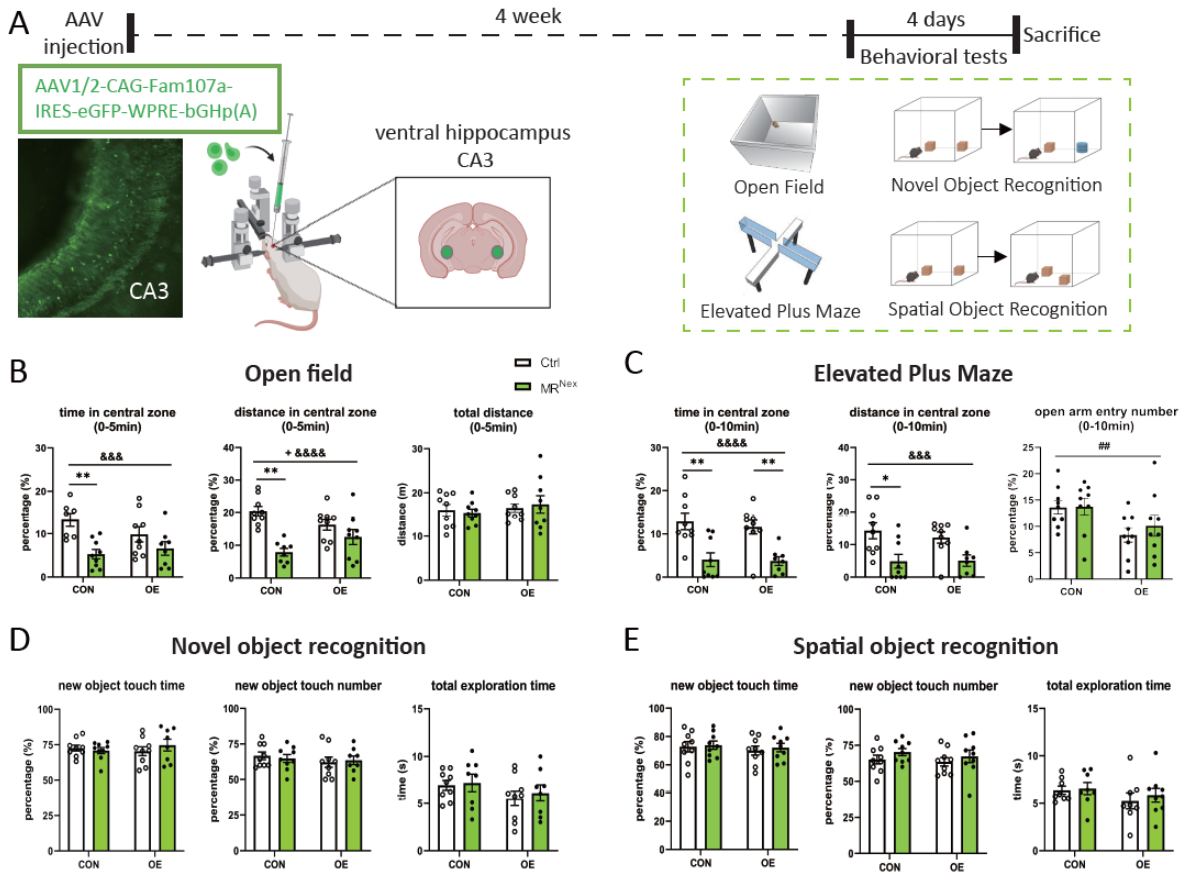


Figure 30 Injection of Fam107a AAV virus partially rescues the genotype (A) Animals received bilateral injections of Fam107a AAV virus or control virus in the ventral hippocampus CA3 region. **(B)** The mice injected with an overexpression of the Fam107a virus spent less time and travelled less in the centre of the arena than mice injected with an empty virus, and there's a significant interaction effect. **(C)** Overexpression of Fam107a reduced the number of times mice entered open arms, stay time, and movement distance. **(D)** and **(E)** In NOR test and SOR test, the performance of those four groups are similar. *$*p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **#** means there's a difference between different genotypes. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. **&** means there's a difference between different treatments. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$. **+** means there is an interaction between those groups. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$.

3.4.4 Overexpression of Fam107a in CA3 glutamatergic neurons partially reverses the anxiety-related phenotype of MR^{Nex} mice.

To establish that the previous results were due to the rescue of Fam107a specifically in glutamatergic neurons, we designed a Cre-dependent Fam107a overexpression virus to specifically target the CA3 glutamatergic cell population.

First, we tested the influence of this cell-type specific Fam107a overexpression under wildtype

conditions using Nex-Cre mice (Figure 31A). The successful and cell-type specific overexpression of Fam107a in the CA3 area of the hippocampus was confirmed by RNAScope ($t_{(105)}=11.8$, $p=0.000$; Figure 31B). Interestingly, in the Nex-cre group, we found that in OF (Figure 31C), even while there was no significant change in the overall distance of mice ($t_{(21)}=0.104$, $p=0.918$), the Fam107a-overexpressing animals had a substantially longer stay time ($t_{(19)}=2.336$, $p=0.030$) and greater movement distance ($t_{(20)}=3.180$, $p=0.005$) in the central arena. In the EPM experiment (Figure 31D), we didn't discover any significant differences (percentage of time in open arm: $t_{(19)}=0.191$, $p=0.850$; percentage of distance in open arm: $t_{(19)}=0.749$, $p=0.463$; open arm entry number: $t_{(22)}=0.452$, $p=0.656$). In NOR test (Figure 31E), we did not see any significant difference in percentage of time to explore the new object ($t_{(20)}=1.367$, $p=0.187$), frequency of exploring new object ($t_{(19)}=0.646$, $p=0.526$), and total time spent exploring new object ($t_{(20)}=1.224$, $p=0.235$). Likewise, in SOR test (Figure 31F), we discovered no statistical difference in percentage of time to explore the new position ($t_{(22)}=1.266$, $p=0.219$), frequency of exploring new position ($t_{(22)}=0.893$, $p=0.382$), and total time spent exploring new position ($t_{(22)}=1.293$, $p=0.209$).

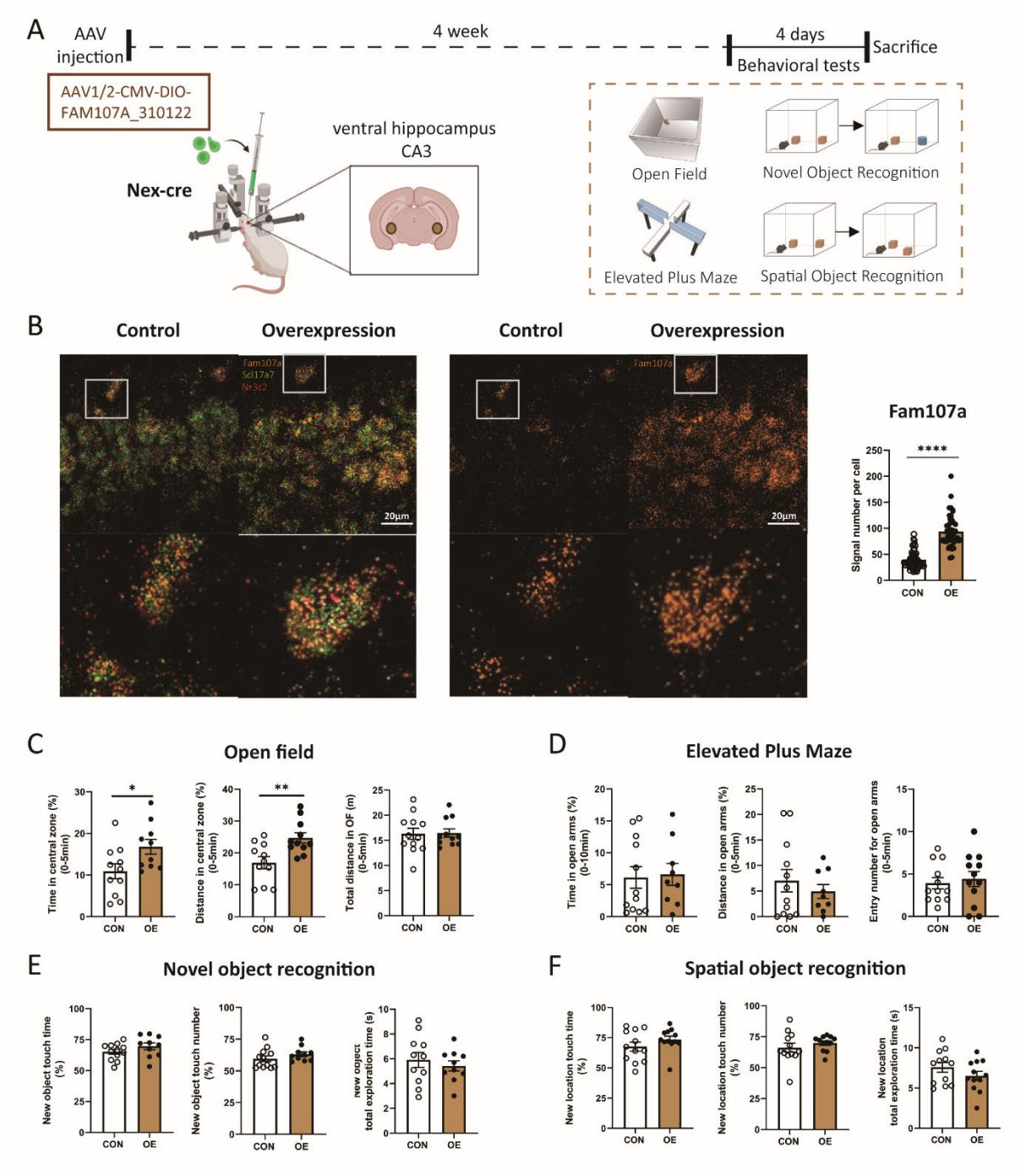


Figure 31 Injection of cre-dependent Fam107a virus on Nex-cre mice leads to reduced anxiety. (A) For Fam107a overexpression, cre-dependent DIO-AAV virus was injected into both sides of the ventral hippocampus CA3 region in mice. **(B)** The expression of Fam107a mRNA in Ctrl mice was significantly increased after virus injection. **(C)** In the OF test, Ctrl animals that overexpressed Fam107a were more active in the central area of open field arena. **(D)** In EPM, no significant behavioral differences were observed before and after virus injection. **(E)** and **(F)** There were no significant differences between two groups in the NOR and SOR tests. **p < 0.05, **p < 0.01, ***p < 0.001.*

Next, we tested whether Fam107a overexpression in glutamatergic CA3 neurons can reverse the anxiolytic phenotype of male MR^{Nex} mice (Figure 32A). The successful and cell-type specific overexpression of Fam107a in the CA3 area of the hippocampus was confirmed by RNAScope ($t_{(98)}=18.01$, $p=0.000$; Figure 32B). In OF (Figure 32C), after overexpression of Fam107a, MR^{Nex} mice had no statistically significant difference in the time spent in the central zone ($t_{(19)}=0.156$, $p=0.877$), the moving distance ($t_{(19)}=0.032$, $p=0.975$), and the moving distance in the whole arena ($t_{(19)}=0.046$, $p=0.964$). In contrast, we discovered that in EPM (Figure 32D), MR^{Nex} animals overexpressing Fam107a were considerably more active on the open arm than MR^{Nex} mice without Fam107a overexpression. Significant statistical variations may be shown in the frequency of entering the open arm ($t_{(17)}=4.401$, $p=0.000$), the duration of time spent there ($t_{(17)}=2.979$, $p=0.008$), and the activity distance ($t_{(17)}=3.232$, $p=0.005$). But we discovered no significant difference in the NOR test (percentage of time to explore the new object: ($t_{(19)}=0.982$, $p=0.338$), frequency of exploring new object: ($t_{(20)}=0.721$, $p=0.479$), total time spent exploring new object: ($t_{(20)}=0.921$, $p=0.368$; Figure 32E) and SOR test (percentage of time to explore the new position ($t_{(20)}=1.400$, $p=0.177$), frequency of exploring new position ($t_{(20)}=0.741$, $p=0.467$), and total time spent exploring new position ($t_{(20)}=1.334$, $p=0.197$; Figure 32F).

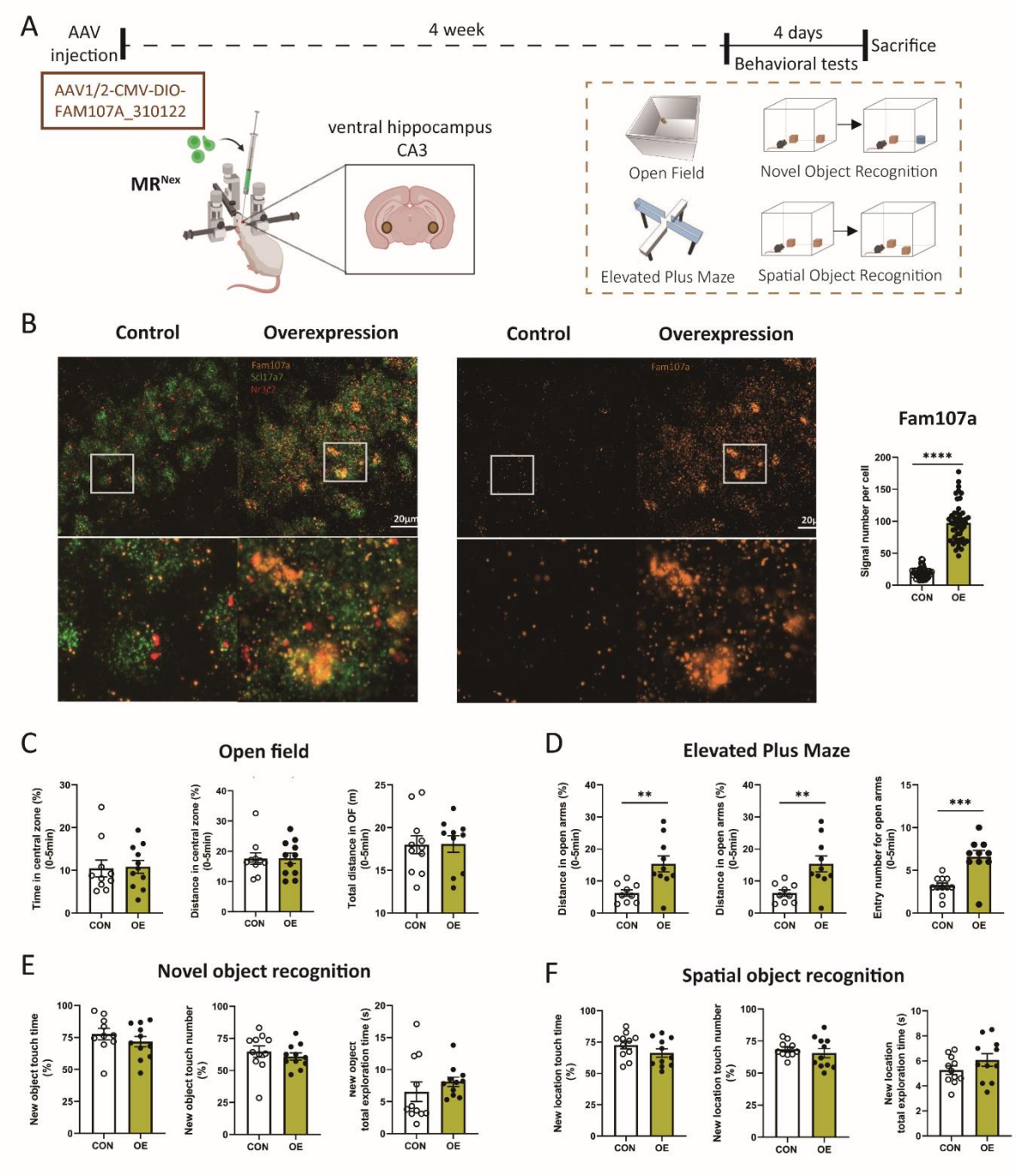


Figure 32 Injection of cre-dependent Fam107a DIO-AAV virus on MR^{Nex} mice can partially rescue the genotype. (A) For Fam107a overexpression, cre-dependent DIO-AAV virus was injected into both sides of the ventral hippocampus CA3 region in mice. **(B)** The expression of Fam107a mRNA in MRNex mice appeared to increase following virus injection. **(C)** In the OF test, viral injections did not significantly affect anxiety behavior. **(D)** In EPM, MR^{Nex} animals that overexpressed Fam107a were more active in open arms. **(E)** and **(F)** The NOR test and SOR test resulted in no significant differences. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.5 Test the baseline on anxiety-related behavior and cognition for the MR^{Dlx}.

To generate mice with specific deletion of the MR in GABAergic neurons, we performed a mating between female $MR^{lox/lox}$ mice and male Dlx-cre mice. In order to confirm the successful knockout of MR in the hippocampus, we employed the ISH technique (Figure 33A). We didn't find a significant reduction of MR in the CA1, CA2, CA3 and DG regions of the hippocampus. This could potentially be attributed to the relatively low abundance of GABAergic neurons in the hippocampus (Pelkey et al., 2017). We employed RNAScope technology for further validation. Compared with ISH, RNAScope technology has the advantages of higher sensitivity and specificity. Through the quantitative analysis of RNAScope, we found that the expression of Nr3c2 in MR^{Dlx} mice GABAergic neurons was significantly less than that of Ctrl ($t_{(49)}=2.651, p=0.011$). the results of genotype detection suggested that the successful mating of mice enabled us to obtain the desired genotype. So we proceeded with a series of behavioral experiments to examine potential variations in emotion, behavior, and cognition among mice with different genotypes.

Figure 33B illustrates the behavioral experiments conducted on male MR^{Dlx} mice to assess their baseline anxiety-related behavior and cognition. The mice used in the experiments were aged between 12 and 20 weeks. Following the behavioral tests, a one-week resting period was provided to allow the mice to return to a stress-free state. Subsequently, acute restraint stress was applied. The findings revealed a significant increase in CORT levels in both MR^{Dlx} mice and Ctrl mice after the restraint ($F_{(2,63)}=191.0, P<0.001$). Additionally, one hour after being returned to their cages, CORT levels in both groups exhibited a significant decrease, with no observable impact of genotype ($F_{(1, 63)}=0.0305, p=0.8619;$ Figure 33C).

During the baseline behavioral experiments, we used the OF and EPM tests to evaluate anxiety-like behavior in mice, and the NOR and SOR tests to evaluate cognitive function in mice (Figure 7B). Our analysis of the OF test showed even though MR^{Dlx} mice moved less total distance across the OF region compared to Ctrl mice ($t_{(22)}=2.509, p=0.020$), there was no significant difference in the percentage of time spent in the central zone ($t_{(22)}=0.675, p=0.507$), the percentage of distance traveled in the central zone ($t_{(22)}=0.235, p=0.816$; Figure 7D). Similar results were observed in the EPM test, where MR^{Dlx} mice exhibited comparable results to Ctrl mice in terms of the time spent in the open arms ($t_{(22)}=2.509, p=0.020$), the distance traveled in the open arms ($t_{(22)}=2.509, p=0.020$), and the number of entries into the open arms

($t_{(22)}=2.509, p=0.020$; Figure 7E). Regarding cognitive function, both the NOR test and SOR test indicated that Ctrl mice spent more time exploring novel objects compared to MR^{Dlx} mice (NOR: $t_{(21)}=3.348, p=0.003$; SOR: $t_{(21)}=2.213, p=0.047;$ Figure 33F, 33G, respectively). Furthermore, not in the SOR ($t_{(21)}=1.835, p=0.081$), but in the NOR test, the Ctrl mice showed a significantly higher frequency of interactions with new objects compared to the MR^{Dlx} mice ($t_{(22)}=2.259, p=0.034$; Figure 33F). However, there was no significant difference between the two groups in terms of the total exploration time of the objects (NOR: $t_{(22)}=0.690, p=0.497$; SOR: $t_{(21)}=1.042, p=0.309$; Figure 33F, 33G, respectively). Thus, it appears that the absence of MR in GABA neurons may impact cognition in male mice, while anxiety levels in male mice do not seem to be affected.

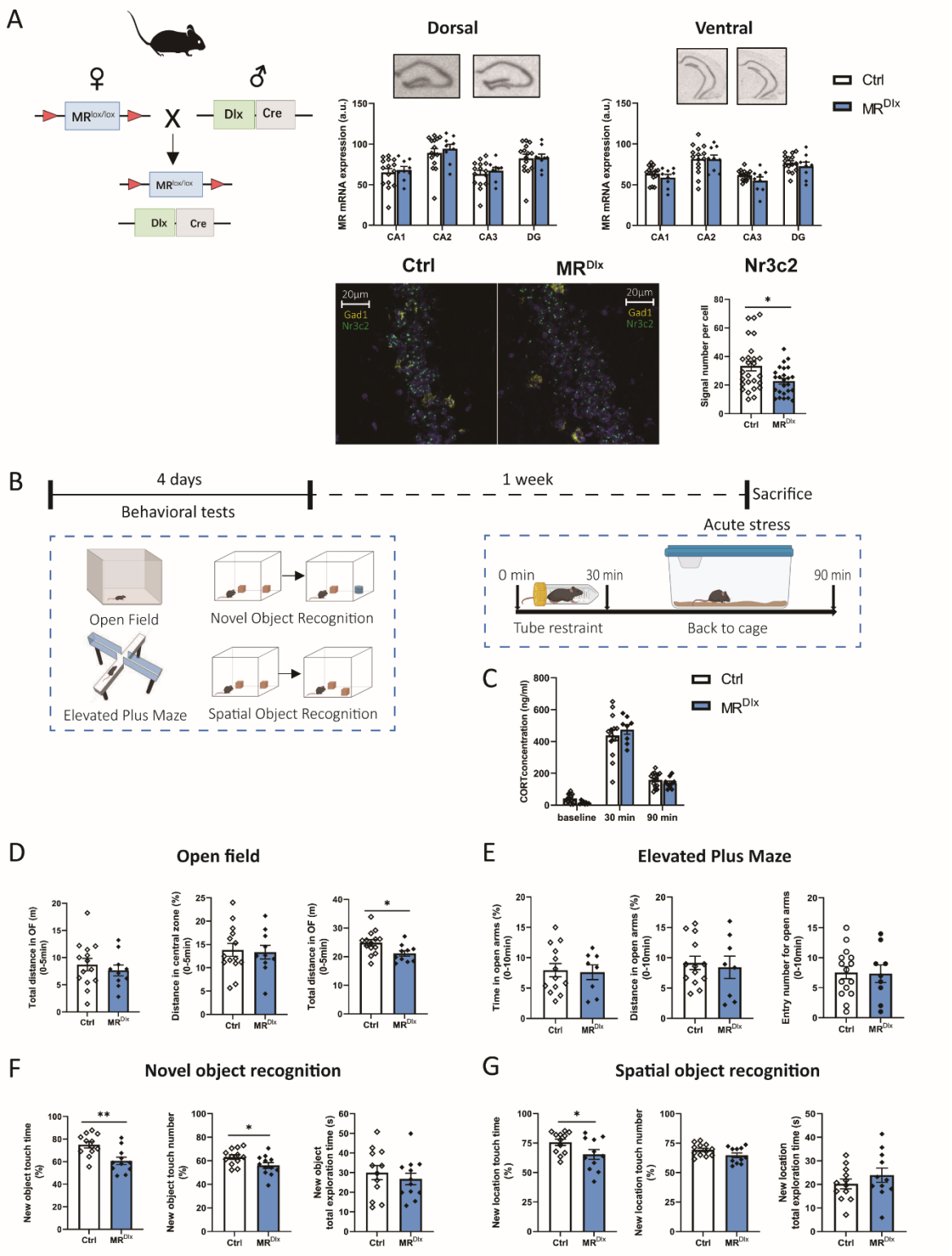


Figure 33 Breeding and validation of MR^{Dlx} mice, baseline behavioral experimental measurements. **(A)** MR^{Dlx} mice were bred by MR$^{lox/lox}$ mice with Dlx-Cre mice, but MR mRNA levels were not significantly reduced in hippocampus. **(B)** Behavioral experiments were conducted on 12-20 week old male MR^{Dlx} and Ctrl mice. One week after the behavioral experiments, these mice were exposed to acute stress paradigms. **(C)** CORT levels were significantly increased in both groups of mice following acute restraint. In OF test **(D)** and EPM test **(E)**, lacking lack of MR in GABAergic neurons has no significant effect on

behavioral performance of mice. The cognitive function of mice in the NOR test **(F)** and SOR test **(G)** was affected by the loss of MR in GABAergic neurons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.6. Spatial memory of mice lacking MR in GABAergic neurons improved under acute stress task.

In the baseline, we found that lacking MR in GABAergic neurons has an effect on the cognition of mice. Therefore, in a different cohort of mice, we evaluated home cage locomotion and stressful learning in the MWM test (Figure 34A). Regarding home cage locomotion, both the Ctrl and MR^{Dlx} mice exhibited a clear circadian rhythm, with increased activity during nighttime compared to daytime. And the results showed that MR^{Dlx} mice were more active than the Ctrl ($F_{(1,37)}=4.417$, $p=0.042$; Figure 34B).

In the MWM test, there were no apparent distinctions between the MR^{Dlx} mice and the Ctrl mice during the four days of training. Both groups showed a gradual decrease in the average time it took them to find the platform, indicating that their learning abilities were not impaired ($F_{(1, 109)}=1.693$, $p=0.196$; Figure 34C). In the probe test, both groups spent significantly more time in the target quadrant ($F_{(3, 88)}=80.35$, $p=0.000$), with the MR^{Dlx} mice exhibiting a notably longer duration ($t_{(20)}=2.453$, $p=0.024$) and distance ($t_{(18)}=2.784$, $p=0.012$) swum in this area compared to the Ctrl group (Figure 34D). Based on these findings, we consider that MR^{Dlx} mice exhibit superior spatial cognition compared to Ctrl mice.

Due to the fact that the MWM experimental procedure is stressful for mice, we questioned whether the enhanced cognitive function of MR^{Dlx} mice was a result of their stressful state. Therefore, we conducted FC experiments on another cohort of mice (Figure 34E). This experiment allows us to assess animals' learning and memory abilities under fear-inducing stress conditions. We observed that both groups of mice demonstrated good learning abilities during the five conditioned plus unconditioned stimuli on the first day, and their performances were comparable ($F_{(1, 104)}=0.161$, $p=0.689$; Figure 34F). In the contextual test conducted on the second day, the freezing time of the MR^{Dlx} group in the chamber was significantly higher than that in the Ctrl group ($t_{(22)}=2.206$,$p=0.038$; Figure 34G). However, in the cued test, the overall differences between the two groups were not evident, significant differences in freezing time were observed between the two groups of mice only during the 2nd ($t_{(24)}=3.164$, $p=0.004$) and 5th ($t_{(24)}=2.104$, $p=0.046$) conditioned stimulus (Figure 34H). These results align with our findings from the MWM test, indicating that MR^{Dlx} mice exhibit enhanced spatial memory when exposed to stress.

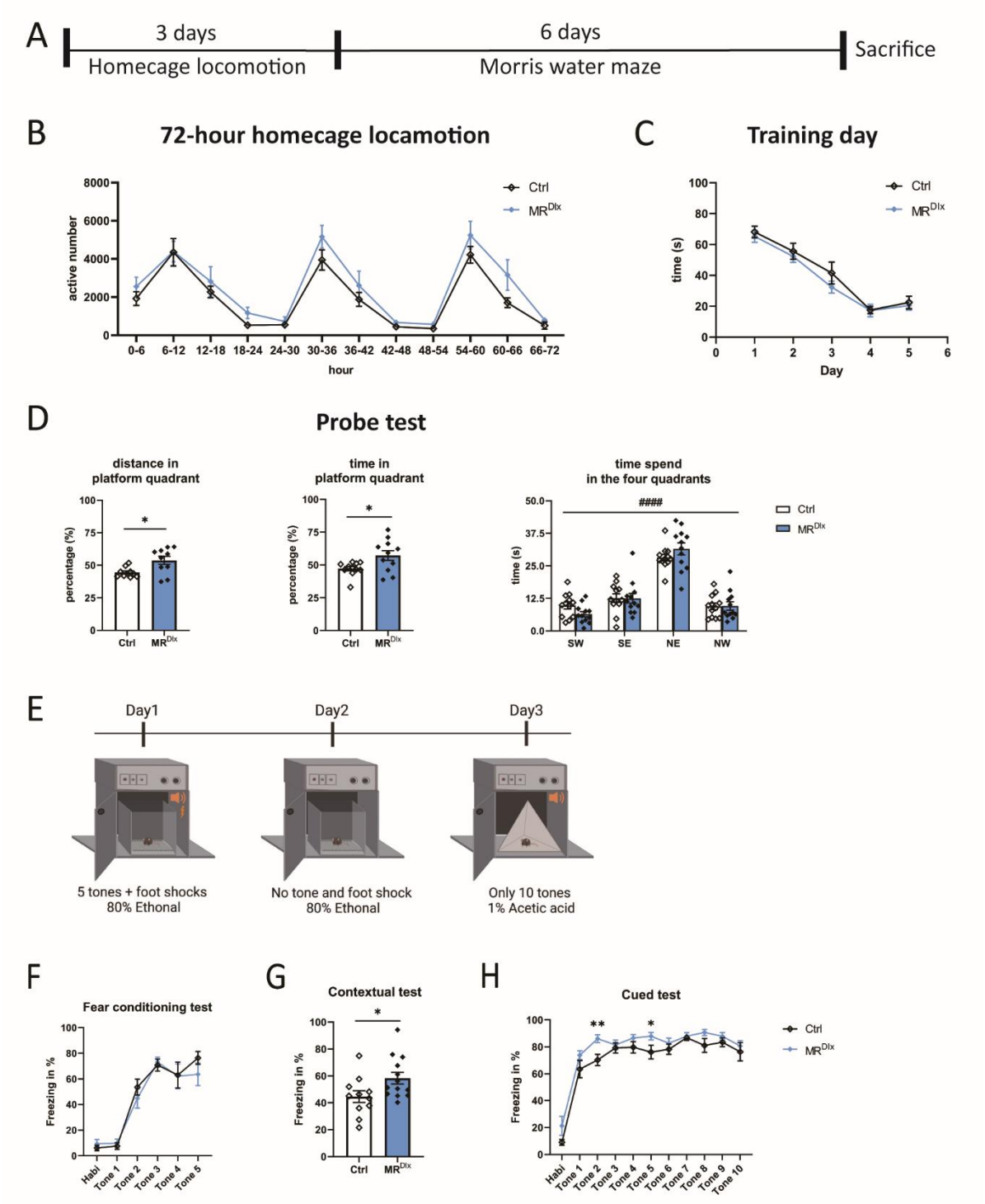


Figure 34 Experimental design and results for assessing home cage activity levels and spatial learning. (A) Experimental design. **(B)** Both MR^{Dlx} mice and Ctrl mice had obvious circadian rhythms in the activities of the home cage, but there was no significant difference between the two groups. **(C)** and **(D)** Loss of MR in GABAergic neurons improved spatial learning in mice. **(E)** Fear conditioning test procedure. **(F)**, **(G)** and **(H)** Improved memory in mice lacking MR in GABAergic neurons was associated with the same context but not the same sound. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.7 The anxiety-related behavior and cognition changes after chronic social defeat stress.

3.7.1 CSDS exerts mild effects in male MR^{Dlx} mice.

Considering that both MWM and FC are short-term stressors for mice, we also investigated whether the emotional behavior and cognitive function of MR^{Dlx} mice were altered under chronic stress conditions. Therefore, mice were exposed to chronic social defeat stress for 21 days, and behavioral tests were performed on days 15 to 18 of this treatment (Figure 35A). After the experiments, we measured the CORT level. Although there were no significant differences between genotypes ($F_{(1,38)}=0.383$, $p=0.539$), between stress treatments ($F_{(1,38)}=0.815$, $p=0.3372$), there was an interaction between genotype and stress ($F_{(1,38)}=5.995$, $p=0.019$; Figure 35B).

In OF, we found that the CSDS group spent significantly less time ($F_{(1,38)}=19.84$, $p=0.000$) and distance ($F_{(1,37)}=28.07$, $p=0.000$) in the central area of the OF arena than the non-stressed group. But there was no apparent difference between genotypes (percentage of time in central zone: $F_{(1,\ 38)}=0.068$, $p=0.796$; percentage of distance in central zone: $F_{(1,\ 37)}=1.248$, $p=0.271$; total distance: $F_{(1,\ 35)}=0.023$, $p=0.879$; Figure 35C).

In EPM, in the Ctrl group, mice subjected to CSDS exhibited a slightly lower percentage of time spent and distance traveled in the open arms compared to the no-stress group. However, there was no significant difference observed between the CSDS mice and the non-stressed mice in the MR^{Dlx} group. We can see the interaction in the percentage of time spent in open arm results ($F_{(1,38)}=4.490$, $p=0.041$; Figure 35D).

During the NOR test, MR^{Dlx} mice that experienced CSDS exhibited a higher percentage of time exploring novel objects compared to CSDS-experienced Ctrl mice, and we can see the interaction between chronic stress and genotype ($F_{(1,39)}=8.890$, $p=0.047$). However, there were no significant differences observed in frequency of exploring new object (stress: $F_{(1,\ 40)}=0.046$, $p=0.832$; geotype: $F_{(1,\ 40)}=0.029$, $p=0.867$), and total time spent exploring new object (stress: $F_{(1,\ 35)}=0.106$, $p=0.746$; geotype: $F_{(1,\ 35)}=0.370$, $p=0.547$; Figure 35E). In SOR, there is presence of an interaction effect between the groups in total object exploring time ($F_{(1,37)}=4.417$, $p=0.042$), but there was no significant difference in the percentage of time spent exploring objects in novel locations (stress: $F_{(1,\ 40)}=1.259$, $p=0.269$; geotype: $F_{(1,\ 40)}=0.075$, $p=0.786$) and the percentage of

times objects were explored in novel locations (stress: $F_{(1, 40)}=3.268$, $p=0.078$; geotype: $F_{(1, 40)}=0.790$, $p=0.379$; Figure 35F). Notably, there were some interactions observed throughout the experiment.

Taken together, we believe a modest interaction exists between chronic stress and genotype in mice lacking MR in GABAergic neurons.

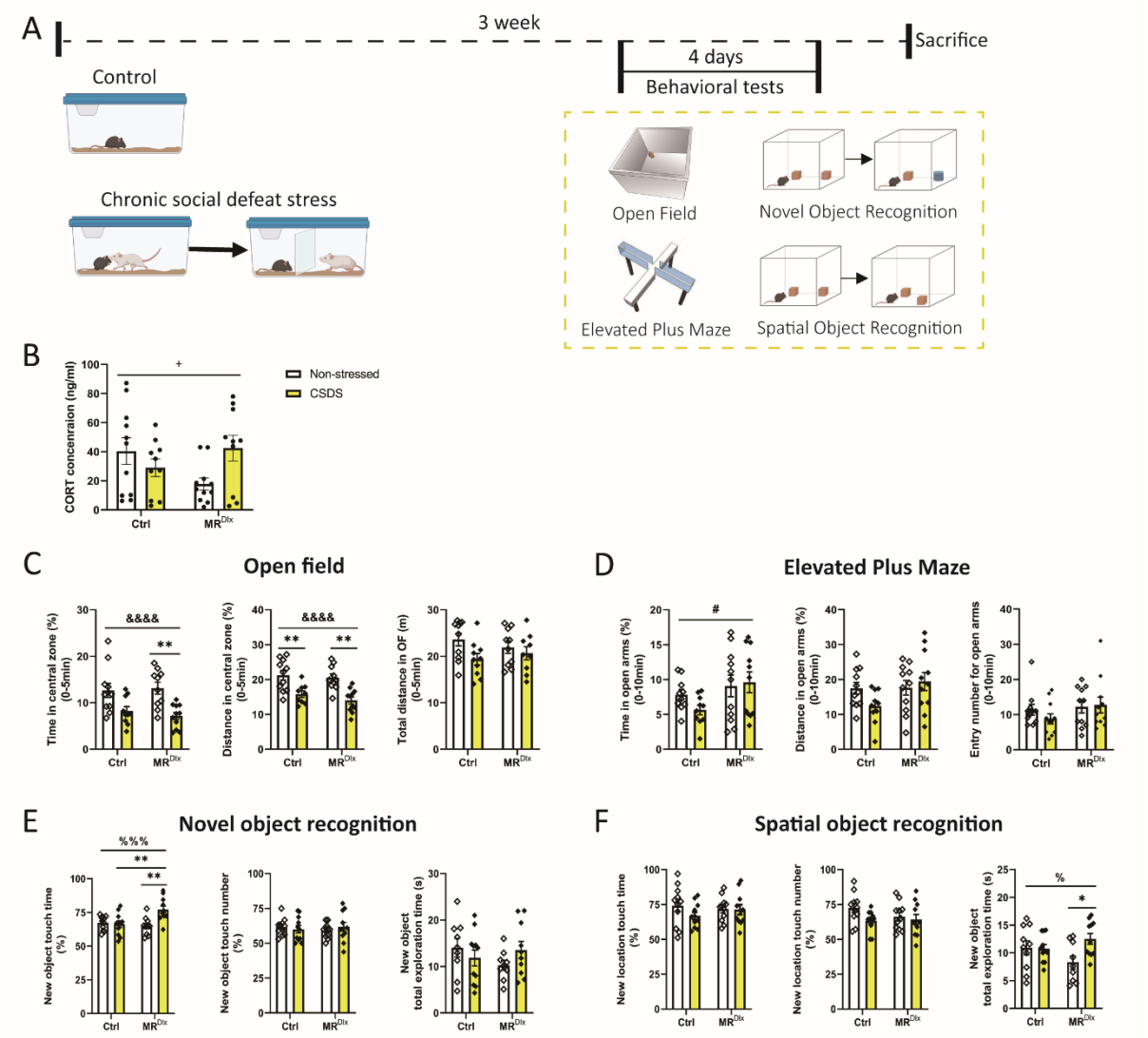


Figure 35 Behavioral studies on male mice under chronic social defeat stress. (A) The duration of the CSDS trial was 21 days, behavioral tests were carried out simultaneously during the third week of CSDS. **(B)** There was an interaction between genotype and stress in CORT level.In the OF test **(C)** After CSDS, both MR^{Dlx} mice and Ctrl mice exhibited decreased activity duration and distance in the centre of the open field arena, with no significant difference between the two groups. In the EPM test **(D)** After CSDS, both MR^{Dlx} mice and Ctrl mice exhibited decreased activity duration and distance in the open arm, with no significant difference between the two groups. In the NOR test **(E)** and SOR test **(F)**, the changes in the two groups after CSDS are not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **#** means there's a difference between different genotypes. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. **&** means there's a difference

between different treatments. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$. **+** means there's an interaction between genotype and stress. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$.

3.7.2 Different phenotype showed in Female MR^{Dlx} mice.

As previously found in studies of MR^{Nex} animals, female mice behave differently in response to stress than male mice. Therefore, in addition to male mice, we also studied female MR^{Dlx} mice. Similar to our approach with male mice, we subjected female MR^{Dlx} and Ctrl mice to CSDS experiments lasting 21 days, with behavioral tests conducted on day 15 to day 18 (Figure 36A). After the experiments, we measured the CORT level, there were no significant differences between genotypes ($F_{(1,35)}=0.001$, $p= 0.971$), between stress treatments ($F_{(1,35)}=3.040$, $p= 0.090$; Figure 36B).

In OF, no variations observed between the genotypes. There were no significant differences between MR^{Dlx} mice and Ctrl mice in the non-stressed group. However, the animals that underwent CSDS displayed reduced time ($F_{(1,34)}=28.09$, $p=0.000$) and shorter movement distance ($F_{(1, 34)}=59.28$, $p=0.000$) in the central area of the open field. In the MR^{Dlx} group, CSDS-experienced female mice also exhibited significantly less movement in the open field compared to the non-stressed ones ($F_{(1, 34)}=5.747$, $p=0.022$; Figure 36B).

In EPM results, Although the percentage of time spent in the open arm ($F_{(1, 35)}=7.637$, $p=0.009$) and the percentage of distance traveled ($F_{(1, 32)}=9.701$, $p=0.004$) spent in the open arm were lower in mice subjected to CSDS than in the non-stressed group, there were no significant differences between mice of the two genotypes. Interestingly, in the non-stress group, the percentage of time ($t_{(16)}=2.964$, $p=0.009$) and the percentage of distance ($t_{(17)}=2.429$, $p=0.026$) MR^{Dlx} mice spent in the open arm were higher than that of Ctrl mice. However, there's no genptype X stress interaction in the results (percentage of time in the open arm: $F_{(1, 35)}=1.925$, $p=0.174$; percentage of distance in open arm: $F_{(1, 32)}=2.296$, $p=0.139$; total travel distance: $F_{(1, 35)}=1.112$, $p=0.299$; Figure 36C).

In NOR test (Figure 36D), the CSDS-experienced mice showed a slightly higher percentage of time ($F_{(1, 32)}=2.296$, $p=0.139$) and number of times ($F_{(1, 32)}=2.296$, $p=0.139$) exploring the novel objects compared to the non-stressed mice. An interaction effect was observed in the overall object exploration time ($F_{(1, 32)}=2.296$, $p=0.139$). In SOR test (Figure 36E), despite percentage of time exploring new location objects and percentage of number exploring new location objects were higher than 50%, no statistical differences were observed in any of the measured

indicators between the non-stress group and CSDS group (percentage of time to explore the new position: $F_{(1, 36)}=0.234$, $p=0.631$; frequency of exploring new position: $F_{(1, 36)}=0.154$, $p=0.697$; total time spent exploring new position: $F_{(1, 36)}=1.632$, $p=0.209$), as well as genotype did not affect the results (percentage of time to explore the new position: $F_{(1, 36)}=0.840$, $p=0.365$; frequency of exploring new position: $F_{(1, 36)}=0.318$, $p=0.576$; total time spent exploring new position: $F_{(1, 36)}=0.592$, $p=0.446$).

Similar to the experimental results of MR^{Nex} mice. Emotional behavior and cognitive performance of female MR^{Dlx} mice are inconsistent with male MR^{Dlx} mice.

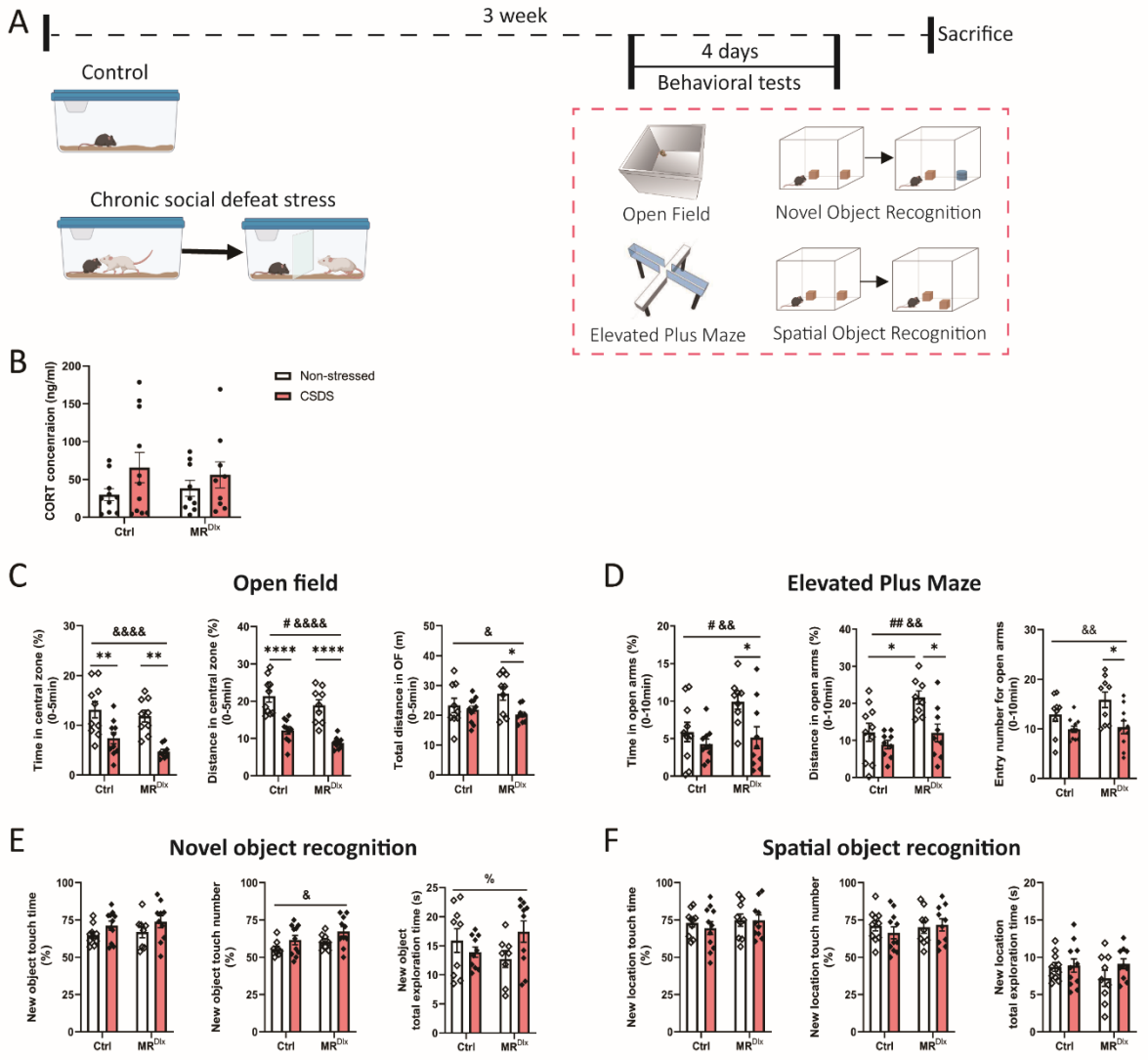


Figure 36 Behavioral studies on female mice under chronic social defeat stress. (A) The duration of the CSDS trial was 21 days, behavioral tests were carried out simultaneously during the third week of CSDS. **(B)** Their CORT levels of female mice did not differ significantly between the groups. **(C)** After CSDS, both MR^{Dlx} mice and Ctrl mice exhibited decreased activity duration and distance in the centre of the open field arena, with no significant difference between the two groups. **(D)** MR^{Dlx} mice were less

anxious than Ctrl under non-stressed conditions, but there was no significant difference between genotypes after CSDS. **(E)** and **(F)** In NOR test and SOR test, the changes in the two groups after CSDS are not significant. *$*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **#** means there's a difference between different genotypes. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. **&** means there's a difference between different treatments. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

3.8 CORT affects LTP in mice lacking MR in GABAergic neurons.

According to previous experiments, we found that the cognitive function of MR^{Dlx} male mice was improved when they were under transient stress, but the cognitive function was not significantly improved when they were under chronic stress. So we tried to perform electrophysiological experiments on male MR^{Dlx} mice and Ctrl mice after stress exposure in vitro. We divided them into vehicle group and CORT group respectively. In the vehicle group (5 Ctrl, 8 brain sections; 6 MR^{Dlx}, 10 brain sections), we used 0.01% EtOH, and in the CORT group (5 Ctrl, 8 brain sections; 5 MR^{Dlx}, 8 brain sections), we use 1 μM CORT (Figure 37A).

Interestingly, we found that in the vehicle group, there was no significant difference in the average fEPSP slope of LTP between MR^{Dlx} mice and Ctrl mice (p=0.9878; Figure 37B). However, after CORT treatment, the average LTP fEPSP slope of Ctrl male mice decreased, while the average LTP fEPSP slope of MR^{Dlx} male mice did not change significantly. Finally, in the CORT group, the average LTP fEPSP slope of Ctrl mice was significantly lower than that of MR^{Dlx} mice (p=0.026), but there was no interaction effect between genotype x CORT ($F_{(1, 30)}$=3.831, p=0.0597; Figure 37C). In addition, there was no significant difference in the paired-pulse ratio between MR^{Dlx} mice and Ctrl mice in either the vehicle group ($F_{(1, 80)}$=0.219, p=0.641) or the CORT group ($F_{(1, 70)}$=0.230, p=0.633; Figure 37D). This may be the reason why the cognitive function of mice lacking MR in GABAergic neurons was better than that of Ctrl mice after short-term stress in previous behavioral experiments.

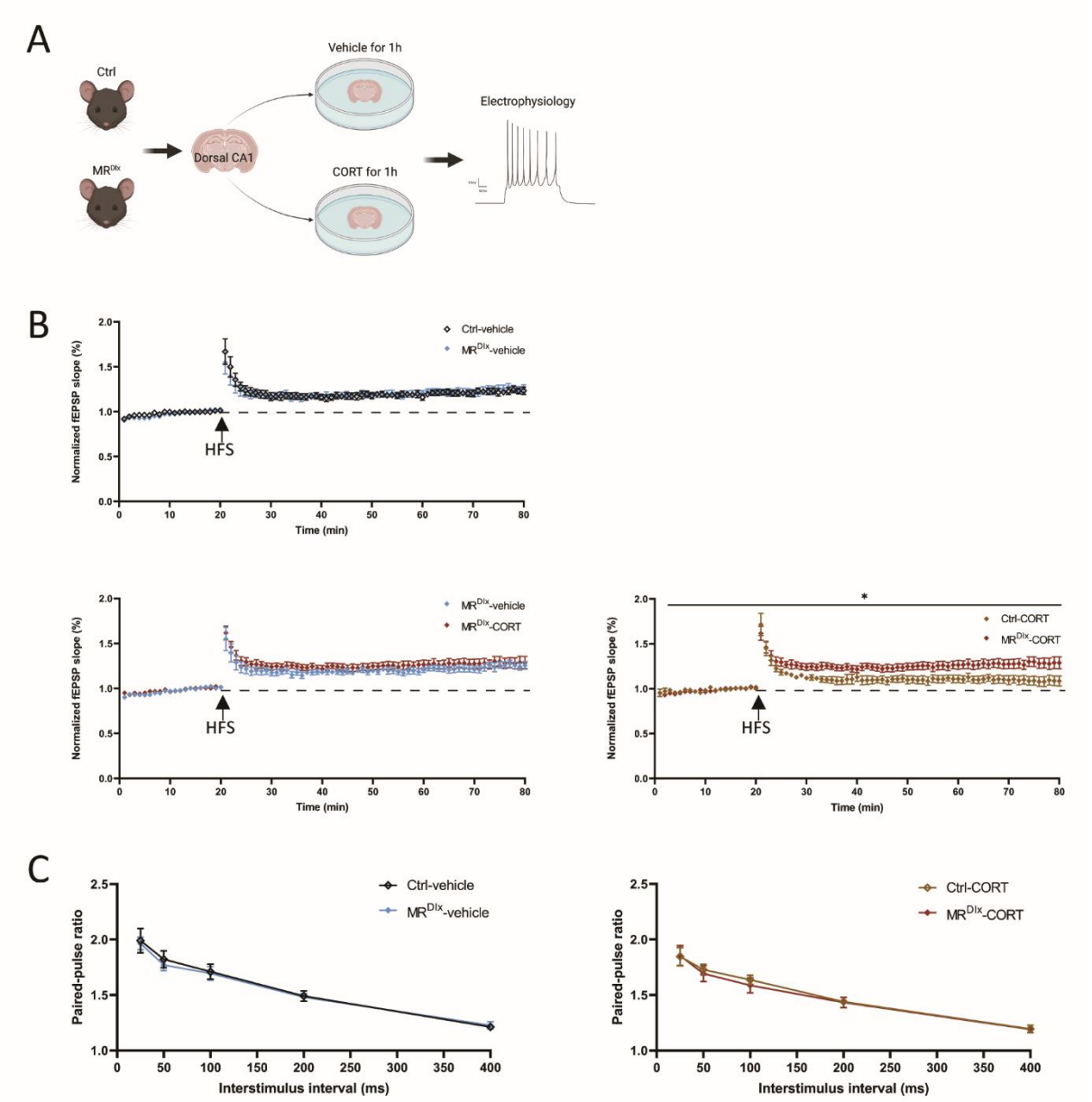


Figure 37 The function and structure changes in MR^{Dlx} mice. (A) Experimental design. **(B)** Average LTP fEPSP slope was reduced in Ctrl male mice but was unaffected in MR^{Dlx} male mice following CORT treatment. **(C)** The paired-pulse ratio of MR^{Dlx} mice and Ctrl mice was not significantly different in vehicle group and CORT group. **p < 0.05, **p < 0.01, ***p < 0.001*.

3.9 Differential genes in single-cell RNA sequencing

For the single-cell RNA sequencing data of MR^{Dlx} mice, the GABAergic neuronal cluster was the focal point of interest. As shown in Figure 38A, there were numerous differentially expressed genes in the baseline group of MR^{Dlx} mice, while only a small number of differentially expressed genes were observed in the stress group.

Within GABA cluster, there were 1040 differentially expressed genes in total. A total of 400 genes were selected based on fold change>|0.5|, and significant differences between Ctrl and MR^{Dlx} at the baseline, or between the non-stressed group and stress group within each genotype. To ensure sufficient expression levels, a threshold>0.5 was set. Ultimately, 24 genes were discovered that exhibited differential expression exclusively in GABAergic neurons and not in other cells. Notably, Npy was identified among these genes (Figure 38C). Npy is a neuropeptide that is highly expressed in certain subsets of GABAergic neurons in the brain and plays a crucial role in regulating stress responses and emotional behavior (Kupcova et al., 2022; Lach & de Lima, 2013). Subsequently, Npy was validated in RNAScope, consistent with single cell RNA sequencing data, the expression of Npy in MR^{Dlx} was significantly less than that in Ctrl ($t_{(42)}=2.866, p=0.006$). This gene will be manipulated in the hippocampus to determine its effects on behavior or cognition.

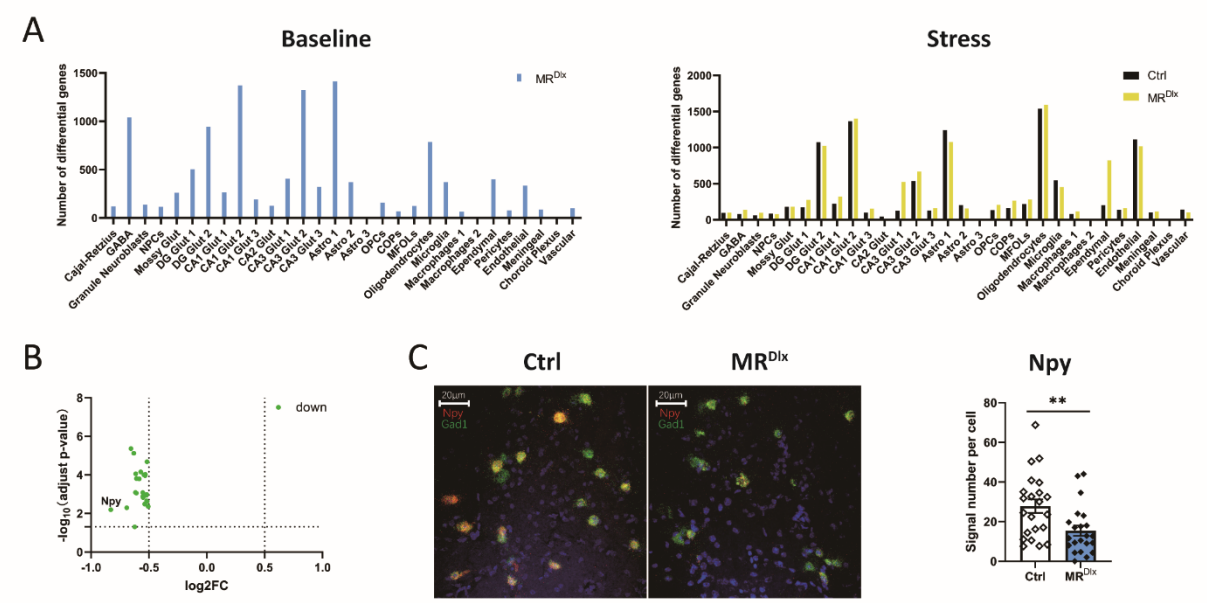


Figure 38 Single-cell RNA sequencing data of MR^{Dlx} animals. (A) Histogram showing the number of differential genes in the baseline of MR^{Dlx} animals and the number of differential genes in MR^{Dlx} animals during stress induction. **(B)** Volcano plot showing the Log2FC pattern of differential genes. Npy was selected for further validation. **(C)** Validation of Npy in MR^{Dlx} mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4 Discussion

In my doctoral study, I conducted several studies utilising the mouse model lacking MR in glutamatergic neurons and the mouse model lacking MR in GABAergic neurons, in order to better understand how the absence of MR in particular neurons in specific brain areas affects emotions, behavior, and cognition. These results provide valuable insights into the potential therapeutic target and biomarker role of MR in stress-related psychiatric disorders (Figure 39). The thesis study showed some interesting differences in the emotional behavior and cognitive function of MR-deficient mice, both in the glutamatergic and GABAergic cell types. Specifically, lack of MR in glutamatergic neurons was shown to result in an anxiety-like phenotype, impact glutamatergic neurotransmission, and alter morphology of pyramidal neurons, leading to reduced presynaptic glutamate neurotransmitter release. However, overexpressing our gene of interest Fam107a with AAV virus led to partial rescue of the phenotype. Conversely, the absence of MR in GABAergic neurons did not increase anxiety-like behavior, and instead enhanced spatial memory ability under stressful conditions. It seems to have a beneficial effect on cognitive function under stressful conditions.

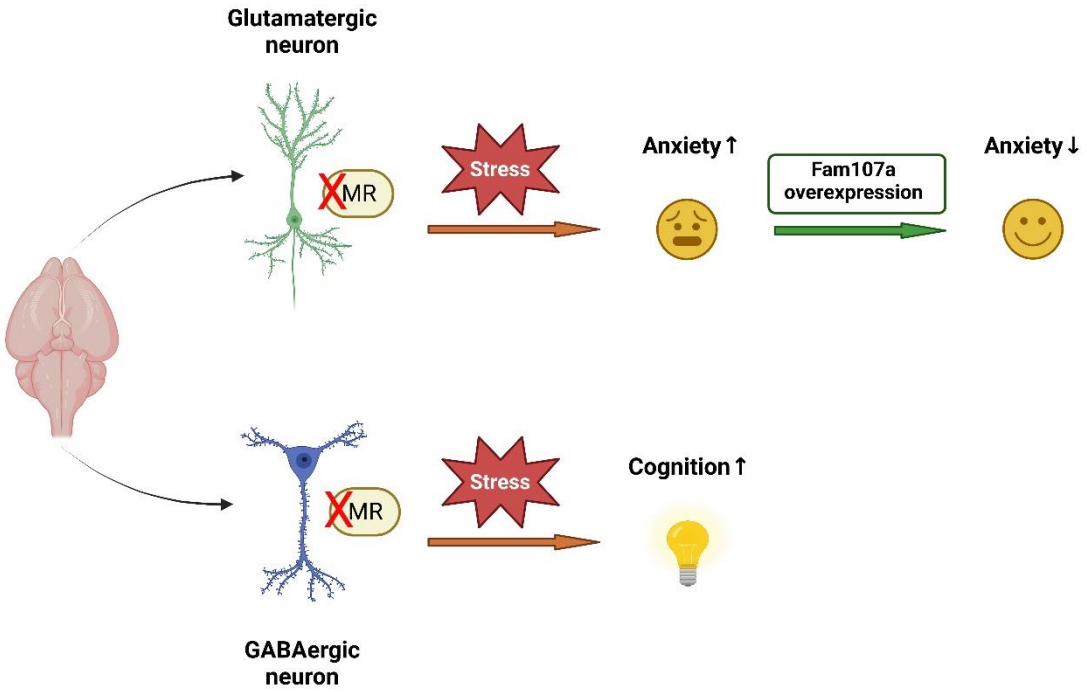


Figure 39 Illustration of thesis study. The absence of MR in glutamatergic neurons results in an anxiety-like phenotype, which is partially rescued by overexpression of Fam107a in glutamatergic neurons. Under stressful conditions, the absence of MR in GABAergic neurons boosts spatial memory.

4.1 MRs play different roles in glutamatergic and GABAergic neurons.

Previous studies have investigated the functional role of MR in the brain. It was found that male mice with embryonic whole-brain deletion of MR exhibited obvious anxiety-like behavior, while male mice with CA2-targeted deletion of MR had no obvious anxiety performance. (McCann et al., 2021). Blocking MR specifically in the hippocampus appears to produce an anxiolytic response (Korte et al., 1995; Smythe et al., 1997). But few studies have gone further to explore the role of MR expression in specific neurons. Therefore, in this thesis, excitatory glutamatergic neurons and inhibitory GABAergic neurons have been selected as research targets to explore the impact of deleting MR in specific neurons on emotional behavior and cognition. Glutamatergic neurons and GABAergic neurons are the two main types of neurons in the central nervous system. Glutamatergic and GABAergic neurons have distinct roles in mediating excitatory and inhibitory signaling, respectively, but a delicate balance between them is crucial for maintaining overall brain homeostasis and ensuring proper function of neuronal networks. Imbalances in this equilibrium can contribute to the development of a variety of neurological and psychiatric disorders (Talebian et al., 2017; Ye et al., 2015).

4.1.1 Lacking MR in glutamatergic neurons showed anxiety-like behavior.

Glutamatergic neurons, characterized by their utilisation of the neurotransmitter glutamate, are excitatory neurons that transmit signals throughout the brain. They are the most abundant neuronal subtype in the central nervous system and are involved in a wide range of physiological processes, including the regulation of emotions, learning, memory, and motor control (Cortese & Phan, 2005; McGrath et al., 2022). Glutamatergic neurons play a crucial role in the pathophysiology of psychiatric disorders. Symptoms of psychiatric disorders such as major depressive disorder, bipolar disorder, anxiety disorders, and schizophrenia are associated with dysregulated glutamate signaling and impaired synaptic plasticity (Henter et al., 2018; Kruse & Bustillo, 2022; J. Liu et al., 2022; Ménard et al., 2014; Nakahara et al., 2022; Sartori & Singewald, 2019). Several glutamatergic drugs have been found to be effective in alleviating mood symptoms in patients with psychiatric disorders (Griebel & Holmes, 2013; Henter et al., 2018).

In this thesis study, I first evaluated the behavior of mice lacking MR in glutamatergic and GABAergic neurons at baseline, aiming to investigate the impact of MR deficiency on mouse behavior in the absence of stress. During the home cage activity test, mice lacking MR in

glutamatergic neurons exhibited similar behavior to control mice, showing no apparent signs of anxiety and displaying clear diurnal rhythms. However, in conventional behavioral tests used to assess animal anxiety, such as the OF test and EPM test, these mice showed significant anxiety-like behaviors. Interestingly, no significant cognitive changes were observed in cognition-related behavioral experiments. This discrepancy may be attributed to the home cage, which is a familiar environment for mice and provides a relatively stable and non-stressful setting that may mask some potential behavioral changes resulting from the lack of MR signaling in these mice. On the other hand, the behavioral testing environments are novel and more restrictive, making them more likely to elicit strong anxiety responses. It is known that MR plays a role in regulating stress responses and is involved in the HPA axis, which is crucial for maintaining internal balance during stress. The increased anxiety phenotype observed in male mice lacking MR in glutamatergic neurons suggests that MR is involved in the modulation of anxiety-related behaviors. While there may be some association between anxiety and cognitive function, the lack of significant cognitive changes suggests that the regulatory pathways and mechanisms of cognitive function may differ from those of anxiety and are relatively independent. The presence of diurnal rhythms indicates that the intrinsic circadian mechanisms in these mice lacking mineralocorticoid receptors remain intact. This suggests that MR may not play a dominant role in regulating diurnal rhythms, and other clock-related molecules and pathways may compensate for their absence, ensuring the normal expression of diurnal rhythms.

Because of the above-mentioned phenotypic differences found in this thesis study, I decided to further explore the effect on the function and structure of specific neurons after loss of MR.

In electrophysiological experiments, LTP is a widely recognized phenomenon associated with learning and memory in the nervous system, where it involves persistent increases in synaptic strength and serves as a crucial mechanism for efficient information transmission between neurons (Bliss & Collingridge, 1993; Lynch, 2004; Nicoll, 2017). In this thesis study, the absence of MR in glutamatergic neurons did not exert a significant impact on the expression of LTP. These findings suggest that MR in glutamatergic neurons may not directly influence the manifestation of LTP. Nevertheless, considering the intricate role of MR in hippocampal function, it is plausible that these results are contingent upon the intricate interplay of multiple signaling pathways. The paired-pulse ratio is a valuable metric employed to assess synaptic transmission between neurons, providing insights into changes in neurotransmitter release

probability and synaptic transmission efficacy (Linders et al., 2022). Increased paired-pulse ratios may implicate inhibition or attenuation of presynaptic neurotransmitter release, indicating a decreased probability of glutamate release in the absence of MR. This observation potentially signifies the involvement of MR in the excitability and signaling of neurons. In addition, lower frequency and amplitude of EPSCs may indicate reduced excitatory synaptic activity. These findings highlight the importance of MR in regulating glutamatergic neurotransmission, which is critical for information processing and neuronal communication in neural circuits. Research has indicated that within the hippocampus, the presence of MRs contributes to the regulation of glutamatergic transmission, ensuring its proper functioning (Karst et al., 2010). Through the nongenomic fast-mediated effect of membrane MR, the stress hormone CORT has the ability to enhance the release of glutamate, resulting in rapid plasticity at glutamatergic synapses and facilitating LTP of glutamatergic neurons (Karst et al., 2005b; Wiegert et al., 2006). This process is thought to support learning, memory formation, and the development of behavioral adaptations (Chaouloff & Groc, 2011; Mikasova et al., 2017; Sarabdjitsingh et al., 2014). In the thesis study, the absence of MR in glutamatergic neurons within the hippocampus resulted in the absence of a substantial increase in LTP, thus confirming the findings of previous research. The current thesis unveiled that the absence of MR in glutamatergic neurons might be associated with reduced frequency and amplitude of presynaptic vesicle release of glutamate neurotransmitters. However, it is important to note that the data obtained through whole-cell patch clamp recordings provide indirect evidence, necessitating further experiments to directly measure neurotransmitter release from extruded vesicles.

Dendritic morphology is critical for synaptic connectivity, plasticity and neuronal communication. Alterations in dendritic arborization and spine density disrupt normal neural circuits, affecting the integration and processing of incoming signals. The structural changes in dendritic morphology observed in this thesis study may help to align with observed behavioral outcomes, such as anxiety-like behavior. Research investigating neuronal morphology has demonstrated that the circadian rhythm of corticosterone can modulate spine morphology. This modulation is likely mediated by membrane-bound MRs and GRs (Liston et al., 2013). Learning training during the peak of the circadian corticosterone levels promotes the formation of dendritic spines, while stability of newly formed spines is facilitated during the troughs of the circadian cycle (Liston et al., 2013). It is noteworthy that MR and GR play distinct roles in synaptic membrane function. Membrane-bound GR primarily influences dendritic spine growth,

whereas MR is involved in regulating dendritic spine structure (Russo et al., 2016). Immunoreactivity studies have revealed that MR is predominantly expressed in dendrites and dendritic spines (Prager et al., 2010). In this thesis study, the absence of MR in glutamatergic neurons led to a reduction in dendrite length and spine number, supporting the previous findings and aligning with the observed electrophysiological properties of these neurons. However, further studies are needed to determine the specific mechanisms by which MR regulates dendrite morphology of glutamatergic neurons.

According to a previous study, anxiety can be caused by hypoactivity of GABAergic neurons and/or an increase in the activity of glutamatergic neurons (Agoglia & Herman, 2018). In this thesis study, mice lacking MR in glutamatergic neurons exhibited increased anxiety-like behavior in behavioral experiments. However, concurrently, there was a reduction in dendritic length and dendritic spine density in pyramidal neurons. Electrophysiological experiments indicated a potential decrease in glutamate release and a reduction in excitatory synaptic activity. These contradictory findings may be attributed to the complementary roles of MR and GR in regulating stress responses, where the absence of MR may enhance GR signaling, leading to alterations in the regulation of neuronal stress responses (de Kloet et al., 2019). MR is typically associated with baseline cortisol levels, while GR responds to higher levels of cortisol (de Kloet et al., 1998; Joëls & de Kloet, 1994). In stress responses, if MR function is inhibited or absent, it can result in increased GR signaling, and CORT may increase glutamate release through GR, thereby enhancing neuronal excitability and promoting anxiety-like behavior (Peng et al., 2021; Treccani et al., 2014).

4.1.2 Lacking MR in GABAergic neurons affected the cognitive function.

On the other hand, GABAergic neurons, which employ gamma-aminobutyric acid (GABA) as their neurotransmitter, are inhibitory neurons. These neurons are predominantly found in the hippocampus, thalamus, basal ganglia, hypothalamus, and brainstem, and they participate in various physiological and behavioral activities, including anxiety and fear regulation, memory, and information processing (Allen et al., 2023). Numerous studies have identified key roles of GABAergic signaling in the pathogenesis and pathology of various psychiatric disorders (Fogaça & Duman, 2019; Mahdavi et al., 2018; Mueller & Meador-Woodruff, 2020; Pizzo et al., 2018; Zhang et al., 2013). GABA is not only the main neurotransmitter required for the action of the classic anti-anxiety drug benzodiazepines, but also the target of many anticonvulsant, anesthetic and cognitive-improving drugs (Ghit et al., 2021; Griebel & Holmes, 2013).

In the experiments with mice lacking MR in GABAergic neurons, I found that their performance in the home cage activity test was similar to that of control mice, and they did not exhibit significant anxiety-like behaviors in the behavioral experiments. However, interesting changes were observed in cognition-related tests. Under non-stressful conditions, the performance of mice lacking MR in GABAergic neurons in cognition-related behavioral experiments was not as good as that of control mice, but in behavioral tests involving a certain level of stress, these mice performed better than the control mice. This suggests that glucocorticoids may act via GABAergic MRs would suppress the ability to remember stress-related spatial contexts. the deficiency of MR in GABAergic neurons may trigger an adaptive response that leads to improved behavioral outcomes when dealing with stress.

However, it should be notice that the purpose and underlying mechanisms of situational fear conditioning and cued testing differ significantly. Contextual fear conditioning involves associating an aversive or fear-inducing stimulus with the environmental context in which it occurs. The neural circuits primarily involved in situational fear regulation are the basolateral amygdala (BLA), which receives information about the situation and integrates it with fear-related signals. Additionally, the hippocampus is critical for contextual fear regulation as it processes spatial and situational information and interacts with the amygdala to encode and retrieve contextually relevant fear memories (W. B. Kim & Cho, 2017, 2020; Tovote et al., 2015). The goal of the extinction phase is to weaken or eliminate the conditioned fear response by repeated presentations of neutral stimuli without an aversive event (Whittle et al., 2021). The extinction phase provides insight into the process by which fear memories are formed, consolidated, and the brain's ability to modify or eliminate fear responses. A key mechanism for this process involves the activation of inhibitory circuits in the amygdala, a region of the prefrontal cortex that is thought to suppress or modulate amygdala activity and facilitate the elimination of fear responses (Bouton, 2002; LeDoux, 2000; Whittle et al., 2021). More experiments are needed to explore the mechanisms underlying the changes in spatial memory due to the absence of GABAergic neurons lacking MR. This investigation should encompass not only the hippocampus but also the amygdala.

This thesis study highlights that the role of MR varies among different types of neurons. MR plays a critical role in regulating stress responses. In glutamatergic neurons, the absence of MR may lead to dysregulation of stress responses, resulting in anxiety-like behavior. However, in GABAergic neurons, the MR deficiency did not exhibit significant changes in anxiety-related

behaviors, which may be attributed to the absence of MR may exert inhibitory effects on stress responses, contributing to stress resilience. Nevertheless, further research is needed to elucidate the specific regulatory and coping mechanisms involved in stress responses.

4.2 Stress type differentially affects the deficit of MR in specific neurons.

Various forms of stress affect our daily lives, and multiple studies have demonstrated that different stressors have distinct effects on individuals (Hammen et al., 2009; Marshall & Garakani, 2002; McGonagle & Kessler, 1990; Sandrini et al., 2020; Yoshida et al., 2021). This thesis focuses on two specific types of stress: acute stress represented by acute restraint, and chronic stress represented by chronic social stress. The aim is to investigate the impact of stress on the absence of mineralocorticoid receptors (MR) in glutamatergic neurons and GABAergic neurons.

Previous studies have consistently indicated that chronic social stress can induce depression- and anxiety-like behaviors (Becker et al., 2008; Keeney & Hogg, 1999; Kinsey et al., 2007; Rygula et al., 2005; Slattery et al., 2012). Decreased expression of hippocampal mineralocorticoid receptors, along with increased anxiety and depression-like behaviors, has been associated with stress vulnerability (Schmidt et al., 2010). This may be due to the overactivation of the HPA axis caused by chronic stress, leading to elevated levels of stress hormones and ultimately resulting in the manifestation of anxiety-like behavior. Additionally, chronic stress exposure can activate limbic systems such as the hippocampus, amygdala, and prefrontal cortex (Deslauriers et al., 2020; Yoshida et al., 2021), potentially resulting in increased glutamate release and elevated expression of glutamate receptors (C.-H. Chang et al., 2015; Moghaddam et al., 1994). Consistent with previous studies, the current research demonstrates that the lack of MR in glutamate neurons is associated with higher levels of anxiety in the chronic social stress group, while the loss of MR in GABAergic neurons does not produce an anxiety-like phenotype at baseline. However, after experiencing chronic social stress, mice lacking MR in GABAergic neurons showed more anxiety behavior in OF, but no significant behavioral changes in the EPM test. There was a moderate interaction between chronic stress and genotype effect. This illustrates that loss of MR may have differential effects on different types of stress responses.

Stress can either enhance or impair learning and memory, depending on whether synapses persist or decline after learning (Arango-Lievano et al., 2019). Chronic stress has been found to

impair spatial reference memory and transiently affect spatial working memory. However, the effects of chronic stress on spatial learning appear to be task-specific (Conrad, 2010). Neuroplasticity in glutamatergic neurons is believed to play a significant role in chronic stress and mood disorders (Pal, 2021). Chronic stress leads to increased glutamate release, impaired LTP, dendritic shrinkage, and deficits in learning and memory in the hippocampus (Reznikov et al., 2009), and also has been shown to impair performance in glutamate-dependent spatial learning tasks (Cortese & Phan, 2005; Isgor et al., 2004; Luine et al., 1994), the behavioral experiments conducted in this thesis revealed that mice lacking MR in glutamate neurons did not exhibit significant cognitive impairment, particularly in terms of spatial memory and learning, regardless of the presence or absence of stress. This suggests that the absence of MR in glutamate neurons may confer protection of spatial cognition.

Although mice lacking MR in GABAergic neurons did not show significant cognitive changes following CSDS compared to the control group in the MWM and FC tasks, their learning and memory improved. For example, these mice exhibited shorter escape latencies in the MWM test and longer freezing times in the FC contextual test. In those two experiments, the mice experienced acute stress by briefly being exposed to water, and exposed to conditioned and unconditioned stimuli. Consequently, in this study, cognitive tests were conducted on mice lacking MR in GABAergic neurons after acute restraint stress, and the results were consistent with the previous findings. Since the elevation of basal CORT levels following chronic social stress was not significant, whereas CORT concentration markedly increased after acute stressors like restraint, this thesis study used CORT treatment in the electrophysiological experiments to simulate acute stress conditions. After CORT treatment, LTP in the control group was significantly decreased, which is consistent with previous studies finding that acute stress or CORT administration blocks hippocampal LTP (Ahmed et al., 2006; Garcia et al., 1997; Pavlides et al., 1996; Shors & Thompson, 1992; Takeda et al., 2012). When GABAergic neurons lacked MR, there was no significant change in LTP. These findings suggest that the lack of MR in GABAergic neurons may actually serve as a protective factor against the effects of stress, particularly in relation to spatial cognitive function.

Unlike chronic stress, numerous studies have indicated that acute stress has negative effects on cognitive processes (Plessow et al., 2011; Raio et al., 2013; Roozendaal et al., 2004; Roozendaal, McEwen, et al., 2009; von Dawans et al., 2021). For example, it can impair working memory (Barsegyan et al., 2010; Duncko et al., 2009; Roozendaal et al., 2004; Schoofs et al.,

2008), decrease cognitive flexibility (Alexander et al., 2007; Plessow et al., 2011; Shields et al., 2016), and weaken goal-directed behavior and self-control (Heatherton & Wagner, 2011; Maier et al., 2015; Plessow et al., 2012; Quaedflieg et al., 2019). Previous research has suggested that the negative impact of acute stress on cognitive function is primarily mediated by the prefrontal cortex, and it is associated with increased cortisol and catecholamine levels (Arnsten, 2009; Arnsten et al., 2012; Datta & Arnsten, 2019; Plessow et al., 2012). However, some studies have indicated that the performance of working memory and cognitive regulation of conditioned fear responses may be impaired independently of cortisol elevation (Langer et al., 2020; Ponce et al., 2019). The time-dependent effects of cortisol on mental performance may explain this variation (Pan et al., 2023). Nongenomic effects mediated by membrane receptors occur immediately when cortisol levels rise rapidly, synergizing with catecholamine effects to activate the prefrontal cortical network (Joëls et al., 2006). Conversely, slower genomic pathways appear to restore PFC function after stress exposure, promoting recovery of cognitive function (Diamond et al., 2007; Henckens et al., 2011; Pan et al., 2023). This may explain the behavioral differences in the acute and chronic stress states in this thesis study.

4.3 Sex specificity of MR function in neurons.

For a considerable period, experiments predominantly focused on male animals due to the estrous cycle in females. As a result, our understanding of potential sex differences underlying the pathogenesis of mental disorders has been limited. But new research points to some misconceptions about sex research, suggesting that female mice are not simply smaller versions of males, and that hormone levels of females do not lead to greater variability in data interpretation than males (White et al., 2021). When compared to male mice, the estrous cycle does not contribute to a larger degree of variety in female behavioral characteristics (Dieterich et al., 2021; Short et al., 2022). Hence, this thesis study aimed to address this gap by conducting experiments on both male and female mice. The study not only examined emotional behavior and cognitive function in male mice, but also performed the same behavioral experiments on female mice to explore whether there is a sex difference in the lack of MR in glutamatergic versus GABAergic neurons. In order to allow a direct comparison of sexes following chronic stress, the CSDS paradigm used by Harris (2018) and Lotte (2020) for female mice was adopted in the research of this thesis. This procedure induces male CD1 mice to attack female mice by smearing the male mouse's urine on the female's body (especially the vaginal opening). The findings of this thesis study revealed that the a of MR in glutamatergic neurons did not result in similar anxiety phenotypes in females. Additionally, the absence of MR in GABAergic neurons

did not produce comparable changes in cognitive function among females.

It is well known that sex is a significant influential factor in behavioral differences, which is the result of complex interplay between genetic, hormonal and environmental factors (Gobinath et al., 2017). Acute stress and chronic stress have different effects on rats of different sexes (Lu et al., 2015). The study of McCormick et al. (2008) found sex differences after experiencing chronic social differences. Chronic social stress given to juvenile rats reduced female anxiety-like behavior during the juvenile period, and had no effect on males. However, when they were adults, female anxiety-like behavior was found to be decreased with age, in contrast to increased anxiety-like behavior in males. Several studies showed that chronic stressors impair cognitive function in males, whereas female may exhibit cognitive resilience to chronic stressors protected by estrogen from the harmful effects of stress (Bowman et al., 2006; Luine, 2016; Luine et al., 2017; Wei et al., 2014). This process may be mediated by estrogens' effects on the HPA-axis (Brand et al., 2021).

There have been some studies on sex differences in MR, but mainly in the direction of cardiovascular disease and renal disease (Barris et al., 2023; Komukai et al., 2010; Moss et al., 2019; Nicolaou, 2021). However, the effects of MR on sex in the brain have been less studied. One study found that when comparing the emotional and cognitive responses of male and female mice, the loss of forebrain MR causes striking disparities (Ter Horst et al., 2012). A study that deleted MR in embryos also found a sex difference, with male mice, but not female mice, showing significantly increased anxiety-like behavior (McCann et al., 2021). GR and MR in different sexes seem to play opposite roles in the HPA axis when in a depression-like state. The HPA axis reactivity in female mice was reduced and was further decreased by the decrease in MR expression (Bonapersona et al., 2019). Extinction of contextual fear was not observed in female mice with a forebrain-specific deletion of MR (Ter Horst et al., 2012). However, the FC test was not performed on female mice in this study, and it is unclear what effect the deletion of MR in glutamatergic neurons and GABAergic neurons will have on sex.

The results obtained from this thesis study suggest that the neurobiological mechanisms underlying anxiety and cognitive processes differ between males and females and that MR signaling is a significant contributor to these behaviors exclusively in males. These sex differences may be influenced by various factors such as hormones and genetics. However, the specific mechanisms leading to these sex differences require further in-depth investigation.

4.4 MR target genes in the hippocampus.

The hippocampus plays a crucial role in emotional memory and learning. The dorsal hippocampus and ventral hippocampus are two distinct functional regions within the hippocampus (J. Wang & Barbas, 2018). The dorsal region of the hippocampus is primarily involved in processing and encoding information related to memory and learning, whereas the ventral hippocampus plays a key role in regulating emotional processes, such as fear and anxiety (Fanselow & Dong, 2010; C. Wang et al., 2020; Xia & Kheirbek, 2020). Notably, the ventral hippocampus is particularly susceptible to the effects of chronic stress and serves as a specific region involved in modulating susceptibility to chronic social defeat stress (Bagot et al., 2015; Bannerman et al., 2004). Therefore, when it was discovered in the thesis study that the absence of MR in glutamatergic neurons can increase anxiety-like behavioral performance, subsequent research focused primarily on the ventral hippocampus. In light of the observed differences in cognitive function following the absence of MR in GABAergic neurons, the focus of the study will be directed towards the dorsal hippocampus.

4.4.1 Manipulation of Fam107a in glutamatergic neurons lacking MR.

It was found that the expression of Fam107a gene was significantly decreased in the ventral hippocampus of mice lacking MR in glutamatergic neurons in the thesis study. The Fam107a gene is located on chromosome 3p21.1, and encodes the DRR1 protein (Wang et al., 2000), binding to F-actin (Schmidt et al., 2011). Fam107a was found to be predominantly expressed in the brain, especially in limbic system neurons (Asano et al., 2010; Le et al., 2010). Initially identified as a downregulated gene in renal cell carcinoma, subsequent studies found it to be involved in a variety of physiological processes, including cell proliferation, differentiation, apoptosis, and stress response (Nakajima & Koizumi, 2014). A conserved N-terminal domain with an unidentified function and a variable C-terminal portion containing a coiled-coil domain are features of the Fam107 family (Nakajima et al., 2012; Nakajima & Koizumi, 2014).It has a similar sequence with the HSP family, and also exhibits similar functions during the stress response of cells, participating in the regulation of gene transcription (Nakajima & Koizumi, 2014).

In multiple studies, it has been discovered that Fam107a has differential expression in psychiatric disorders such as schizophrenia, bipolar disorder and Alzheimer's disease (S. Kim et

al., 2007; Stankiewicz et al., 2014; Sun et al., 2022), suggesting its involvement in the pathophysiology of these disorders. It is also reported that the protein expression of Fam107a in the brain is affected by glucocorticoids (Masana et al., 2018), Various stressors significantly increase Fam107a mRNA expression, especially in the paraventricular nucleus (PVN) of the hypothalamus and the CA3 region of the hippocampus (Schmidt et al., 2011; Stankiewicz et al., 2014). Therefore, Fam107a is involved in stress-induced in vivo changes and plays a role in buffering the consequences of stress, potentially restoring brain homeostasis.

In this thesis study, I hypothesize that downregulation of Fam107a is associated with the anxiety-like phenotype in mice lacking MR in glutamatergic neurons. Viral vectors offer an effective approach to significantly enhance gene expression and protein production without negative effects on organismal development. Thus, I initially employed a fluorescent Fam107a virus to overexpress Fam107a in all hippocampal cells, and then used a Cre-dependent Fam107a overexpression virus that can be used to specifically target the glutamatergic cell population, to increase its expression levels bilaterally in the hippocampus of mice lacking MR in glutamatergic neurons. I then assessed the animals' behavioral performance at baseline levels. The study results revealed that Fam107a gene overexpression partially alleviated the anxiety phenotype induced by MR loss in glutamatergic neurons. This partial rescue, observed specifically in the ventral hippocampus, implies a potential interaction between Fam107a and the MR signaling pathway. This suggests that Fam107a may regulate or compensate for the absence of MR in glutamatergic neurons. Fam107a gene is likely regulated by MR, positioning Fam107a downstream in the MR signaling pathway. The partial alleviation of anxiety-like behaviors suggests that Fam107a could play a role in modulating anxiety, potentially acting as a downstream molecular mediator in the relationship between MR and anxiety. However, as the rescue of the behavioral phenotype was only partial, it's likely that other factors also contribute to the effects of MR on anxiety. However, it is important to note that while the open field test primarily measures animal exploration and movement in a novel environment (Prut & Belzung, 2003), the elevated plus maze test focuses on the conflict between an animal's natural inclination to explore and its aversion to open and elevated spaces (Komada et al., 2008). The lack of improvement in the elevated plus maze test indicates the complex relationship between this gene and anxiety-related behaviors, with the exact mechanisms requiring further elucidation. These findings serve as a compelling starting point to investigate the potential role of Fam107a in anxiety disorders and its interplay with MRs. Such research may lay the groundwork for future studies on the molecular mechanisms underlying anxiety

and the development of novel therapeutic strategies.

4.4.2 Potential therapeutic target gene Npy in GABAergic neurons lacking MR.

Single-cell sequencing identified a significant and cell-type specific decrease in the expression of the Npy gene in the hippocampus of mice lacking MR in GABAergic neurons. The Npy gene encodes neuropeptide Y, a widely distributed neurotransmitter in the brain (Allen et al., 1983), with particularly high concentrations in areas involved in cognition such as the hippocampus, amygdala, and prefrontal cortex (Stanić et al., 2011), affecting multiple brain functions including neurotransmission, neuroprotection, regulation of stress responses, and learning and memory (Lach & de Lima, 2013). Studies found that in humans, Npy is widely acknowledged as a significant neurochemical contributor to posttraumatic resilience and recovery (Heilig, 2004; Kupcova et al., 2022; Redrobe et al., 2002; Yehuda et al., 2006). It plays a crucial role in the regulation of stress, anxiety, and the HPA axis, while also being associated with the pathogenesis of psychiatric disorders (Eaton et al., 2007). Npy is synthesized in GABAergic neurons and is often co-released with GABA in various types of interneurons (McDonald & Pearson, 1989; Oberto et al., 2001). Within the amygdala, terminals containing Npy are closely linked to CaMKII-positive neurons that express GR and MR receptors (Cui et al., 2008). This suggests a potential functional relationship between Npy signaling and MR receptors.

This thesis study indicates that the absence of MR in GABAergic neurons is associated with a decline in cognitive function, and in this condition, the expression of Npy in the hippocampus decreases. Previous research has indicated that chronic repeated stress stimuli result in gradual adaptive changes in behavior and endocrine responses, accompanied by upregulation of Npy expression in the amygdala (Thorsell et al., 1999). Therefore, the upregulation of Npy expression may contribute to behavioral adaptation to stress. Furthermore, in other studies, Npy expression has been shown to decrease in both vCA1-vCA3 and dCA1-dCA3 regions of the hippocampus after chronic stress (Czéh et al., 2015; Sergeyev et al., 2005; Sweerts et al., 2001). This contrasts with the upregulation of Npy in the amygdala and suggests that different brain regions may respond differently to chronic stress. In the absence of MR in GABAergic neurons, GR may be overactivated due to unopposed effects of corticosteroids, which may lead to reduced Npy expression and cognitive decline.

Understanding the role of MR in regulating Npy gene expression could shed light on the mechanisms underlying the behavioral responses to stress. Further investigations will focus on

manipulating the Npy gene to examine its impact on the phenotype of mice lacking MR in GABAergic neurons. Additionally, the specific pathway through which MRs regulate Npy expression remains unclear and warrants further exploration. Since both MR and Npy are involved in stress responses and are co-expressed in the hippocampus, one possibility is that MR directly influences Npy synthesis or release in response to stress stimuli. However, this remains a hypothesis that requires experimental confirmation. While our study demonstrated reduced Npy expression in the hippocampus of mice lacking MR in GABAergic neurons, it remains uncertain whether the reduction in Npy is a direct consequence of the absence of MR or a secondary effect. Future studies should aim to investigate whether similar effects are observed when MR is pharmacologically inhibited, as this would strengthen the association between MR and Npy expression.

4.5 Future outlook

Identifying potential biomarkers for stress-related psychiatric disorders holds significant importance in facilitating early detection, prognostic assessment, and personalized treatment strategies. The present thesis study, investigating the impact of MR deficiency on specific neuronal populations and the consequential behavioral and structural alterations, has the potential to contribute to the identification of such biomarkers. By shedding light on the functionality of MR in distinct neuronal subtypes under diverse stress conditions, this research brings us closer to utilising MR as a promising therapeutic target. Notably, the divergent responses of male and female mice to MR deficiency highlight the potential for sex-specific biomarkers. Additionally, the partial rescue of anxiety-related phenotypes in glutamatergic neurons lacking MR through Fam107a overexpression suggests that modulating the expression of this gene or its downstream signaling pathways could offer alternative approaches for the development of anxiety disorder treatments. In summary, this thesis study advances our comprehension of the functional roles of MR within distinct neuronal populations and their relevance to symptoms associated with stress-related psychiatric disorders. It is anticipated that these research findings can be translated to human studies in the future, thereby fostering the development of MR-targeted interventions for psychiatric diseases.

Although this paper provides some insights into MR, certain limitations should be acknowledged, including: 1. The use of a small sample size in some experiments, which may restrict the statistical power and limit the generalizability of the results. Increasing the sample

size could enhance the reliability and validity of the findings related to the effect of MR. 2. Mice are social animals, but the mice in the experiment reside alone in cages. I have not thoroughly investigated how this single-cage feeding method will affect the emotional behavior and cognition of the mice. 3. Female animals have an estrus cycle, which affects hormone secretion. However, this factor was not controlled in our experiments. 4. In the thesis study, female mice were only subjected to CSDS paradigm without acute restraint stress, and the emotional/behavioral and cognitive performance of female mice after acute stress in relation to MR signaling is unclear.

In addition to the aforementioned limitations, in future experiments the following research goals can be considered: 1. Further exploration of the molecular mechanisms and cellular pathways underlying MR as a stress response system would enhance our understanding of how observed phenotypes are modulated. 2. Investigating the effects of age on MR and examining the consequences of long-term MR deprivation in specific neurons in aged mice could provide valuable insights into the age-dependent aspects of MR function. 3. Expanding the scope of stressors beyond acute restraint stress and CSDS by incorporating early-life stress paradigms in mice would allow for the examination of the long-term impact of early stress experiences on adult phenotypes. 4. Whether manipulating the Npy gene can rescue the phenotype of lacking MR in GABAergic neurons.

Given the findings of this thesis study, addressing its limitations and pursuing the aforementioned future research directions can achieve a comprehensive understanding of the role of MR in stress-related psychiatric disorders.

List of figures and tables

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Curriculum Vitae

Personal details

Name	Huanqing Yang
Date of Birth	01.10.1987
Place of Birth	Henan, China

Education

2019 - At present	Ludwig-Maximilians-Universität München
	Max Planck Institute of Psychiatry
	PhD in neurobiology
2016 - 2019	Shanghai Jiao Tong University School of Medicine
	Joint education program in Shanghai Institute of Materia Medica, Chinese Academy of Sciences
	Master of medical science
2005 – 2010	Qiqihar medical university
	Bachelor of clinical medicine

Working experience

2016 - 2019	Research assistant
	Shanghai Mental Health Centre
2010 - 2015	Psychiatrist
	Kangning Hospital Affiliated to Wenzhou Medical University

Publications

1. van Doeselaar, L., Stark, T., Mitra, S., **Yang, H.**, Bordes, J., Stolwijk, L., Engelhardt, C., Kovarova, V., Narayan, S., Brix, L. M., Springer, M., Deussing, J. M., Lopez, J. P., Czisch, M., & Schmidt, M. V. (2023). Sex-specific and opposed effects of FKBP51 in glutamatergic and GABAergic neurons: Implications for stress susceptibility and resilience. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(23), e2300722120. https://doi.org/10.1073/pnas.2300722120

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6. van Doeselaar, L., **Yang, H.**, Bordes, J., Brix, L., Engelhardt, C., Tang, F., & Schmidt, M. V. (2021). Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. *Stress (Amsterdam, Netherlands)*, *24*(2), 168–180. https://doi.org/10.1080/10253890.2020.1864319

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Affidavit/Eidesstattliche Erklärung

Hiermit versichere ich eidesstattlich, dass ich die vorliegende Dissertation selbststandig und nur mit den angegebenen Quellen und Hilfsmitteln angefertigt habe. Alle Ausfuhrungen, die wortlich oder sinngemas ubernommen wurden, sind als solche gekennzeichnet.

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München, den 26.07.2023
Huanqing Yang