UNIVERSITY OF CRETE
SCHOOL OF SCIENCE AND ENGINEERING
BIOLOGY DEPARTMENT

PhD THESIS

The role of corticotropin releasing factor in the pathophysiology of schizophrenia

CHALKIADAKI KLEANTHI

SUPERVISOR: ASSISTANT PROFESSOR KYRIAKI SIDIROPOULOU

HERAKLION, CRETE, JUNE 2018

ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΚΑΙ ΤΕΧΝΟΛΟΓΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Ο ρόλος του εκλυτικού παράγοντα κορτικοτροπίνης στην παθοφυσιολογία της σχιζοφρένειας

ΧΑΛΚΙΑΔΑΚΗ ΚΛΕΑΝΘΗ

ΕΠΙΒΛΕΠΟΥΣΑ ΚΑΘΗΓΗΤΡΙΑ: ΕΠΙΚΟΥΡΗ ΚΑΘΗΓΗΤΡΙΑ ΚΥΡΙΑΚΗ ΣΙΔΗΡΟΠΟΥΛΟΥ

ΗΡΑΚΛΕΙΟ, ΚΡΗΤΗΣ, ΙΟΥΝΙΟΣ 2018

Table of Contents

Abstract

Schizophrenia (SZ) is a chronic, severe mental disorder, which affects 1% of the global population. Its main symptoms are categorized in positive, negative and cognitive deficits and arise after late adolescence/early adulthood. The main brain regions affected are the hippocampus (HPC) and the prefrontal cortex (PFC). Its biological basis still remains unknown, with several hypotheses trying to shed light on the underlying mechanisms. The leading hypothesis for the etiopathology of SZ is the neurodevelopmental hypothesis, which supports that SZ may originate from abnormalities that occur during the early stages of fetal brain development. Additionally, there are several lines of evidence supporting a crucial role of stress on SZ, while an important but rarely studied fact is the sexual dimorphism observed in the emergence, symptomatology and response to treatment.

The multifactorial character of SZ has become a barrier for the identification of its biological basis and has magnified the need for developing more complex models for the study of the disorder. A very well-characterized animal model of SZ, based on the neurodevelopmental hypothesis, in rats, is the gestational MAM model. It is generated by prenatal exposure to the mitotoxin MAM, during Gestational day (GD) 17. Our study aimed to develop and validate the MAM model in mice, in both sexes, and investigate the role of corticotropin releasing factor (CRF) system, the neuroendocrine modulator of stress response, in the symptoms of the model. We further investigated the effects of acute stress in the cognitive function of control animals, in both sexes. Because of its good validity, we believe that developing the MAM model in mice, would provide us with a strong tool to create more complex models for the study of SZ, given the increased number of genetic mouse models. In addition, we believe that studying both sexes would further elucidate the complexity of SZ, as the majority of the studies have been conducted in male subjects.

We showed that the MAM model in mice is better reproduced by exposure to the mitotoxin on GD16. The validation experiments revealed histological adaptations in both PFC and HPC, and indications of positive

symptoms in both male and female mice, such as enhanced locomotor activity in response to MK-801 administration in female mice and reduced pre-pulse inhibition in male mice. With regards to cognitive deficits, HPC function was found impaired in both sexes, as shown by reduced contextual fear memory and decreased long-term potentiation (LTP). However, this was not the case for PFC-dependent cognitive function or the Parvalbumin protein expression. Only male mice exposed to MAM exhibited impaired PFC function, as indicated by reduced performance in the delayed alternation task in the T-maze and reduced LTP. Furthermore, male, but not female, MAM mice exhibited reduced Parvalbumin expression in HPC and PFC, which is a marker of SZ phenotype in humans.

We extended the sex comparison of MAM-exposed mice in the basal anxiety levels and CRF1 receptor levels on PFC, which was the region showing sexual dimorphism. We revealed heightened levels of trait anxiety in MAM-exposed female mice but decreased levels in males, which were only observed during adulthood. The quantification of CRF1 protein levels from PFC samples revealed a significant decrease only in male MAM-exposed mice. These results intrigued us to hypothesize that CRF system could have a strong influence on the sex-biased PFC-dysfunction, through CRF1 activation, as our results supported normal PFC function in female MAM-exposed mice. We therefore, systemically blocked CRF1 in female MAM-16 mice, using antalarmin, which is a CRF1 specific antagonist. Our aim was to test whether CRF1 inactivation could induce PFC-dysfunction. We observed a disruption of LTP in the PFC of female MAM-exposed mice, in contrast to the facilitation we recorded in saline-exposed animals treated with antalarmin. Finally, we acutely stressed control animals, in order to study PFC-cognitive function after enhancing CRF system activation, mimicking the alterations that we observed in CRF system of male MAM-16 mice. We found increased vulnerability of females, in terms of anxiety levels, but no significant alterations were found in the PFC-dependent cognitive function.

In conclusion, our study reveals that the MAM model of SZ can be successfully reproduced in mice, showing sex-dimorphic alterations observed also in the human condition, and supports a significant contribution of CRF

system in the pathology of the disorder. We speculate that SZ could be a condition of sustained CRF over-expression and the altered levels of CRF1 in male MAM-expose mice could reflect a homeostatic mechanism developed to counterbalance this abnormal increase, with the cost of impaired PFC function. An alternative mechanism in female MAM-exposed mice, through the activation of different cellular signaling pathways could protect PFC, without affecting CRF1 availability.

Περίληψη

Η σχιζοφρένεια είναι μία χρόνια, σοβαρή νοητική διαταραχή, η οποία επηρεάζει το 1% του παγκόσμιου πληθυσμού. Τα κύρια συμπτώματα κατηγοριοποιούνται σε θετικά, αρνητικά και γνωσιακά ελλείμματα και εμφανίζονται μετά το τέλος της εφηβείας/ αρχή της ενηλικίωσης. Οι κύριες περιοχές που επηρεάζονται είναι ο ιππόκαμπος και ο προμετωπιαίος φλοιός. Η βιολογική της βάση παραμένει ακόμα άγνωστη, με διάφορες υποθέσεις να προσπαθούν να ρίξουν φως στους υποκείμενους μηχανισμούς. Η επικρατούσα υπόθεση για την αιτιοπαθολογία της σχιζοφρένειας είναι η νευροαναπτυξιακή υπόθεση, η οποία υποστηρίζει ότι η σχιζοφρένεια προέρχεται από ανωμαλίες που λαμβάνουν χώρα κατά τα πρώτα στάδια της ανάπτυξης του εγκεφάλου του εμβρύου. Επιπλέον, υπάρχουν αρκετές ενδείξεις που υποστηρίζουν ότι το στρες παίζει πολύ σημαντικό ρόλο στη σχιζοφρένεια, ενώ ένα πολύ σημαντικό στοιχείο που σπάνια μελετάται είναι ο διμορφισμός που σχετίζεται με το φύλο και παρατηρείται στην εμφάνιση, τη συμπτωματολογία και την απόκριση στη θεραπεία.

Ο πολυπαραγοντικός χαρακτήρας της σχιζοφρένειας αποτελεί εμπόδιο για την ταυτοποίηση της βιολογικής βάσης της διαταραχής και έχει μεγεθύνει την ανάγκη για την ανάπτυξη πολύπλοκων μοντέλων για τη μελέτη της διαταραχής. Ένα πολύ καλά χαρακτηρισμένο ζωικό μοντέλο της σχιζοφρένειας, το οποίο βασίζεται στη νευροαναπτυξιακή υπόθεση, στους αρουραίους, είναι το νευροαναπτυξιακό μοντέλο MAM. Δημιουργείται μετά από προγεννητική έκθεση στη μιτοτοξίνη MAM, κατά τη 17^{η} μέρα κύησης. Ο στόχος μας στη μελέτη αυτή ήταν να αναπτύξουμε και να επικυρώσουμε το μοντέλο MAM στα ποντίκια και στα δύο φύλα, και να διερευνήσουμε το ρόλο του εκλυτικού παράγοντα κορτικοτροπίνης, του νευροενδοκρινικού ρυθμιστή της απόκρισης στο στρες, στα συμπτώματα του μοντέλου. Επιπλέον, μελετήσαμε τις επιδράσεις του οξέος στρες στη γνωστική λειτουργία, σε φυσιολογικά ζωα και των δύο φύλων. Λόγω της καλής εγκυτότητας του μοντέλου, πιστεύουμε ότι η ανάπτυξη του μοντέλου MAM σε ποντίκια , θα μας παράσχει ένα δυνατό εργαλείο για να δημιουργήσουμε πιο πολύπλοκα μοντέλα για τη μελέτη της σχιζοφρένειας, δεδομένου και του αυξημένου αριθμού γενετικών μοντέλων που έχουν αναπτυχθεί σε ποντίκια. Επιπλέον,

πιστεύουμε ότι η μελέτη και των δύο φύλων θα διασαφηνίσει περαιτέρω την πολυπλοκότητα της διαταραχής, καθώς οι περισσότερες μελέτες έχουν διεξαχθεί σε αρσενικά άτομα.

Τα αποτελέσματά μας έδειξαν ότι το μοντέλο MAM στα ποντίκια αναπαράγεται καλύτερα με έκθεση στη μιτοτοξίνη κατά τη 16η μέρα της κύηση. Τα πειράματα για την επικύρωση αποκάλυψαν ιστολογικές προσαρμογές τόσο στον προμετωπιαίο όσο και στον ιππόκαμπο, και ενδείξεις για θετικά συμπτώματα και στα δύο φύλα, όπως αυξημένη κινητική δραστηριότητα ως απόκριση στη χορήγηση του MK-801, στα θηλυκά MAM ποντίκια, και μειωμένη προπαλμική αναστολή στα αρσενικά MAM ποντίκια. Όσον αφορά στα γνωσιακά ελλείμματα, η λειτουργία του ιπποκάμπου βρέθηκε μειωμένη και στα δύο φύλα, όπως έδειξε η μειωμένη μνήμη φόβουκαι η μειωμένη μακρόχρονη ενδυνάμωση. Ωστόσο, δεν παρατηρήθηκε κάτι ανάλογο στην εξαρτώμενη από τον προμετωπιαίο φλοιό γνωσιακή λειτουργία ή την έκφραση της πρωτεΐνης παρβαλβουμίνης. Μόνο τα αρσενικά MAM ποντίκια παρουσίασαν ελλείμματα στη λειτουργία του προμετωπιαίου φλοιού, όπως έδειξε η μειωμένη απόδοση στη δοκιμασία εναλλαγής βραχίονα με καθυστέρηση στο λαβύρινθο σχήματος «Τ», καθώς και η μειωμένη μακρόχρονη ενδυνάμωση. Επιπλέον, τα αρσενικά, αλλά όχι τα θηλυκά MAM ποντίκια παρουσίασαν μειωμένη έκφραση της πρωτεΐνης παρβαλβουμίνης εύρημα το οποίο αποτελεί δείκτη του φαινότυπου σχιζοφρένειας στους ανθρώπους.

Επεκτείναμε τη σύγκριση των δύο φύλων στα ζώα MAM και στην απόκριση στο στρες, εστιάζοντας στον προμετωπιαίο φλοιό, η οποία έδειξε διμορφισμό που σχετίζεται με το φύλο. Αποκαλύψαμε υψηλά επίπεδα άγχους στα θηλυκά MAM ποντίκια και χαμηλά επίπεδα στα αντίστοιχα αρσενικά, το οποία ήταν εμφανή μόνο κατά την ενηλικίωση. Η ποσοτικοποίηση των επιπέδων της πρωτεΐνης του υποδοχέα 1 του εκλυτικού παράγοντα κορτικοτροπίνης στον προμετωπιαίο φλοιό αποκάλυψε σημαντική μείωση μόνο στα αρσενικά MAM ποντίκια. Τα αποτελέσματα αυτά μας οδήγησαν στο να υποθέσουμε ότι το σύστημα του εκλυτικού παράγοντα κορτικοτροπίνης θα μπορούσε να επηρεάζει τη λειτουργία του προμετωπιαίου φλοιού με διαφορετικό τρόπο σε κάθε φύλο, μέσω της ενεργοποίησης του υποδοχέα 1. Έτσι, προχωρήσαμε σε αποκλεισμό του υποδοχέα στα θηλυκά MAM

ποντίκια, με συστημική χορήγηση, χρησιμοποιώντας ανταλαρμίνη, η οποία είναι ένας ειδικός ανταγωνιστής του υποδοχέα 1. Ο στόχος μας ήταν να ελέγξουμε εάν η απενεργοποίηση του υποδοχέα 1 του εκλυτικού παράγοντα κορτικοτροπίνης, θα μπορούσε να προκαλέσει δυσλειτουργία στον προμτεωπιαίο φλοιό. Παρατηρήσαμε μείωση της μακρόχρονης ενδυνάμωσης στον προμετωπιαίο φλοιό των θηλυκών MAM ποντικών, σε αντίθεση με την ενίσχυση της μακρόχρονης ενδυνάμωση που καταγράψαμε στα ζώα ελέγχου που έλαβαν ανταλαρμίνη. Τέλος, πραγματοποιήθηκε οξεία έκθεση ζώων ελέγχου σε στρες περιορισμού, με σκόπο τη μελέτη της γνωσιακής λειτουργίας του προμετωπιαίου φλοιού, μετά από ενίσχυση της ενεργότητας του συστήματος του εκλυτικού παραγοντα κορτικοτροπίνης, μιμούμενοι έτσι τις αλλαγές που παρατηρήσαμε στο σύστημα αυτό στα αρσενικά MAM ποντίκια. Βρήκαμε αυξημένα επίπεδα άγχους στα θηλυκά ζώα, αλλά καμία σημαντική διαφορά στη γνωσιακή λειτουργία του προμετωπιαίου φλοιού.

Συμπερασματικά, η μελέτη μας αποκαλύπτει ότι το μοντέλο MAM της σχιζοφρένειας μπορεί να αναπαραχθεί με επιτυχία στα ποντίκια, εμφανίζοντας διαφορές ανάμεσα στα δύο φύλα που παρατηρούνται και στους ανθρώπους, και υποστηρίζει τη σημαντική συνεισφορά του συστήματος του εκλυτικού παράγοντα κορτικοτροπίνης στην παθολογία της διαταραχής. Υποθέτουμε ότι η σχιζοφρένεια θα μπορούσε να αποτελεί μία κατάσταση παρατεταμένης υπερέκφρασης του εκλυτικού παράγοντα κορτικοτροπίνης και οι αλλαγές στα επίπεδα του υποδοχέα 1 στα MAM αρσενικά ποντίκια να αντικατοπτρίζουν έναν ομοιοστατικό μηχανισμό που έχει αναπτυχθεί για να εξισορροπήσει τη μη φυσιολογική αύξηση, με κόστος τη μειωμένη λειτουργία του προμετωπιαίου φλοιού. Ένας εναλλακτικός μηχανισμός στα θηλυκά MAM ποντίκια, μέσω ενεργοποίησης διαφορετικών σηματοδοτικών μονοπατιών, θα μπορούσε να προστατεύει τον προμετωπιαίο φλοιό, χωρίς να επηρεάζει τη διαθεσιμότητα του υποδοχέα 1.

Chapter 1

1. **General Introduction**

1.1The Brain

1.1.1 Brain development and plasticity

Gestational period in humans normally lasts for 40 weeks, while in rodents birth occurs on 20-21st day of gestation. Human brain starts developing during the third gestational week (GW), when the neural progenitor cells begin to differentiate, forming the first brain structure, the neural tube. In rats and mice, the neural tube formation occurs on gestation day (GD) 10.5-11 and 9.9.5, respectively. By the end of the embryonic period (GW8), a five-segment primitive nervous system has been formed(Stiles, 2008), which continues to evolve into differentiated and organized structures, until the end of the fetal period. In humans, the fetal period lasts from the 9th GW to the end of gestation and is characterized by extreme changes in the developing brain. The smooth 'lissencephalic' brain structure transforms into a mature structure with gyri and sulci (Stiles and Jernigan, 2010). The anatomical changes observed in the fetal brain reflect the extended cellular changes that take place during development.

There are three key processes that occur during fetal period: neuron production, neuron migration and neuron differentiation. Neuron production starts on embryonic day 42 (E42) (Bystron, Blakemore and Rakic, 2008; Stiles and Jernigan, 2010). Before this time point, neural progenitor cells undergo multiple rounds of symmetrical cell divisions, increasing their numbers. After E42 the cell division becomes asymmetrical; each neural progenitor divides into a new neural progenitor and one neuron (Wodarz and Huttner, 2003). The newborn neurons leave the proliferative zone and migrate to the developing neocortex, where they form layers and differentiate into different types of mature neurons. The excessive connectivity (synapse formation) observed during that period, is much

higher than that seen in adults (Innocenti and Price, 2005), but is gradually suppressed by processes, like synaptic pruning and cell death. At least 50% of the neurons of a brain region die, while up to 50% of synapses are eliminated(Stiles and Jernigan, 2010).These processes are considered crucial for the normal and effective function of neural circuits (Buss, Sun and Oppenheim, 2006). However, the structural and functional changes extend after birth, predominantly, during the early postnatal period.

Studies have identified that key developmental processes and behavioral phenotypes are conserved between mammalian species. However, several prenatal processes of the human brain development, take place postnatally in rodents, such as the establishment of the blood-brain barrier (BBB) and the peak in gliogenesis, which occur between 23-40 GW in humans, but during the first 10 days after birth in rodents (as reviewed in (Semple *et al.*, 2013)). Results from MRI studies have shown that human brain volume reaches 90-95% of its adult size by the age of 6, while in rodents on postnatal day (PND) 20-21(Giedd *et al.*, 1999; Chuang *et al.*, 2011).

The most impressive characteristic of the brain that can be both beneficial and disadvantageous is plasticity, which is the ability to adapt in environmental stimuli and change its structure and function. Early developing brain is even more capable of adaptation and plasticity. While genetic programming firstly determines the brain networks, there is a second phase in which brain development is strongly influenced by environmental factors, experiences and epigenetic changes. Dendrites and spines show high plasticity (synaptic plasticity) in response to external stimuli. Synapses can be formed or lost within hours or minutes (Garner *et al.*, 2002; Harvey and Svoboda, 2007). However, different developmental stages show different levels of synaptic plasticity. Several studies have identified time windows of heightened plasticity in brain development, namely critical periods. As reviewed in (Hensch and Bilimoria, 2012), in humans the peak level of plasticity for sensory pathways is early, during the first years after birth, followed by a critical period of language and motor skills acquisition, during childhood. The critical period for higher cognitive

functions appears later in development, peaks after puberty and lasts throughout adulthood (Hensch, 2004, 2005).

Synaptic plasticity can be described as the modification of the strength or efficacy of synaptic transmission at synapses. The modification can be either enhancement or depression of synaptic transmission and can last from milliseconds to hours, days or longer (Citri and Malenka, 2008). Synaptic plasticity can be categorized in short-term and long-term, based on the maintenance of synaptic transmission alterations. Short-term synaptic plasticity lasts for milliseconds to several minutes. It can be provoked by short bursts of activity which increase the calcium concentration in presynaptic neurons, altering the probability of neurotransmitter release in the synaptic cleft. This type of synaptic plasticity allows synapses to filter the information they receive (Citri and Malenka, 2008). In contrast, long-term synaptic plasticity describes longer lasting modifications of synaptic strength, which can either enhance synaptic activity, referred to as Long-term potentiation (LTP), or decrease synaptic strength, a phenomenon called Long-term depression (LTD). Importantly, the ability of an organism to learn and remember depends on the existence of long-term synaptic plasticity. In particular, the synaptic plasticity and memory hypothesis supports the notion that synaptic plasticity is induced in synapses during memory formation and it is both necessary and sufficient for the encoding and storage of the memory (Takeuchi, Duszkiewicz and Morris, 2013).

1.1.2 The hippocampal formation

Hippocampal formation is a subcortical region found in all vertebrates. In primates, it is located in the medial temporal lobe and belongs to the limbic system of the brain (Fig 1.1). It has a crucial role in memory storage, consolidation and recall and deficits in this structure can lead to impaired long-term memory. Hippocampal formation can be divided into 3 parts: hippocampus proper (HPC) or Ammon's horn, dentate gyrus (DG) and subiculum (Fig.1.1). HPC can be further subdivided into four distinct subregions, known as CA1-CA4 (CA from *cornu ammonis*) (Fig.1.1)(Stephan and Manolescu, 1980). Although hippocampal formation is considered a

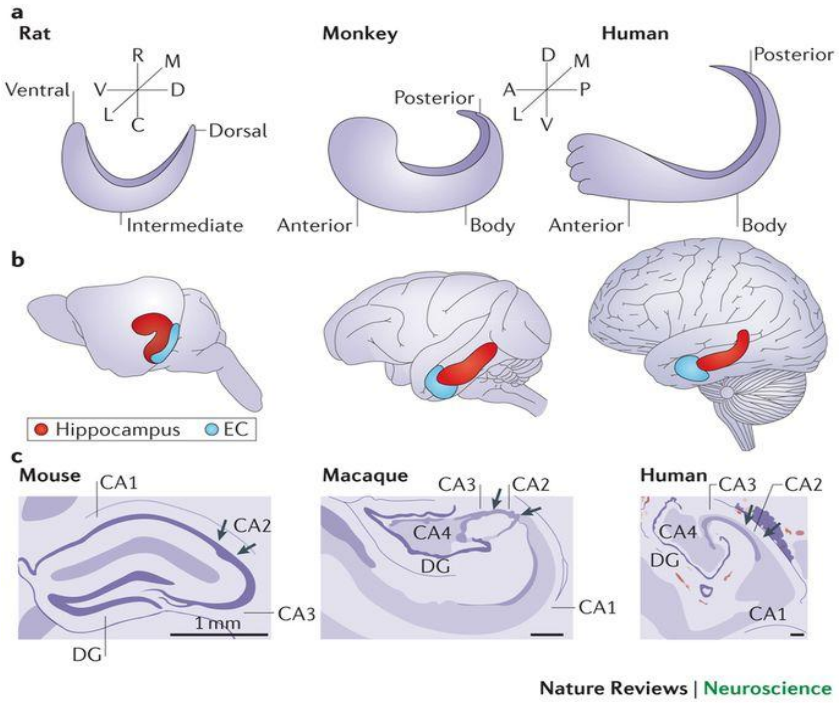


Figure 1.1: Hippocampal formation anatomy in rodents, monkeys and humans. a) Schematic illustration of the orientation of the hippocampal axis. b) Schematic representation of the precise position of hippocampal formation in the brains of rats, macaque monkeys and humans. c) Drawings of Nissl cross-sections of mouse, rhesus and human hippocampi. A, anterior; C, caudal; D, dorsal; DG, dentate gyrus; EC, entorhinal cortex; L, lateral; M, medial; P, posterior; R, rostral; V, ventral;

(Strange *et al.*, 2014)

phylogenetically primitive brain region, several species differences have been found, in terms of cytoarchitecture and connectivity. The main neuronal cell types of hippocampal formation are the excitatory pyramidal cells and the inhibitory interneurons, which are distributed in layers. HPC contains 6 layers (starting from the surface): the *alveus* (pyramidal neurons axons), the *stratum oriens* (inhibitory cells), the *stratum pyramidale* (pyramidal neurons and some types of interneurons) and three more layers, mostly containing neuronal fibers, the *stratum lucidum, radiutum and lacunosum-moleculare*(D., P. and G., 1995) (Fig.1.2). DG consists of 3 cell layers: the polymorphic, the granule cell and a cell-free layer, the molecular layer (Amaral, Scharfman and Lavenex, 2007). Subiculum is a three-layer structure, which contains a deep, polymorphic, a

pyramidal and a molecular layer, and constitutes a continuation of the stratum *lacunosum-moleculare* of CA1.

In terms of connectivity, hippocampal formation sends and receives signals through three fiber systems: the angular bundle, which connects it with the entorhinal cortex (EC), the fimbria-fornix, which provides connection with the forebrain, the hypothalamus and the brain stem, and the hippocampal commissure, which connects the two parts of hippocampal formation of the two hemispheres (Andersen et al. 2007). In its simplest form, the hippocampal formation circuit is known as the 'trisynaptic circuit' (Andersen, Bliss and Skrede, 1971) (Fig.1. 3). Layers 2 and 3 from EC project to the DG granule cells (perforant pathway) which are connected to CA3 subfield pyramidal neurons (mossy fibers). There is also a direct projection from EC to CA3 (alvear pathway). The circuit is completed with a third projection, from CA3 to CA1 subfield (schaffer collaterals) and back to layer 5 of EC, creating a

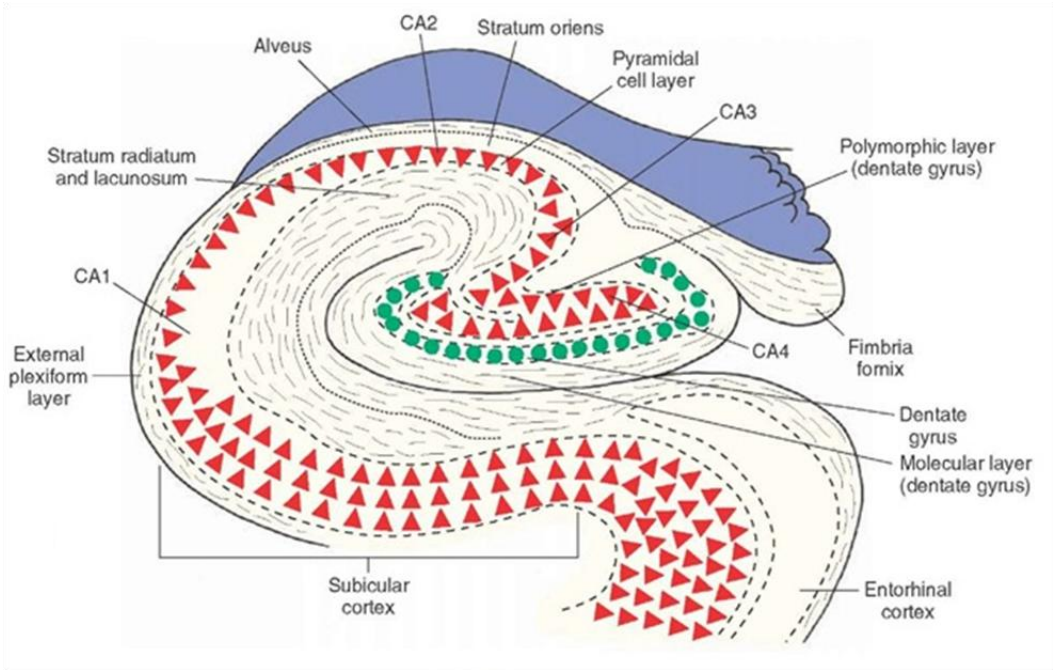


Figure 1.2: Schematic illustration of the histological appearance of the cell layers within the hippocampal formation.

http://what-when-how.com/neuroscience/the-limbic-system-integrative-systems-part-1/

feedback loop (Fig.1.3).

Hippocampal formation is one of the most important structures for the initial encoding of memories and the navigation in space. The memory impairments after lesions in the hippocampal formation of the famous patient H.M. (Milner and Scoville, 1957), and the discovery of the 'place cells' in the HPC of rats (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976) have been milestones in the study of hippocampal formation. Early studies in rodents with specific lesions in HPC recapitulated the H.M.'s amnesic symptom in a lesser extent. However it was proved that hippocampal formation has a crucial role in memory consolidation (Hölscher, 2003) . Both H.M. patient and animals had memories from before the lesions, but had difficulty or were unable to learn or remember recent information. In other words, normal hippocampal function is required for generating personal or episodic memories (Milner and Scoville, 1957). On the other hand, the theory of a cognitive map in the 'place cells' supports that hippocampal cells can hold environmental representations, encoding at the same time information regarding the direction or the speed of the movement (Hölscher, 2003). Additionally, other studies have shown that hippocampal formation is also involved in the storage of timing information, which are important for learning sequences. In any case, it has been suggested that the mechanisms supporting navigation in space are the same with those underlying episodic memory. Hippocampal formation receives information from all senses, through the EC, and creates episodic memories (autobiographical memory of events) or self-referenced maps of the environments one has explored. Importantly, using the same mechanisms, the episodic memories and the self-referenced maps can turn into allocentric, context independent memories (Buzsáki and Moser, 2013).

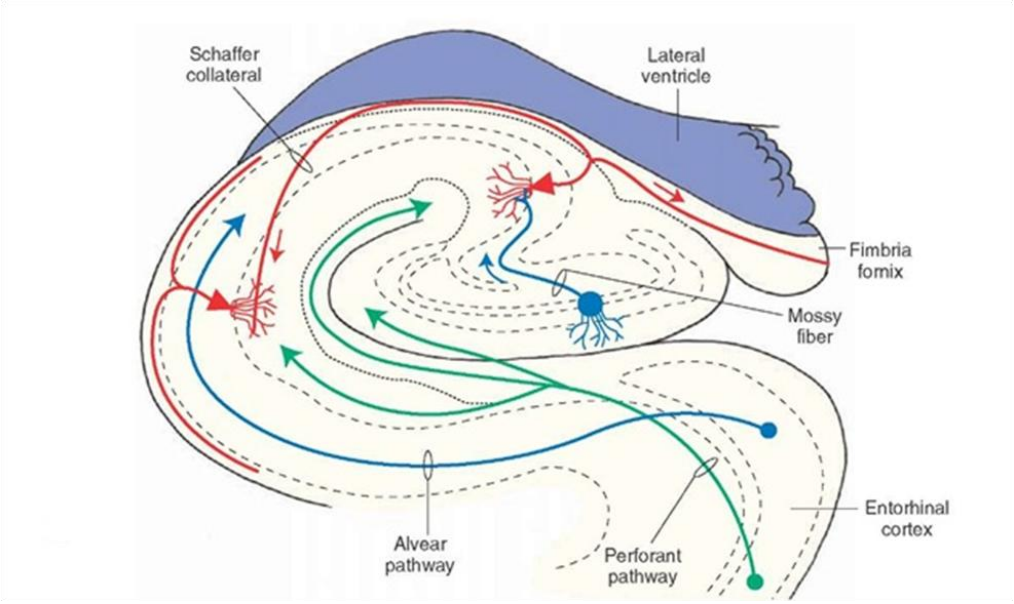


Figure 1.3: Schematic diagram illustrating the afferent fibers from the entorhinal cortex, (perforant and alvear pathways), the internal circuitry, which includes the connections of the mossy fibers and Schaffer collaterals and the efferent projections of the hippocampal formation through the fimbria-fornix system of fibers.

http://what-when-how.com/neuroscience/the-limbic-system-integrative-systems-part-1/

1.1.3 The prefrontal cortex

The early days of fetal life period are critical in the development of one of the most important parts of information processing in the brain, the neocortex. Neocortex sends and receives information from subcortical nuclei. It is part of the cerebral cortex of mammals and is the biggest part of the human brain. The largest neocortical area in humans is the prefrontal cortex (PFC) (Fig.1.4) which is considered to be a very complex brain area, in terms of connectivity with other regions, but mostly in terms of function. It is strategically positioned to coordinate many neural processes, via efferent and afferent projections to sensory and motor systems and subcortical structures. Like all cortical regions, PFC has two neuronal cell types: the excitatory pyramidal cells and the inhibitory interneurons. They are distributed in five layers, in contrast to other cortical regions which have 6 layers, as layer 4 is absent in PFC (Van De Werd *et al.*, 2010). Interneurons

can be categorized into different subclasses, based on their morphology (e.g. basket or chandelier cells) and the location of their target (e.g. perisomatic region or dendrites) or based on their electrophysiological profile (fast spiking, adapting, irregular spiking or intrinsic bursting). A very common classification is the molecular classification, which divides interneurons based on the calcium-binding proteins they express (Pravalbumin-, Somatostatin- Calretinin- expressing) (Defelipe *et al.*, 2013).

Human PFC is divided in many areas, each one of them appears to be responsible for distinct aspects of PFC functions, but at the same time, all together have to cooperate for normal behaviors. Human PFC can be roughly divided into lateral, medial and orbitofrontal PFC. As part of the neocortex, PFC also makes reciprocal connections with thalamus, hypothalamus, hippocampal formation, amygdala and other associative regions of the brain. As reviewed by Seamans and colleagues (Seamans, Lapish and Durstewitz, 2008), the corresponding regions of the rat PFC with regards to the anatomy, function and connectivity, appear to be the medial PFC (mPFC), which can be divided into the dorsal part of the anterior cingulate cortex (dACC), the Prelimbic cortex (PL) (medial part) and the Infralimbic cortex (IL) (ventral part) (Fig.1.4). Many studies have revealed the conservation of PFC circuitry across species. Generally, the rat medial PFC resembles to the primate ACC and dlPFC. Dorsal ACC in rodents is functionally distinguished by the ventral part, as it is activated during tasks that require attention and awareness (Margulies *et al.*, 2007), in contrast to the ventral part which is linked to affective behaviors (Vogt *et al.*, 2005). PL and IL cortices are high interconnected regions in rodents, which share different roles. PL projects mainly to associative and sensory-motor areas of the neocortex, and limbic structures, such as amygdala, while IL projects mainly to areas involved in autonomic and visceral activity but in general, they have been associated with cognitive and emotional processes and anatomically linked with the limbic system (Vertes, 2004).

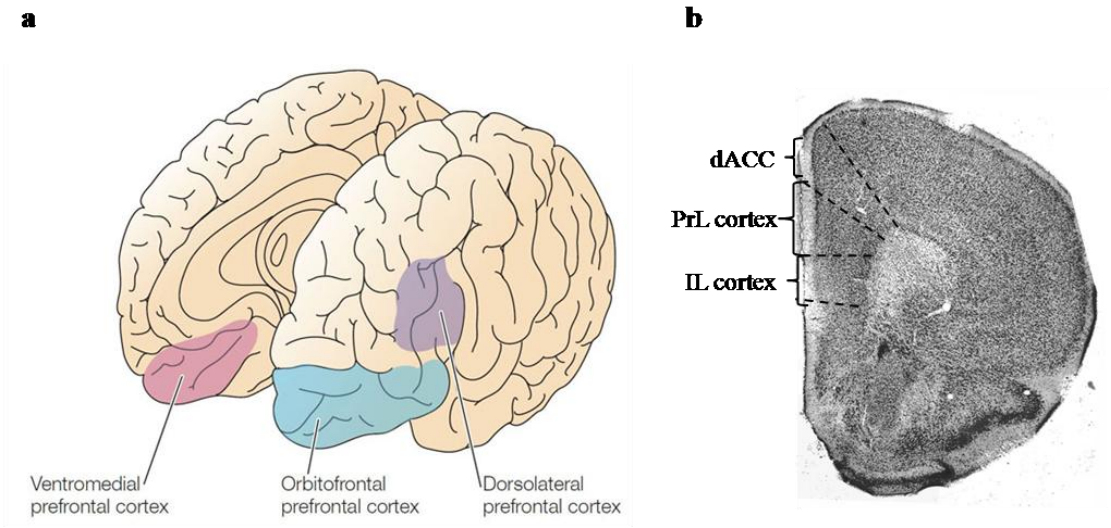


Figure 1.4: Prefrontal cortex in human and mouse brain. a) Schematic illustration of prefrontal cortex division in the human brain. b) Representative image of a coronal section of the mouse brain, containing the prefrontal cortex. The corresponding regions of the human prefrontal cortex are the dorsal part of the ACC, the PrL and the IL cortices. dACC, dorsal anterior cingulate cortex; PrL, Prelimbic; IL, Infralimbic

PFC is not the brain structure responsible for simple, automatic behaviors, but rather for more complicated aspects of behavior (executive functions), such as social and emotional control, thinking, judging and decision making. One of the most important functions of PFC is working memory, which enables us to hold active representations in mind for a few seconds. Working memory is not only important as a simple type of memory storage. It supports other cognitive functions, including language ability, learning, attention, planning and imagining (Goldman-Rakic, 1996). Because of its critical role in intelligence, consciousness and in personality formation, PFC is often referred as the ‘’CEO of the brain’’. Despite its great ‘power’, PFC has been characterized as one of the most vulnerable brain areas and due to its association with psychiatric disorders the prefrontal neuron has been given the name of ‘psychic cell’, by Dr Patricia Goldman-Rakic (Goldman-Rakic, 1999).

1.1.4 Sex differences in normal brain function

Sex is rarely incorporated as a variable by researchers and the vast majority of research and experiments are being conducted in male subjects. However, several sex differences have been observed in the brain, both in normal and pathological function. For example, recently, it was found that synaptic pruning of medial PFC is documented in both male and female adolescent rats, whereas only female rats lose a significant number of dendrites (Koss *et al.*, 2014). Similarly, the increase in the volume of white matter under PFC continues in both male and female rats as they reach adulthood. However, at Postnatal day 90, white matter is larger in males than females (Markham, Morris and Juraska, 2007). Finally, it has been found that female but not male rats lose a significant number of neurons in the mPFC between PD 35 and 45(onset of puberty), suggesting that pubertal hormones may influence the anatomical changes observed in PFC.

1.2 Schizophrenia

1.2.1 Schizophrenia pathology

Schizophrenia (SZ) belongs to the broader family of schizophrenia spectrum and other psychotic disorders. It is a chronic, severe mental disorder with more than 21 millions of people affected worldwide, according to the latest data from the World Health Organization (http://www.who.int/). People who suffer from SZ lose contact with reality and are incapable of everyday functioning (Harvey *et al.*, 2012). Its symptoms are mainly categorized in positive (e.g. delusions, hallucinations), negative (e.g. avolition, blunted emotion) and cognitive (e.g. working memory deficits), and arise after late adolescence/early adulthood (Arguello *et al.*, 2010; Stahl, 2013). The most frequently reported neural deficits of SZ are thinning of the cortex, especially of the PFC (Cannon *et al.*, 2002), down-regulated expression of the γ-aminobutyric acid (GABA) synthesis enzyme GAD-1 in several cortical regions, including the PFC (Mitchell *et al.*, 2015) and excessive striatal Dopamine release (Kegeles *et al.*, 2010).

During the last 50 years, extensive research concerning the pathophysiology and the underling mechanisms has revealed the multi-factorial character of SZ. Twin studies and Genome-wide studies have revealed several susceptible genetic loci for the risk of schizophrenia, as well as possible epigenetic mechanisms that might contribute to its manifestation, whereas epidemiological results have linked the risk of SZ with environmental factors (reviewed in (Gejman, Sanders and Duan, 2010). Most recent findings (Hilker *et al.*, 2017) revealed a 79% heritability of SZ, suggesting a high genetic risk. However the low concordance rate of 33% reported in monozygotic twins, could imply that an environmental dimension might be needed to trigger the emergence of the disorder.

1.2.2 Schizophrenia hypotheses

Despite the great effort of scientific community, the precise contributions and interactions of genetic, environmental or other stochastic factors remain unclear. However, several hypotheses have been proposed for the pathophysiology of SZ. First, the dopamine (DA) hypothesis proposed initially that excessive DA transmission in the brain was responsible for schizophrenic symptoms, and was reconceptualized (Davis *et al.*, 1991) and formulated as a subcortical hyper-dopaminergic and prefrontal hypo-dopaminergic condition (reviewed in (Howes and Kapur, 2009; Brisch, 2014). When the Glutamate hypothesis started to emerge (Carlsson and Carlsson, 1990), it was proposed that SZ is a state of NMDA receptor hypofunction in the brain (Olney, Newcomer and Farber, 1999), shifting the scientific community towards another neurotransmitter system for the etiology of the disease (reviewed in (Nakazawa *et al.*, 2012; Snyder and Gao, 2013). But growing evidence strengthens the neurodevelopmental hypothesis of SZ which supports the notion that SZ arises as a consequence of disturbed nervous system development, leading to inadequate or poorly innervated brain. The theory was formulated thirty years ago (Weinberger, 1987) and has gained a lot of support since then. It does not exclude a genetic dimension, while it involves environmental and other stochastic factors, like prenatal viral infections or obstetric complications, including hypoxia and ischemia, all affecting the

normal brain development and function, possibly synergistically (Fatemi and Folsom, 2009). The fact that SZ symptoms arise after late adolescence/ early adulthood is in agreement with a neurodevelopmental model of the disorder, where deficits occur early in development, but impact function and behavior when the maturation of the brain is complete. As a consequence of the complexity of SZ, the current approved pharmacotherapy includes anti-psychotic drugs which reduce some positive symptoms of psychosis, at a cost of several side effects, and with minimal improvement on cognitive or negative symptoms. In some cases, the intolerable side effects and the toxicity from medications increase the number of non-compliant patients, emphasizing, at the same time, how crucial is to better understand the complexity of SZ and design new therapeutic approaches and more effective medication.

1.2.3 Sex differences in schizophrenia

As reviewed by McCarthy and colleagues (McCarthy *et al.*, 2012), there are important sex differences in cognitive and emotional responses relevant to learning and memory, fear, anxiety, as well as the risk and consequences of several neurological disorders, such as Parkinson's and Huntington's disease. With regards to SZ, it is well reported that the risk of developing SZ is up to 4 times higher in males, with more severe symptoms and an earlier onset reported (Abel, Drake and Goldstein, 2010). In particular, men exhibit more negative symptoms and cognitive deficits, compared to women (Leung and P Chue, 2000; Abel, Drake and Goldstein, 2010). Studies have reported poor performance in tasks evaluating attention and executive functions, in male patients (Goldstein *et al.*, 1994; Seidman *et al.*, 1997; Hoff *et al.*, 1998). On the other hand, female patients show more affective symptoms (Szymanski *et al.*, 1995; Koster *et al.*, 2008; Morgan, Castle and Jablensky, 2008) and tend to exhibit more intense auditory hallucinations and delusions, compared to men(Goldstein *et al.*, 1998; Leung and Pierre Chue, 2000). However, it has been shown that women with SZ perform better at cognitive tasks, compared to males (Fiszdon *et al.*, 2003; Bozikas *et al.*, 2010) and tend to respond better in anti-psychotic treatments, at lower doses, although their

response seems to depend on several factors (e.g. treatment type, menopause stage) (Abel, Drake and Goldstein, 2010). The precise mechanisms underlying the sex differences in symptomatology, emergence and treatment of SZ still have not been elucidated, leaving great gaps in our understanding of SZ pathology and the development of more effective treatments for both men and women.

Chapter 2

2.1 Introduction

2.1.1 Modeling Schizophrenia

2.1.1.1 Animal models of Schizophrenia

Despite the extended research on humans, the scientific community has obtained significant information concerning SZ etiopathology from animal model studies. A reliable animal model should present symptom homology (face validity), replicate the theoretical neurobiological base of the disease (construct validity) and show the expected pharmacological response or lack of it, after treatment with known antipsychotics or novel drugs tested (predictive validity). All animal models try to recapitulate the core symptoms of the disorder and the timing of the anomalies manifestation and are sorted in four categories: drug-induced, genetic manipulation, lesion and neurodevelopmental models. Drug-induced models use substances like amphetamine, phencyclidine or dizocilpine (MK-801) which act on dysfunctional-in-SZ neurotransmitter systems, like dopamine and glutamate systems (C. Jones, Watson and Fone, 2011). A significant number of studies support the validity and superiority of the PCP over the Amphetamine model, even though a few discrepancies weaken its power (C. Jones, Watson and

Fone, 2011). Genetic mouse models have been invaluable in understanding the genetic dimension of SZ. In most cases, they replicate the mRNA or protein alterations observed in the human condition, while helping to connect individual schizophrenia endophenotypes with underlying risk genes. Four well-known genetic mouse models lack or carry mutations in genes expressing synaptic proteins: the Disrupted in schizophrenia-1 (DISC-1) mouse model, the Reeler mouse, the Dysbindin mutant and the Neuregulin 1 mutant mouse (C. Jones, Watson and Fone, 2011). The neonatal ventral hippocampal lesion model of SZ is the most widely used lesion model. It is produced by injecting the excitotoxin, ibotenic acid locally, during postnatal day 7 (Lipska, Jaskiw and Weinberger, 1993).. Neurodevelopmental models can be generated by prenatal or perinatal exposure to adverse environmental factors, such as stress (post-weaning social isolation stress model) (Fone and Porkess, 2008), maternal immune activation (Poly I:C prenatal exposure model) (Reisinger *et al.*, 2015)or neuroblasts proliferation disruption (gestational MAM model)(Moore *et al.*, 2006; Lodge, Behrens and A. A. Grace, 2009). Importantly, the majority of the behavioral and cellular alterations resembling SZ phenotype appear progressively after puberty, resembling the human condition.

2.1.1.2Gestational MAM 17 model in rats

The MAM model of SZ has been developed and characterized in rats. It utilizes the anti-mitotic (anti-proliferative) agent Methylazoxymethanol acetate (MAM), in pregnant rat dams at gestation day 17 (GD17). It targets specifically neuroblasts development without affecting glial cells, while disturbing the normal brain development of offspring. As reviewed by Jones and colleagues (C. Jones, Watson and Fone, 2011), different gestation days of MAM treatment have been tested, finally concluding that when MAM is injected before GD15, the behavioral and histological deficits are too extensive and that GD17 constitutes the optimal day in rats. Prenatally-exposed rats (MAM-17 rats) show behavioral, histological and molecular abnormalities which resemble to SZ phenotype and arise after puberty. In particular, at the

behavioral level, MAM-17 rats show enhanced locomotor activity in response to amphetamine (Moore *et al.*, 2006) or MK-801, and spontaneous hyperactivity when placed in a novel environment (Le Pen *et al.*, 2006), all considered as equivalent to positive symptoms of SZ. Additionally, these animals show sensorimotor gating problems, as revealed by impaired Prepulse inhibition (PPI) of the acoustic startle reflex (Le Pen *et al.*, 2006; Moore *et al.*, 2006; Hazane *et al.*, 2009), a cross-species measure, also disrupted in human condition, and deficits in social behavior (Flagstad *et al.*, 2004; Gourevitch *et al.*, 2004; Le Pen *et al.*, 2006; Hazane *et al.*, 2009), which is interpreted as the equivalent of negative symptoms of SZ. Several studies have revealed cognitive deficiencies in MAM-17 rats, including impairments in spatial learning and memory (Gourevitch *et al.*, 2004; Hazane *et al.*, 2009; Gastambide *et al.*, 2015a; Ratajczak *et al.*, 2015a) contextual discrimination deficits(Gill, Miller and Grace, 2017), impaired cognitive flexibility (Moore *et al.*, 2006) and deficits in problem-solving procedure (Robert E Featherstone *et al.*, 2007). Despite the fact that MAM-17 rats show difficulties in learning working memory tasks (Howe *et al.*, 2015), their performance is found normal, as well as their sustained attention ability (Robert E Featherstone *et al.*, 2007). Histological findings have shown decreased total brain weight in MAM-17 rats (Flagstad *et al.*, 2004) and reduced thickness in cortical and subcortical regions, including HPC, PFC, dorsal striatum and hypothalamus(Flagstad *et al.*, 2004; Moore *et al.*, 2006; Robert E Featherstone *et al.*, 2007; Matricon, Bellon, Frieling, Kebir, Le Pen, Beuvon, Daumas-Duport, Thérèse M Jay, *et al.*, 2010; Sanderson *et al.*, 2012), which have been observed in human patients. Also, laminar disorganization in the entorhinal cortex, heretopias in CA3 subregion of HPC, along with decreased neuronal soma size has been reported (Matricon, Bellon, Frieling, Kebir, Le Pen, Beuvon, Daumas-Duport, Thérèse M Jay, *et al.*, 2010) in the brain of MAM-17 rats. The face validity of the MAM 17 model in rats is further supported by evidence of dysregulated Dopamine system (reviewed (Grace, 2017)) and Parvalbumin loss in ventral subicullum, dorsal hippocampus regions and medial PFC (Penschuck *et al.*, 2006; Lodge, Behrens and A. a Grace, 2009; Kathryn M Gill and Grace, 2014). With regards to synaptic transmission, a few electrophysiological deficits have been found in MAM-17

rats, including hyperactivity of Ventral tegmental area dopamine neurons (Lodge and Grace, 2007), alterations in synaptic plasticity of Nucleus accumbens after HPC neurons activation, but unaltered synaptic plasticity (Sanderson *et al.*, 2012). The predictive validity of GD17 MAM model has recently started to be tested by few studies, using anti-psychotic drugs(Valenti *et al.*, 2011; Belujon, Patton and Grace, 2014) or treating animals with a novel a5GABA$_A$ benzodiazepine-positive allosteric –modulator (Gill, Miller and Grace, 2017), to ameliorate psychotic-like symptoms, or using a novel mGluR5positive allosteric modulator to restore cognitive deficits (Gastambide *et al.*, 2012). Most recently, peripubertal diazepam treatment was tested for its efficacy in attenuating/restoring specific behavioral and physiological phenotypes of MAM-17 rats (Du and Grace, 2013, 2016).

2.1.2 Aim of the study

As mentioned earlier, a reliable animal model should meet the triad of validity criteria and it seems that the GD17 MAM model in rats fulfills them. The fact that it is based on the neurodevelopmental hypothesis of SZ, the leading theory for the pathophysiology of the disorder, together with the absence of confounding drugs or surgical interventions in the offspring, as seen in pharmacological and lesion models, can be considered important advantages of this model (C. Jones, Watson and Fone, 2011). On the other hand, genetic models have largely contributed in understanding specific genes impact on certain endophenotypes of SZ, but cannot replicate the complexity of the disorder. The need of creating more advanced animal models to study complex psychiatric disease, such as SZ, is growing. Genetic and environmental factors need to be integrated by developing animal models that will be based on gene-environment interaction (Ayhan *et al.*, 2009; Papaleo, Lipska and Weinberger, 2012; McOmish, Burrows and Hannan, 2014). Therefore, we pursued the development aim of MAM model in mice. Given the difficulties in the development of genetic rat models, along with the increased number of genetic mouse models that already exist, we believe that this would give us in the near future, the possibility to create an advanced animal model, by incorporating genetic and environmental dimensions in one

model. This would help to better understand the complexity of SZ, the potential pathogenic or protective function of certain genes and gene mutations, even at specific time points of development. In addition, we aim to validate the MAM model in both sexes, as SZ affects both men and women. Although a higher incidence is observed in men, along with an earlier age of onset and a more severe phenotype, women patients also suffer from the disorder. Hence, it is important to investigate both sexes in an effort to elucidate the underlying mechanisms that lead to the sexual dimorphism, which could help the development of more effective therapeutics.

2.2 Materials and Methods

2.2.1 Animals and MAM treatment

All experiments were conducted in adult (>3 months old) C57BL/6 male and female offspring of pregnant dams treated with either saline or MAM. Mice were housed in groups (3-4 per cage) and provided with standard mouse chow and water ad libitum, under a 12 h light/dark cycle (light on at 7:00 am) with controlled temperature (23 +/- 1 Celsius). All procedures were performed according to the Guidelines of the Research Ethics Committee of the University of Crete and the European Union ethical standards outlined in the Council Directive 2010/63EU of the European Parliament on the protection of animals used for scientific purposes.

Time pregnant dams received intraperitoneal (i.p.) injections of MAM (MRI global, Kansas City, MO) (26mg/kg) or saline (1ml/kg) on GD 16 or 17. According to species comparison of Carnegie stages of embryonic development(https://embryology.med.unsw.edu.au/embryology/index.php/Carnegie_Stage_Comparison), GD16 rather than GD17 of mice corresponds better to the GD17 of rats, regarding the morphological development of the embryo. In addition, the latest predictive model (Workman *et al.*, 2013) proposes that equivalent maturation stages of brain development between mouse and rat differ 1-2 days, with the latter maturing later. Based on these data, we initially

tested mice treated on both GDs in validation experiments, referred from now on as MAM-16 and MAM-17mice. We continued our study using the offspring with the most intense phenotype to reduce the number of animals used. All injections were conducted between 01.00-03.00p.m. Time pregnant BALB/c dams were used as foster mothers, until pups were weaned on day 25. All behavioral experiments were conducted between 10.00a.m.-5.00p.m..

2.2.2 MK-801 challenge

Locomotor hyperactivity in response to MK-801 was assessed in a total of 30 female animals, 10 mice per group (saline, MAM-16, MAM-17). Animals were moved to the experimentation room, 1 h prior the test, for acclimation. The experiment took place under low illumination conditions. Animals were placed in an open field arena (45x45x45cm) for a 3-hour habituation period. After subcutaneous (s.c.) saline injection (1ml/kg), they were placed back to the arena for 30 minutes, and finally they received an i.p. injection of MK-801 and placed back for 90 more minutes. Locomotor activity was video-recorded during the whole task, using a recording camera. All videos were analyzed with ANY-maze tracking software. For each animal, total distance traveled was measured throughout the experiment in 10min bins.

2.2.3 Prepulse Inhibition (PPI) of the acoustic startle reflex

The expression of PPI of the acoustic startle reflex was assessed in a total of 27 male mice (10 saline, 12 MAM-16 and 7 MAM-17). We used a custom-made PPI apparatus, which consisted of a mini-chamber (3x7x2cm), located inside a ventilated plywood sound attenuating box, dimly lit. Animals were habituated to the mini-chamber prior to testing for 3 non-consecutive days, 10 minutes each day, to reduce anxiety levels. All acoustic noise bursts and background noise were delivered through a computer, connected to a speaker located inside the box. Throughout the habituation sessions a background noise level of 68dB was maintained. At testing day, after a 7-minute acclimation period (68dB background noise), animals were submitted to a series of 5 startle stimuli, referred as pulse-alone (115dB, duration 50ms),

with inter-trial intervals of 20-25 seconds, aiming to accustom the animals to the startle pulses. Subsequently, animals received 15 pulse-alone (115dB, 50ms), 15 prepulse+pulse, where the prepulse was 6-12 dB above background, had duration of 20ms and inter-stimulus interval between the prepulse and the pulse 40ms, and 10 prepulse-alone stimuli, pseudo-randomly presented every 20-25 sec. The behavior of the animal was monitored throughout the task, using a camera placed inside the box. Animal response to each noise burst was tracked with OpenVision control tracking software, exported as image processing data and analyzed with custom-made Matlab code (http://github.com/NBLab/PPI). In order to calculate the startle response, 5 image frames before each sound (1 sec) and 1 image frame after each sound (20msec)were analyzed for animal movement. Animal movement was counted as the number of pixels that changed from one frame to the other. Startle amplitude was calculated as the ratio of the number of moved pixels after the sound to the number of moved pixels before the sound. Mean amplitude of startle response to pulse alone (P) and Prepulse+pulse (PP+P) trials, was calculated for each animal. The level of PPI was assessed by expressing the Prepulse+pulse response amplitude as a percentage decrease from pulse alone response amplitude, using the following formula: %PPI=100-[100 x (PP/P)].

2.2.4 Contextual fear conditioning

The contextual fear conditioning paradigm was used to assess fear memory formation, as outlined before (Nikoletopoulou *et al.*, 2017), in 17 female (8 saline and 9 MAM-16) and 19 male (8 saline and 11 MAM-16) mice. Specifically, animals were transferred to the experimentation area 1 h prior the experiment for acclimation and at every single day of the task, both groups of animals (saline and MAM-treated) were used. On the 1st day of the experiment (training day), mice were placed in the fear-conditioning chamber (MedAssociates, St Albans, VT, USA), which was controlled through a custom-made interface connected to the computer, for 10 minutes. After a 7 min of habituation to the chamber, a mild electrical foot shock (0.7mA, 1sec)

was delivered and the animal remained for another 3 minutes. The following day, mice were returned to the training chamber but did not receive any electrical shock. The activity of each animal was recorded and freezing behavior was analyzed with the JWatcher software (http://www.jwatcher.ucla.edu/). Every 5 seconds the observer scored the behavior of the animal (moving or not moving-freezing) and the freezing behavior was calculated using the formula: %freezing=[not moving/(moving + not moving)].

2.2.5 Delayed alternation task in the T-maze

The T-maze apparatus includes a start arm and two goal arms (45X5cm each). The delayed alternation task in the T-maze is a classic behavioral task used for the study of working memory(Konstantoudaki *et al.*, 2018). 15 female (7 saline and 8 MAM-16) and 16 male (8 saline and 8 MAM-16) were tested. Mice were initially handled by the experimenter for about a week, food-restricted so that animals maintained 85-90% of their initial weight and then habituated in the T-maze apparatus for 2 days. Mice were subjected to 10-trial sessions, 3 sessions per day. At the first trial of each session, mice were allowed to freely choose between the right or left goal arms. In the following trials, mice had to alternate the goal arms in order to receive reward, initially with no temporal delay between the trials. Once they reached a pre-defined criterion for the alternation procedure (i.e., 2 consecutive sessions of ≥70% correct choices (performance), delays were introduced starting at 5 seconds and increasing by 5 seconds when the criterion for each delay was achieved, until mice completed the alternation procedure with a 15 seconds delay. Upon successful completion of the above mentioned trials, mice were tested for 2 days, 3 sessions a day, in a random set of delays ranging from 5 seconds to 25 seconds in order to better test performance in the working memory task.

2.2.6 Tissue preparation

For the histological experiments 3-6 animals per group were used. Mice were deeply anesthetized with 20mg/ml avertin (250mg/kg, i.p.), perfused with phosphate buffer saline (PBS) and subsequently withice cold phosphate-buffered 4% paraformaldehyde. Brains were removed, post-fixed for 24 hours and preserved in PBS-azide at 4^{o}C, until slicing with a vibratome (VT1000S, Leica Microsystems, Wetzlar, Germany). 40-μm-thick coronal slices were cut sequentially in sets of four sections. 3-5 sections per animal were used for neuroanatomy and immunohistochemistry experiments, which corresponded to different rostro-caudal levels of the brain.

2.2.7 Neuroanatomy based on Nissl staining

Slices containing the PFC, HPC or barrel cortex (BC) were stained with cresyl violet, as previously described (Konstantoudaki *et al.*, 2016). Briefly, sections were incubated in xylene (5min), 90% and 70% ethanol solutions (3min), dH_2O, followed by 10-min incubation in 0.1% Cresyl Violet solution. Sections were then dehydrated with increasing concentrations of ethanol (70%, 90%,100%), incubated in xylene for 5min and coverslipped with permount. Sections containing PFC were taken from Bregma 2.22 to 1.70 mm, sections containing dorsal HPC were taken from Bregma -1.34 to -2.06mm and finally, sections containing BC corresponded to three different levels: Bregma -0.94 to -1.22mm, -1.34 to -1.70mm and -1.82 to -2.06mm. This categorization aimed to minimize the bias in the measurements, if BC thickness changed along the rostro-caudal axis. Images from whole sections were obtained in 5x magnification of a light microscope (Axioskop2FS, Carl Zeiss AG, Oberkochen, Germany). Multiple overlapped pictures were taken for each slice and merged using Adobe Photoshop CS6 software. We measured cortical thickness for PFC and BC as well as the horizontal and vertical dimensions of HPC. According to the literature, three subdivisions of PFC have been recognized, in terms of cyto-architecture, chemo-anatomy, connectivity and function (Heidbreder and Groenewegen, 2003; Etkin, Egner and Kalisch, 2011; Giustino and Maren, 2015), namely the anterior cingulate

(ACC), prelimbic (PrL) and infralimbic (IL) cortices. The width of each different subdivision was measured from midline to the beginning of the white matter. For HPC, a horizontal straight line was taken from the dorsal tip of the third ventricle, reaching the corpus callosum, for the horizontal plane measurement and a vertical straight line was taken in the middle of the natural curve the structure forms, extending until the beginning of thalamus. The exact position of the BC was identified using the online mouse brain atlas (http://www.brain-map.org/). Two vertical lines from the edge of corpus callosum, reaching layer I of the cortex (650μm in between distance) were used for thickness measurement. To ensure the accuracy of the measurements, the two lines were taken 260μm inwards the approximate borders of BC. The average of the two measurements was calculated and used for the analysis. All area measurements were conducted manually, using Adobe Photoshop CS6 software.

2.2.8 Fluorescent immunohistochemistry

Free floating sections, adjacent to the Nissl-stained sections, containing PFC or HPC (as described above) were stained using indirect fluorescent immunohistochemistry for detection of PV–containing interneurons. Briefly, sections were rinsed with Tris-buffered saline (TBS, 1M), blocked for 90 minutes with 10% fetal bovine serum (FBS), 0.4% Triton in TBS-Tween 0.01% and incubated with primary antibody (rabbit-polyclonal anti-PV, 1:3000, PV27, Swant, Inc., Switzerland) in 5% FBS, 0.2% Triton in TBS-Tween 0.01%, overnight at 4^{o}C. Sections werethen incubated in secondary antibody (Goat-anti-rabbit, Alexa-488 conjugated, 1:500, Thermo Fisher Scientific, Inc., U.S.A.) for 2 hours at room temperature. They were subsequently rinsed with TBS-Tween 0.01% and incubated with Propidium Iodide in TBS for 7 minutes, after a 30 minute incubation with RNAseA (Quiagen, Inc., U.S.A.) in TBS. Finally, sections were rinsed with TBS, mounted onto slides and coverslipped with Mowiol solution. After PV immunostaining, images were obtained with a confocal microscope (Leica TCS, SP1, Leica Microsystems, Mannheim, Germany) using the 10x objective. Multiple overlapped pictures were taken for

eachregion of interest and merged using Adobe Photoshop CS6 software. For HPC containing sections, PV-positive cells were counted manually for the CA1 area. For PFC-containing sections, PV-positive cells were summed in the ACC and PrL areas. Merged images were cropped in the regions of interest, according to mouse brain atlas and the background color of each cropped image was converted to black, while the cells were colored green. Images were then loaded into Matlab, where the number of green cells was counted in each image, regardless of staining intensity.

2.2.9 Electrophysiology

Electrophysiological experiments were performed using the *in vitro* slice preparation as previously described (Konstantoudaki *et al.*, 2016). Mice were decapitated under halothane anesthesia. The brain was removed immediately and placed in cold, oxygenated (95% O_2 / 5% CO_2) artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 1 $MgCl_2$ and 10 glucose (pH= 7.4, 315 mOsm/l). The brain was blocked and glued onto the stage of a vibratome (Leica, VT1000S, Leica Biosystems GmbH, Wetzlar, Germany). 400-μm-thick brain slices containing either the PFC or the HPC were taken and were transferred to a submerged chamber, which was continuously superfused with oxygenated (95% O_2 / 5% CO_2) aCSF containing (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, $2CaCl_2$, 1 $MgCl_2$ and 10 glucose (pH= 7.4, 315 mOsm/l) at room temperature. After 1-2 hours of equilibration, slices were transferred to a submerged recording chamber, which was continuously superfused with oxygenated (95% O_2 / 5% CO_2) aCSF(same as constitution as the one used for maintenance of brain slices), at room temperature. Extracellular recordings were conducted in one or two slices per animal for each region studied.

For HPC recordings, extracellular recording electrodes filled with NaCl (2M) were placed on pyramidal layer of CA1 subregion of dHPC containing slices (Bregma -1.34 to -2.06mm). Platinum/iridium metal microelectrodes (Harvard apparatus UK, Cambridge, UK) were placed on CA1 subregion, about 300μm away from the recording electrode and near CA3 subregion. For

PFC recordings, both the recording and stimulating electrodes were placed on layer II/III, (in-between distance 300μm), of PFC-containing slices (Bregma 2.22 to 1.70 mm). Stimulation of the regions evoked field excitatory postsynaptic potentials (fEPSPs) that were amplified using a Dagan BVC-700A amplifier (Dagan Corporation, Minneapolis, MN, USA), digitized using the ITC-18 board (Instrutech, Inc, Longmont, CO, USA) on a PC, using custom-made procedures in IgorPro (Wavemetrics, Inc, Lake Oswego, OR, USA). The electrical stimulus consisted of a single square waveform of 100μsec duration given at intensities of 0.05-0.3mA, generated by a stimulator equipped with a stimulus isolation unit (World Precision Instruments, Inc, Sarasota, FL, USA).

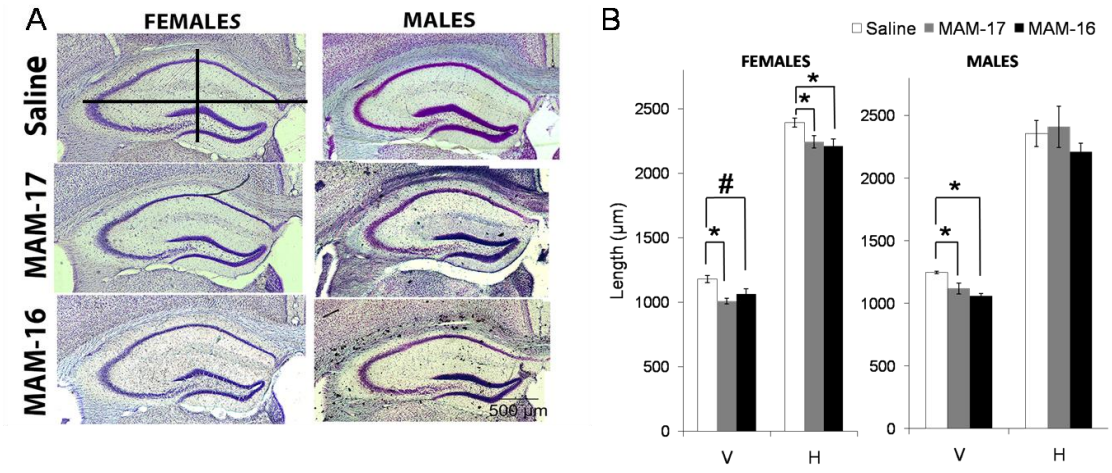
2.2.10 Data acquisition and analysis

Data were acquired and analyzed using custom-written procedures in IgorPro software. The fEPSP amplitude was measured from the minimum value of the synaptic response (4-5 ms following stimulation) compared to the baseline value prior to stimulation. Both parameters were monitored in real-time in every experiment. A stimulus-response curve was then determined using stimulation intensities between 0.05 and 0.3mA. For each different intensity level, two traces were acquired and averaged. Baseline stimulation parameters were selected to evoke a response of 1mV. For the long-term potentiation experiments, baseline responses were acquired for at least 20 minutes. Then two theta-burst stimulation trains (5 bursts of 100Hz/5Hz) were applied in dHPC-containing slices, while three 1second tetanic stimuli (100Hz) with an inter-stimulus interval of 20 seconds were applied in PFC containing slices. Finally, responses were acquired for at least 30 minutes and 50 minutes post theta-burst and post-tetanus, for HPC and PFC recordings, respectively. Synaptic responses were normalized to the average 10 minutes pre-theta burst/pre-tetanic fEPSP.

2.3 Results

The MAM model has not been previously studied in mice. Therefore, we first conducted validation experiments, in three groups of animals: a) mice that were in injected with saline at GD16 (saline group), b) mice that were injected with MAM at GD17 (MAM-17 group), which is the same injection time-point as in rats, and c) mice that were injected with MAM at GD16 (MAM-16 group), the time point that we predict will have a stronger phenotype and similar to the rat MAM model. The validation experiments included the investigation of histological alterations of the HPC, PFC and BC, behavioral tests of 'schizotypic-like' symptoms (MK-801-induced hyperlocomotion in female mice and PPI of the acoustic startle reflex test in male mice), due to sexual dimorphic results reported in the literature (see discussion below), as well as immunostaining for the PV protein, in both sexes.

2.3.1 Morphological alterations in brain regions after MAM exposure



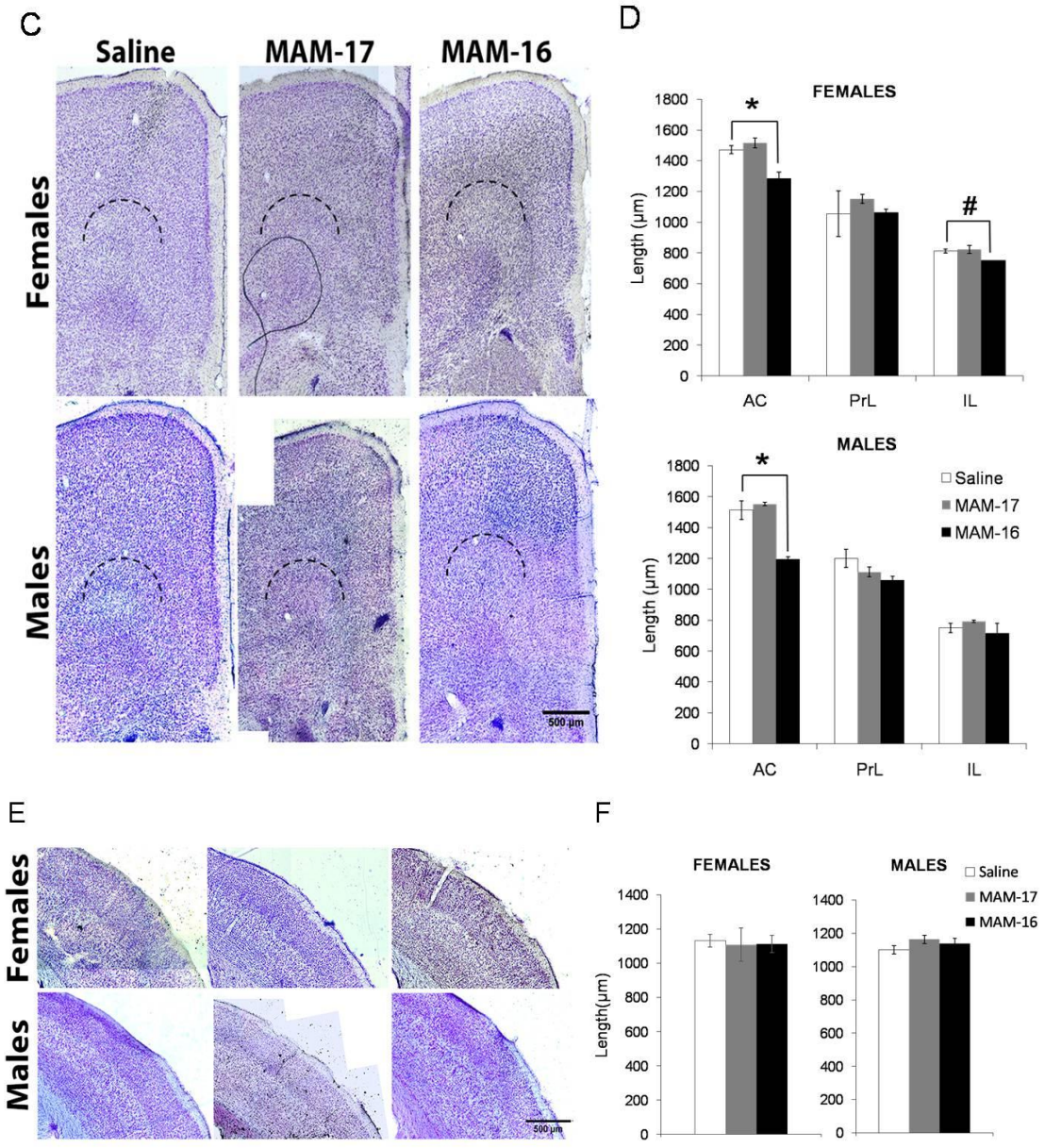


Figure 2.1: Anatomical alterations in cortical and subcortical brain regions. A) Representative images of coronal sections containing the dorsal hippocampus of Saline- and MAM- exposed male and female mice. B) Bar graphs showing the vertical (V) and horizontal (H) plane measurements in each group. C) Representative images of coronal sections containing the prefrontal cortex. D) Bar graphs showing the width of each subregion. E) Representative images of coronal sections containing the Barrel cortex. F) Bar graphs showing the absence of differences in barrel cortex thickness between saline and MAM-exposed mice, in both sexes. MAM, methylazoxymethanol acetate; ACC, Anterior cingulate cortex; PrL, Prelimbic; IL, Infralimbic).

Histological analysis, using Nissl staining, revealed anatomical alterations

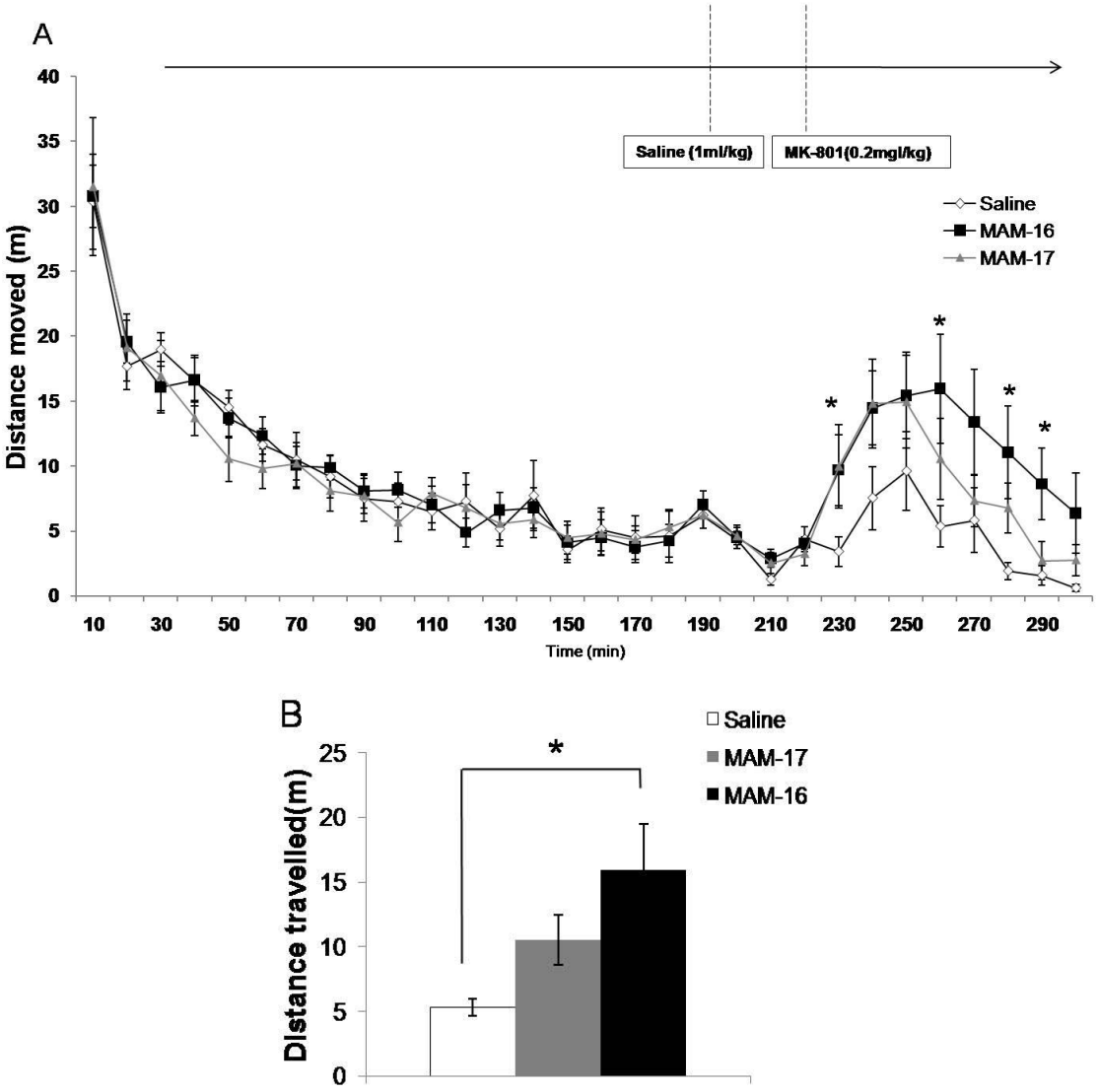
in cortical and subcortical brain regions in MAM treated mice. For HPC, a decrease in the vertical plane of the hippocampal formation in female MAM-17 mice (Kruskal-Wallis test, *p*=0.03) and a trend towards a decrease in female MAM-16 mice (Kruskal-Wallis test, *p*=0.08) were observed (Fig. 2.1A, B). Similar effects were identified in male mice, in which both MAM-16 and MAM-17 mice exhibit significantly reduced vertical dimension, compared to saline-treated mice (Kruskal-Wallis test, *p*=0.01 and p=0.03, respectively). At the horizontal plane, analysis revealed a significant effect of MAM treatment in female mice for both MAM-16 (Kruskal-Wallis test, *p=0.006*) and MAM-17 group (Kruskal-Wallis, *p*=0.02) but no significant alterations were observed in either group of MAM-treated male mice, compared to saline-treated mice (Kruskal-Wallis test, *p*=0.70and *p*=0.30, for MAM-17 and MAM-16, respectively) (Fig. 2.1A, B).

For the PFC, we analyzed the width of ACC, PrL and IL. ACC width was found statistically decreased in MAM-16 female and in MAM-16 male mice, compared to their respective saline-treated mice (Kruskal-Wallis test, *p*=0.02and *p*=0.006, respectively), whereas no alterations were observed in MAM-17 female and male mice (Kruskal-Wallis test, *p*=0.43) (Fig. 2.1C, D). No significant alterations were found in the width of PrL subregion between MAM-treated and saline-treated animals (Kruskal-Wallis test, *p*=0.20 for both females and males [MAM-16/MAM-17]). Additionally, the analysis of the IL cortex width showed a trend for thinning only in female MAM-16 mice (Kruskal-Wallis test, *p*=0.06) and no alterations in male MAM-16 mice (Kruskal-Wallis test, *p*=0.35) or MAM-17 mice groups (Kruskal-Wallis test, *p*=0.71 and *p*=0.60, for females and males, respectively). Finally, no significant differences were identified between MAM-16, nor MAM-17 and saline-treated mice in BC of females (Kruskal-Wallis test, *p*=0.80 and 0.60, MAM-16 and MAM-17, respectively) and or males (Kruskal-Wallis test, *p*=0.60 and.1 for MAM-16 and MAM-17, respectively) (Fig. 2.1E, F).

2.3.2 Schizotypic-like symptoms in MAM mice

We subsequently examined the possible schizotypic-like symptoms of the MAM-treated mice at the behavioral level, such as the enhanced locomotor activity in response to an MK- 801 challenge and the reduced PPI of the acoustic startle reflex. Animals were separated by sex, in an effort to reduce the number of subjects used. In particular, female mice were examined for their locomotor activity in response to MK-801, since females are more sensitive in this task (Andiné *et al.*, 1999). Male mice were used in the PPI task, as there have been indications for control females exhibiting reduced levels of PPI (Kumari, Aasen and Sharma, 2004; Matsuo *et al.*, 2016).

In female mice, no differences were observed among the groups with regards to locomotor activity during the first three hours of the test or the



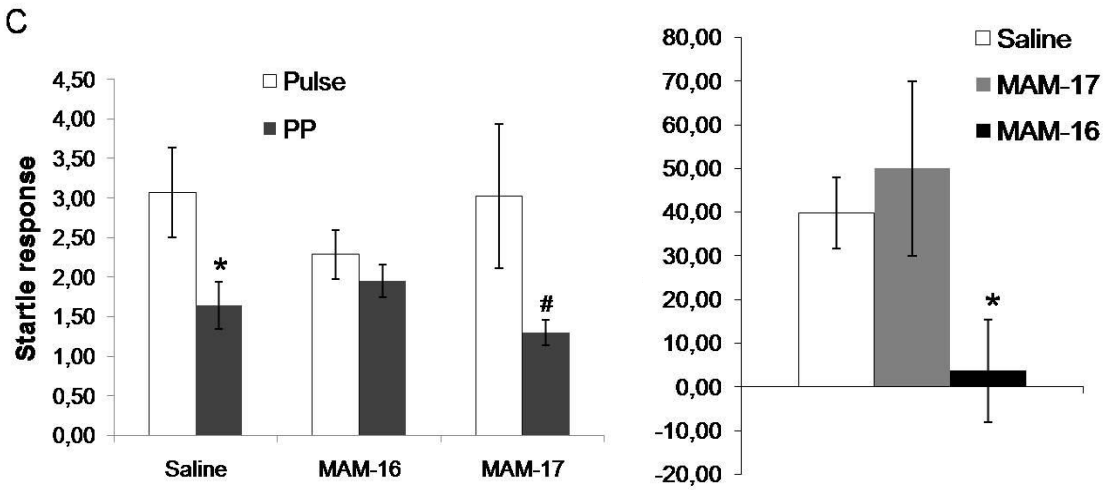


Figure 2.2: **Schizotypic-like' phenotype of male and female MAM-treated mice.** A-B. Locomotor hyper-activity of female mice after systemic treatment with MK-801. A) Line plot showing the locomotor activity in the open field, expressed as distance (m) travelled, during habituation phase (0-180min), after saline injection (180min-210min) and after treatment with 0.2mg/kg of MK-801 (210min-300min). B) Bar graph showing the mean total distance travelled for each group, during the first 50 minutes after the MK-801 injection. C-D. Prepulse inhibition of the acoustic startle reflex of male mice. C) Bar graphs showing the mean startle response of each group at pulse alone (Pulse) and prepulse – pulse (PP) trials. D) Percent of Prepulse inhibition.

30min after saline (s.c.) injection. In all groups, locomotor activity decreased over time, indicative of normal habituation in the apparatus. Administration of MK-801 (0.2 mg/kg, i.p.) resulted in enhanced locomotor activity in all groups (repeated measures ANOVA, $F_{(2,27)}$=3.47,p=0.04). Post-hoc analysis showed a significant effect of MAM treatment in the MAM-16 group (p=0.04), but no significant effect of MAM exposure in the MAM-17 group (p=0.5) (Fig. 2.2A). In addition, analysis of the total distance travelled, showed that MAM-16 mice had already travelled significantly longer inside the apparatus 50 minutes after the MK-801 injection (post-hoc LSD test, p=0.02), compared to saline-treated mice, while MAM-17 mice did not seem to differ significantly from the control group (post hoc, p= 0.15) (Fig.2.2B).

In male mice, no significant differences were found in the startle response among the three groups (Kruskal-Wallis test, p=0.43) (Fig.2.2C). Saline-

treated mice significantly inhibited their startle reflex in response to the prepulse-pulse tone (p=0.01), and so did the MAM-17 mice (p=0.06). However, MAM-16 group did not inhibit the startle reflex in response to the prepulse-pulse tone (p=0.20). When all three groups were tested for their PPI index, we found a significant decrease in MAM-16 group, compared to saline (Kruskal-Wallis test, p= 0.03), but normal expression of PPI in MAM-17 mice (Kruskal-Wallis test, p= 0.14) (Fig.2.2D).

2.3.3 PV expression in MAM-16 mice

Based on the behavioral and anatomical results, that showed a stronger 'schizotypic' phenotype of MAM-16, compared to MAM-17 mice we continued our experiments with MAM-16 mice, because they had a stronger phenotype compared to MAM-17 mice. Decreased PV expression has been identified as a histological marker of SZ from post-mortem studies (Lewis *et al.*, 2001; Zhang and Reynolds, 2002; A. Y. Wang *et al.*, 2011). Using fluorescent immunohistochemistry for the PV protein, we counted the number of PV-positive cells in brain slices containing the HPC or PFC. Our analysis, revealed a sex- selective decrease in PV-positive interneurons in the brain of MAM-16 mice. In the CA1 HPC region, the analysis showed no alterations in the number of PV-positive cells in female MAM-16 mice (Mann-Whitney test, p=0.52), while in male MAM-16 mice a statistically significant decrease was observed in the number of PV-positive cells (Mann-Whitney test, p=0.02) (Fig. 2.3A, B). Similarly, no alteration in the number of PV-positive cells of PFC was observed in MAM-16 female mice (Mann-Whitney test, p=0.50), but decreased number of PV-positive cells in MAM-16 male mice (Mann-Whitney test, p=0.05) (Fig. 2.3C,D).

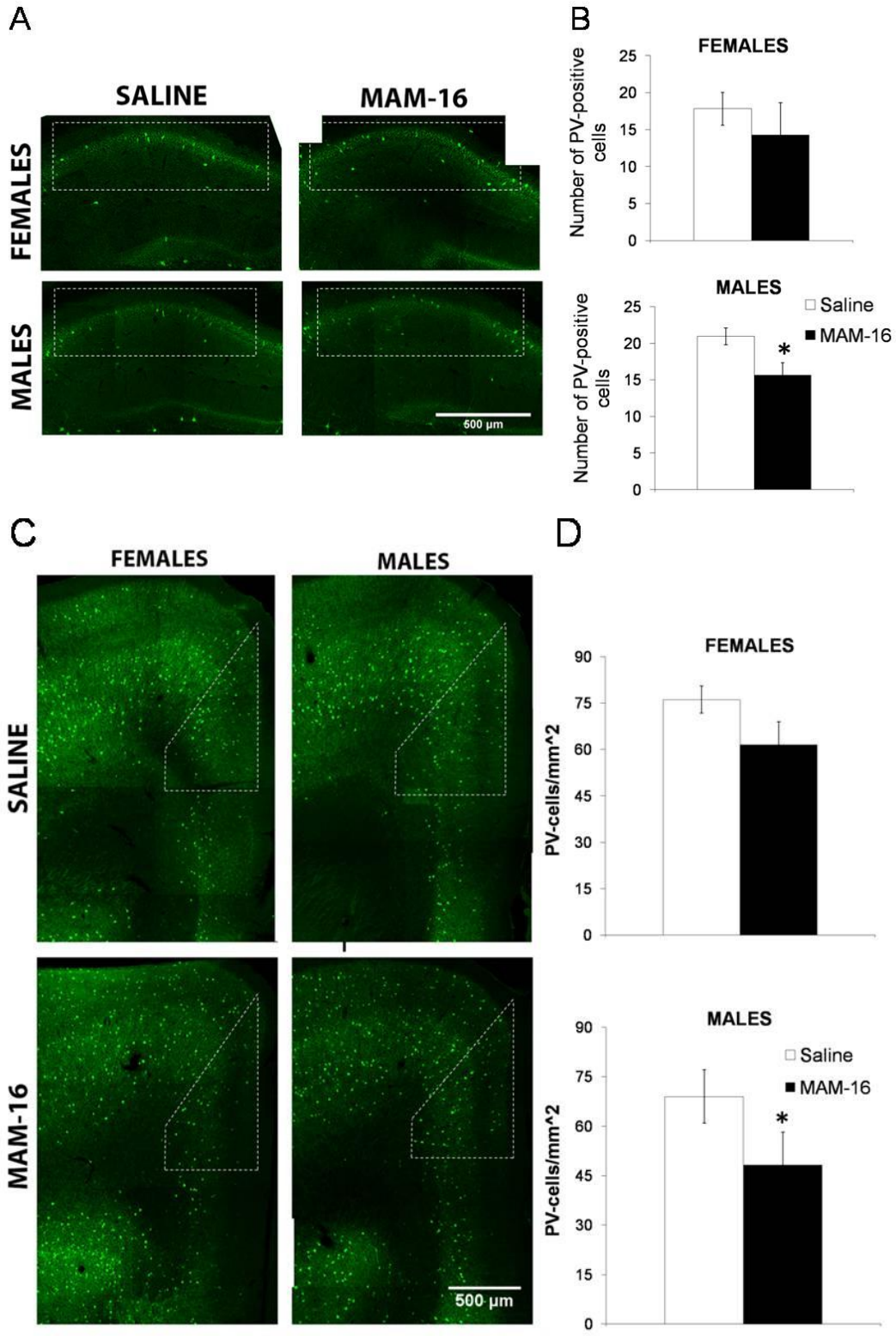


Figure 2.3: **Parvalbumin expression in HPC and PFC of MAM-16 mice** A) Representative images of coronal hippocampal sections from saline and MAM-16 mice. White frames indicate the borders of CA1 region. B) Bar graphs showing a significant reduction in the number of PV-positive cells in CA1 of male MAM-16 mice C) Representative images of coronal sections containing PFC, from saline and MAM-16 mice. D) Bar graphs showing a significant reduction in the number of PV-positive cells in PFC of male MAM-16 mice

2.3.4HPC function deficits in both Male and Female MAM-16 mice

The MAM model in rats has also been shown to affect HPC function (Moore *et al.*, 2006; Penschuck *et al.*, 2006; Matricon, Bellon, Frieling, Kebir, Le Pen, Beuvon, Daumas-Duport, Thérèse M. Jay, *et al.*, 2010; Hradetzky *et al.*, 2012; Snyder, Adelman and Gao, 2013; Kathryn M. Gill and Grace, 2014). To assess HPC function in our model, the contextual-fear conditioning paradigm was used in both female and male mice. Mice were trained and tested for fear memory 24 hours later. Both female and male MAM-16 mice showed a statistically significant decrease of their freezing behavior, compared to saline mice (*t*-test, *p* =0.03 and *p*=0.02, females and males, respectively) (Fig. 2.4A), suggesting that prenatal MAM exposure can cause deficits in contextual fear memory of both male and female mice. We next investigated synaptic transmission and plasticity in hippocampal CA3 to CA1 synapses. We found that the fEPSP was similar for both females and males in response to increasing current stimulation (repeated measures ANOVA, $F_{(1,14)}$=0.7, p=0.2 for females, $F_{(1,14)}$= 0.5, p=0.4 for males) (Fig.2.4B). Theta-burst stimulation induced LTP in both male and female saline-treated mice. MAM-16-treated mice, both males and females, exhibited significant reduction in the LTP (repeated measures ANOVA, $F_{(1,14)}$=10.1, p=0.001 for females and $F_{(1,14)}$= 8.2, p=0.001 for males) (Fig. 2.4C).

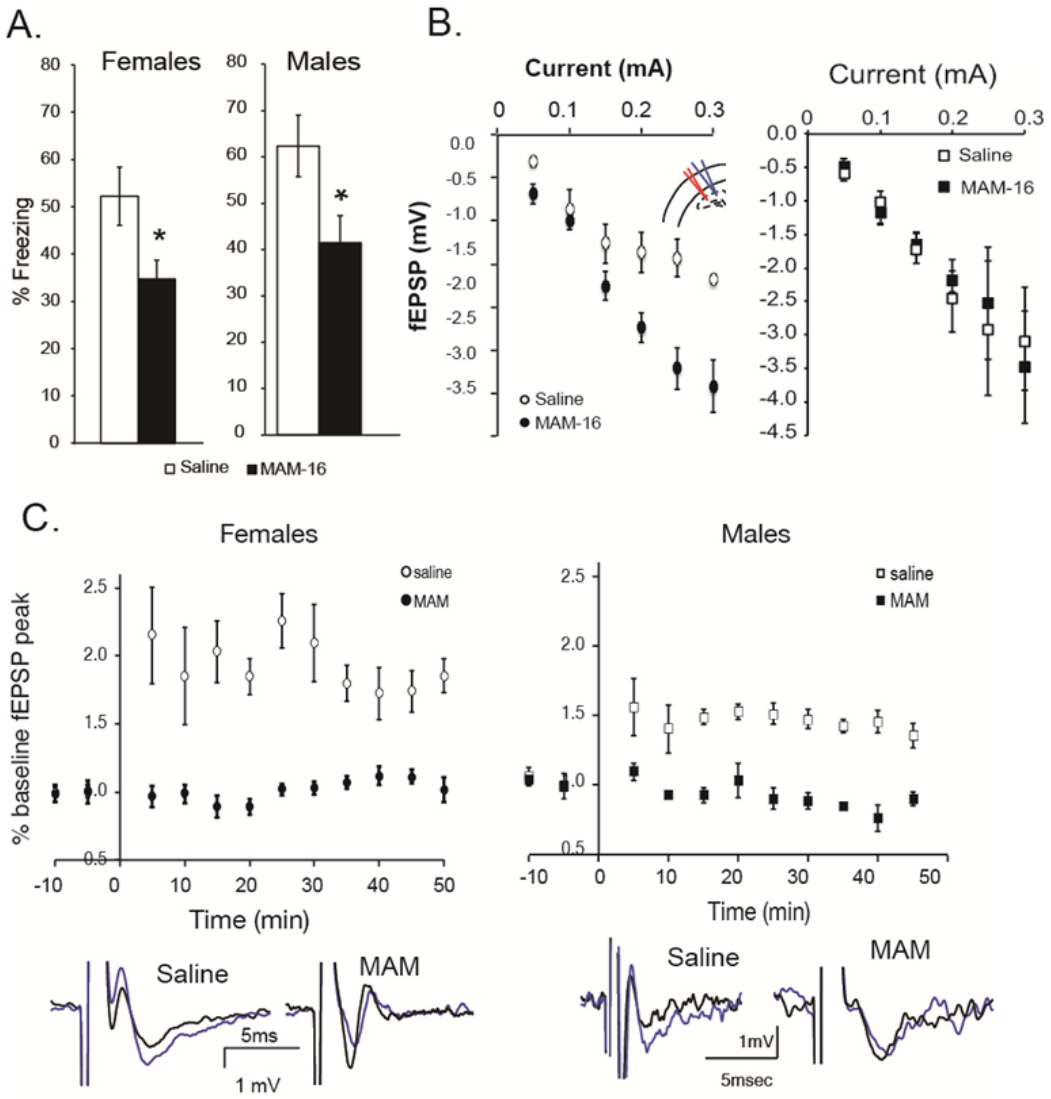


Figure 2.4: HPC function in male and female MAM-16 mice. A) Graphs showing reduced freezing behavior 24hours following training in the contextual fear conditioning task. B) Graphs showing the fEPSP in response to increasing current stimulation (left-females, right-males). C) Graphs (top) and representative traces (bottom) showing LTP following theta-burst stimulation in HPC in females and male.

2.3.5 Male but not female MAM-16 mice exhibit PFC function deficits

SZ patients and several animal models of SZ exhibit PFC deficits (Weinberger, 1987; Barch *et al.*, 2003; Keedy *et al.*, 2006; C. a Jones, Watson and Fone, 2011). Therefore, we investigated PFC function by examining working memory function as well as synaptic transmission and plasticity in

PFC brain slices. Saline and MAM-16 treated mice were subjected to the delayed alternation task, which examines spatial working memory function. Saline and MAM-16 mice were initially trained to alternate the left and right arm in the T-maze in order to receive their food reward. No significant difference was observed in the training for the alternation procedure, for both the female and male mice (one-way ANOVA,$F_{(1,14)}$=0.80,*p*=0.20). Once delays were introduced in the alternating procedure, female MAM-16 required the same number of trials to reach criterion compared to female saline-treated mice (Fig. 2.5A)(one-way ANOVA, $F_{(1,14)}$=0.20,*p*=0.30). However, this was not the case for male MAM-16 mice that required increased number of trials in order to reach criterion (one-way ANOVA, $F_{(1,14)}$= 5.7,*p*=0.02). In addition, performance in the T-maze did not differ between female MAM-16 mice and their respective saline-treated mice (t-test, *p*=0.3), while in male MAM-16 a significant reduction was observed (t-test, *p*=0.04) compared to their respective saline-treated mice (Fig. 2.5B).

Working memory function is supported by both synaptic transmission and synaptic plasticity in the PFC (Miller and Cohen, 2001; Blumenfeld, 2006; Konstantoudaki *et al.*, 2018). Therefore, we recorded fEPSPs from layer II/III of the PFC of saline or MAM-16 female and male treated mice (Fig. 2.5C). We found no alterations in the fEPSP peak in female MAM-16 mice (Fig.2.5D) (repeated measures ANOVA, $F_{(1,14)}$=0.7, *p*=0.5). However, in male MAM-16 mice the fEPSP peak was found reduced at various intensities of current stimulation ($F_{(1,14)}$=6.2, *p*=0.01) (Fig. 2.5E).

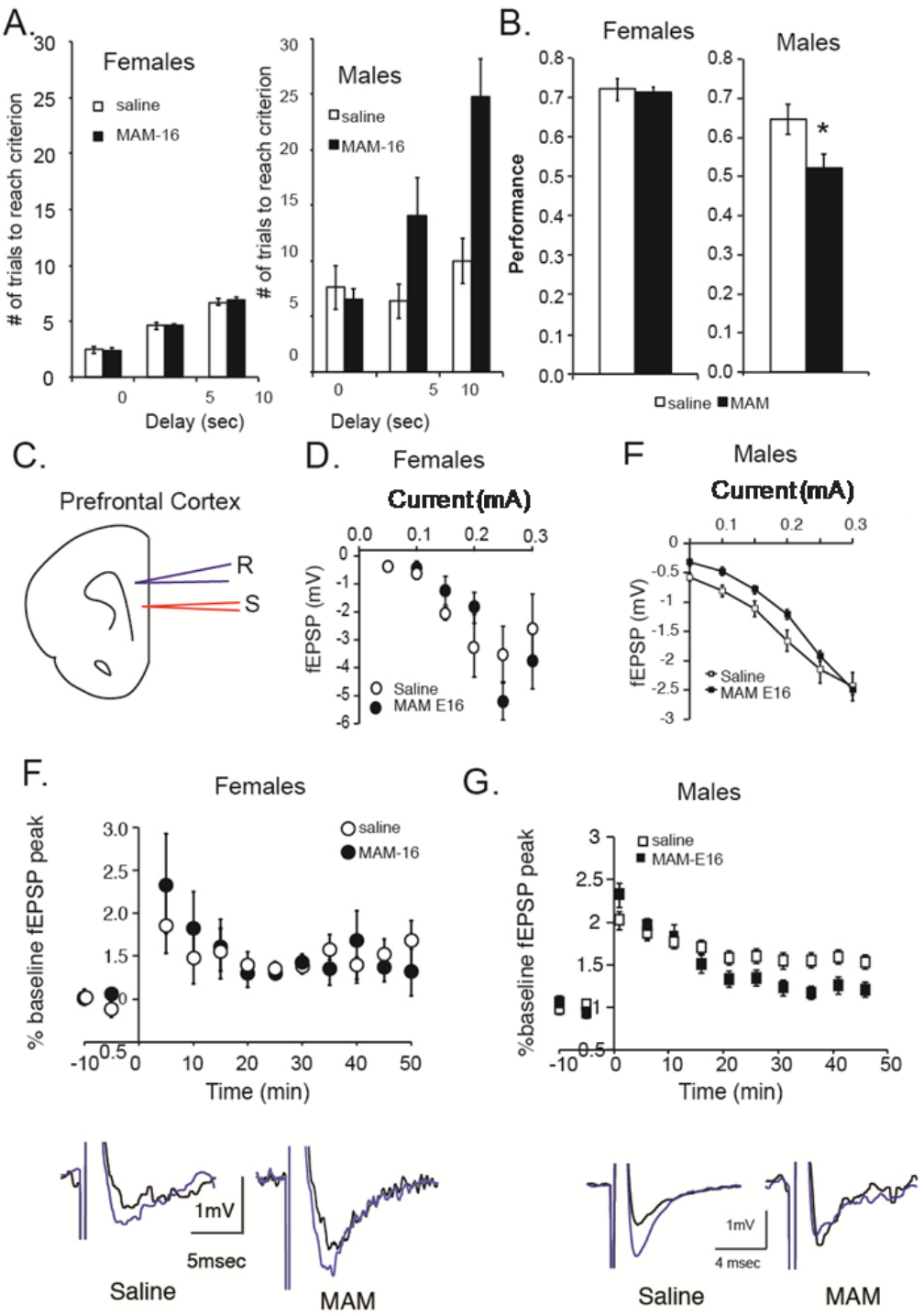


Figure 2.5: PFC function male and female MAM-16 mice. A) Graphs showing the number of cumulative trials in the delayed alternation task in the T-maze at 0, 5 and 10sec delays. B) Graphs showing the performance (% correct trials) at the 20sec delay. C) Illustrations indicating the position of the electrodes for the recordings in the PFC. D-E) Graphs showing the fEPSP in response to increasing current stimulation for male and female mice. F-G) Graphs (top) and representative traces (bottom) showing LTP following tetanic stimulation.

To determine whether synaptic plasticity in the PFC is affected, we induced

LTP by tetanic stimulation. Brain slices from both saline and MAM-16 treated female mice enhanced the fEPSP following tetanic stimulation that lasted for at least 50min (repeated measures ANOVA, $F_{(1,14)}$=0.6, p=0.6) (Fig. 2.5F). On the other hand, brain slices from saline-treated male mice exhibited enhancement of the fEPSP that lasted for at least 50min, while brain slices from male MAM-16-treated mice exhibited an initial enhancement of the fEPSP, which was significantly reduced and approached baseline levels about 15 min following the tetanus (repeated measure ANOVA, $F_{(1,14)}$=2.2, p=0.03)(Fig.2.5G). Therefore, it seems that LTP in the PFC is impaired in male MAM-16 mice but not in female MAM-16 treated mice, similar to the working memory deficits.

2.4 DISCUSSION

This part of the study describes the SZ neurodevelopmental MAM model in mice and identifies sex differences in prefrontal cortical and hippocampal function deficits. Regarding the model, we find that the MAM model in mice is better reproduced by exposure to the mitotoxin on the 16th day of gestation, compared to the 17th day of gestation in rats. Both male and female mice exhibit thinning of the cortex and the hippocampus and indications of positive symptoms (reduced PPI in males and increased locomotor activity in response to MK-801 in females). PV reduction is only observed in male mice. HPC-dependent cognitive function is equally affected in both male and female mice, while PFC-dependent cognitive function is differentially adapted in MAM-16-treated male and female mice.

2.4.1 Comparison with the MAM model in male rats

The MAM model has been established and extensively studied in male rats (Modinos *et al.*, 2015). Our results in mice indicate that the MAM model can also be used in mice by performing the MAM treatment one-day earlier, compared to rats. Species comparison of embryonic development has indicated that the mouse embryo brain development mouse occurs faster by

1-2 days compared to the rat. Therefore, the GD 16th in mice is the equivalent to GD 17th of rats and corresponds to a peak proliferation stage of neural cells, which then migrate particularly in the HPC and cortex (Bayer and Altman, 2004).It is expected that MAM administration after this peak stage of proliferation, would affect brain development in a lesser degree; possibly affecting only those regions that are formed later in brain development.

Our histological analysis shows thinning of the ACC in both male and female MAM-16 mice, but not MAM-17 mice. On the other hand, the HPC is affected in both MAM-16 and MAM-17 male and female mice. These findings are in agreement with the histopathological deficits found in MAM-17 rats, where both mPFC and HPC (dorsal and ventral part) have decreased thickness or total size, compared to control animals(Flagstad *et al.*, 2004; Moore *et al.*, 2006; Robert E. Featherstone *et al.*, 2007; Matricon, Bellon, Frieling, Kebir, Le Pen, Beuvon, Daumas-Duport, Thérèse M. Jay, *et al.*, 2010; Hradetzky *et al.*, 2012). Finally, no alterations in the width of BC, a sensory cortex, are found, in agreement to Moore et al. (Moore *et al.*, 2006). However, there are reports of shrinkage in the sensory-motor cortex (Robert E Featherstone *et al.*, 2007; Matricon *et al.*, 2010).

Several studies have shown decreased PPI of the acoustic startle reflex in male MAM-17 rats(Le Pen *et al.*, 2006; Moore *et al.*, 2006; Hazane *et al.*, 2009), which is one of the positive symptoms also observed in SZ patients. PPI is characterized as a cross species measure of sensory-motor gating and in animals can be indicative of both hyper-dopaminergic and hypo-glutamatergic function of the brain(Van Den Buuse, 2010). We also find decreased PPI in MAM-16, but not in MAM-17 treated mice. Both MAM-treated groups exhibit normal responses in startle stimuli, indicating that MAM exposure has not affected either the auditory pathway or the motor output of the acoustic startle circuit; therefore the observed decrease in PPI is due to a sensorimotor gating deficit. These experiments imply a stronger effect of prenatal MAM exposure on GD16.

Another consistent finding regarding the deficiency in post-mortem studies of SZ patients is the decreased PV-immunoreactivity or mRNA levels of the

PV expressing gene in the cortex, including the dorsolateral PFC, ACC, and HPC(Zhang and Reynolds, 2002; Torrey *et al.*, 2005; A. Y. Wang *et al.*, 2011; Konradi *et al.*, 2011). Our findings showing reduced PV expression in male PFC and HPC are in agreement with human reports and with studies of MAM-17 male rats (Penschuck *et al.*, 2006; Lodge, Behrens and A. A. Grace, 2009). Therefore, our study proposes that the MAM-16 model in mice is comparable to the MAM-17 rat model with regards to 'schizotypic-like' alterations.

Both SZ patients and the MAM-17 rats exhibit significant cognitive deficits, including working memory and spatial memory deficits. Specifically, MAM-17 rats have deficits in the water-maze and Y-maze spontaneous alternation tasks(Gourevitch *et al.*, 2004; Snyder, Adelman and Gao, 2013; Gastambide *et al.*, 2015b; Ratajczak *et al.*, 2015b; Gill, Miller and Grace, 2017). In our study, male MAM-16 mice exhibit deficits in learning and performance of the delayed alternation task and in fear memory. In addition, we find significant decrease in both the fEPSP and LTP in male HPC. Studies in rats have shown reduced intrinsic excitability in MAM-17 rats, which could account for the reduced fEPSP observed in our study, but no difference in LTP (Sanderson *et al.*, 2012). For LTP, a slightly different protocol of theta-burst was used which could account for the difference in our findings (three trains instead of 2, 10sec instead of 20sec). Furthermore, adaptations in up-down states have been observed in male MAM rats, indicative of impaired PFC function(Moore *et al.*, 2006).

2.4.2 Sex differences possibly observed in humans and other animal models of schizophrenia

Our study revealed significant sex differences in PV-expression, PFC-dependent but not HPC-dependent cognitive deficits. While the number of PV-expressing neurons decreased in male MAM-16 mice, no alterations were identified in either PFC or HPC in female mice. Since we have not measured the total number of cells in each region of interest, we cannot exclude the possibility that there is a total loss of neuronal cells in MAM-16 mice PFC and HPC, which could result in a concomitant reduction of PV-positive neurons.

However, our results show that reductions in PFC and HPC occurred in both males and females, suggesting that the reduction of PV in males is a sex specific adaptation. In humans, there is also indication of female patients that do not show alterations in PV-positive interneurons of HPC (Zhang and Reynolds, 2002).

Although SZ affects both men and women, a higher incidence is observed in men, along with an earlier age of onset and a more severe phenotype. Particularly, men exhibit more negative and cognitive symptoms compared to females and show more severe structural brain deficits(Leung and Pierre Chue, 2000; Abel, Drake and Goldstein, 2010; Ochoa *et al.*, 2012).However, sex is not always incorporated as a variable and the vast majority of research is conducted in male subjects, leaving gaps in our knowledge regarding sex-related manifestations of the disease that could affect management.

With regards to MAM model in rats, the majority of the studies have been conducted in male rats. To our knowledge, there are three studies that evaluated female rats at the behavioral and electrophysiological level(Hazane *et al.*, 2009; Pen, Jay and Krebs, 2011; Snyder, Adelman and Gao, 2013) indicating the presence of positive symptoms and reduced HPC function, in agreement to our findings. Sex differences have also been observed in other developmental animal models studies, including the Poly I:C exposure model and the neonatal ventral hippocampal lesion model (for review see (Hill, 2016)). Our study further reports that female MAM-16 mice do not exhibit significant impairments in PFC function, as indicated by similar performance in the delayed alternation task in the T-maze, same LTP emergence and maintenance. Stronger deficits in PFC function have also been found in the Disc1 mouse model, showing enhanced excitation-to-inhibition ratio in male but not in female PFC(Holley *et al.*, 2013). Although there is a lack of a significant number of studies that include both male and female subjects, the data so far indicate increased vulnerability of the male PFC to neurodevelopmental insults(Hill, 2016). Our study further shows that working memory function and synaptic transmission and plasticity are rescued in female MAM-16 mice. Overall, our description of the mouse MAM SZ model,

from the viewpoint of sex comparisons, proves that our model mirror disease sex-specific phenotypes in human and could offer an ideal platform to study differences in pathophysiology and treatment responses to the direction of tailoring management.

2.4.3 Possible mechanisms of PFC vulnerability in males but similar vulnerability of female HPC

Estrogens have been shown to exert a protective effect on the hippocampus, particularly in response to stress during adulthood. It is likely that due to the early development of the hippocampus (i.e. before adolescence), estrogens are not able to rescue HPC function in prenatal insults. On the other hand, the PFC continues to develop through adolescence at which time estrogens in females could exert their possible protective effect (McEwen, Nasca and Gray, 2015; Davis *et al.*, 2016). Furthermore, male sensitivity to perinatal testosterone may be interrupted by MAM exposure in a way that the brain, and particularly the PFC, is affected in adulthood (McCARTHY, 2008). The MAM mouse model having a sex differences phenotype similar to that of the human population could be used in conjunction with investigation of developmental events to shed light on different developmental changes that could be affected in males and females.

Chapter 3

3.1 Introduction

3.1.1 Stress, CRF and brain malfunction

3.1.1.1 Defining stress

The concept of stress has been strongly linked to negative and unpleasant situations that can disturb the normal function of an organism, permanently or temporarily, causing uncomfortable feelings. However, the idea of a beneficial type of stress, the eustress, a term created by Hans Selye in 1976 (Selye, 1976), has given a second dimension in the concept of stress, the one that can promote the progress and the well-being. The first and most common definition of stress, given by Selye states, is that "*Stress is the non-specific response of the body to any demand*". Although this concept was criticized as too general, it incorporates both the biological/physiological and psychological/cognitive dimensions, while at the same time, it promotes the molecular, genotypic and phenotypic analysis of stress response in every organism (Fink, 2010).

Thus stress represents the normal response of an organism in any stressful stimulus (stressor). It includes the activation of the nervous system, the endocrine system and the immune system. When we perceive something as a potential threat, our brain sends activating signals to our sympathetic nervous system, that prepare our body to respond to the threat; the so-called "fight or flight" response (Canon 1915), that alert the body. Another principal effector of stress response includes the hypothalamic-pituitary-adrenal (HPA) axis (Fig.3.1). In the presence of a stressful stimulus, the activation of HPA axis starts with the secretion of the corticotropin-releasing-factor (CRF) from the paraventricular nucleus of the hypothalamus, which is then released into the portal circulation. It accesses the anterior pituitary gland and subsequently

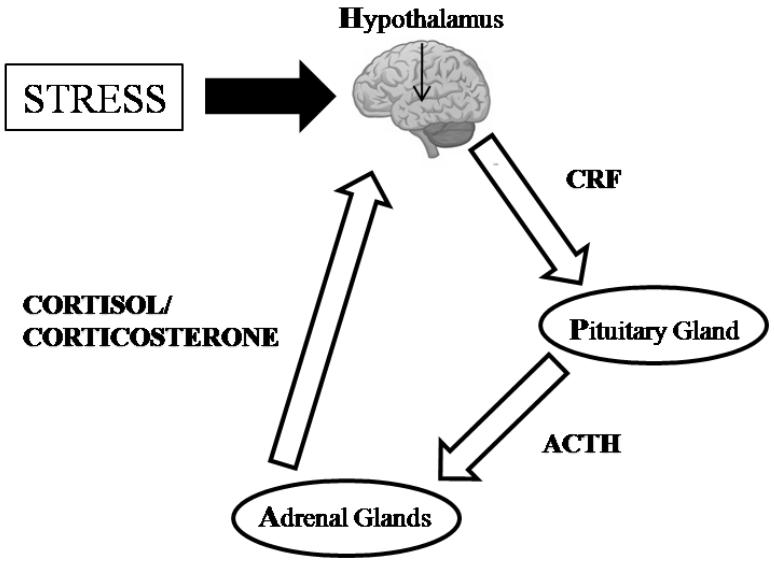


Figure 3.1: Schematic diagram of Hypothalamus-Pituitary-Adrenals (HPA) axis**.**

mediates the release of pro-opiomelanocortin (POMC)-derived peptides, such as adrenocorticotropic hormone (ACTH) and β-endorphin. ACTH is delivered through systemic circulation to the adrenal gland cortex, where it stimulates glucocorticoids (GCs) synthesis and secretion (cortisol in humans and fish and corticosterone in rodents) (Smith and Vale, 2006). GCs are the final mediators of stress response, acting on several systems and controlling the HPA axis activity, by creating a negative feedback loop. However, the HPA axis can also be activated by non-aversive, positive, stimuli, such as sexual encounter, which also result in GCs secretion (Dedic *et al.*, 2017). In any case, the ultimate goal of the response is to adapt through physiological alterations, in order to reinstate homeostasis and promote survival of the organism.

3.1.1.2 CRF system components

CRF (also referred to as corticotropin-releasing-hormone – CRH) has been characterized as the neuropeptide of stress, as it is the basic physiological activator of the HPA axis. It is a 41-amino-acid polypeptide, produced after the proteolytic cleavage of the C-terminal region of a pre-proCRF precursor (Dautzenberg and Hauger, 2002). Since its isolation and

identification in 1981 from the ovine hypothalamus (Vale *et al.*, 1981)a great number of studies have revealed the existence of a whole family of CRF-related peptides, highly conserved across species (Lederis *et al.*, 1990). Their actions are detected in both brain and periphery, and are linked to various physiological processes, including feeding, reproduction-related behaviors, learning and memory, regulation of the autonomic nervous system (Smith and Vale, 2006).CRF is the mostly studied member of its family, which includes the non-mammalian urotensin (teleost fish) and sauvagine (frog), and the mammalian urocortins 1,2 and 3 (UCN). Among UCNs, UCN 1 shares the higher homology with CRF (43%). It was first described by Vaughan et al. in 1995(Vaughan *et al.*, 1995)and is now considered to be more conserved than CRF across species (Dautzenberg and Hauger, 2002).

CRF is highly expressed throughout the brain and periphery. Numerous studies on mRNA or protein levels, mostly in rats, have consistently shown that apart from the paraventricular nucleus of hypothalamus, CRF immuno-reactive cells are found in limbic regions, such as the bed nucleus of the stria terminalis, the central amygdaloid nucleus and the hippocampus, in the brain stem, but also in the cerebral cortex (Olschowka et al. 1982; Swanson et al. 1983; Palkovits et al. 1985;Delville et al. 1992; I. et al. 2005) (Fig.3.2). Most recently, with the use of genetic techniques and advanced whole-brain optical imaging, CRF protein expression has been mapped in the mouse brain, revealing consistent results with rat studies and adding important details for the specific site of expression (soma, fibers) and the morphology of CRF-positive cells in the mouse brain (Kono *et al.*, 2017; Peng *et al.*, 2017). CRF is also expressed in peripheral tissues, including adrenal glands, placenta, gastrointestinal tract, testis, thymus and skin (Smith and Vale, 2006). On the other hand, the distribution of UCN1 in the mammalian brain is more restricted; high expression has been found mostly in the Edinger –Westphal nucleus, while low levels have been observed in other regions, such as the lateral septal area (Tamás, Hitoshi and Akira, 1998; Morin *et al.*, 1999; Weitemier, Tsivkovskaia and Ryabinin, 2005). In periphery, UCN1 is highly expressed in the glands (pituitary, thymus, testis), the gastrointestinal tract, the spleen and the cardiac myocytes (Dautzenberg and Hauger, 2002).

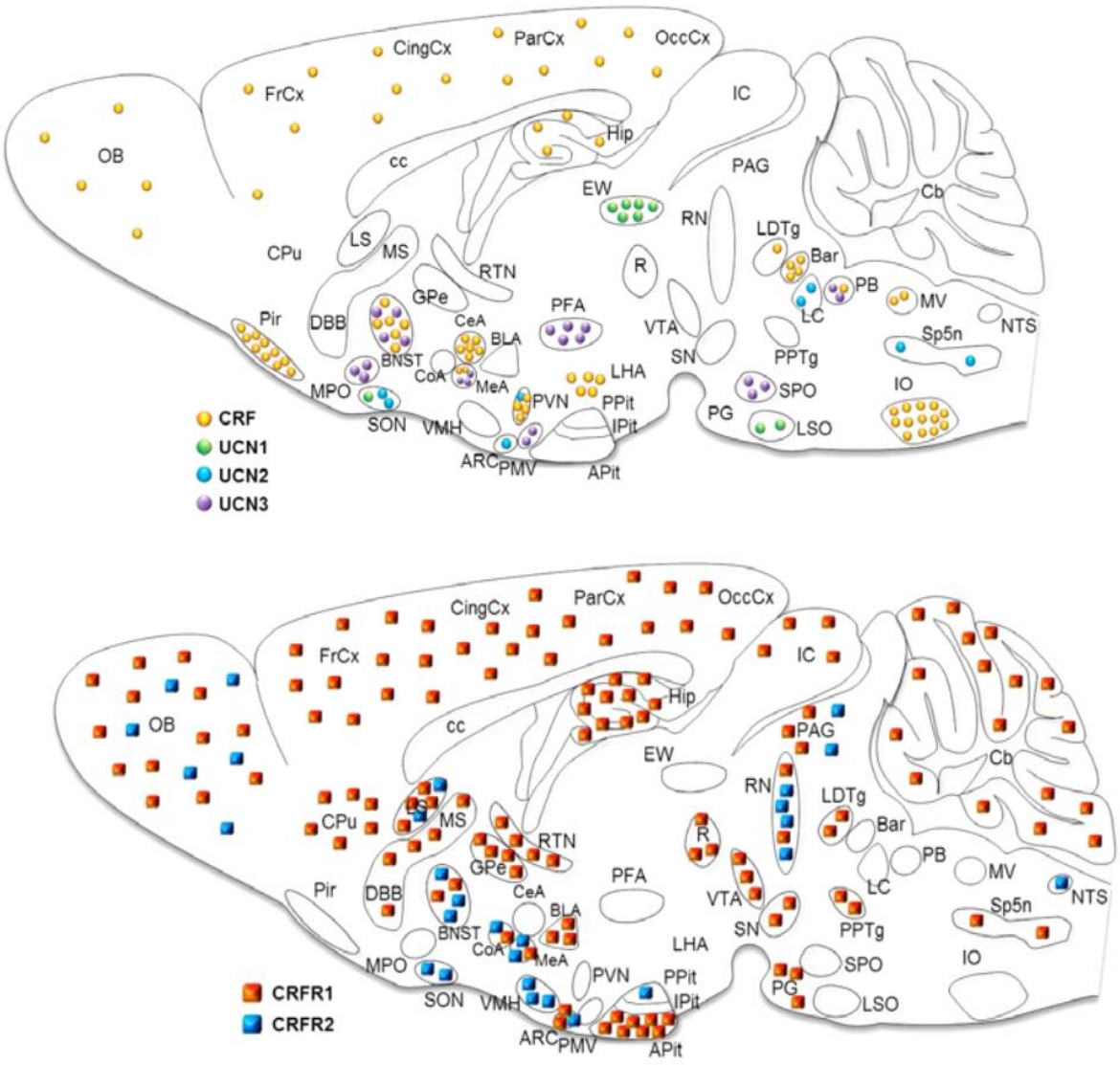


Figure 3.2: Schematic illustrations of the spatial distribution and relative expression of CRF family peptides and their receptors in the mouse brain. Abbreviations: Anterior pituitary (APit), arcuate nucleus (ARC), basolateral nucleus of the amygdala (BLA), bed nucleus ofthe stria terminalis (BNST), caudate putamen (CPu), central nucleus of the amygdala (CeA), cerebellum (Cb), cingulate cortex (CingCx),corpus callosum (cc), cortical nucleus of the amygdala (CoA), Barrington's nucleus (Bar), diagonal band of Broca (DBB), Edinger Westphal nucleus (EW), frontal cortex (FrCx), globus pallidus (GPe), inferior colliculi (IC), inferior olive (IO), intermediate lobe of the pituitary (IPit), locus coeruleus (LC), lateral septum (LS), laterodorsal tegmental nucleus (LDTg), lateral hypothalamic area (LHA), lateral superior olive (LSO), medial nucleus of the amygdala (MeA), medial preoptic area (MPO), medial septum (MS), medial vestibular nucleus (MV), nucleustractus solitarii (NTS), olfactory bulb (OB), occipital cortex (OccCx), parietal cortex (ParCx), parabrachial nucleus (PB), periaquaductal gray (PAG), perifornical area (PFA), piriform cortex (Pir), pontine gray (PG), posterior pituitary (Ppit), pedunculopontine tegmental nucleus (PPTg), premammillary nucleus (PMV), paraventricular nucleus of the hypothalamus (PVN), red nucleus (R), raphe nuclei (RN), reticular thalamic nucleus (RTN), superior colliculi (SC), substantia nigra (SN), supraoptic nucleus (SON), spinal trigeminal nucleus (Sp5n), superiorparaolivary nucleus (SPO), ventral medial hypothalamus (VMH), ventral tegmental area (VTA).

(Dedic *et al.*, 2017)

CRF and UCNs action is mediated through two receptors, CRF1 and CRF2, which belong to the class B family of G-protein coupled receptors and share 70% amino acid homology (Chen *et al.*, 1993; Souza and Grigoriadis, 2002; Grammatopoulos and Chrousos, 2018). Both CRF1 andCRF2 genes encode more than one splice variants. One functional splice variant has been identified for CRF1 in humans and rodents, while for CRF2 there are three and two functional isoforms in humans and rodents, respectively (as reviewed in (Smith and Vale, 2006). In rodents, CRF1 has a widespread distribution in the brain. Increased mRNA levels and protein are expressed in the pyramidal cells of the cortex, in hippocampal formation and in the basal nucleus of amygdala, but also in the anterior pituitary, the hypothalamus and the brain stem (Chalmers, Lovenberg and De Souza, 1995; Chen *et al*., 2000, 2004; Kühne *et al.*, 2012; Primus *et al*., 1997) (Fig.3.2). In periphery, CRF1 is expressed in the skin and the gastrointestinal track (E Chatzaki *et al.*, 2004; E Chatzaki *et al.*, 2004; Pisarchik and Slominski, 2004). In contrast, CRF2 shows a differential distribution in the brain, compared to CRF1. It is expressed in subcortical structures, with high levels found in the lateral septum, the hypothalamus, the olfactory bulb and the dorsal raphe nucleus (Chalmers, Lovenberg and De Souza, 1995; Lovenberg *et al.*, 1995; Primus *et al.*, 1997; Lukkes *et al.*, 2011; A. *et al.*, 2017) (Fig.3.2). In periphery CRF2 expression is mostly observed in the heart and skeletal muscles (Chalmers, Lovenberg and De Souza, 1995; Perrin *et al.*, 1995).

Another important component of CRF system is the soluble glycoprotein, CRF-binding protein (CRF-BP). It was first isolated in maternal plasma (Linton *et al.*, 1988). As reviewed in Kemp et al. 1998 (Kemp, Woods and Lowry, 1998), it was discovered that, despite the increased levels of CRF peptide during pregnancy, the ACTH concentrations remained at normal levels, due to a specific protein that blocked the ACTH-releasing activity of CRF. Today, two soluble proteins are known, which bind both CRF and UCN1 with high affinity, acting possibly as regulators of the free available peptides (for review see (Smith and Vale, 2006; Dedic *et al.*, 2017). Interestingly, 40-60% of CRF in the human brain is bound to the CRF-BP (Behan, Heinrichs, *et al.*, 1995). CRF-BP is expressed throughout the brain, including cerebral cortex and

subcortical structures, such as amygdala, bed nucleus of the stria terminalis and raphe nuclei, while high levels are found in the anterior pituitary(Kemp, Woods and Lowry, 1998; Smith and Vale, 2006). CRF-BP expression outside the brain has also been reported in the human liver (Behan, De Souza, *et al.*, 1995) and in the adrenal glands of rats (Chatzaki, Margioris and Gravanis, 2002). When CRF and UCN1 are not bound to CRF-BP they can activate either CRF1 or CRF2 receptor. CRF shows higher affinity for CRF1 than CRF2, while UCN1 binds with high affinity in both receptors. On the other hand, UCN2 and UCN3 display high selectivity for CRF2 and bind to CRF1with low affinity (as reviewed in (Kemp, Woods and Lowry, 1998; Smith and Vale, 2006; Dedic *et al.*, 2017).

Basic research and several clinical pharmacological studies have focused on the intracellular effects of CRF and its related peptide action. The results have revealed important information concerning the signal transduction upon CRF receptors activation. Two major signaling pathways can be activated upon CRF/UCN1 binding to CRF1 or CRF2: the Gs-coupling (PKA pathway) and the Gq-coupling (PKC pathway) pathways, both leading to gene transcription events. CRF/UCN1 binding to CRF1 alters its conformation and increases its affinity to $G\alpha_s$ subunit. The subsequent stimulation of adenylyl cyclase, leads to cyclic AMP (cAMP) production and the cAMP-dependent protein kinase A (PKA) activation. Similarly, CRF2 activation either by UCNs 2 & 3 or CRF and UCN1 stimulates the cAMP-PKA pathway, leading to downstream events of signal transduction, like CREB phosphorylation(Hauger *et al.*, 2006). Despite the fact that both CRF1 and CRF2 are capable to activate the phospholipase C – Protein kinase C (PLC-PKC) signaling pathway, possibly through Gq-coupling, the choice of which pathway will be selected, depends on yet unclarified factors (Hauger *et al.*, 2009). Data from cell culture studies have revealed additional signaling pathways linked to CRF system activation, including the Nitric oxide synthase – guanylyl cyclase pathway, the Caspase pro-apoptotic pathway and the nuclear factor-kappaB (NF-κB) transcription factor pathway ((Hauger *et al.*, 2006). Another very-well studied signaling cascade linked to CRF system is the extracellular signal-regulated kinase (ERK) – Mitogen-activated protein (MAP) kinase (ERK-

MAPK) cascade. The MAP kinase pathway is considered a very crucial signaling pathway, as it is involved in the regulation of synaptic plasticity, by

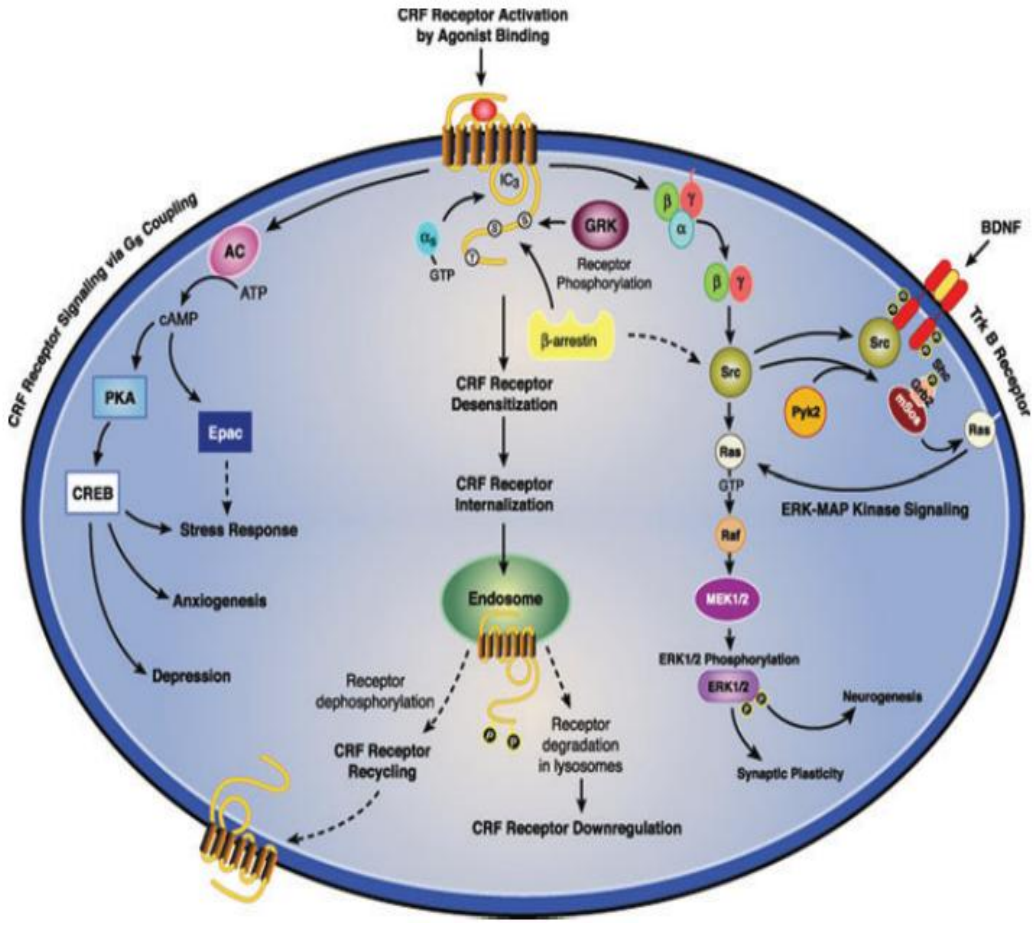


Figure 3.3: Major intracellular signal transduction pathways for CRF1 and CRF2. The dominant mode of signaling for both receptors signaling is Gs-coupled AC-PKA cascade. AC, Adenylyl cyclase; PKA, Protein kinase A

(Hauger *et al.*, 2009)

stabilizing dendritic spine structures, regulating ion channel transmission and transcription of genes, but also, in receptor scaffolding and trafficking (Sweatt, 2004).Besides the functional differentiation of the two CRF receptors, as indicated by their different distribution, it seems that the same peptide acting on the same CRF receptor can activate different signaling pathways in specific areas of the brain or in different cell types. Similarly, different peptides can activate distinct cellular signaling pathways via the same CRF receptor. (Fig.3.3)

There are the mechanisms that regulate the CRF receptors signaling. The general regulatory pathway of receptors signaling includes three steps: desensitization and phosphorylation of the receptor, endosomal internalization and finally, dephosphorylation (recycling) or degradation (down-regulation). Desensitization takes place after continuous or prolonged exposure to high levels of a ligand (e.g. CRF). The excessive stimulation of the CRF receptor activates cellular mechanisms which aim to reduce its responsiveness. The agonist-dependent desensitization (homologous desensitization) includes the phosphorylation of the CRF receptor by G-protein coupled receptor kinases (GKRs), at specific residues of serine/threonine, followed by β-arrestins recruitment and binding to the receptor, which terminates the signal transduction (by Ga protein uncoupling) (DeWire *et al.*, 2007; Hauger *et al.*, 2009; Slater, Yarur and Gysling, 2016). B- arrestins and clathrins together with other adapter proteins mediate the receptor internalization (early endosomes formation) (Luttrell and Lefkowitz, 2002). The sequestration of CRF receptor is followed by either dephosphorylation of the receptor, which can return to the cell membrane (recycling), or by degradation in the lysosomes (Fig.1.3). However, second-messenger-dependent kinases, like PKA and PKC, can also phosphorylate CRF receptors in serine/threonine residues, causing desensitization (heterologous desensitization). In this case, β-arrestins contribution is not required and the agonist occupancy is not a prerequisite (Luttrell and Lefkowitz, 2002; Hauger *et al.*, 2006, 2009).

3.1.1.3 Anxiety and CRF: Function or malfunction?

The term anxiety has been used to describe two different states; a normal and a pathologic. When referring to normal state, anxiety is considered as an adaptive response. It is the normal negative emotion we feel for a potential threatening situation, which alerts the organism to cope with the stressful stimulus. In other words, anxiety can be matched to the concept of stress response. Given the natural diversity among organisms, including humans, a differential perception of what is stressful is expected; what makes someone anxious may not affect others. However, in a pathological condition, there is

excessive negative feeling, which remains after the potential threat has disappeared, inducing a positive feedback, in which anxiety produces more anxiety(Perkins and Corr, 2014).As proposed by two independent scientist groups (Trimmer *et al.*, 2015; Bergstrom and Meacham, 2016), it is possible that anxiety arises from adaptive, homeostatic mechanisms, which have become dysregulated by unknown factors, leading to maladaptive behaviors.

As a psychiatric disorder, anxiety is characterized by two core symptoms: uncontrollable fear and worry (Stahl, 2013). Fear expression has been linked to the amygdala function. Amygdala is a subcortical region near the hippocampal formation, divided into many subnuclei, which have distinct anatomical connections with other brain regions. Both animal and human studies have revealed the reciprocal connections of amygdala and PFC (reviewed in (Kim, Loucks, *et al.*, 2011). Amygdala integrates sensory and cognitive information and then determines the fear response, through connections with the hypothalamus. Several studies have shown that in this interplay, the role of PFC is related to the emotion regulation, by either suppression of the feeling, or by reappraisal of the situation/stimulus (Banks *et al.*, 2007; Erk *et al.*, 2010; Kim, Gee, *et al.*, 2011). As reviewed by Martin and Ressler (Martin and Ressler, 2009), anatomical and neuroimaging data have revealed that a very common functional deficit observed in the brains of people suffering from anxiety disorders, like Generalized anxiety disorder and post-traumatic stress disorder (PTSD), is the hyperactivity of amygdala and the hypoactivity of PFC. Several neurotransmitter systems are connected to anxiety/fear symptoms; among them are GABA, Serotonin (5-HT), Glutamate and Nor-adrenaline. On the other hand, the feeling of worry, which is the second core symptom of anxiety, has been linked to the cortico-striato-thalamo-cortical circuit (CSTC). Striatum is part of the basal ganglia circuit. It receives inputs from both cortex and thalamus. The CSTC loop is completed through thalamic projections which are sent back to the cortex (Haber, 2016). There is strong evidence that malfunction of this circuit loop underlies the symptoms of worry in anxiety, implicating several neurotransmitter systems, including GABA, DA and 5-HT (Felmingham *et al.*, 2014; Lee *et al.*, 2015; Sareen *et al.*, 2018).

The CRF system seems to have a discrete relation to anxiety. Being the neuropeptide of stress and acting as a neurotransmitter, CRF together with all the components of CRF system have been extensively studied, with regards to their involvement in the pathophysiology of anxiety. Pharmacological studies have given valuable insights into the CRF system contribution in anxiety behaviors, although additional information has been obtained by genetic approaches, which can mimic long-lasting CRF system dysregulations. A comprehensive review by Dedic and colleagues (Dedic *et al.*, 2017) reports a remarkable number of studies on genetically engineered mice with gain or loss of function of CRF-family members and their receptors. The first CRF over-expressing (CRF-OE) mice were generated by Stenzel-Poore and colleagues (Stenzel-Poore *et al.*, 1992). They expressed CRF in a ubiquitous manner, had high levels of plasma corticosterone and exhibited increased anxiety-related behavior. Interestingly, one of the several conditional over-expressing mice that specifically over-expressed CRF in the forebrain pyramidal cells showed a mild anxiogenic phenotype (Vicentini *et al.*, 2009). When CRF1 gene was totally ablated or conditionally knocked out from the forebrain cells, mice exhibited reduced anxiety-related behavior(Timpl *et al.*, 1998; Muller *et al.*, 2003), implying that CRF1 is implicated in the modulation of anxiety. In order to unravel the specific neurotransmitter systems, which are controlled by CRF1, specific deletion of CRF1 gene from Glutamatergic, GABAergic, Dopaminergic or Serotonergic neurons was tested. Deletion of CRF1 from forebrain glutamatergic circuits resulted in reduced anxiety, while deletion from midbrain dopaminergic cells enhanced anxiety levels (Refojo *et al.*, 2011; Kratzer *et al.*, 2013), revealing a possible antagonistic function of these systems, controlled by CRF system (Dedic 2017). In contrast, deletion of CRF2 gene, has given conflicting data so far, with regards to anxiety phenotype, subverting the initial simplistic view that CRF2 has anxiolytic function.

3.1.2 Stress and CRF system in cognition: sex matters

3.1.2.1 Stress effects at different developmental stages

An organism can receive stressful stimuli from the very beginning of its life, even before its birth (prenatal period), until the late stages of its development (adulthood) and during ageing. Although the basic mechanism of stress response is the same, the developmental stage of the organism plays a crucial role on how this response affects brain, and especially cognitive function. Lupien and colleagues have proposed *the life cycle model of stress* that shows how the different developmental stages can shape the effects of stress on different brain areas (Lupien *et al.*, 2009).

During pregnancy, acute or repeated activation of mother's stress axis can increase the fetal HPA axis activity, through placenta, affecting the development of cortical and subcortical regions. For example, decreased dendritic spine density has been observed in the ACC and the orbitofrontal (OB) cortex of rats exposed to stress, during the last week of gestation (Murmu *et al.*, 2006), while in another study ,the offspring of stressed pregnant rats showed alterations in synaptic plasticity of HPC, which underlied the observed impaired learning and memory (Jianli *et al.*, 2006). In humans, maternal stress exposure (cortisol increases), especially during the early stages of pregnancy, has been linked with low cognitive function and deficits in the emotional processing of the child (Laplante *et al.*, 2004; P. and A., 2010; Buss *et al.*, 2012). On the other hand, early post-natal life, in both rodents and humans, is considered a stress hypo-responsive period (Levine, 1994; R. and L., 2003). Several paradigms have been developed for the induction of post-natal stress and studies have revealed confounding results. The most commonly used paradigm for induction of post-natal stress is the maternal separation stress. Prolonged maternal separation has been shown to increase the CRF-binding sites in the brain, including PFC, HPC and hypothalamus (Anisman *et al.*, 1998), leading to morphological changes and cognitive impairments in adulthood (Hulshof *et al.*, 2011; Aisa *et al.*, 2018). However, recent studies have proposed a rather positive impact of postnatal stress in the adult brain. It seems that during this period of development,

stress exposure may lead to adaptive programming in adulthood; that is, better coping behavior under similar stressful situations (Novais *et al.*, 2017).

Transition to adolescence coincides with the sexual maturation period in humans and marks the beginning of another critical period of brain development. The significant structural and cellular alterations that take place, during adolescence seem to render the brain more vulnerable to stress and its effects in cognitive function. Indeed, studies in rodents and humans have shown heightened activation of HPA axis under stress(Gunnar *et al.*, 2009; Klein and Romeo, 2013), while human studies have revealed increased baseline levels of GCs in adolescents with early-life stress experiences (Evans and English, 2002; Halligan *et al.*, 2007).Furthermore, according to animal and human studies in adolescent subjects, regions like amygdala, HPC and PFC show alterations after chronic exposure to stress, including structural remodeling of and reductions in synaptic-plasticity protein markers, which could account for the observed deficits in learning and memory or the increased emotional reactivity seen in adolescents (reviewed in (Romeo,

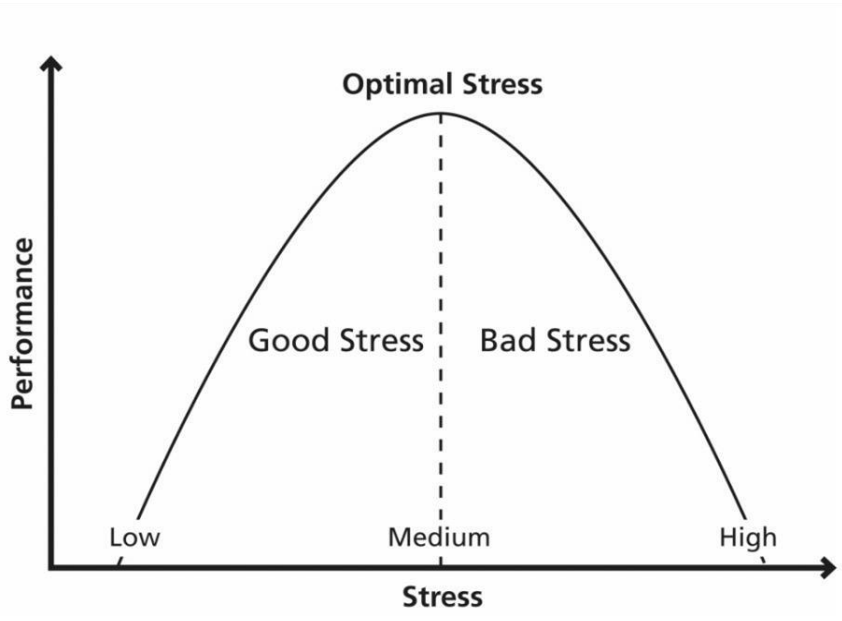


Figure 3.4: Schematic illustration of Yerkes Dodson Law.

2017). It is not surprising, that several psychiatric disorders, including anxiety and schizophrenia, emerge during adolescence.

The effect of stress in adult subjects holds the greatest number of studies, compared to the other developmental stages. Studies have revealed differential effects between acute and chronic or prolonged stress, often reporting conflicting results. In particular, an inverted U-shaped relationship of stress and cognitive function has been proposed. In was initially introduced by Yerkes and Dodson in 1908 (M. and D., 2004), known also as the Yerkes-Dodson law (Fig.3.4), who suggested that the optimal cognitive performance can be achieved under particular (optimal) stress conditions, but can be impaired under less or excessive stress. Studies in rodents have shown both beneficial and adverse effects of acute stress in cognitive function. Acute immobilization stress in mice improved their cognitive function, possibly due to increased cholinergic activity in the brain (Das *et al.*, 2000), while enhanced working memory function was observed in acutely stressed rats, which was accompanied by a high and sustained expression of AMPA and NMDA receptors in pyramidal neurons of PFC (Yuen *et al.*, 2009). Salehi and colleagues (Salehi, Cordero and Sandi, 2010) were the first to confirm the inverted U-shaped relationship between acute stress (of different intensities) and the early learning and memory phases, using an HPC-dependent task. Earlier findings have also shown an inverted U-shaped effect of GCs elevations and hippocampal function (Diamond *et al.*, 1992). However, converging evidence support that acute stress impairs spatial recognition memory in adult animals (Cazakoff, Johnson and Howland, 2010). Recently, it was found that acute stress resulted in disruption of spatial memory, through overactivation of CA1 interneurons of dorsal HPC (Yu *et al.*, 2018). Interestingly, Conrad and colleagues(Conrad *et al.*, 2004) have shown that only male rats exposed to acute stress exhibited impairments in spatial memory, while an opposite effect (facilitation) was observed in females. In addition, another study revealed impaired decision-making processes in acutely stressed animals (Bryce and Floresco, 2016). Human studies have also reported an inverted U-shaped relationship between acute GCs elevations and cognitive performance, but the favorable effect of acute stress

seems to be paired with emotional memories, while the adverse effect with the retrieval of neutral memories (Lupien *et al.*, 2009). Beneficial effect of acute stress on attentional performance has been supported (Chajut and Algom, 2003; Hu *et al.*, 2012; Plieger *et al.*, 2017), while other studies have shown impaired extinction memory retrieval and working memory function (Qin *et al.*, 2009; Raio *et al.*, 2014).

Several lines of evidence support that chronic stress exposure has damaging effects of structure and function of brain regions, such as HPC, PFC and amygdala, which translate into behavioral deficits. For example, dendritic atrophy and loss of synapses have been observed in CA1 (Sousa *et al.*, 2000) and CA3(Watanabe, Gould and McEwen, 1992) subfields of HPC and in PFC (J. *et al.*, 2007). In contrast, hypertrophy in the basolateral amygdala has been reported in adult rodents, after chronic stress(Cohen *et al.*, 2006). It has been suggested that chronic stress affects the glucocorticoid receptors (GRs) levels in PFC and HPC, dampening the GCs negative feedback mechanism (Mizoguchi *et al.*, 2003). These structural alterations underlie the behavioral deficits, including impairments in attention (Bondi *et al.*, 2007), in decision-making (Dias-Ferreira *et al.*, 2009) and spatial learning and memory performance (Sousa *et al.*, 2000; Conrad *et al.*, 2003). Notably, the effects of chronic stress in adult subjects can be totally or partially reversed after a few weeks of non-stress(Sousa *et al.*, 2000). In humans, most of the studies concerning chronic stress exposure effects on adult brain have focused mostly in stress-related disorders or the impact of early-life stress in adulthood. Increased basal cortisol levels in depressed patients (or decreased in PTSD), hippocampal atrophy, accompanied by deficits in hippocampal function are common features of depression and PTSD (Lupien *et al.*, 2009; Novais *et al.*, 2017). With regards to PFC function, a link between working memory function and poverty during childhood has been reported. It particular, it was found that the longer the period of poverty, the higher the levels of stress during childhood and the lower the working memory function in adults (Evans and Schamberg, 2009).

3.1.2.2 Sex differences in stress response

One of the most intriguing, but less studied observations has been the differential manner that exposure to stress affects male and female brain. These differences are observed at all stages of life, starting from embryonic life. During gestation, males show higher risk for short-term and long-term deficits, while in childhood, adverse events appear to preferentially affect women, increasing the risk for affective disorders (Bale and Epperson, 2015).

Sex differences are initially observed in the neuroendocrine response. In rodents, higher levels of GCs have been reported in females compared to males, under acute or chronic stress conditions (Wilson and Biscardi, 1994; Seale *et al.*, 2004). It is widely supported that gonadal hormones (estrogens and testosterone) display opposite effects on ACTH secretion; estrogens increase ACTH and GCs levels, in response to acute stress, while testosterone shows an inhibitory effect on HPA activity (Viau and Meaney, 1991).Other studies have also reported similar effects, after exogenous replacement of estrogens and testosterone (Handa *et al.*, 1994; D. *et al.*, 2004; Viau *et al.*, 2005). However, increased HPA activity in response to restraint stress, has been reported, after systemic blockade of estrogens (Young *et al.*, 2001). In humans, differences in stress response between men and women seem to be more complicated. Although basal levels of cortisol do not show significant differences, ACTH secretion is found higher in men, implying an increased sensitivity of the adrenal gland in women (Roelfsema *et al.*, 1993), which has been also observed after systemic stimulation of HPA axis, using human CRF and vasopressin peptides (Born *et al.*, 1995). Similarly, Kirschbaum and colleagues (Kirschbaum *et al.*, 1999) have shown that after a psychosocial stress test, the hypothalamic response of men was significantly higher, compared to women, while the salivary cortisol levels did not reveal a similar pattern.

Sex differences have been also found at the behavioral level of stress response. Different types of stressors (duration, intensity, etc.) can evoke different behavioral stress responses. For example, it has been shown that acute uncontrollable stress facilitates associative learning and induces helplessness in male, but not female rats (Wood and Shors, 1998; Dalla *et al.*,

2007), while after chronic stress exposure, female rats were found more vulnerable than males (Paré *et al.*, 1999). Interestingly, sex differences have been also observed in cognitive function, after stress. Acute exposure can improve PFC-mediated cognitive function, by enhancing glutamatergic transmission in PFC, whereas chronic stress exposure can decrease glutamatergic transmission, leading to impaired PFC function (Yuen *et al.*, 2012; Yuen, Wei and Yan, 2016). This inverted U relationship of stress to cognitive function, that has been proposed, applies only for males. Furthermore, previous studies assessing the effects of chronic stress in learning and memory, both hippocampal-dependent and PFC-dependent, in rats, have shown impaired performance only in male animals, unaltered performance in females and a concomitant dimorphic activity of several neurotransmitter systems(reviewed in (Luine, 2002; Luine *et al.*, 2017). Interestingly, there are a few studies reporting even enhanced performance of females in spatial memory tasks, after chronic stress(Conrad *et al.*, 2003; Luine *et al.*, 2017). It seems that estrogens make females more resilient to repeated stress. As shown by Wei and colleagues (Wei *et al.*, 2014), estradiol protected glutamatergic transmission and PFC-cognitive function from the effects of repeated stress, in female rats and reversed the impairments seen in male animals.

Apart from gonadal hormones and especially the role of estradiol in shaping the differential response between men and women, there are other factors that could potentially underlie the sexually dimorphic stress response, either independently or in a synergistic manner. For example, the sex differences could reflect anatomical differences observed in the brains of males and females, such as the increased size of LC which has been found in females (Pinos *et al.*, 2001) or the dimorphic distribution of GRs (Kitraki *et al.*, 2004). Alternatively, they could be attributed to activation of different brain structures, as neuroimaging studies have revealed. For example, it has been reported that women show increased activation of the limbic system and the dorsal ACC, in response to psychological stress, while men exhibit high activation in the right PFC (Wang *et al.*, 2007). The fact that these structures are implicated in different functions of the brain, could explain the difference in

behavioral stress responses between men and women (Goldstein *et al.*, 2010; Verma, Balhara and Gupta, 2011).

Recently, a lot of attention has been given to CRF, the neuropeptide of stress, as there are several lines of evidence supporting a potential role in the sexual dimorphism in stress response. First of all, a direct link exists between CRF and gonadal hormones. It has been shown that CRF gene promoter contains estrogen and testosterone responsive elements, allowing gonadal hormones to regulate CRF expression in asexually dimorphic manner (Vamvakopoulos and Chrousos, 1993; Shapiro, Xu and Dorsa, 2000; Bao *et al.*, 2006).Other findings supporting CRF contribution in stress response have been found in the context of the arousal system. Studies have shown that LC neurons activation under stress conditions is mediated by CRF. Interestingly, electrophysiological recordings have revealed increased sensitivity of noradrenergic neurons of LC upon CRF exposure, in female rats, compared to male rats(Curtis, Bethea and Valentino, 2005), suggesting a mechanism that could explain the sexually dimorphic stress response (Bangasser and Valentino, 2014). With regards to CRF and its receptors distribution in the brain, studies have revealed increased CRF mRNA expression in female mice hypothalamus. Although no significant sex difference in CRF1 mRNA or protein expression has been reported in brain cortex, including PFC (Bangasser *et al.*, 2010; Li *et al.*, 2016), CRF1 binding in the ACC has been found greater in female rats, both juvenile and adult animals(Jill M Weathington, Hamki and Cooke, 2014)(Jill M. Weathington, Hamki and Cooke, 2014). These differences could explain the increased HPA activity/activation observed in females, in response to stressors.

Recent studies have identified sex differences in CRF1 signaling and trafficking. In particular, Bangasser and colleagues (Bangasser *et al.*, 2010)revealed increased CRF1-Gs coupling in unstressed females compared to male rats, which remained at the same levels for females, but increased in males, after exposure to the same stressor, which was attributed to inadequate internalization of CRF1 in female mice, after stress. Indeed, immuno-precipitation experiments in cortical tissue revealed increased βarrestin2 coupling with CRF1 in stressed male, but not female rats

(Bangasser *et al.*, 2010), suggesting that if the βarrestin2 pathway is not preferred by females, then there is lack of CRF1 internalization, which could render females more vulnerable to hyper-arousal conditions (Bangasser and Valentino, 2014). Expanding this proposed sex-biased CRF1 signaling, it could be hypothesized that it predisposes females or males to certain stress-related psychiatric disorders, which show different prevalence in each sex.

3.1.2.3 Restraint stress and cognitive function

Among the behavioral tasks that have been developed to induce stress, restraint stress is a commonly psychical stressor used in rodents. The animal is placed in a ventilated, plastic tube, where it is restrained, but not totally immobilized. Several different paradigms have been developed, with regards to the intensity and the duration of the restraint procedure. Depending on the time the animal remains restrained, the task can be mild (a few minutes), moderate (a few hours) or severe (several hours), while depending on the duration it can be acute or chronic (lasting for days or weeks). Each of these manipulations aims to activate the HPA axis, several neurotransmitters systems and finally, to alter the emotional and cognitive processes in the brain.

As discussed in section 1.2.1 acute and chronic exposure to stress can produce opposite effects on cognitive function. Acute restraint stress for 2 hours increased corticosterone levels, enhanced working memory, lasting for 1 day after the test, and increased glutamatergic transmission in PFC of rats (Yuen *et al.*, 2009) . In another study, the effects of mild restraint stress (30minutes) on long-term spatial memory were tested. Mice were restrained immediately after the acquisition phase of a spatial object recognition task and 24 hours later, their hippocampal-dependent long term memory was tested. It was found that acute restraint stress was able to disrupt long-term memory, possibly through the observed over-activation of CA1 interneurons, which could in turn reduce the activity of pyramidal neurons(Yu *et al.*, 2018). Similarly, Drouet and colleagues have shown that 1 hour of restraint stress in rats resulted in higher HPA activation (high corticosterone levels) and blunted

PFC activity (e.g. decreased cFos expression),only in animals showing high PFC GABA/glutamate ratio (Drouet *et al.*, 2015). These results suggested a possible association of the enhanced reactivity to acute stressors with impaired PFC function and the excitation/inhibition balance. In accordance with other studies, the use of restraint stress in both male and female subjects have revealed opposite effects between the two sexes. For example, Shansky and colleagues (Shansky *et al.*, 2006) evaluated the effects of stress on working memory, utilizing two different intensities of acute restraint stress (60 or 120 minutes), in male and female rats. The low intensity restraint stress (60minutes) affected PFC function only in females with high levels of estrogens (proestrus phase), while the high intensity restraint stress, produced significant impairments in PFC function in both sexes. Studies with chronic restraint stress have been extensively used. The results are in accordance with other studies of chronic stress. They suggest a potential protective role of estrogens against the effects of chronic stress in anxiety and cognitive function, and a differential impact of chronic stress in the two sexes, during adolescence (Bowman, Ferguson and Luine, 2002; K. *et al.*, 2010).

Restraint stress has been also tested in transgenic mice for CRF system components(Muller *et al.*, 2003; Makino *et al.*, 2005), while in a lot of studies pharmacological approaches have been combined with restraint stress, utilizing CRF receptors antagonists, in order to ameliorate anxiety or depressive symptoms. A commonly used non-selective CRF receptor antagonist is the synthetic peptide a-helical CRF_{9-41} (Rivier, Rivier and Vale, 1984). This peptide does not cross the blood-brain barrier (BBB), and its use is restricted to in vitro experiments or local infusions, usually intra-cerebro-ventricular (i.c.v.).However, there are several studies supporting its anxiolytic effects (Krahn *et al.*, 1986; Adamec and McKay, 1993; Heinrichs *et al.*, 1994; Wierońska *et al.*, 2003). Another widely used CRF receptor antagonist is antalarmin (Webster *et al.*, 1996), which belongs to the family of non-peptide CRF1 antagonists. Antalarmin has the advantage of being able to cross the (BBB), allowing *per os* administration. Its effects on stress-induced psychological and behavioral changes have been extensively investigated in rodents and in primates. For example, it has been shown that acute

intraperitoneal (i.p.) administration of antalarmin in rodents has an anxiolytic effect, either reversing the effects of immobilization stress or the effects of CRF local infusion in the brain (Zorrilla *et al.*, 2002; X.-D. Wang *et al.*, 2011). On the other hand, chronic treatment with antalarmin, decreased baseline ACTH and corticosterone levels in rats, (Bornstein *et al.*, 1998), without affecting the HPA axis response in acute stress (Wong *et al.*, 1999) and reversed the physical effects of chronic mild stress in mice (Ducottet, Griebel and Belzung, 2003).

3.1.2.4 Role of CRF system in HPC and PFC function

Hippocampal formation and PFC have been two of the most attracting brain regions for neuroscientists, not only because of their crucial role in cognition and behavior, but also because of the significant deficits they show in several common neurologic and neuropsychiatric disorders, such as Alzheimer's and Parkinson's disease, Autism Spectrum Disorder (ASD) and SZ. Their role in learning and memory requires their integrity and their continuous normal function, which is based on the balanced interplay of several neurotransmitter systems. The link of CRF system with stress and stress-related neuropsychiatric disorders, along with the spatial distribution of CRF family peptides and their receptors, in brain regions affected in neuropsychiatric disorders, constitute strong evidence supporting its role in HPC and PFC function. As described earlier in this chapter (section **1.1.2**.), CRF peptide is expressed throughout the brain, including HPC and PFC regions, where none of its related peptides has been described to be expressed. Between the two CRF receptors, studies have revealed that CRF1 expression prevails against CRF2 in the rodent HPC and cortex, including frontal, cingulate, parietal and occipital cortices, and only orbito-frontal cortex shows a concomitant expression of both receptors (Dedic *et al.*, 2017).

With regards to HPC function, several studies have implicated CRF and CRF1in learning and memory and the underlying cellular mechanisms of these functions. It has been shown that short-lasting raise of CRF levels can be beneficial for HPC-dependent learning and memory, while prolonged

elevations disrupt its function, leading to cognitive deficits (reviewed in (Chen *et al.*, 2012)). Data from *in vitro* studies have revealed that CRF acts through CRF1 receptor in HPC to facilitate synaptic transmission and LTP expression through modulation of voltage-gated ion channels (Aldenhoff *et al.*, 1983; Wang *et al.*, 1998; von Wolff *et al.*, 2011; Kratzer *et al.*, 2013). Furthermore, several lines of evidence support that the observed dendritic atrophy in HPC after prolonged exposure to stress, is CRF-induced and underlies the synaptic plasticity and memory deficits, seen in anxiety and stress-related disorders (Chen *et al.*, 2008, 2010, 2013; Ivy *et al.*, 2010). Pharmacological approaches have revealed that the use of CRF1 antagonists could prevent the morphological and LTP impairments in HPC, restoring the HPC-dependent memory impairments(Chen *et al.*, 2010; Ivy *et al.*, 2010). Additionally, studies using conditional knockout mice with CRF1 forebrain deficiency or postnatal forebrain CRF over-expression have shown impaired HPC neurotransmission and deficits spatial learning and memory function (Refojo *et al.*, 2011; X.-D. Wang *et al.*, 2011). Interestingly, these deficits, as well as, dendritic atrophy in HPC subregions, were restored in stressed mice with forebrain CRF1 deficiency (X.-D. Wang *et al.*, 2011; Xiao-Dong Wang *et al.*, 2011; Wang *et al.*, 2013).

As in HPC, CRF-CRF1 signaling is expected to affect PFC plasticity and PFC-dependent cognitive functions. However, very little is known for the direct role of CRF system in PFC function, compared to HPC or other brain regions and only a few studies have shown the neuronal effects after manipulations of CRF system signaling. For example, one study have shown that, CRF peptide injection in the rat frontal cortex at high dose, had a significant anxiolytic-like effect and induced a transient decrease of excitatory synaptic transmission in frontal cortex slices, in an NMDA receptor-dependent manner (Zieba *et al.*, 2008). One of the latest studies on CRF system role in PFC functions showed that subjecting mice to acute stress increases CRF1 mRNA levels and leads to PFC dysfunction. Similarly, intra PFC CRF injections mimic the acute-stress executive dysfunction. Both manipulations resulted in PKA signaling pathway activation, while either intra-PFC CRF1 deletion or PKA blockade reversed the executive dysfunction (Uribe-Mariño *et al.*, 2016). Yang and

colleagues have recently studied the effects of postnatal stress exposure on PFC structure and the role of CRF1 in modulating these effects. The stress-induced dendritic remodeling in PFC pyramidal neurons was prevented by concurrent blockade of CRF1 with systemic antalarmin administration, abolishing the PFC- cognitive impairments, but not anxiety-related behavior(Yang *et al.*, 2015).

3.1.3 Stress and CRF system in neuropsychiatric disorders

3.1.3.1 Stress-related neuropsychiatric disorders

Neuropsychiatric disorders constitute a group of serious, chronic and often disabling brain diseases, including SZ, Major Depressive disorder, ASD, Alzheimer's disease and Epilepsy (Taber, Hurley and Yudofsky, 2010). Among the various symptoms that have been characterized for each of them, cognitive impairments constitute a common symptom (Miyoshi and Morimura, 2010). On the other hand, stress sensitivity is a significant converging point, proposed as a key factor in their onset and their progression. As discussed in section **1.1.1**, stress response is a normal function, which is activated in order to reinstate the homeostasis of an organism. CRF activates HPA axis leading to GCs release, which in turn act in the brain, creating a negative feedback loop. However, in several neuropsychiatric disorders stress system is found dysregulated. Increased HPA activity including high levels of cortisol, ACTH and/or cerebrospinal fluid (CSF) CRF have been reported, with a few exceptions, in patients with depression, in several cases of anxiety disorders, in Obsessive-compulsive disorder, in SZ and ASD, while decreased HPA activity has been found in patients suffering from PTSD (reviewed in (Jacobson, 2014). Furthermore, although stress response activates the same mechanism, the effects of stress differ across the lifespan (Lupien *et al.*, 2009). For example, the HPA axis of adolescent rodents shows prolonged activation in response to stressors, compared to adult animals. This observation is also suggested by human studies, which have shown that adolescent period is associated with heightened basal and stress-induced activity of the HPA axis. Additionally, the adolescence is a critical period for

the brain development as some regions including PFC and amygdala continue to mature. Thus, it is not surprising that many neuropsychiatric disorders, such as SZ emerge during adolescence or early adulthood, when the brain is more vulnerable to environmental factors.

Based on animal and human studies, there have been developed two different but not mutually exclusive hypotheses: the neurotoxicity hypothesis (Sapolsky, Krey and McEwen, 1986) and the vulnerability hypothesis (Gilbertson *et al.*, 2002). According to neurotoxicity hypothesis, prolonged exposure to stress hormones may affect brain regions such as HPC and PFC, by reducing their ability to resist in other insults or the normal aging processes. In contrast, vulnerability hypothesis suggests that the brain is already sensitive due to other factors, such as genetic factors or early-life stress exposure and the histological, cellular or molecular deficits are already present.

3.1.3.2 Stress and schizophrenia –the link

As discussed in chapter 1 (section **1.2**) SZ has been characterized as a multifactorial disease, since both genetic and environmental factors contribute to its manifestation. There are several lines of evidence that support the notion that stress is a crucial risk factor for the disease manifestation and progression. As reviewed by Caceda and colleagues (Cáceda, Kinkead and Nemeroff, 2007) studies have shown that stressful situations lead in exacerbation of the symptoms or increase the relapse frequency of patients. These reports support an increased HPA axis activity in SZ patients, resulting in high cortisol levels (Phillips *et al.*, 2006). However, it seems that the elevated HPA activity is more pronounced in first-episode patients (Ryan *et al.*, 2004; Guest *et al.*, 2011) and there are studies reporting inconsistent results. It is rather believed that this HPA increase reflects a higher stress sensitivity in SZ (Bradley and Dinan, 2010). Interestingly, it has been consistently found a reduced HPA activity (decreased levels of cortisol and ACTH) in response to psychological stress, but in physical stressors (reviewed in (Jacobson, 2014)). The precise mechanisms that lead to

dysregulated HPA axis in schizophrenia are still not clear. What is clearly suggested, though, is that genetic predisposition for SZ might not be enough to result in a clinical phenotype, without an environmental contribution. On the other hand, we cannot ignore the fact that several genes linked with SZ have been identified, including DISC1, COMT and PDE4A. These genes are also linked to stress response and Dopaminergic function (Howes *et al.*, 2017). Integrating genes, DA and stress, it could be proposed that dysregulated Dopaminergic function, due to genetic risk variants, could render DA neurons more vulnerable to stress or other environmental insults.

3.1.3.3 CRF system in SZ

Evidence of a strong link between SZ and stress response reasonably implies a potential link of SZ with CRF. Indeed, there is evidence from human and animal studies, supporting a role of CRF system in the pathophysiology of the disease. Postmortem studies have shown decreased CRF immuno-reactivity in Cingulate Gyrus of SZ with cognitive deficits(Gabriel *et al.*, 1996), while Herringa and colleagues (Herringa, Roseboom and Kalin, 2006) have reported decreased expression of CRF-BP mRNA in the basolateral amygdala of male schizophrenic patients. Additionally, genetic studies have revealed that interactions between CRF-BP and CRF1 polymorphisms lead to increased suicide behavior and risk for alcoholism in schizophrenia patients, suggesting a possible hyperactive CRF transmission that may lead to HPA dysregulations (De Luca *et al.*, 2010; Ribbe *et al.*, 2011). With regards to animal studies, it has been shown that CRF-OE mice have similar deficits to SZ phenotype (Groenink *et al.*, 2002; Dirks *et al.*, 2003). Interestingly, it was shown that the PPI deficits observed in these mice are mediated via CRF1 and not GC receptors, suggesting a possible role of CRF1 in the emergence of psychotic symptoms in stress-related psychiatric disorders (Groenink *et al.*, 2008). Further support towards s potential role of CRF in SZ is given by several studies, reviewed by Bangasser and Kawasumi (Bangasser and Kawasumi, 2015) which implicate CRF system in PFC- and HPC-dependent

cognitive functions, including working memory, attention, declarative memory and fear learning.

3.1.4 Aim of the study

As discussed in the previous sections, anxiety behavior has been linked with several psychiatric disorders, such as SZ, while stress has been heavily linked to the emergence and progression, as a significant risk factor. It is now widely accepted that exposure to stress conditions can either exacerbate the symptoms of SZ or lead to relapse. Importantly, it seems that stress exposure can lead to deterioration of the cognitive function, by affecting both cortical and subcortical regions. Furthermore, there is evidence of a strong link between CRF system and SZ pathology, especially with regards to the cognitive deficits seen in PFC and HPC. However, CRF system contribution has not been clarified yet. Based on the above and on our intriguing finding of the sex-biased PFC-cognitive impairment in MAM-16 mice, we aim to identify the contribution of CRF system in the cognitive deficits of PFC. We explore the stress response and the CRF system function in control and MAM-exposed mice, in order to clarify the link between stress, CRF system and MAM model pathology. We believe that this will provide significant information that will guide us to explore how CRF system interventions may improve cognitive deficits, observed in the MAM mouse model of schizophrenia.

3.2 Materials and Methods

3.2.1 Animals and treatment

The experiments were conducted in adolescent (40-45 days old) and/or adult (>3 months old) C57BL/6 male and female offspring of pregnant dams treated with either saline or MAM on GD16 (as described in chapter 1, section 2.1). Mice were housed in groups (3-4 per cage) and provided with standard mouse chow and water ad libitum, under a 12 h light/dark cycle (light on at 7:00 am) with controlled temperature (23 +/- 1 Celsius). All procedures were

performed according to the Guidelines of the Research Ethics Committee of the University of Crete and the European Union ethical standards outlined in the Council Directive 2010/63EU of the European Parliament on the protection of animals used for scientific purposes.
All behavioral experiments were conducted between 10.00a.m.-5.00p.m. .

3.2.2 Elevated plus maze

Elevated plus maze task (EPM) is a standardized behavioral paradigm for the evaluation of trait anxiety levels in rodents, based on the conflict of the innate curiosity of the animal to explore a new environment and the fear of the open arms. It consists of two open arms (5x35cm), two closed arms (5x35x15cm) and one intersection (5x5cm) compartment. Adolescent and adult mice, MAM-16 and Saline, from both sexes (8-10 mice from each group) were tested. After 1 hour of acclimation in the experimentation area, each animal was placed in the intersection, facing a closed arm, opposite the experimenter and allowed to explore the apparatus for 5 minutes. The activity of the animal was recorded during the task, using a camera above the apparatus, and was analyzed with the JWatcher software (http://www.jwatcher.ucla.edu/). The number of entries in the different compartments and the time spent in each compartment were scored. The basal anxiety levels were evaluated by calculating the ratio of open arms/closed arms entries/time.

3.2.3 CRF1 protein expression

For the quantification of CRF1 protein levels in PFC and HPC, total protein extraction was conducted, followed by SDS-PAGE procedure and western blotting using specific anti-CRF1 antibody. In particular, adult saline and MAM-16 mice of both sexes (4 animals per group) were decapitated following cervical dislocation, the brains were quickly placed in ice-cold PBS and then positioned on a brain mold, where 1.5-mm slices were taken, containing the PFC or the HPC. The slices were placed on dry ice and stored

at -80 °C, until homogenization in lysis buffer (50mM HEPES, 150mM NaCl, 1% Glycerol, 1% Triton, 1,5 mM MgCl2, 5mM EGTA, 1:1000 inhibitor cocktail). Protein extracts were fractionated by SDS-PAGE (40 μg of sample protein in each lane, 10% acrylamide separating gel, 4% acrylamide stacking gel, electrophoresis at 140 V for 80 min) and transferred onto nitrocellulose membrane. The membrane was blocked with 5% BSA (Bovine Serum Albumin) in TBS-Tween or PBS-Tween 0.1%, incubated O/N in goat polyclonal anti-CRF1 (AA 107-117) (Sigma-Aldrich, Darmstadt, Germany, 1:3000) or anti-actin (Chemicon, 1:2000), washed, incubated in secondary goat anti-rabbit or anti-goat IgG Horseradish Peroxidase Conjugate antibody (Invitrogen, 1:5000) and finally incubated in Pierce ECL Western Blotting Substrate. The visualization of proteins of interest was conducted with Chemiluminescence, which was detected by a Gel imaging system. Analysis of the CRF1 expression was performed with ImageJ software, and the raw values of CRF1from each sample were normalized to their respective a-Actin values.

3.2.4 Chronic antalarmin treatment

The effects of chronic antalarmin treatment on synaptic plasticity of female PFC were tested in adult female MAM-16 and control mice. 7 MAM-16 and 3 control mice were i.p. injected twice a day (every 12 hours) for 5 consecutive days with 20mg/kg of antalarmin hydrochloride (Sigma-Aldrich), dissolved in 5% DMSO and 95% sterile saline. Two groups of 2 MAM-16 and 3 control mice received i.p. injections of saline (1ml/kg), twice a day for 5 days. 16 hours after the last injections of the 5th day, animals were decapitated under halothane anesthesia and brains were used for in vitro electrophysiological experiments in the PFC region (as described in chapter 2, section **2.9**).

3.2.5 Cognitive function under stress

The evaluation of cognitive function after exposure to stress was conducted at the behavioral level. First, animals were prepared for the object

recognition for temporal order cognitive task (described in **3.2.8)** followed by two hours exposure to the stressor, and immediately after, their anxiety levels were evaluated using the Light/Dark Box task. Finally, their cognitive function was assessed, using the object recognition task.

3.2.6 Restraint stress

Restraint stress is a common physical stressor, used in rodents for the induction of stress response and the study of drug effects upon the response. Animals are restrained in ventilated plastic tubes (e.g. 50ml falcons) for a few minutes to hours, depending on the intensity of the task. Restraint stress task was utilized for the induction of stress response in male and female animals, in order to assess their cognitive function after stress.14 animals (7 males and 7 females) were restrained (RS group) for 2 hours and 14 animals were kept in their home cages, as the control group (No RS). Anxiety levels were measured in the Light/Dark box, immediately after the restrained stress procedure and in No RS group.

3.2.7 Light dark box task

Light/Dark test is one of the most widely used tasks for the assessment of anxiety behavior in mice. It is based on their natural aversion to illuminated areas and their spontaneous exploratory behavior in novel environments. The apparatus consists of two chambers of equal dimensions (27x26x24 cm), one dark and one illuminated, divided by a partition and a door. Mice were placed in the dark part, while the door was kept closed for 10 seconds. Then, mice were allowed to explore freely the two chambers for 5 minutes and their activity was monitored throughout the task, using a camera placed above the box. The latency to enter the illuminated chamber, as well as the number of transitions and the time spent in each compartment were analyzed using the JWatcher software.

3.2.8 Object recognition for temporal order

The object recognition task for temporal order was used to assess the recency memory of mice (Konstantoudaki *et al.*, 2018)Animals were initially handled by the experimenter for about a week and then they were habituated to the apparatus (open field arena) devoid of any objects for 10 minutes, each day, for three consecutive days before the beginning of the behavioral test. The task comprised two 5-minute sample trials and one 5-minute test trial, with an inter-trial interval of 25 minutes. In each sample trial, mice were allowed to explore two identical copies of the same object. Different objects were used for the two sample trials, which had been confirmed that had no ethological significance to the mice and also that mice did not show specific preference to any of them. During the test phase one object from sample trial 1 (old) and one object from sample trial 2 (recent) were presented and the animals were allowed to explore the apparatus. The behavior of each animal was recorded throughout the task (trial and test phases) and was analyzed with JWatcher software. Object recognition memory for temporal order was defined as an increase in time exploring the old familiar object compared to the time exploring the recent familiar object (Mitchell and Laiacona, 1998).

3.3 Results

3.3.1 Altered trait-anxiety levels after prenatal mam exposure, observed in adulthood

Adolescent and adult, male –female and saline or MAM-treated mice were tested in the elevated plus maze task. The analysis revealed sex- and age-related differences in trait anxiety in saline and MAM-treated mice. For adult females, analysis revealed that MAM-16 mice tend to be more anxious compared to saline-treated mice, as shown by the Open/Closed arms ratio for the entries (O/C entries) (t-test, *p*= 0.08) and the Open/Closed arms ratio for the time spent (O/C time) (t-test, *p*= 0.09)(Fig.3.5A). However, no differences were observed between adolescent saline and MAM-treated animals (t-test, *p* = 0.5 and 0.7, O/C entries and O/C time, respectively) (Fig.3.5C). For adult males, analysis showed that MAM-16 mice have decreased trait anxiety levels, compared to saline-treated animals, as shown by the increased time spent in the open arms (t-test, p = 0.05) and the decreased time spent in the intersection (t-test, p = 0.039) (Fig.3.5B). On the other hand, adolescent saline and MAM-16 mice exhibited no significant differences in their basal anxiety levels (t-test, p =0.97, 0.3, O/C entries and O/C time, respectively) (Fig.3.5D).

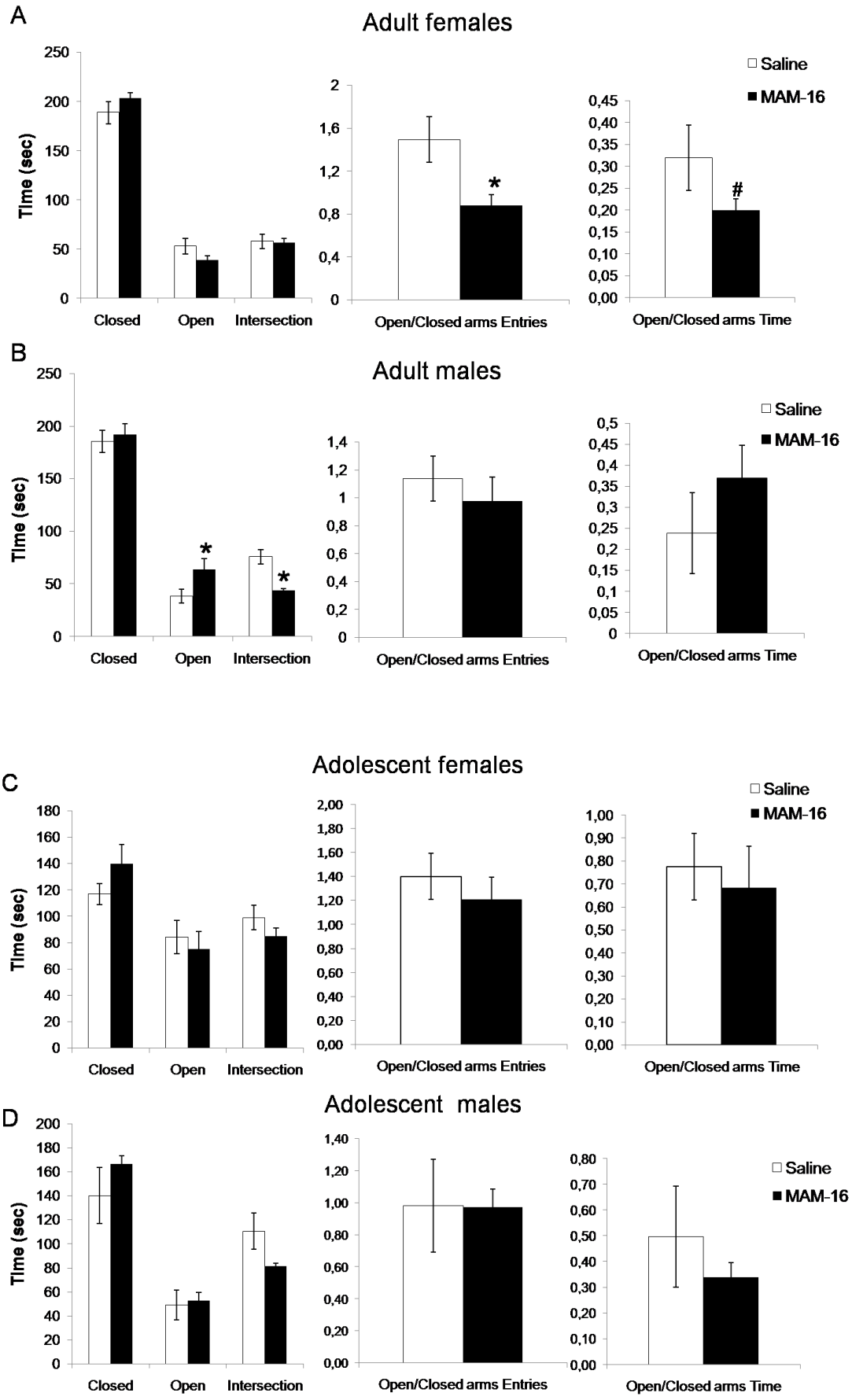

A
Adult females
Time (sec)
Closed
Open
Intersection
Open/Closed arms Entries
Open/Closed arms Time
Saline
MAM-16
B
Adult males
C
Adolescent females
D
Adolescent males

Figure 3.5: Trait-anxiety levels in adult and adolescent, female - male and saline or MAM-treated mice. A-D) Bar graphs showing (left) the time spent in each compartment of the EPM apparatus, during the 5 minutes of the task, (middle) the ratio of Open/Closed arms for entries and (right) the ratio of Open/Closed arms for time. EPM, Elevated plus maze

3.3.2 Sexually dimorphic CRF1 expression in PFC, after prenatal MAM exposure - Adults

As described in Chapter 2 (sections **3.4** & **3.5**), both cognitive evaluation and electrophysiological recordings showed that prenatal MAM exposure, affected PFC function only in male mice, while deficits in HPC were observed in both sexes. Using western blotting technique, we measured CRF1 protein levels in lysates of PFC samples from 4 saline- and 4 MAM-16 mice, for both sexes. The SDS-PAGE procedure was conducted separately for male and female samples. Analysis revealed a significant reduction of CRF1 expression in the PFC of male MAM-16 mice, compared to saline-treated mice (t-test, p = 0.004). However, this was not the case for female mice, where no alteration was observed between control and MAM-treated animals

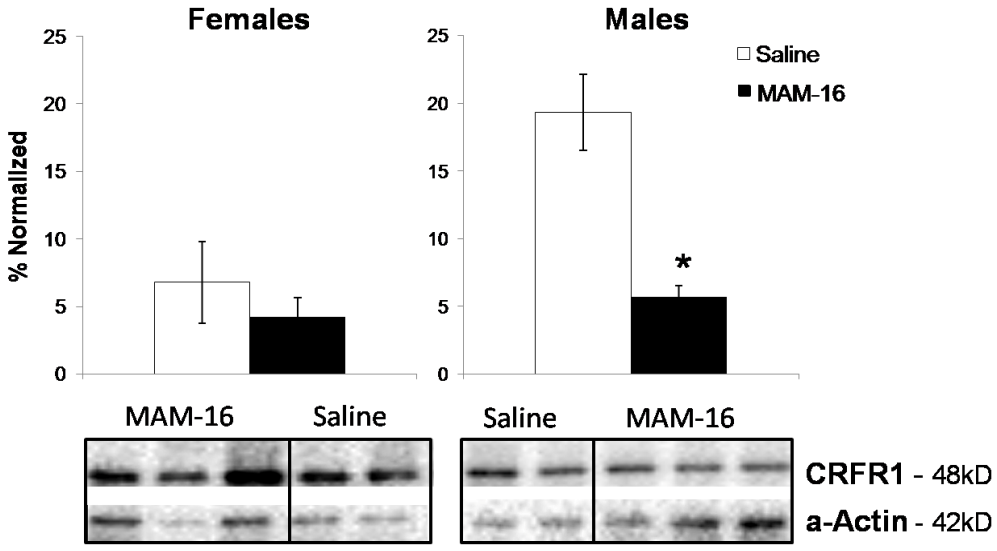
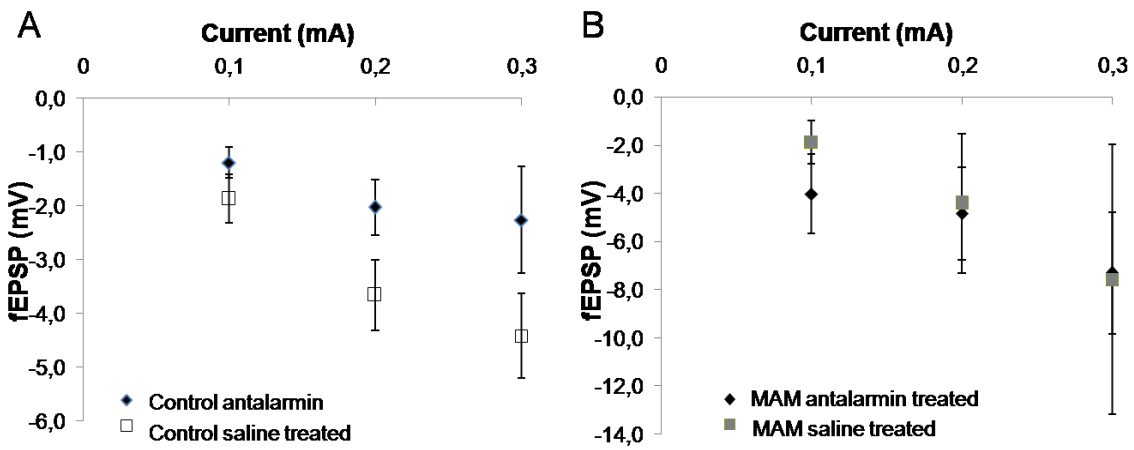


Figure 3.6: Western blot analysis of CRF1 expression in PFC samples of female-male MAM-16 and saline mice. (Top) Bar graphs showing the normalized expression levels of CRF1 protein and (bottom) representative protein immuno-blots of CRF1 and a-Actin as a loading control.

(t-test, p = 0.4) (Fig. 3.6).

3.3.3 Chronic antalarmin treatment effects on synaptic transmission and plasticity in PFC of female mice

Based on literature, animal studies have shown that females are more resilient than males to chronic stress, with regards to PFC-cognitive function (Yuen *et al.*, 2012; Wei *et al.*, 2014; Yuen, Wei and Yan, 2016). Furthermore, an inadequate internalization of CRF1 in females after stress has been found (Bangasser *et al.*, 2010). Since our results from synaptic plasticity in the PFC of MAM-exposed mice revealed no alterations in MAM-16 female mice, while the trait anxiety of these animals was found increased, we hypothesized that CRF1 signaling could potentially protect PFC-cognitive function of MAM-16 female mice. So, we blocked CRF1 signaling with chronic antalarmin treatment in control and MAM-16 female mice and evaluated the effects on synaptic transmission and plasticity in layer II synapses of PFC. Analysis of the fEPSPs showed that synaptic transmission was reduced in increasing intensity stimulation, in control antalarmin-treated animals (repeated measures ANOVA, $F_{(1,10)}$=4.2, p = 0.02) (Fig.3.7A). In contrast, synaptic transmission in MAM-16 antalarmin-treated animals seemed to be increased in low intensity stimulations (repeated measures ANOVA, $F_{(1,10)}$=1.5, p = 0.2) (Fig.3.7B). To determine the effects on synaptic plasticity in the PFC, we induced LTP by tetanic stimulation. Our results revealed opposite effects of antalarmin in control and MAM-16 mice. In particular, LTP was found increased in control animals that received antalarmin (repeated measures ANOVA, $F_{(1,10)}$=5.7, p = 0.02), while in MAM-16 animals that received



antalarmin, LTP was significantly decreased (repeated measures ANOVA, $F_{(1,10)}$=6.2, p = 0.01) (Fig. 3.7).

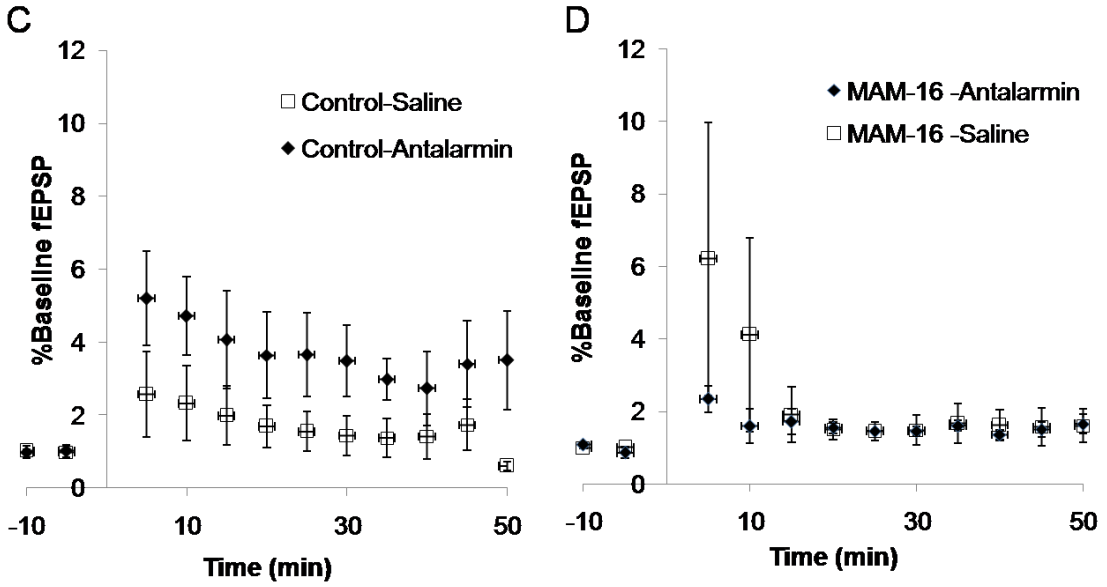


Figure 3.7: Effects of antalarmin treatment on synaptic transmission and plasticity of PFC. A-B) Graphs showing the fEPSP in response to increasing current stimulation for control (A) and MAM-exposed (B) female mice treated with saline or antalarmin. C-D) Graphs showing LTP following tetanic stimulation in control (C) and MAM-exposed (D) female mice treated with saline or antalarmin.

3.3.4 Female mice are more vulnerable to two hours of restraint stress

Control male and female mice were restrained for two hours in plastic tubes, in order to activate HPA axis, and test their cognitive function. Based in our previous results from MAM-treated male and female animals with regards to trait anxiety and CRF system alterations (CRF1 receptor protein levels), we tried to induce a condition of altered CRF system activity in control animals. We evaluated the restraint stress-induced anxiety levels, using the Light-Dark box task. Analysis of the latency to enter for the first time the illuminated compartment and the time spent in each part of the device showed that restrained (RS) female mice spent significantly less time in the illuminated area (t-test, p = 0.01) and significantly more time in the dark compartment, compared to their respective controls (t-test, p = 0.01), indicating increased

anxiety levels (Fig.3.8). RS female mice exhibited also decreased number of transitions between the two compartments, implying decreased locomotor activity (t-test, p <0.001) (Fig.3.8). In contrast with females, no significant alterations were observed in the time spent in each compartment between RS and No RS male mice. However, the number of transitions between the two compartments was found significantly decreased in RS animals, which could

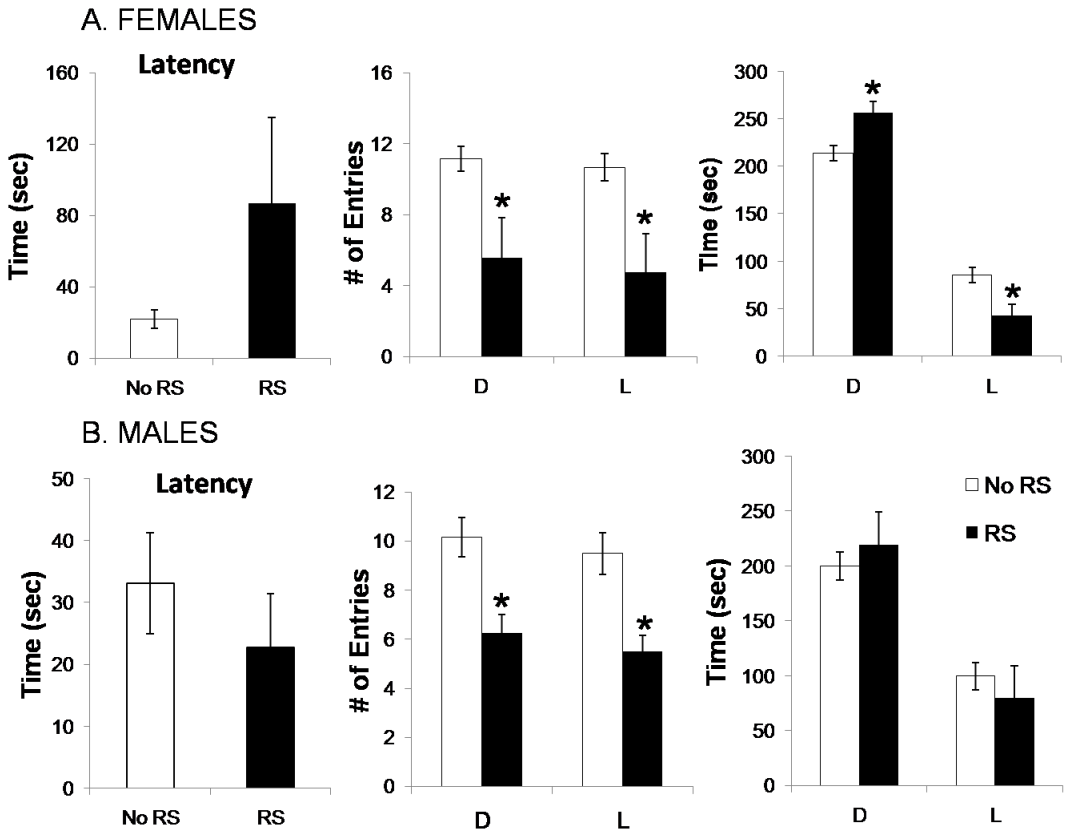


Figure 3.8: Stress-induced anxiety evaluation in the Light-Dark box in female and male mice. A-B) Bar graphs showing the latency to enter the illuminated compartment for the first time, the number of transitions between the two compartments and the time spent in each compartment, in restrained (RS) and non-restrained (NoRS) female (A) and male (B) mice. D, Dark; L, Light

imply a decrease in locomotor activity of this group.

With regards to cognitive function of PFC, we evaluated the recency memory, using the recognition task for temporal order. The discrimination ratio (DR) between the old and the recent familiar object was measured for the test phase of the task. Our results showed no significant differences in the discrimination ratio (DR) between restrained animals and their respective controls, either in female mice (t-test, p =0.3), or male mice (t-test, p =0.5) (Fig.3.9). However, it is possible that increasing the number of animals tested, could unravel interesting differences between the groups.

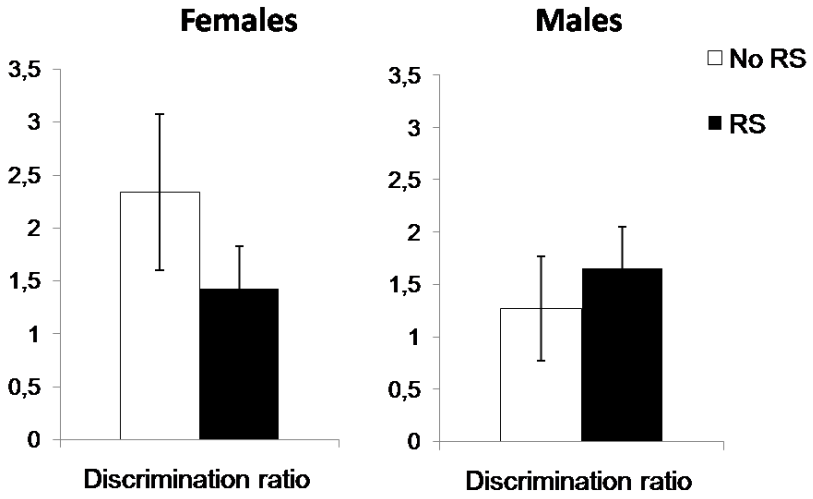


Figure 3.9: Recency memory function in the recognition task for temporal order. Graphs showing the discrimination index of female and male mice after two hours of restraint stress, compared to their respective control groups.

3.4 DISCUSSION

This part of the study extends the sex comparison of MAM-exposed mice, with regards to trait anxiety and CRF system involvement in PFC function, and explores the effects of acute restraint stress in cognitive function of adult male and female mice, at the behavioral level. We find that MAM-exposure has no effect on adolescent mice of either sex, while it affects in an opposite way the two sexes, during adulthood; heightened levels of trait anxiety in MAM-16 female mice and decreased levels in MAM-16 male mice, compared to their respective controls. Results from the quantification of CRF1 protein expression in PFC samples of MAM-16 and control mice reveal a significant decrease only in MAM-16 male mice, while chronic blockade of CRF1 in females, abolishes the LTP induction, after tetanic stimulation in PFC of MAM-16 group. When we induce stress response in control animals (2 hours of restraint stress), our results reveal that female mice are more vulnerable to restraint stress in terms of anxiety levels and PFC-dependent cognitive function.

3.4.1 Comparison with the MAM model in rats and other SZ models

The assessment of basal anxiety levels was conducted using the EPM task, in two age groups, for both sexes. We find no differences in adolescent animals, between MAM-16 and control groups of either sex. However, increased anxiety has been reported in male MAM-17 rats during adolescence, which could be reversed by chronic diazepam treatment). In adult animals, we find increased anxiety levels in female MAM-16 mice and decreased anxiety levels in male MAM-16 mice. To our knowledge, there are no previous studies that have evaluated anxiety behavior in female MAM-17 rats, while for males, results have been conflicting. The first report from adult MAM-17 rats had shown decreased basal anxiety levels (Gastambide *et al.*, 2015b), as measured by the EPM task, which is in line with our results for male MAM-16 mice. However, a recent study by the same group that supported increased anxiety in adolescent MAM-17 rats, found identical results (increased anxiety) in adult animals(Du and Grace, 2016). In addition,

other studies have shown increased stress response in MAM-17 juvenile animals(Zimmerman *et al.*, 2013), after acute footshock stress, while increased vulnerability to stress exposure (cold stress) was found in adult MAM-17 rats with regards to PFC function (Goto and Grace, 2006). It is possible that alterations in stress response occurred later in MAM-16 mice, compared to MAM-17 rats, and at the time of the test they were not apparent.

In other neurodevelopmental models of schizophrenia, sexual dimorphism has been also observed in anxiety behavior, as well as some discrepancies, which could reflect strain differences or variations in experimental procedures. For example, in a rat model of prenatal immune challenge, IL-1β exposure during gestation (GD17-21) caused a decrease in anxiety-related behavior only in female animals (Paris *et al.*, 2011). Studies using the prenatal stress model, have found conflicting results. One study reported increased anxiety levels only in female rats prenatally exposed to stress (Schulz *et al.*, 2014), while others have shown that anxiety occurs in both sexes (Wilson, Schade and Terry, 2012; Palacios-García *et al.*, 2015). Furthermore, our results regarding the decreased anxiety levels in MAM-16 male mice are in line with results from another developmental animal model, the social isolation rearing model. Anxiogenic behavior has been reported in male isolation reared rats and mice, but not in female animals (Guo 2004, Weiss 2004). However, results from the phencyclidine (PCP) model of SZ have shown anxiogenic behavior in male and anxiolytic behavior in female mice (Turgeon, Anderson and O'Loughlin, 2010). Diverse results have been also revealed from genetic animal models. Reduced anxiety levels in male mice or in both sexes have been reported, or even increased anxiety-behavior in female animals (O'Tuathaigh *et al.*, 2008; Kuroda *et al.*, 2011).

Despite the variability in the results concerning the rates in comorbidity of anxiety and SZ in humans, the general idea suggests a high prevalence of anxiety in SZ (Achim *et al.*, 2011). Our results are partially consistent with human reports which suggest an elevated HPA axis activity in SZ patients. In particular, increased HPA axis activity is more pronounced in first-episode patients (Ryan *et al.*, 2004; Guest *et al.*, 2011) (, while increased anxiety often exacerbates the psychotic symptoms (Lysaker and Salyers, 2007).

Importantly, human studies report that women with SZ show more severe anxiety and depression symptoms, compared to men (Emsley *et al.*, 1999; Abel, Drake and Goldstein, 2010).

3.4.2 Sexually dimorphic CRF1 expression

We measured the protein levels of CRF1 in PFC samples of MAM-16 and control animals in order to identify possible alterations after prenatal MAM exposure in the CRF system which could support a link between the MAM model of SZ in mice and CRF system alterations, as well as with the basal anxiety levels. Analysis reveals decreased levels of CRF1 only the PFC of male MAM-16 mice, which show decreased levels of trait anxiety, as measured in the EPM task. Our results are in line with studies from conditional knocked out animals. Specifically, reduced anxiety behavior has been recorded in mice from which CRF1 gene was totally deleted or ablated from glutamatergic forebrain neurons (Timpl *et al.*, 1998; Refojo *et al.*, 2011; Wang *et al.*, 2013). In contrast, we do not find alterations in CRF1 levels in female MAM-16 mice, despite their increased basal anxiety, which could suggest altered CRF1 expression in PFC. However we cannot exclude the possibility that CRF1 expression in other brain regions has been affected. It has been shown that specific deletion of CRF1 from dopaminergic neurons of the midbrain leads to high anxiety levels(Kratzer *et al.*, 2013). The observed increased anxiety behavior of female MAM-16 mice in the EPM task may reflect an increased sensitivity in their stress response, which could be attributed to dysfunction of other brain areas, such as amygdala or the cortico-striatal-thalamo-cortical loop, or alterations in other neurotransmitter systems. Alternatively, the observed alterations in CRF1 protein levels could be the result of increased β-arrestin-2 coupling of the receptors in male but not female MAM-16 mice which leads to desensitization of the receptor. Based on recent findings from Bangasser and colleagues (Bangasser *et al.*, 2010; Bangasser and Valentino, 2014), the β-arrestin-2 pathway is preferred by stressed male but not female rats, leading to CRF1 internalization. If we hypothesize that prenatal exposure to MAM mimics the effects of a stressful condition, it could be possible that in male mice the β-arrestin-2 pathway is

activated as a compensatory mechanism, leading to CRF1 internalization, degradation and finally, in decreased levels. However, according to the above studies, this is not the case for female MAM-exposed mice, giving support to the hypothesis of increased predisposition of females to certain stress-related psychiatric disorders.

3.4.3 Does CRF system protect PFC function in females?

The results from CRF1 protein measurements led us to further hypothesize that the sex-biased PFC-dysfunction observed in MAM-16 mice (described in chapter **2**) could be at least partially influenced by CRF1 signaling/activity or that PFC function in female MAM-16 mice is somehow protected through CRF1 function. We blocked CRF1 signaling in MAM-16 and control female mice, with chronic antalarmin systemic administration. Analysis reveals opposite effects on synaptic transmission and plasticity between the two groups. We report enhanced LTP induction in control mice and absence of LTP in MAM-16 mice. Despite the restricted knowledge concerning the direct role of CRF system in PFC function our results are in line with other studies, with regards to the effects of antalarmin in control animals. Recently, it was shown that systemic administration of antalarmin during early-life stress exposure prevented both stress-induced dendritic shrinkage of pyramidal cells in layers II/III and V of PFC and the related PFC cognitive deficits (Yang *et al.*, 2015), which could be compared to the enhanced synaptic plasticity of PFC we find in control animals. Similarly, antalarmin treatment has been reported to restore HPC structural and functional deficits induced after early life-stress exposure(Ivy *et al.*, 2010).

On the other hand, several studies have shown both facilitating and negative effects of CRF on cognitive function. It seems that CRF through CRF1 affects differently PFC and HPC function. Specifically, acute elevations of CRF can be beneficial for HPC(Wang *et al.*, 1998; Orozco-Cabal *et al.*, 2006), enhancing synaptic transmission, but not for PFC-dependent cognitive function (Zieba *et al.*, 2008; Uribe-Mariño *et al.*, 2016), while prolonged exposure to high levels of CRF seems to be detrimental for both structures

(Chen *et al.*, 2008, 2010, 2012; Hains *et al.*, 2009; Ivy *et al.*, 2010). Interestingly, we find that blockade of CRF1 abolishes LTP induction in layer II/III of PFC in MAM-16 mice, resembling the observed deficit found in male MAM-16 mice. It has been previously shown that *in vivo* intra-cortical CRF infusion into rat sensorimotor cortex induced long-lasting depression of synaptic transmission, measured in layers II/III and V(Froc and Christie, 2005) Similarly, in rat frontal cortex slices, the CRF-induced depression of synaptic transmission was evident only after the simultaneous blockade of GABa and GABAb receptors, which had a dis-inhibiting effect on the slice (Zieba *et al.*, 2008). Based on these observations, we can hypothesize that the decreased number of PV interneurons in the PFC of male MAM-16 mice (chapter 2) could have a dis-inhibiting effect, which could be combined with the decreased number of CRF1 receptors, leading to LTP deficits and PFC dysfunction. Furthermore, chronic antalarmin treatment in females seems to mimic the decreased levels/activity of CRF1 seen in males, leading to LTP deficits.

3.4.4 Cognitive function after restraint stress – does sex matter?

In this part of the study our first goal was to evaluate the anxiety-related behavior after exposure to acute restraint stress, in control mice of both sexes. Analysis of their activity inside the Light-Dark box, after 2 hours of restraint stress, reveals increased anxiety levels in RS females, compared to their control group, as indicated by the increased time spent in the dark compartment. In RS males we only report decreased number of transitions and no differences in the time spent in each compartment. The decreased number of transitions is also observed in RS females and is considered an index of decreased exploratory behavior, which could be interpreted as anxiety-related behavior (Belzung, Misslin and Vogel, 1987; Bourin and Hascoët, 2003). As reviewed in Horst and colleagues (Ter Horst *et al.*, 2012), studies have shown inconsistent results with regards to the behavioral/emotional effects of acute stress exposure between male and female animals. For example, increased anxiety levels following restraint stress have been reported in male mice, but not in females (Bowman *et al.*,

2009), while exposure to isolation stress reduced anxiety levels in male and had no effect on female mice (Guo *et al.*, 2004). However, exposure to a psycho-emotional stressor has shown increased anxiety-related behavior in both sexes(Avgustinovich and Kovalenko, 2010). The general concept proposed is that stress tends to induce more anxiety in male rodents, while in females anxiety behavior depends on the type of stressor (Ter Horst *et al.*, 2012). Based on the above, our results from the Light/Dark task evaluation are in line with other studies.

The second part of this experiment includes the cognitive evaluation of the animals after restraint stress exposure. The recognition task for temporal order is extensively used for the assessment of the PFC function. Analysis shows no differences with regards to recency memory function after two hours of restraint stress, in either sex. However, the low number of animals tested so far, might be insufficient to unravel possible effects of restraint stress in cognitive function of mice. There are studies supporting a favorable effect of acute stress on cognitive function, with the majority of the reports referring to PFC cognitive function of male subjects(Wood and Shors, 1998; Das *et al.*, 2000; Conrad *et al.*, 2004; Dalla *et al.*, 2007; Yuen *et al.*, 2009; Bryce and Floresco, 2016). Furthermore, Drouet and colleagues have shown weak PFC activity after 1 hour of restraint stress in rats with high PFC GAB/Glutamate ratio, suggesting a possible association of increased emotional reactivity in acute stress with PFC deficits(Drouet *et al.*, 2015). However, a similar study conducted in rats have found impaired PFC function in both male and female animals after exposure to two hours of restraint(Shansky *et al.*, 2006).

Based on literature and taking into consideration our own results, we can conclude that acute restraint stress can affect in a different way the two sexes. Females seem to be more vulnerable, at the emotional level, while our results reveal higher tolerance of male mice against the effects of restraint stress. Further experiments would give us a clearer view on how restraint stress can affect recency memory of female and male mice.

Chapter 4

General discussion

Taking into consideration that SZ is one of the commonest and most serious mental disorders, often debilitating for the patients and their families, but still incurable, the need to understand its multifactorial character and unravel the underlying mechanisms of its pathology is imperative. Another important aspect that is rarely incorporated in SZ studies, are the sex differences related to the emergence and the symptomatology of the disorder (Abel, Drake and Goldstein, 2010), which can be an additional obstacle, in developing effective treatment. Based on the above considerations, and in support to the two-hit hypothesis (Maynard *et al.*, 2001; Monte *et al.*, 2017) for the emergence of SZ, we describe the development of the SZ neurodevelopmental MAM model in mice, which has been established and extensively studied in rats(Modinos *et al.*, 2015), mostly in male animals and we validate it in both sexes. Furthermore, we investigate whether and how CRF system is involved in the pathology of the MAM model of SZ, as a strong link between SZ, stress and CRF system seems to exist(Cáceda, Kinkead and Nemeroff, 2007; De Luca *et al.*, 2010; Ribbe *et al.*, 2011).

The first part of the study reveals that GD 16 is the optimal time point for the prenatal exposure to the mitotoxin and that both male and female mice show ‘schizotypic-like’ alterations, as adults (positive symptoms and histological deficits), suggesting that MAM-16 model in mice is comparable to the MAM-17 rat model. The concurrent study of both sexes demonstrates significant differences in PV protein expression and in PFC-dependent cognitive deficits, which are also observed in humans and other animal models (Leung and Pierre Chue, 2000; Zhang and Reynolds, 2002; Abel, Drake and Goldstein, 2010; Ochoa *et al.*, 2012; Holley *et al.*, 2013; Hill, 2016). Specifically, we find decreased number of PV-interneurons in HPC and PFC only of male MAM-16 mice, accompanied by impaired synaptic plasticity in both HPC and PFC and concomitant cognitive deficits. While HPC function is

impaired in both sexes, PFC seems to be more vulnerable in males. A possible explanation for these observations, already discussed in chapter 2, could be given by the gonadal hormones function. Recent studies suggest a protective role of estrogens on hippocampal function, in response to stress. However, here, it could be possible that estrogens can only protect the late developing PFC of female MAM-exposed mice. The second part of the study reveals alterations in basal anxiety levels of MAM-exposed mice, along with a potential critical role of CRF system in PFC cognitive deficits of the MAM mouse model. In particular, we find increased anxiety levels in females and decreased in male mice prenatally exposed to MAM, observed only in adulthood. Furthermore, asexually dimorphic expression of CRF1 protein in the PFC is added in the observed sex-biased deficits. Alterations in both anxiety levels and CRF system components have been reported in the rat MAM mode, in other animal models of SZ and in the human condition (Herringa, Roseboom and Kalin, 2006; Turgeon, Anderson and O'Loughlin, 2010; Achim *et al.*, 2011; Ribbe *et al.*, 2011; Schulz *et al.*, 2014; Gastambide *et al.*, 2015b; Du and Grace, 2016),despite the several discrepancies that exist in the literature, which complicate our conclusions.

Our first hypothesis that the sex-biased PFC-dysfunction observed in MAM-16 mice could be partially influenced by CRF1 signaling/activity or that PFC function in female MAM-16 mice might be protected through CRF1 function is supported by the systemic antalarmin treatment results, which show impaired PFC synaptic plasticity in MAM-16 female mice after chronic blockade of CRF1. However, the question to be answered is which are the underlying mechanisms for this sexual dimorphism in CRF system function that might contribute to men's increased vulnerability to develop SZ? According to our results, the decreased number of PV-positive interneurons in the PFC of male MAM-16 mice could have a double negative effect on PFC function: 1) they could disturb the balance between excitation and inhibition, by dis-inhibiting PFC circuits, leading consequently to impaired synaptic transmission and plasticity and 2) they could lead to decreased CRF1 expression levels as a homeostatic mechanism for the decreased CRF-IR neurons, which are interneurons. Since PV interneurons are not affected in

the PFC of female MAM-16 mice, but chronic blockage of CRF1 reproduces the deficits found in male PFC, the second hypothesis does not seem possible. However, it is possible that there is excessive CRF in both male and female MAM-exposed animals, probably existing prior to adulthood, which leads to the activation of different homeostatic mechanisms between the two sexes.

As discussed earlier, recent findings have shown that β-arrestin pathway is preferred by stressed male but not female rats. If we hypothesize that this is the case for male MAM-expose mice, then the observed decreased CRF1 levels could be the result of internalization and degradation processes that take place in the PFC, as a compensatory mechanism for the excessive CRF. On the other hand, the unaltered levels of CRF1 in the PFC of female MAM-16 mice imply that β-arrestin pathway is not preferred by females and suggest that MAM-16 females develop a different homeostatic mechanism to compensate for the increased CRF levels. We hypothesize that this compensatory mechanism could be the activation of a different signaling pathway in response to CRF1 binding. There are several signaling pathways that can be initiated upon CRF1 activation, and the choice of the pathway to activated, probably depends on the 'needs' and the state of the organism(Hauger *et al.*, 2009). The AC-PKA signaling pathway is considered the dominant pathway for both CRF1 and CRF2 (Hauger *et al.*, 2009). Interestingly, it has been shown that acute stress or intra-PFC CRF injections lead to PFC dysfunction, which can be reversed with either intra-PFC CRF1 deletion or after PKA blockade (Uribe-Mariño *et al.*, 2016). An alternative signaling pathway that could be activated is the Ras- PI3K –PKB kinase signaling pathway. Ras-MAPK and cAMP/PKA are considered competing pathways that regulate the expression of plasticity-associated genes(Waltereit and Weller, 2003). We hypothesize that in female MAM-16 mice PFC, the AC-PKA signaling cascade is suppressed and the Ras signaling pathway is activated. Several lines of evidence support that Ras signaling pathway has a crucial role in LTP and memory function(Jin and Feig, 2010; Liu *et al.*, 2011)by controlling the synaptic trafficking of AMPA receptors, through phosphorylation of GluR1 (Zhu *et al.*, 2002; McCormack, Stornetta and Zhu,

2006; Hu *et al.*, 2008; Kielland *et al.*, 2009). This compensatory mechanism could reinstate the normal PFC function and rescue PFC function of female MAM mice. Consequently, when we chronically block CRF1 with systemic antalarmin treatment, it seems that homeostasis is again disturbed. With regards to control animals treated with antalarmin, the observed LTP facilitation

The last part of the study constitutes the first step to our ultimate goal, which would be to test the cognitive function of MAM-mice after acute stress and identify possible sexually dimorphic adaptations in both behavioral stress response and PFC-function. This would further help us elucidate the role of CRF system in SZ pathology and the underlying cellular mechanism responsible for the observed sexual dimorphism.

References

A., T. L. *et al.* (2017) ‘Distribution of corticotropin- releasing factor (CRF) receptor binding in the mouse brain using a new, high- affinity radioligand, [125I]- PD- Sauvagine’, *Journal of Comparative Neurology*. Wiley-Blackwell, 525(18), pp. 3840–3864. doi: 10.1002/cne.24307.

Abel, K. M., Drake, R. and Goldstein, J. M. (2010) ‘Sex differences in schizophrenia’, *International Review of Psychiatry*, 22(5), pp. 417–428. doi: 10.3109/09540261.2010.515205.

Achim, A. M. *et al.* (2011) ‘How prevalent are anxiety disorders in schizophrenia? a meta-analysis and critical review on a significant association’, *Schizophrenia Bulletin*, 37(4), pp. 811–821. doi: 10.1093/schbul/sbp148.

Adamec, R. E. and McKay, D. (1993) ‘The effects of CRF and alpha-helical CRF on anxiety in normal and hypophysectomized rats.’, *Journal of psychopharmacology (Oxford, England)*. United States, 7(4), pp. 346–354. doi: 10.1177/026988119300700406.

Aisa, B. *et al.* (2018) ‘Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats’, *Psychoneuroendocrinology*. Elsevier, 32(3), pp. 256–266. doi: 10.1016/j.psyneuen.2006.12.013.

Aldenhoff, J. B. *et al.* (1983) ‘Corticotropin releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons.’, *Science (New York, N.Y.)*. United States, 221(4613), pp. 875–877.

Amaral, D. G., Scharfman, H. E. and Lavenex, P. (2007) ‘The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies)’, *Progress in brain research*, 163, pp. 3–22. doi: 10.1016/S0079-6123(07)63001-5.

Andersen, P., Bliss, T. V and Skrede, K. K. (1971) ‘Lamellar organization of hippocampal pathways.’, *Experimental brain research*. Germany, 13(2), pp. 222–238.

Andiné, P. *et al.* (1999) ‘Characterization of MK-801-induced behavior as a putative rat model of psychosis.’, *The Journal of pharmacology and experimental therapeutics*, 290(3), pp. 1393–1408.

Anisman, H. *et al.* (1998) ‘Do early-life events permanently alter behavioral and hormonal responses to stressors?’, *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*. England, 16(3–4), pp. 149–164.

Arguello, P. A. *et al.* (2010) ‘Development of animal models for schizophrenia’, *Disease Models & Mechanisms*, 3(1–2), pp. 22–26. doi: 10.1242/dmm.003996.

‘Autoradiographic localization of CRF1and2 in rat brain_Primus 1997.pdf’ (no date).

Avgustinovich, D. F. and Kovalenko, I. L. (2010) 'Gender-related characteristics of responding to prolonged psychoemotional stress in mice.', *Neuroscience and behavioral physiology*. United States, 40(3), pp. 257–262. doi: 10.1007/s11055-010-9252-1.

Ayhan, Y. *et al.* (2009) 'Animal models of gene–environment interactions in schizophrenia', *Behavioural Brain Research*. Elsevier, 204(2), pp. 274–281. doi: 10.1016/J.BBR.2009.04.010.

Bale, T. L. and Epperson, C. N. (2015) 'Sex differences and stress across the lifespan', *Nature Neuroscience*, 18(10), pp. 1413–1420. doi: 10.1038/nn.4112.

Bangasser, D. A. *et al.* (2010) 'Sex differences in corticotropin-releasing factor receptor signaling and trafficking: Potential role in female vulnerability to stress-related psychopathology', *Molecular Psychiatry*. Nature Publishing Group, 15(9), pp. 896–904. doi: 10.1038/mp.2010.66.

Bangasser, D. A. and Kawasumi, Y. (2015) 'Cognitive disruptions in stress-related psychiatric disorders: A role for corticotropin releasing factor (CRF)', *Hormones and Behavior*, 76, pp. 125–135. Available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L603964686%5Cnhttp://dx.doi.org/10.1016/j.yhbeh.2015.04.003%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=10956867&id=doi:10.1016%2Fj.yhbeh.2015.04.003&atitle=Cognitive+disrupt.

Bangasser, D. A. and Valentino, R. J. (2014) 'Sex differences in stress-related psychiatric disorders: Neurobiological perspectives', *Frontiers in Neuroendocrinology*. Elsevier Inc., 35(3), pp. 303–319. doi: 10.1016/j.yfrne.2014.03.008.

Banks, S. J. *et al.* (2007) 'Amygdala-frontal connectivity during emotion regulation.', *Social cognitive and affective neuroscience*. England, 2(4), pp. 303–312. doi: 10.1093/scan/nsm029.

Bao, A.-M. *et al.* (2006) 'A direct androgenic involvement in the expression of human corticotropin-releasing hormone', *Molecular Psychiatry*. Nature Publishing Group, 11, p. 567. Available at: http://dx.doi.org/10.1038/sj.mp.4001800.

Barch, D. M. *et al.* (2003) 'Working memory and prefrontal cortex dysfunction: Specificity to schizophrenia compared with major depression', *Biological Psychiatry*, 53(5), pp. 376–384. doi: 10.1016/S0006-3223(02)01674-8.

Bayer, S. A. and Altman, J. (2004) 'Development of the telencephalon: neural stem cells, neurogenesis, and neuronal migration', in *The Rat Nervous System (Third Edition)*. Elsevier, pp. 27–73.

Behan, D. P., De Souza, E. B., *et al.* (1995) 'Corticotropin releasing factor (CRF) binding protein: a novel regulator of CRF and related peptides.', *Frontiers in neuroendocrinology*, pp. 362–382. doi: 10.1006/frne.1995.1013.

Behan, D. P., Heinrichs, S. C., *et al.* (1995) 'Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease.', *Nature*. England, 378(6554), pp. 284–287. doi: 10.1038/378284a0.

Belujon, P., Patton, M. H. and Grace, A. A. (2014) 'Role of the prefrontal

cortex in altered hippocampal-accumbens synaptic plasticity in a developmental animal model of schizophrenia', *Cerebral Cortex*, 24(4), pp. 968–977. doi: 10.1093/cercor/bhs380.

Belzung, C., Misslin, R. and Vogel, E. (1987) 'Anxiogenic Effects of in a Light / Dark Choice Situation', *Pharmacology Biochemistry and Behavior*, 28(1), pp. 29–33.

Bergstrom, C. T. and Meacham, F. (2016) 'Depression and anxiety: maladaptive byproducts of adaptive mechanisms', *Evolution, Medicine, and Public Health*, 2016(1), pp. 214–218. doi: 10.1093/emph/eow019.

Blumenfeld, R. S. (2006) 'Dorsolateral Prefrontal Cortex Promotes Long-Term Memory Formation through Its Role in Working Memory Organization', *Journal of Neuroscience*, 26(3), pp. 916–925. doi: 10.1523/JNEUROSCI.2353-05.2006.

Bondi, C. O. *et al.* (2007) 'Chronic Unpredictable Stress Induces a Cognitive Deficit and Anxiety-Like Behavior in Rats that is Prevented by Chronic Antidepressant Drug Treatment', *Neuropsychopharmacology*. American College of Neuropsychopharmacology, 33, p. 320. Available at: http://dx.doi.org/10.1038/sj.npp.1301410.

Born, J. *et al.* (1995) 'Effects of age and gender on pituitary-adrenocortical responsiveness in humans.', *European journal of endocrinology*. England, 132(6), pp. 705–711.

Bornstein, S. R. *et al.* (1998) 'Chronic effects of a nonpeptide corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation.', *Endocrinology*. United States, 139(4), pp. 1546–1555. doi: 10.1210/endo.139.4.5938.

Bourin, M. and Hascoët, M. (2003) 'The mouse light / dark box test', *European Journal of Pharmacology*, 463(1), pp. 55–65. doi: 10.1016/S0014-2999(03)01274-3.

Bowman, R. E. *et al.* (2009) 'Sex-dependent changes in anxiety, memory, and monoamines following one week of stress', *Physiology and Behavior*. Elsevier B.V., 97(1), pp. 21–29. doi: 10.1016/j.physbeh.2009.01.012.

Bowman, R. E., Ferguson, D. and Luine, V. N. (2002) 'Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats', *Neuroscience*, 113(2), pp. 401–410. doi: https://doi.org/10.1016/S0306-4522(02)00156-2.

Bozikas, V. P. *et al.* (2010) 'Sex differences in neuropsychological functioning among schizophrenia patients.', *The Australian and New Zealand journal of psychiatry*. England, 44(4), pp. 333–341. doi: 10.3109/00048670903489833.

Bradley, A. J. and Dinan, T. G. (2010) 'Review: A systematic review of hypothalamic-pituitary-adrenal axis function in schizophrenia: implications for mortality', *Journal of Psychopharmacology*, 24(4_suppl), pp. 91–118. doi: 10.1177/1359786810385491.

Brisch, R. (2014) 'The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: Old fashioned, but still in vogue', *Frontiers in Psychiatry*, 5(APR), pp. 1–11. doi:

10.3389/fpsyt.2014.00047.

Bryce, C. A. and Floresco, S. B. (2016) ‘Perturbations in Effort-Related Decision-Making Driven by Acute Stress and Corticotropin-Releasing Factor’, *Neuropsychopharmacology*. Nature Publishing Group, 41(8), pp. 2147–2159. doi: 10.1038/npp.2016.15.

Buss, C. *et al.* (2012) ‘Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems’, *Proceedings of the National Academy of Sciences*, 109(20), p. E1312 LP-E1319. Available at: http://www.pnas.org/content/109/20/E1312.abstract.

Buss, R. R., Sun, W. and Oppenheim, R. W. (2006) ‘Adaptive roles of programmed cell death during nervous system development.’, *Annual review of neuroscience*. United States, 29, pp. 1–35. doi: 10.1146/annurev.neuro.29.051605.112800.

Van Den Buuse, M. (2010) ‘Modeling the positive symptoms of schizophrenia in genetically modified mice: Pharmacology and methodology aspects’, *Schizophrenia Bulletin*, 36(2), pp. 246–270. doi: 10.1093/schbul/sbp132.

Buzsáki, G. and Moser, E. I. (2013) ‘Memory, navigation and theta rhythm in the hippocampal-entorhinal system’, *Nature Neuroscience*, 16(2), pp. 130–138. doi: 10.1038/nn.3304.

Bystron, I., Blakemore, C. and Rakic, P. (2008) ‘Development of the human cerebral cortex: Boulder Committee revisited.’, *Nature reviews. Neuroscience*. England, 9(2), pp. 110–122. doi: 10.1038/nrn2252.

Cáceda, R., Kinkead, B. and Nemeroff, C. B. (2007) ‘Involvement of neuropeptide systems in schizophrenia: human studies.’, *International review of neurobiology*, 78(06), pp. 327–76. doi: 10.1016/S0074-7742(06)78011-4.

Cannon, T. D. *et al.* (2002) ‘Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia’, *Proceedings of the National Academy of Sciences*, 99(5), pp. 3228–3233. doi: 10.1073/pnas.052023499.

Carlsson, M. and Carlsson, A. (1990) ‘Schizophrenia: a subcortical neurotransmitter imbalance syndrome?’, *Schizophrenia Bulletin*, 16(3), pp. 425–432. doi: 10.1093/schbul/16.3.425.

Cazakoff, B. N., Johnson, K. J. and Howland, J. G. (2010) ‘Converging effects of acute stress on spatial and recognition memory in rodents: A review of recent behavioural and pharmacological findings’, *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(5), pp. 733–741. doi: https://doi.org/10.1016/j.pnpbp.2010.04.002.

Chajut, E. and Algom, D. (2003) ‘Selective attention improves under stress: implications for theories of social cognition.’, *Journal of personality and social psychology*. United States, 85(2), pp. 231–248.

Chalmers, D. T., Lovenberg, T. W. and De Souza, E. B. (1995) ‘Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression.’, *The Journal of neuroscience : the official journal of the Society*

for Neuroscience, 15(10), pp. 6340–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7472399.

Chatzaki, E. *et al.* (2004) 'CRF receptor type 1 and 2 expression and anatomical distribution in the rat colon', *Journal of Neurochemistry*, 90(2), pp. 309–316. doi: 10.1111/j.1471-4159.2004.02490.x.

Chatzaki, E. *et al.* (2004) 'Differential profile of CRF receptor distribution in the rat stomach and duodenum assessed by newly developed CRF receptor antibodies', *Journal of Neurochemistry*, 88(1), pp. 1–11. doi: 10.1046/j.1471-4159.2003.02078.x.

Chatzaki, E., Margioris, A. N. and Gravanis, A. (2002) 'Expression and regulation of corticotropin-releasing hormone binding protein (CRH-BP) in rat adrenals', *Journal of Neurochemistry*, 80(1), pp. 81–90. doi: 10.1046/j.0022-3042.2001.00667.x.

Chen, R. *et al.* (1993) 'Expression cloning of a human corticotropin-releasing-factor receptor.', *Proceedings of the National Academy of Sciences of the United States of America*. United States, 90(19), pp. 8967–8971.

Chen, Y. *et al.* (2000) 'Immunocytochemical distribution of corticotrophin-releasing hormone receptor type-1 (CRF(1))-like immunoreactivity in the mouse brain: light microscopy analysis using an antibody directed against the C-terminus', *Journal of Comparative Neurology*, 420(December 1999), pp. 305–323.

Chen, Y. *et al.* (2004) 'Hippocampal corticotropin releasing hormone: pre- and postsynaptic location and release by stress', *Neuroscience*, 126(3), pp. 533–540. doi: https://doi.org/10.1016/j.neuroscience.2004.03.036.

Chen, Y. *et al.* (2008) 'Rapid loss of dendritic spines after stress involves derangement of spine dynamics by corticotropin-releasing hormone.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. United States, 28(11), pp. 2903–2911. doi: 10.1523/JNEUROSCI.0225-08.2008.

Chen, Y. *et al.* (2010) 'Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling', *Proceedings of the National Academy of Sciences*, 107(29), pp. 13123–13128. doi: 10.1073/pnas.1003825107.

Chen, Y. *et al.* (2012) 'Tuning synaptic transmission in the hippocampus by stress: the CRH system', *Frontiers in Cellular Neuroscience*, 6(April), pp. 1–7. doi: 10.3389/fncel.2012.00013.

Chen, Y. *et al.* (2013) 'Impairment of synaptic plasticity by the stress mediator CRH involves selective destruction of thin dendritic spines via RhoA signaling', *Molecular psychiatry*, 18(4), pp. 485–496. doi: 10.1038/mp.2012.17.

Chuang, N. *et al.* (2011) 'An MRI-based atlas and database of the developing mouse brain', *NeuroImage*, 54(1), pp. 80–89. doi: https://doi.org/10.1016/j.neuroimage.2010.07.043.

Citri, A. and Malenka, R. C. (2008) 'Synaptic plasticity: Multiple forms, functions, and mechanisms', *Neuropsychopharmacology*, 33(1), pp. 18–41.

doi: 10.1038/sj.npp.1301559.

Cohen, R. A. *et al.* (2006) ‘Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei.’, *Biological psychiatry*. United States, 59(10), pp. 975–982. doi: 10.1016/j.biopsych.2005.12.016.

Conrad, C. D. *et al.* (2003) ‘Sex differences in spatial and non-spatial Y-maze performance after chronic stress’, *Neurobiology of Learning and Memory*, 79(1), pp. 32–40. doi: https://doi.org/10.1016/S1074-7427(02)00018-7.

Conrad, C. D. *et al.* (2004) ‘Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle’, *Pharmacology Biochemistry and Behavior*, 78(3), pp. 569–579. doi: https://doi.org/10.1016/j.pbb.2004.04.025.

Curtis, A. L., Bethea, T. and Valentino, R. J. (2005) ‘Sexually Dimorphic Responses of the Brain Norepinephrine System to Stress and Corticotropin-Releasing Factor’, *Neuropsychopharmacology*. American College of Neuropsychopharmacology, 31, p. 544. Available at: http://dx.doi.org/10.1038/sj.npp.1300875.

D., B. R., P., W. M. and G., A. D. (1995) ‘Perirhinal and postrhinal cortices of the rat: A review of the neuroanatomical literature and comparison with findings from the monkey brain’, *Hippocampus*. Wiley-Blackwell, 5(5), pp. 390–408. doi: 10.1002/hipo.450050503.

D., L. T. *et al.* (2004) ‘Androgen Inhibits, While Oestrogen Enhances, Restraint-Induced Activation of Neuropeptide Neurones in the Paraventricular Nucleus of the Hypothalamus’, *Journal of Neuroendocrinology*. Wiley/Blackwell (10.1111), 16(3), pp. 272–278. doi: 10.1111/j.0953-8194.2004.01167.x.

Dalla, C. *et al.* (2007) ‘Females do not Express Learned Helplessness like Males do’, *Neuropsychopharmacology*. American College of Neuropsychopharmacology, 33, p. 1559. Available at: http://dx.doi.org/10.1038/sj.npp.1301533.

Das, A. *et al.* (2000) ‘Immobilization stress-induced changes in brain acetylcholinesterase activity and cognitive function in mice’, *Pharmacological Research*, 42(3), pp. 213–217. doi: https://doi.org/10.1006/phrs.2000.0678.

Dautzenberg, F. M. and Hauger, R. L. (2002) ‘The CRF peptide family and their receptors: Yet more partners discovered’, *Trends in Pharmacological Sciences*, 23(2), pp. 71–77. doi: 10.1016/S0165-6147(02)01946-6.

Davis, J. *et al.* (2016) ‘A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis’, *Neuroscience and Biobehavioral Reviews*. Elsevier Ltd, 65, pp. 185–194. doi: 10.1016/j.neubiorev.2016.03.017.

Davis, K. L. *et al.* (1991) ‘Dopamine in schizophrenia: A review and reconceptualization’, *American Journal of Psychiatry*, 148(11), pp. 1474–1486. doi: 10.1176/ajp.148.11.1474.

Dedic, N. *et al.* (2017) ‘The CRF Family of Neuropeptides and their Receptors - Mediators of the Central Stress Response’, *Current Molecular Pharmacology*, 11(1), pp. 1–28. doi: http://dx.doi.org/10.2174/1874467210666170302104053.

Defelipe, J. *et al.* (2013) 'New insights into the classification and nomenclature of cortical GABAergic interneurons', *Nature Reviews Neuroscience*. Nature Publishing Group, 14(3), pp. 202–216. doi: 10.1038/nrn3444.

Delville, Y., Stires, C. and Ferris, C. F. (1992) 'Distribution of corticotropin-releasing hormone immunoreactivity in golden hamster brain', *Brain Research Bulletin*, 29(5), pp. 681–684. doi: https://doi.org/10.1016/0361-9230(92)90138-N.

DeWire, S. M. *et al.* (2007) 'β-Arrestins and Cell Signaling', *Annual Review of Physiology*. Annual Reviews, 69(1), pp. 483–510. doi: 10.1146/annurev.physiol.69.022405.154749.

Diamond, D. M. *et al.* (1992) 'Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation.', *Hippocampus*. United States, 2(4), pp. 421–430. doi: 10.1002/hipo.450020409.

Dias-Ferreira, E. *et al.* (2009) 'Chronic Stress Causes Frontostriatal Reorganization and Affects Decision-Making', *Science*, 325(5940), p. 621 LP-625. Available at: http://science.sciencemag.org/content/325/5940/621.abstract.

Dirks, A. *et al.* (2003) 'Reversal of startle gating deficits in transgenic mice overexpressing corticotropin-releasing factor by antipsychotic drugs.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 28(10), pp. 1790–8. doi: 10.1038/sj.npp.1300256.

Drouet, J. B. *et al.* (2015) 'Differences in prefrontal cortex GABA/glutamate ratio after acute restraint stress in rats are associated with specific behavioral and neurobiological patterns', *Neuroscience*. IBRO, 285, pp. 155–165. doi: 10.1016/j.neuroscience.2014.10.058.

Du, Y. and Grace, A. A. (2013) 'Peripubertal diazepam administration prevents the emergence of dopamine system hyperresponsivity in the MAM developmental disruption model of schizophrenia', *Neuropsychopharmacology*. Nature Publishing Group, 38(10), pp. 1881–1888. doi: 10.1038/npp.2013.101.

Du, Y. and Grace, A. A. (2016) 'Amygdala Hyperactivity in MAM Model of Schizophrenia is Normalized by Peripubertal Diazepam Administration', *Neuropsychopharmacology*. Nature Publishing Group, 41(10), pp. 2455–2462. doi: 10.1038/npp.2016.42.

Ducottet, C., Griebel, G. and Belzung, C. (2003) 'Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice.', *Progress in neuro-psychopharmacology & biological psychiatry*. England, 27(4), pp. 625–631. doi: 10.1016/S0278-5846(03)00051-4.

Emsley, R. A. *et al.* (1999) 'Depressive and anxiety symptoms in patients with schizophrenia and schizophreniform disorder.', *The Journal of clinical psychiatry*. United States, 60(11), pp. 747–751.

Erk, S. *et al.* (2010) 'Acute and Sustained Effects of Cognitive Emotion

Regulation in Major Depression', *The Journal of Neuroscience*, 30(47), p. 15726 LP-15734. Available at: http://www.jneurosci.org/content/30/47/15726.abstract.

Etkin, A., Egner, T. and Kalisch, R. (2011) 'Emotional processing in anterior cingulate and medial prefrontal cortex', *Trends in Cognitive Sciences*, 15(2), pp. 85–93. doi: 10.1016/j.tics.2010.11.004.

Evans, G. W. and English, K. (2002) 'The environment of poverty: multiple stressor exposure, psychophysiological stress, and socioemotional adjustment.', *Child development*. United States, 73(4), pp. 1238–1248.

Evans, G. W. and Schamberg, M. A. (2009) 'Childhood poverty, chronic stress, and adult working memory', *Proceedings of the National Academy of Sciences*, 106(16), pp. 6545–6549. doi: 10.1073/pnas.0811910106.

Fatemi, S. H. and Folsom, T. D. (2009) 'The neurodevelopmental hypothesis of Schizophrenia, revisited', *Schizophrenia Bulletin*, 35(3), pp. 528–548. doi: 10.1093/schbul/sbn187.

Featherstone, R. E. *et al.* (2007) 'Gestational methylazoxymethanol acetate treatment impairs select cognitive functions: parallels to schizophrenia.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 32(2), pp. 483–492. doi: 10.1038/sj.npp.1301223.

Felmingham, K. L. *et al.* (2014) 'Reduced Amygdala and Ventral Striatal Activity to Happy Faces in PTSD Is Associated with Emotional Numbing', *Reviews in the Neurosciences* . England: Elsevier, 9(3), p. e103653. doi: 10.1515/REVNEURO.2010.21.2.119.

Fink, G. (2010) *Stress Science: Neuroendocrinology*. Elsevier Science. Available at: https://books.google.nl/books?id=HJwqWQhQELMC.

Fiszdon, J. M. *et al.* (2003) 'Verbal memory in schizophrenia: sex differences over repeated assessments', *Schizophrenia Research*. Elsevier, 61(2), pp. 235–243. doi: 10.1016/S0920-9964(02)00285-2.

Flagstad, P. *et al.* (2004) 'Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens', *Neuropsychopharmacology*, 29(11), pp. 2052–2064. doi: 10.1038/sj.npp.1300516.

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

Froc, D. J. and Christie, B. R. (2005) 'Corticotrophin-releasing hormone decreases synaptic transmission in rat sensorimotor cortex in vivo.', *Neuroscience*. United States, 134(3), pp. 965–973. doi: 10.1016/j.neuroscience.2005.05.004.

Gabriel, S. M. *et al.* (1996) 'Neuropeptide deficits in schizophrenia vs. Alzheimer's disease cerebral cortex.', *Biological psychiatry*, 39(2), pp. 82–91. doi: 10.1016/0006-3223(95)00066-6.

Garner, C. C. *et al.* (2002) ‘Molecular mechanisms of CNS synaptogenesis’, *Trends in Neurosciences*. Elsevier, 25(5), pp. 243–250. doi: 10.1016/S0166-2236(02)02152-5.

Gastambide, F. *et al.* (2012) ‘Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator’, *Neuropsychopharmacology*, 37(4), pp. 1057–1066. doi: 10.1038/npp.2011.298.

Gastambide, F. *et al.* (2015a) ‘Alterations in spatial memory and anxiety in the MAM E17 rat model of hippocampal pathology in schizophrenia’, *Psychopharmacology*, 232(21–22), pp. 4099–4112. doi: 10.1007/s00213-014-3862-1.

Gastambide, F. *et al.* (2015b) ‘Alterations in spatial memory and anxiety in the MAM E17 rat model of hippocampal pathology in schizophrenia’, *Psychopharmacology*, 232(21–22), pp. 4099–4112. doi: 10.1007/s00213-014-3862-1.

Gejman, P. V., Sanders, A. R. and Duan, J. (2010) ‘The role of genetics in the etiology of schizophrenia’, *Psychiatric Clinics of North America*, 33(1), pp. 35–66. doi: 10.1016/j.psc.2009.12.003.

Giedd, J. N. *et al.* (1999) ‘Brain development during childhood and adolescence: a longitudinal MRI study’, *Nature Neuroscience*. Nature America Inc., 2, p. 861. Available at: http://dx.doi.org/10.1038/13158.

Gilbertson, M. W. *et al.* (2002) ‘Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma.’, *Nature neuroscience*. United States, 5(11), pp. 1242–1247. doi: 10.1038/nn958.

Gill, K. M. and Grace, A. A. (2014) ‘Corresponding decrease in neuronal markers signals progressive parvalbumin neuron loss in MAM schizophrenia model’, *International Journal of Neuropsychopharmacology*, 29(3), pp. 1–11. doi: 10.1017/S146114571400056X.

Gill, K. M. and Grace, A. a (2014) ‘Corresponding decrease in neuronal markers signals progressive parvalbumin neuron loss in MAM schizophrenia model.’, *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, pp. 1–11. doi: 10.1017/S146114571400056X.

Gill, K. M., Miller, S. A. and Grace, A. A. (2017) ‘Impaired contextual fear-conditioning in MAM rodent model of schizophrenia’, *Schizophrenia Research*. doi: 10.1016/j.schres.2017.08.064.

Giustino, T. F. and Maren, S. (2015) ‘The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear’, *Frontiers in Behavioral Neuroscience*, 9(November), pp. 1–20. doi: 10.3389/fnbeh.2015.00298.

Goldman-Rakic, P. S. (1996) ‘The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive.’, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. England, 351(1346), pp. 1445–1453. doi: 10.1098/rstb.1996.0129.

Goldman-Rakic, P. S. (1999) ‘The “psychic” neuron of the cerebral cortex.’, *Annals of the New York Academy of Sciences*, 868, pp. 13–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10414278.

Goldstein, J. M. *et al.* (1994) ‘Are schizophrenic men at higher risk for developmental deficits than schizophrenic women? Implications for adult neuropsychological functions’, *Journal of Psychiatric Research*, 28(6), pp. 483–498. doi: https://doi.org/10.1016/0022-3956(94)90039-6.

Goldstein, J. M. *et al.* (1998) ‘Are there sex differences in neuropsychological functions among patients with schizophrenia?’, *Am J Psychiatry*, 155(10), pp. 1358–1364. doi: 10.1176/ajp.155.10.1358.

Goldstein, J. M. *et al.* (2010) ‘Sex Differences in Stress Response Circuitry Activation Dependent on Female Hormonal Cycle’, *The Journal of Neuroscience*, 30(2), p. 431 LP-438. Available at: http://www.jneurosci.org/content/30/2/431.abstract.

Goto, Y. and Grace, A. A. (2006) ‘Alterations in Medial Prefrontal Cortical Activity and Plasticity in Rats with Disruption of Cortical Development’, *Biological Psychiatry*, 60(11), pp. 1259–1267. doi: 10.1016/j.biopsych.2006.05.046.

Gourevitch, R. *et al.* (2004) ‘Working memory deficits in adult rats after prenatal disruption of neurogenesis’, *Behavioural Pharmacology*, 15(4), pp. 287–292. doi: 10.1097/01.fbp.0000135703.48799.71.

Grace, A. A. (2017) ‘Dopamine System Dysregulation and the Pathophysiology of Schizophrenia: Insights From the Methylazoxymethanol Acetate Model’, *Biological Psychiatry*, 81(1), pp. 5–8. doi: 10.1016/j.biopsych.2015.11.007.

Grammatopoulos, D. K. and Chrousos, G. P. (2018) ‘Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists’, *Trends in Endocrinology & Metabolism*. Elsevier, 13(10), pp. 436–444. doi: 10.1016/S1043-2760(02)00670-7.

Groenink, L. *et al.* (2002) ‘HPA axis dysregulation in mice overexpressing corticotropin releasing hormone.’, *Biological psychiatry*. United States, 51(11), pp. 875–881.

Groenink, L. *et al.* (2008) ‘CRF_1 Not Glucocorticoid Receptors Mediate Prepulse Inhibition Deficits in Mice Overexpressing CRF’, *Biological Psychiatry*. Elsevier, 63(4), pp. 360–368. doi: 10.1016/j.biopsych.2007.06.002.

Guest, P. C. *et al.* (2011) ‘Altered levels of circulating insulin and other neuroendocrine hormones associated with the onset of schizophrenia.’, *Psychoneuroendocrinology*. England, 36(7), pp. 1092–1096. doi: 10.1016/j.psyneuen.2010.12.018.

Gunnar, M. R. *et al.* (2009) ‘Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty.’, *Development and psychopathology*. United States, 21(1), pp. 69–85. doi: 10.1017/S0954579409000054.

Guo, M. *et al.* (2004) ‘Sex difference in psychological behavior changes

induced by long-term social isolation in mice', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 28(1), pp. 115–121. doi: https://doi.org/10.1016/j.pnpbp.2003.09.027.

Haber, S. N. (2016) 'Corticostriatal circuitry', *Dialogues in Clinical Neuroscience*. France: Les Laboratoires Servier, 18(1), pp. 7–21. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4826773/.

Hains, A. B. *et al.* (2009) 'Inhibition of protein kinase C signaling protects prefrontal cortex dendritic spines and cognition from the effects of chronic stress.', *Proceedings of the National Academy of Sciences of the United States of America*. United States, 106(42), pp. 17957–17962. doi: 10.1073/pnas.0908563106.

Halligan, S. L. *et al.* (2007) 'Disturbances in morning cortisol secretion in association with maternal postnatal depression predict subsequent depressive symptomatology in adolescents.', *Biological psychiatry*. United States, 62(1), pp. 40–46. doi: 10.1016/j.biopsych.2006.09.011.

Handa, R. J. *et al.* (1994) 'Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors', *Physiology & Behavior*, 55(1), pp. 117–124. doi: https://doi.org/10.1016/0031-9384(94)90018-3.

Harvey, C. D. and Svoboda, K. (2007) 'Locally dynamic synaptic learning rules in pyramidal neuron dendrites', *Nature*, 450(7173), pp. 1195–1200. doi: 10.1038/nature06416.

Harvey, P. D. *et al.* (2012) 'Functional impairment in people with schizophrenia: Focus on employability and eligibility for disability compensation', *Schizophrenia Research*. Elsevier B.V., 140(1–3), pp. 1–8. doi: 10.1016/j.schres.2012.03.025.

Hauger, R. L. *et al.* (2006) 'Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets.', *CNS & neurological disorders drug targets*, 5(4), pp. 453–79. doi: 10.2174/187152706777950684.

Hauger, R. L. *et al.* (2009) 'Role of CRF receptor signaling in stress vulnerability, anxiety, and depression', *Annals of the New York Academy of Sciences*, 1179, pp. 120–143. doi: 10.1111/j.1749-6632.2009.05011.x.

Hazane, F. *et al.* (2009) 'Behavioral Perturbations after Prenatal Neurogenesis Disturbance in Female Rat', *Neurotoxicity Research*, 15(4), pp. 311–320. doi: 10.1007/s12640-009-9035-z.

Heidbreder, C. A. and Groenewegen, H. J. (2003) 'The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics', *Neuroscience and Biobehavioral Reviews*, 27(6), pp. 555–579. doi: 10.1016/j.neubiorev.2003.09.003.

Heinrichs, S. C. *et al.* (1994) 'Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. England, 11(3), pp. 179–186. doi: 10.1038/sj.npp.1380104.

Hensch, T. K. (2004) ‘Critical period regulation.’, *Annual review of neuroscience*. United States, 27, pp. 549–579. doi: 10.1146/annurev.neuro.27.070203.144327.

Hensch, T. K. (2005) ‘Critical period plasticity in local cortical circuits.’, *Nature reviews. Neuroscience*. England, 6(11), pp. 877–888. doi: 10.1038/nrn1787.

Hensch, T. K. and Bilimoria, P. M. (2012) ‘Re-opening Windows: Manipulating Critical Periods for Brain Development.’, *Cerebrum : the Dana forum on brain science*, 2012(August), p. 11.

Herringa, R. J., Roseboom, P. H. and Kalin, N. H. (2006) ‘Decreased amygdala CRF-binding protein mRNA in post-mortem tissue from male but not female bipolar and schizophrenic subjects.’, *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 31(8), pp. 1822–31. doi: 10.1038/sj.npp.1301038.

Hilker, R. *et al.* (2017) ‘Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register’, *Biological Psychiatry*. Society of Biological Psychiatry. doi: 10.1016/j.biopsych.2017.08.017.

Hill, R. A. (2016) ‘Neuroscience and Biobehavioral Reviews Sex differences in animal models of schizophrenia shed light on the underlying pathophysiology’, *Neuroscience and Biobehavioral Reviews*, 67, pp. 41–56. doi: 10.1016/j.neubiorev.2015.10.014.

Hoff, A. L. *et al.* (1998) ‘Sex differences in neuropsychological functioning of first-episode and chronically ill schizophrenic patients.’, *The American journal of psychiatry*. United States, 155(10), pp. 1437–1439. doi: 10.1176/ajp.155.10.1437.

Holley, S. M. *et al.* (2013) ‘Frontal cortical synaptic communication is abnormal in Disc1 genetic mouse models of schizophrenia.’, *Schizophrenia research*. Netherlands, 146(1–3), pp. 264–272. doi: 10.1016/j.schres.2013.02.007.

Hölscher, C. (2003) ‘Time, space and hippocampal functions.’, *Reviews in the neurosciences*, 14(3), pp. 253–84. doi: 10.1017/CBO9781107415324.004.

Ter Horst, J. P. *et al.* (2012) ‘Relevance of stress and female sex hormones for emotion and cognition’, *Cellular and Molecular Neurobiology*, 32(5), pp. 725–735. doi: 10.1007/s10571-011-9774-2.

Howe, W. M. *et al.* (2015) ‘MAM (E17) rodent developmental model of neuropsychiatric disease: Disruptions in learning and dysregulation of nucleus accumbens dopamine release, but spared executive function’, *Psychopharmacology*, 232(21–22), pp. 4113–4127. doi: 10.1007/s00213-015-3955-5.

Howes, O. D. *et al.* (2017) ‘The Role of Genes, Stress, and Dopamine in the Development of Schizophrenia.’, *Biological psychiatry*. United States, 81(1), pp. 9–20. doi: 10.1016/j.biopsych.2016.07.014.

Howes, O. D. and Kapur, S. (2009) ‘The dopamine hypothesis of schizophrenia: Version III - The final common pathway’, *Schizophrenia Bulletin*, 35(3), pp. 549–562. doi: 10.1093/schbul/sbp006.

Hradetzky, E. *et al.* (2012) 'The methylazoxymethanol acetate (MAM-E17) rat model: Molecular and functional effects in the hippocampus', *Neuropsychopharmacology*, 37(2), pp. 364–377. doi: 10.1038/npp.2011.219.

Hu, H. *et al.* (2008) 'Ras signaling mechanisms underlying impaired GluR1-dependent plasticity associated with fragile X syndrome.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. United States, 28(31), pp. 7847–7862. doi: 10.1523/JNEUROSCI.1496-08.2008.

Hu, K. *et al.* (2012) 'Threat of bodily harm has opposing effects on cognition.', *Emotion (Washington, D.C.)*. United States, 12(1), pp. 28–32. doi: 10.1037/a0024345.

Hulshof, H. J. *et al.* (2011) 'Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats', *Behavioural Brain Research*, 216(2), pp. 552–560. doi: https://doi.org/10.1016/j.bbr.2010.08.038.

I., M. *et al.* (2005) 'Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain', *American Journal of Anatomy*. Wiley-Blackwell, 165(4), pp. 385–396. doi: 10.1002/aja.1001650404.

Innocenti, G. M. and Price, D. J. (2005) 'Exuberance in the development of cortical networks', *Nat Rev Neurosci*, 6(12), pp. 955–965. Available at: http://dx.doi.org/10.1038/nrn1790.

Ivy, A. S. *et al.* (2010) 'Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. United States, 30(39), pp. 13005–13015. doi: 10.1523/JNEUROSCI.1784-10.2010.

J., R. J. *et al.* (2007) 'Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex', *Journal of Comparative Neurology*, 507(1), pp. 1141–1150. doi: doi:10.1002/cne.21588.

Jacobson, L. (2014) 'Hypothalamic-pituitary-adrenocortical axis: Neuropsychiatric aspects', *Comprehensive Physiology*, 4(2), pp. 715–738. doi: 10.1002/cphy.c130036.

Jianli, Y. *et al.* (2006) 'Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring', *Hippocampus*. Wiley-Blackwell, 16(5), pp. 431–436. doi: 10.1002/hipo.20181.

Jin, S. and Feig, L. A. (2010) 'Long-Term Potentiation in the CA1 Hippocampus Induced by NR2A Subunit-Containing NMDA Glutamate Receptors Is Mediated by Ras-GRF2/Erk Map Kinase Signaling', *PLOS ONE*. Public Library of Science, 5(7), p. e11732. Available at: https://doi.org/10.1371/journal.pone.0011732.

Jones, C. a, Watson, D. J. G. and Fone, K. C. F. (2011) 'Animal models of schizophrenia.', *British journal of pharmacology*, 164(4), pp. 1162–94. doi: 10.1111/j.1476-5381.2011.01386.x.

Jones, C., Watson, D. and Fone, K. (2011) 'Animal models of schizophrenia', *British Journal of Pharmacology*, 164(4), pp. 1162–1194. doi: 10.1111/j.1476-5381.2011.01386.x.

K., B. C. *et al.* (2010) ‘Chronic restraint stress in adolescence differentially influences hypothalamic-pituitary-adrenal axis function and adult hippocampal neurogenesis in male and female rats’, *Hippocampus*. Wiley-Blackwell, 21(11), pp. 1216–1227. doi: 10.1002/hipo.20829.

Keedy, S. K. *et al.* (2006) ‘Functional magnetic resonance imaging studies of eye movements in first episode schizophrenia: smooth pursuit, visually guided saccades and the oculomotor delayed response task.’, *Psychiatry research*. Ireland, 146(3), pp. 199–211. doi: 10.1016/j.pscychresns.2006.01.003.

Kegeles, L. S. *et al.* (2010) ‘Increased synaptic dopamine function in associative regions of the striatum in schizophrenia.’, *Archives of general psychiatry*. United States, 67(3), pp. 231–239. doi: 10.1001/archgenpsychiatry.2010.10.

Kemp, C. F., Woods, R. J. and Lowry, P. J. (1998) ‘The corticotrophin-releasing factor-binding protein: An act of several parts’, *Peptides*, 19(6), pp. 1119–1128. doi: 10.1016/S0196-9781(98)00057-6.

Kielland, A. *et al.* (2009) ‘Activity patterns govern synapse-specific AMPA receptor trafficking between deliverable and synaptic pools.’, *Neuron*. United States, 62(1), pp. 84–101. doi: 10.1016/j.neuron.2009.03.001.

Kim, M. J., Gee, D. G., *et al.* (2011) ‘Anxiety Dissociates Dorsal and Ventral Medial Prefrontal Cortex Functional Connectivity with the Amygdala at Rest’, *Cerebral Cortex*, 21(7), pp. 1667–1673. Available at: http://dx.doi.org/10.1093/cercor/bhq237.

Kim, M. J., Loucks, R. A., *et al.* (2011) ‘The structural and functional connectivity of the amygdala : From normal emotion to pathological anxiety’, *Behavioural Brain Research*. Elsevier B.V., 223(2), pp. 403–410. doi: 10.1016/j.bbr.2011.04.025.

Kirschbaum, C. *et al.* (1999) ‘Impact of Gender, Menstrual Cycle Phase, and Oral Contraceptives on the Activity of the Hypothalamus-Pituitary-Adrenal Axis’, *Psychosomatic Medicine*, 61(2). Available at: https://journals.lww.com/psychosomaticmedicine/Fulltext/1999/03000/Impact_of_Gender,_Menstrual_Cycle_Phase,_and_Oral.6.aspx.

Kitraki, E. *et al.* (2004) ‘Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress’, *Neuroscience*, 125(1), pp. 47–55. doi: https://doi.org/10.1016/j.neuroscience.2003.12.024.

Klein, Z. A. and Romeo, R. D. (2013) ‘Changes in hypothalamic-pituitary-adrenal stress responsiveness before and after puberty in rats.’, *Hormones and behavior*. United States, 64(2), pp. 357–363. doi: 10.1016/j.yhbeh.2013.01.012.

Kono, J. *et al.* (2017) ‘Distribution of corticotropin-releasing factor neurons in the mouse brain: a study using corticotropin-releasing factor-modified yellow fluorescent protein knock-in mouse’, *Brain Structure and Function*. Springer Berlin Heidelberg, 222(4), pp. 1705–1732. doi: 10.1007/s00429-016-1303-0.

Konradi, C. *et al.* (2011) ‘Hippocampal interneurons are abnormal in schizophrenia’, *Schizophrenia Research*. Elsevier B.V., 131(1–3), pp. 165–

173. doi: 10.1016/j.schres.2011.06.007.

Konstantoudaki, X. *et al.* (2016) 'Impaired synaptic plasticity in the prefrontal cortex of mice with developmentally decreased number of interneurons', *Neuroscience*. IBRO, 322, pp. 333–345. doi: 10.1016/j.neuroscience.2016.02.048.

Konstantoudaki, X. *et al.* (2018) 'Prefrontal cortical-specific differences in behavior and synaptic plasticity between adolescent and adult mice.', *Journal of neurophysiology*. United States, 119(3), pp. 822–833. doi: 10.1152/jn.00189.2017.

Koss, W. a *et al.* (2014) 'Dendritic remodeling in the adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats.', *Synapse (New York, N.Y.)*, 68(2), pp. 61–72. doi: 10.1002/syn.21716.

Koster, A. *et al.* (2008) 'Gender differences in first episode psychosis.', *Social psychiatry and psychiatric epidemiology*. Germany, 43(12), pp. 940–946. doi: 10.1007/s00127-008-0384-3.

Krahn, D. D. *et al.* (1986) 'CRF antagonist partially reverses CRF- and stress-induced effects on feeding', *Brain Research Bulletin*, 17(3), pp. 285–289. doi: https://doi.org/10.1016/0361-9230(86)90233-9.

Kratzer, S. *et al.* (2013) 'Activation of CRH receptor type 1 expressed on glutamatergic neurons increases excitability of CA1 pyramidal neurons by the modulation of voltage-gated ion channels.', *Frontiers in cellular neuroscience*, 7(July), p. 91. doi: 10.3389/fncel.2013.00091.

Kühne, C. *et al.* (2012) 'Visualizing corticotropin-releasing hormone receptor type 1 expression and neuronal connectivities in the mouse using a novel multifunctional allele', *Journal of Comparative Neurology*, 520(14), pp. 3150–3180. doi: 10.1002/cne.23082.

Kumari, V., Aasen, I. and Sharma, T. (2004) 'Sex differences in prepulse inhibition deficits in chronic schizophrenia', *Schizophrenia Research*, 69(2–3), pp. 219–235. doi: 10.1016/j.schres.2003.09.010.

Kuroda, K. *et al.* (2011) 'Behavioral alterations associated with targeted disruption of exons 2 and 3 of the Disc1 gene in the mouse', *Human Molecular Genetics*, 20(23), pp. 4666–4683. Available at: http://dx.doi.org/10.1093/hmg/ddr400.

Laplante, D. P. *et al.* (2004) 'Stress During Pregnancy Affects General Intellectual and Language Functioning in Human Toddlers', *Pediatric Research*. International Pediatrics Research Foundation, Inc., 56, p. 400. Available at: http://dx.doi.org/10.1203/01.PDR.0000136281.34035.44.

Lederis, K. P. *et al.* (1990) 'Evolutionary aspects of corticotropin releasing hormones.', *Progress in clinical and biological research*. United States, 342, pp. 467–472.

Lee, L.-T. *et al.* (2015) 'Lower availability of striatal dopamine transporter in generalized anxiety disorder: a preliminary two-ligand SPECT study.', *International clinical psychopharmacology*. England, 30(3), pp. 175–178. doi: 10.1097/YIC.0000000000000067.

Leung, A. and Chue, P. (2000) 'Sex differences in schizophrenia, a review of the literature.', *Acta psychiatrica Scandinavica. Supplementum*. Denmark, 401, pp. 3–38.

Leung, A. and Chue, P. (2000) 'Sex differences in schizophrenia, a review of the literature.', *Acta Psychiatrica Scandinavica*, 101, pp. 3–38. doi: 10.1111/j.0065-1591.2000.0ap25.x.

Levine, S. (1994) 'The ontogeny of the hypothalamic-pituitary-adrenal axis. The influence of maternal factors.', *Annals of the New York Academy of Sciences*. United States, 746, pp. 275–293.

Lewis, D. A. *et al.* (2001) 'Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: Evidence for fewer projections from the thalamus', *American Journal of Psychiatry*, 158(9), pp. 1411–1422. doi: 10.1176/appi.ajp.158.9.1411.

Li, K. *et al.* (2016) 'Article A Cortical Circuit for Sexually Dimorphic Oxytocin-Dependent Anxiety Behaviors Article A Cortical Circuit for Sexually Dimorphic Oxytocin-Dependent Anxiety Behaviors', *Cell*, (1), pp. 60–72. doi: 10.1016/j.cell.2016.08.067.

Linton, E. A. *et al.* (1988) 'A specific carrier substance for human corticotrophin releasing factor in late gestational maternal plasma which could mask the ACTH-releasing activity.', *Clinical endocrinology*. England, 28(3), pp. 315–324.

Lipska, B. K., Jaskiw, G. E. and Weinberger, D. R. (1993) 'Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: A potential animal model of schizophrenia', *Neuropsychopharmacology*, 9(1), pp. 67–75. doi: 10.1038/npp.1993.44.

Liu, M.-G. *et al.* (2011) 'Differential roles of ERK, JNK and p38 MAPK in pain-related spatial and temporal enhancement of synaptic responses in the hippocampal formation of rats: Multi-electrode array recordings', *Brain Research*, 1382, pp. 57–69. doi: https://doi.org/10.1016/j.brainres.2011.01.076.

Lodge, D. J., Behrens, M. M. and Grace, A. A. (2009) 'A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(8), pp. 2344–54. doi: 10.1523/JNEUROSCI.5419-08.2009.

Lodge, D. J., Behrens, M. M. and Grace, A. a (2009) 'A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(8), pp. 2344–54. doi: 10.1523/JNEUROSCI.5419-08.2009.

Lodge, D. J. and Grace, A. A. (2007) 'Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. United States, 27(42), pp. 11424–11430. doi: 10.1523/JNEUROSCI.2847-

07.2007.

Lovenberg, T. W. *et al.* (1995) ‘CRF2 alpha and CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues’, *Endocrinology*, 136(9), pp. 4139–4142. Available at: http://dx.doi.org/10.1210/endo.136.9.7544278.

De Luca, V. *et al.* (2010) ‘Association of HPA axis genes with suicidal behaviour in schizophrenia.’, *Journal of psychopharmacology (Oxford, England)*. United States, 24(5), pp. 677–682. doi: 10.1177/0269881108097817.

Luine, V. (2002) ‘Sex Differences in Chronic Stress Effects on Memory in Rats’, *Stress*. Taylor & Francis, 5(3), pp. 205–216. doi: 10.1080/1025389021000010549.

Luine, V. *et al.* (2017) ‘Sex differences in chronic stress effects on cognition in rodents’, *Pharmacology Biochemistry and Behavior*, 152, pp. 13–19. doi: https://doi.org/10.1016/j.pbb.2016.08.005.

Lukkes, J. L. *et al.* (2011) ‘Topographical distribution of corticotropin-releasing factor type 2 receptor-like immunoreactivity in the rat dorsal raphe nucleus: co-localization with tryptophan hydroxylase.’, *Neuroscience*. United States, 183, pp. 47–63. doi: 10.1016/j.neuroscience.2011.03.047.

Lupien, S. J. *et al.* (2009) ‘Effects of stress throughout the lifespan on the brain, behaviour and cognition’, *Nature Reviews Neuroscience*, 10(6), pp. 434–445. doi: 10.1038/nrn2639.

Luttrell, L. M. and Lefkowitz, R. J. (2002) ‘The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals.’, *Journal of cell science*, 115(Pt 3), pp. 455–65. doi: 10.1074/jbc.274.3.1185.

Lysaker, P. H. and Salyers, M. P. (2007) ‘Anxiety symptoms in schizophrenia spectrum disorders: associations with social function, positive and negative symptoms, hope and trauma history.’, *Acta psychiatrica Scandinavica*. United States, 116(4), pp. 290–298. doi: 10.1111/j.1600-0447.2007.01067.x.

M., Y. R. and D., D. J. (2004) ‘The relation of strength of stimulus to rapidity of habit-formation’, *Journal of Comparative Neurology and Psychology*. Wiley-Blackwell, 18(5), pp. 459–482. doi: 10.1002/cne.920180503.

Makino, S. *et al.* (2005) ‘Expression of type 1 corticotropin-releasing hormone (CRH) receptor mRNA in the hypothalamic paraventricular nucleus following restraint stress in CRH-deficient mice.’, *Brain research*. Netherlands, 1048(1–2), pp. 131–137. doi: 10.1016/j.brainres.2005.04.065.

Margulies, D. S. *et al.* (2007) ‘Mapping the functional connectivity of anterior cingulate cortex.’, *NeuroImage*. United States, 37(2), pp. 579–588. doi: 10.1016/j.neuroimage.2007.05.019.

Markham, J. A., Morris, J. R. and Juraska, J. M. (2007) ‘Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood.’, *Neuroscience*, 144(3), pp. 961–8. doi: 10.1016/j.neuroscience.2006.10.015.

Martin, E. I. and Ressler, K. (2009) ‘The Neurobiology of Anxiet y Disorders :

Brain I maging , Genetics , a nd Psychoneuro endo crinology', 32, pp. 549–575. doi: 10.1016/j.psc.2009.05.004.

Martins-de-Souza, D. *et al.* (2010) 'The Role of Energy Metabolism Dysfunction and Oxidative Stress in Schizophrenia Revealed by Proteomics', *Antioxidants & Redox Signaling*. Mary Ann Liebert, Inc., publishers, 15(7), pp. 2067–2079. doi: 10.1089/ars.2010.3459.

Matricon, J., Bellon, A., Frieling, H., Kebir, O., Le Pen, G., Beuvon, F., Daumas-Duport, C., Jay, T. M., *et al.* (2010) 'Neuropathological and Reelin deficiencies in the hippocampal formation of rats exposed to MAM; differences and similarities with schizophrenia.', *PloS one*, 5(4), p. e10291. doi: 10.1371/journal.pone.0010291.

Matricon, J., Bellon, A., Frieling, H., Kebir, O., Le Pen, G., Beuvon, F., Daumas-Duport, C., Jay, T. M., *et al.* (2010) 'Neuropathological and Reelin Deficiencies in the Hippocampal Formation of Rats Exposed to MAM; Differences and Similarities with Schizophrenia', *PLoS ONE*, 5(4), p. e10291. doi: 10.1371/journal.pone.0010291.

Matsuo, J. *et al.* (2016) 'A large single ethnicity study of prepulse inhibition in schizophrenia: Separate analysis by sex focusing on effect of symptoms', *Journal of Psychiatric Research*. Elsevier Ltd, 82, pp. 155–162. doi: 10.1016/j.jpsychires.2016.07.026.

Maynard, T. M. *et al.* (2001) 'Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia.', *Schizophrenia bulletin*, 27(3), pp. 457–76. doi: 10.1093/oxfordjournals.schbul.a006887.

McCarthy, M. M. *et al.* (2012) 'Sex differences in the brain: the not so inconvenient truth.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. United States, 32(7), pp. 2241–2247. doi: 10.1523/JNEUROSCI.5372-11.2012.

McCARTHY, M. M. (2008) 'Estradiol and the Developing Brain', *Physiological Reviews*, 88(1), pp. 91–134. doi: 10.1152/physrev.00010.2007.

McCormack, S. G., Stornetta, R. L. and Zhu, J. J. (2006) 'Synaptic AMPA receptor exchange maintains bidirectional plasticity.', *Neuron*. United States, 50(1), pp. 75–88. doi: 10.1016/j.neuron.2006.02.027.

McEwen, B. S., Nasca, C. and Gray, J. D. (2015) 'Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex', *Neuropsychopharmacology*. American College of Neuropsychopharmacology, 41, p. 3. Available at: http://dx.doi.org/10.1038/npp.2015.171.

McOmish, C. E., Burrows, E. L. and Hannan, A. J. (2014) 'Identifying novel interventional strategies for psychiatric disorders: integrating genomics, "enviromics" and gene-environment interactions in valid preclinical models', *British journal of pharmacology*, 171(20), pp. 4719–4728. doi: 10.1111/bph.12783.

Miller, E. K. and Cohen, J. D. (2001) 'An integrative theory of prefrontal cortex function.', *Annual review of neuroscience*, 24, pp. 167–202. doi: 10.1146/annurev.neuro.24.1.167.

Mitchell, A. C. *et al.* (2015) 'Transcriptional regulation of GAD1 GABA

synthesis gene in the prefrontal cortex of subjects with schizophrenia', *Schizophrenia Research*. Elsevier B.V., 167(1–3), pp. 28–34. doi: 10.1016/j.schres.2014.10.020.

Mitchell, J. B. and Laiacona, J. (1998) 'The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat.', *Behavioural brain research*. Netherlands, 97(1–2), pp. 107–113.

Miyoshi, K. and Morimura, Y. (2010) 'Neuropsychiatric Disorders'. doi: 10.1007/978-4-431-53871-4.

Mizoguchi, K. *et al.* (2003) 'Chronic stress attenuates glucocorticoid negative feedback: Involvement of the prefrontal cortex and hippocampus', *Neuroscience*, 119(3), pp. 887–897. doi: 10.1016/S0306-4522(03)00105-2.

Modinos, G. *et al.* (2015) 'Translating the MAM model of psychosis to humans', *Trends in Neurosciences*. Elsevier Ltd, 38(3), pp. 129–138. doi: 10.1016/j.tins.2014.12.005.

Monte, A. S. *et al.* (2017) 'Two-hit model of schizophrenia induced by neonatal immune activation and peripubertal stress in rats: Study of sex differences and brain oxidative alterations.', *Behavioural brain research*. Netherlands, 331, pp. 30–37. doi: 10.1016/j.bbr.2017.04.057.

Moore, H. *et al.* (2006) 'A Neurobehavioral Systems Analysis of Adult Rats Exposed to Methylazoxymethanol Acetate on E17: Implications for the Neuropathology of Schizophrenia', *Biological Psychiatry*, 60(3), pp. 253–264. doi: 10.1016/j.biopsych.2006.01.003.

Morgan, V. A., Castle, D. J. and Jablensky, A. V (2008) 'Do women express and experience psychosis differently from men? Epidemiological evidence from the Australian National Study of Low Prevalence (Psychotic) Disorders.', *The Australian and New Zealand journal of psychiatry*. England, 42(1), pp. 74–82. doi: 10.1080/00048670701732699.

Morin, S. M. *et al.* (1999) 'Differential distribution of urocortin- and corticotropin-releasing factor-like immunoreactivities in the rat brain', *Neuroscience*, 92(1), pp. 281–291. doi: https://doi.org/10.1016/S0306-4522(98)00732-5.

Muller, M. B. *et al.* (2003) 'Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress.', *Nature neuroscience*. United States, 6(10), pp. 1100–1107. doi: 10.1038/nn1123.

Murmu, M. S. *et al.* (2006) 'Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy.', *The European journal of neuroscience*. France, 24(5), pp. 1477–1487. doi: 10.1111/j.1460-9568.2006.05024.x.

Nakazawa, K. *et al.* (2012) 'GABAergic interneuron origin of schizophrenia pathophysiology', *Neuropharmacology*, 62(3), pp. 1574–1583. doi: 10.1016/j.neuropharm.2011.01.022.

Nascimento, J. M. and Martins-de-Souza, D. (2015) 'The proteome of schizophrenia', *npj Schizophrenia*, 1(1), p. 14003. doi: 10.1038/npjschz.2014.3.

Nikoletopoulou, V. *et al.* (2017) 'Modulation of Autophagy by BDNF Underlies Synaptic Plasticity', *Cell Metabolism*. Elsevier Inc., 26(1), p. 230–242.e5. doi: 10.1016/j.cmet.2017.06.005.

Novais, A. *et al.* (2017) 'How age, sex and genotype shape the stress response', *Neurobiology of Stress*. Elsevier Inc, 6, pp. 44–56. doi: 10.1016/j.ynstr.2016.11.004.

O'Keefe, J. (1976) 'Place units in the hippocampus of the freely moving rat', *Experimental Neurology*, 51(1), pp. 78–109. doi: https://doi.org/10.1016/0014-4886(76)90055-8.

O'Keefe, J. and Dostrovsky, J. (1971) 'The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat', *Brain Research*, 34(1), pp. 171–175. doi: https://doi.org/10.1016/0006-8993(71)90358-1.

O'Tuathaigh, C. M. P. *et al.* (2008) 'Disruption to social dyadic interactions but not emotional/anxiety-related behaviour in mice with heterozygous "knockout" of the schizophrenia risk gene neuregulin-1', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(2), pp. 462–466. doi: https://doi.org/10.1016/j.pnpbp.2007.09.018.

Ochoa, S. *et al.* (2012) 'Gender differences in schizophrenia and first-episode psychosis: a comprehensive literature review', *Schizophr Res Treatment*, 2012, p. 916198. doi: 10.1155/2012/916198.

Olney, J. W., Newcomer, J. W. and Farber, N. B. (1999) 'NMDA receptor hypofunction model of schizophrenia', *Journal of Psychiatric Research*, 33(6), pp. 523–533. doi: 10.1016/S0022-3956(99)00029-1.

Olschowka, J. A. *et al.* (1982) 'The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain', *Peptides*, 3(6), pp. 995–1015. doi: https://doi.org/10.1016/0196-9781(82)90071-7.

Orozco-Cabal, L. *et al.* (2006) 'Regulation of synaptic transmission by CRF receptors.', *Reviews in the neurosciences*, 17(3), pp. 279–307. doi: 10.1515/REVNEURO.2006.17.3.279.

P., D. E. and A., S. C. (2010) 'The Timing of Prenatal Exposure to Maternal Cortisol and Psychosocial Stress Is Associated With Human Infant Cognitive Development', *Child Development*. Wiley/Blackwell (10.1111), 81(1), pp. 131–148. doi: 10.1111/j.1467-8624.2009.01385.x.

Palacios-García, I. *et al.* (2015) 'Prenatal Stress Down-Regulates Reelin Expression by Methylation of Its Promoter and Induces Adult Behavioral Impairments in Rats', *PLOS ONE*. Public Library of Science, 10(2), p. e0117680. Available at: https://doi.org/10.1371/journal.pone.0117680.

Palkovits, M., Brownstein, M. J. and Vale, W. (1985) 'Distribution of corticotropin-releasing factor in rat brain.', *Federation proceedings*. United States, 44(1 Pt 2), pp. 215–219.

Papaleo, F., Lipska, B. K. and Weinberger, D. R. (2012) 'Mouse models of genetic effects on cognition: Relevance to schizophrenia', *Neuropharmacology*, 62(3), pp. 1204–1220. doi: 10.1016/j.neuropharm.2011.04.025.

Paré, W. P. *et al.* (1999) ‘Gender Differences in Acute and Chronic Stress in Wistar Kyoto (WKY) Rats’, *Integrative Physiological and Behavioral Science*, 34(4), pp. 227–241. doi: 10.1007/BF02688691.

Paris, J. J. *et al.* (2011) ‘Immune stress in late pregnant rats decreases length of gestation and fecundity, and alters later cognitive and affective behaviour of surviving pre-adolescent offspring’, *Stress*. Taylor & Francis, 14(6), pp. 652–664. doi: 10.3109/10253890.2011.628719.

Le Pen, G. *et al.* (2006) ‘Peri-pubertal maturation after developmental disturbance: a model for psychosis onset in the rat.’, *Neuroscience*, 143(2), pp. 395–405. doi: 10.1016/j.neuroscience.2006.08.004.

Pen, G. Le, Jay, T. M. and Krebs, M. O. (2011) ‘Effect of antipsychotics on spontaneous hyperactivity and hypersensitivity to MK-801-induced hyperactivity in rats prenatally exposed to methylazoxymethanol’, *Journal of Psychopharmacology*, 25(6), pp. 822–835. doi: 10.1177/0269881110387839.

Peng, J. *et al.* (2017) ‘A Quantitative Analysis of the Distribution of CRH Neurons in Whole Mouse Brain’, *Frontiers in Neuroanatomy*, 11(July), pp. 1–12. doi: 10.3389/fnana.2017.00063.

Penschuck, S. *et al.* (2006) ‘Decrease in parvalbumin-expressing neurons in the hippocampus and increased phencyclidine-induced locomotor activity in the rat methylazoxymethanol (MAM) model of schizophrenia’, *European Journal of Neuroscience*, 23(1), pp. 279–284. doi: 10.1111/j.1460-9568.2005.04536.x.

Perkins, A. M. and Corr, P. J. (2014) ‘Anxiety as an adaptive emotion’, *The Positive Side of Negative Emotions*, pp. 37–54. doi: 10.5772/53223.

Perrin, M. *et al.* (1995) ‘Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart.’, *Proceedings of the National Academy of Sciences*, 92(7), p. 2969 LP-2973. Available at: http://www.pnas.org/content/92/7/2969.abstract.

Phillips, L. J. *et al.* (2006) ‘Stress, the hippocampus and the hypothalamic pituitary adrenal axis: implications for the development of psychotic disorders’, *Australian and New Zealand Journal of Psychiatry*, 40(9), pp. 725–741.

Pinos, H. *et al.* (2001) ‘The development of sex differences in the locus coeruleus of the rat’, *Brain Research Bulletin*, 56(1), pp. 73–78. doi: https://doi.org/10.1016/S0361-9230(01)00540-8.

Pisarchik, A. and Slominski, A. (2004) ‘Molecular and functional characterization of novel CRFR1 isoforms from the skin.’, *European journal of biochemistry*. England, 271(13), pp. 2821–2830. doi: 10.1111/j.1432-1033.2004.04216.x.

Plieger, T. *et al.* (2017) ‘The impact of acute stress on cognitive functioning: a matter of cognitive demands?’, *Cognitive Neuropsychiatry*. Routledge, 22(1), pp. 69–82. doi: 10.1080/13546805.2016.1261014.

Primus, R. J. *et al.* (1997) ‘Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain.’, *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. England, 17(5), pp. 308–316. doi: 10.1016/S0893-133X(97)00071-7.

Qin, S. *et al.* (2009) 'Acute Psychological Stress Reduces Working Memory-Related Activity in the Dorsolateral Prefrontal Cortex', *Biological Psychiatry*. Society of Biological Psychiatry, 66(1), pp. 25–32. doi: 10.1016/j.biopsych.2009.03.006.

R., G. M. and L., C. C. (2003) 'Brain and behavior interface: Stress and the developing brain', *Infant Mental Health Journal*. Wiley-Blackwell, 24(3), pp. 195–211. doi: 10.1002/imhj.10052.

Raio, C. M. *et al.* (2014) 'Acute stress impairs the retrieval of extinction memory in humans', *Neurobiology of Learning and Memory*, 112, pp. 212–221. doi: https://doi.org/10.1016/j.nlm.2014.01.015.

Ratajczak, P. *et al.* (2015a) 'Biochemical and cognitive impairments observed in animal models of schizophrenia induced by prenatal stress paradigm or methylazoxymethanol acetate administration', *Acta Neurobiologiae Experimentalis*, 75(3), pp. 314–325.

Ratajczak, P. *et al.* (2015b) 'Biochemical and cognitive impairments observed in animal models of schizophrenia induced by prenatal stress paradigm or methylazoxymethanol acetate administration', *Acta Neurobiologiae Experimentalis*, 75(3), pp. 314–325.

Refojo, D. *et al.* (2011) 'Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1.', *Science (New York, N.Y.)*, 333(6051), pp. 1903–7. doi: 10.1126/science.1202107.

Reisinger, S. *et al.* (2015) 'Pharmacology & Therapeutics The Poly (I : C) -induced maternal immune activation model in preclinical neuropsychiatric drug discovery', *Pharmacology and Therapeutics*, 149, pp. 213–226. doi: 10.1016/j.pharmthera.2015.01.001.

Ribbe, K. *et al.* (2011) 'Prediction of the risk of comorbid alcoholism in schizophrenia by interaction of common genetic variants in the corticotropin-releasing factor system.', *Archives of general psychiatry*. United States, 68(12), pp. 1247–1256. doi: 10.1001/archgenpsychiatry.2011.100.

Rivier, J., Rivier, C. and Vale, W. (1984) 'Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat.', *Science (New York, N.Y.)*. United States, 224(4651), pp. 889–891.

Roelfsema, F. *et al.* (1993) 'Sex-dependent alteration in cortisol response to endogenous adrenocorticotropin.', *The Journal of clinical endocrinology and metabolism*. United States, 77(1), pp. 234–240. doi: 10.1210/jcem.77.1.8392084.

Romeo, R. D. (2017) 'The impact of stress on the structure of the adolescent brain: Implications for adolescent mental health', *Brain Research*, 1654, pp. 185–191. doi: https://doi.org/10.1016/j.brainres.2016.03.021.

Ryan, M. C. M. *et al.* (2004) 'Evidence of basal pituitary-adrenal overactivity in first episode, drug naive patients with schizophrenia.', *Psychoneuroendocrinology*. England, 29(8), pp. 1065–1070. doi: 10.1016/j.psyneuen.2003.08.011.

Salehi, B., Cordero, M. I. and Sandi, C. (2010) 'Learning under stress: The inverted-U-shape function revisited', *Learning & Memory*, 17(10), pp. 522–

530. doi: 10.1101/lm.1914110.

Sanderson, T. M. *et al.* (2012) 'Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia', *Neuropharmacology*, 62(3), pp. 1349–1358. doi: 10.1016/j.neuropharm.2011.08.005.

Sapolsky, R. M., Krey, L. C. and McEwen, B. S. (1986) 'The Neuroendocrinology of Stress and Aging: The Glucocorticoid Cascade Hypothesis*', *Endocrine Reviews*, 7(3), pp. 284–301. Available at: http://dx.doi.org/10.1210/edrv-7-3-284.

Sareen, J. *et al.* (2018) 'Striatal Function in Generalized Social Phobia: A Functional Magnetic Resonance Imaging Study', *Biological Psychiatry*. Elsevier, 61(3), pp. 396–404. doi: 10.1016/j.biopsych.2006.05.043.

Schulz, K. M. *et al.* (2014) 'Dietary choline supplementation to dams during pregnancy and lactation mitigates the effects of in utero stress exposure on adult anxiety-related behaviors.', *Behavioural brain research*. Netherlands, 268, pp. 104–110. doi: 10.1016/j.bbr.2014.03.031.

Seale, J. V *et al.* (2004) 'Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats.', *Journal of neuroendocrinology*. United States, 16(6), pp. 516–524. doi: 10.1111/j.1365-2826.2004.01195.x.

Seamans, J. K., Lapish, C. C. and Durstewitz, D. (2008) 'Comparing the prefrontal cortex of rats and primates: insights from electrophysiology.', *Neurotoxicity research*, 14(2–3), pp. 249–62. doi: 10.1007/BF03033814.

Seidman, L. J. *et al.* (1997) 'Sex differences in olfactory identification and Wisconsin Card Sorting performance in schizophrenia: relationship to attention and verbal ability.', *Biological psychiatry*. United States, 42(2), pp. 104–115. doi: 10.1016/S0006-3223(96)00300-9.

Selye, H. (1976) 'Stress without Distress BT - Psychopathology of Human Adaptation', in Serban, G. (ed.). Boston, MA: Springer US, pp. 137–146. doi: 10.1007/978-1-4684-2238-2_9.

Semple, B. D. *et al.* (2013) 'Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species', *Progress in Neurobiology*, 106–107, pp. 1–16. doi: 10.1016/j.pneurobio.2013.04.001.

Shansky, R. M. *et al.* (2006) 'The effects of sex and hormonal status on restraint-stress-induced working memory impairment', *Behavioral and Brain Functions*, 2(1), p. 8. doi: 10.1186/1744-9081-2-8.

Shapiro, R. A., Xu, C. and Dorsa, D. M. (2000) 'Differential Transcriptional Regulation of Rat Vasopressin Gene Expression by Estrogen Receptor α and β**This work was supported by Public Health Service Grant NS20311 and the Alzheimer's Disease Research Center of the University of Washington, AG-05136. ', *Endocrinology*, 141(11), pp. 4056–4064. Available at: http://dx.doi.org/10.1210/endo.141.11.7796.

Slater, P. G., Yarur, H. E. and Gysling, K. (2016) 'Corticotropin-Releasing Factor Receptors and Their Interacting Proteins: Functional Consequences',

Molecular Pharmacology, 90(5), pp. 627–632. doi: 10.1124/mol.116.104927.

Smith, S. M. and Vale, W. W. (2006) 'The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress', *Dialogues in Clinical Neuroscience*, 8(4), pp. 383–395. doi: 10.1038/nrendo.2011.222.

Snyder, M. A. and Gao, W.-J. (2013) 'NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia', *Frontiers in Cellular Neuroscience*, 7(March), pp. 1–12. doi: 10.3389/fncel.2013.00031.

Snyder, M. a, Adelman, A. E. and Gao, W.-J. (2013) 'Gestational methylazoxymethanol exposure leads to NMDAR dysfunction in hippocampus during early development and lasting deficits in learning.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. Nature Publishing Group, 38(2), pp. 328–40. doi: 10.1038/npp.2012.180.

Sousa, N. *et al.* (2000) 'Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement', *Neuroscience*, 97(2), pp. 253–266. doi: https://doi.org/10.1016/S0306-4522(00)00050-6.

Souza, E. B. De and Grigoriadis, D. E. (2002) 'Corticotropin-Releasing Factor: Physiology, Pharmacology and Role in Central Nervous System Disorders', *Psychoneuroendocrinology*, 20(8), pp. 789–819. Available at: http://www.acnp.org/asset.axd?id=f5f809f4-f81c-40e7-8a19-ccdbb4b95392.

Stahl, S. M. (2013) *Stahls essential psycopharmacology*.

Stenzel-Poore, M. P. *et al.* (1992) 'Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice.', *Endocrinology*. United States, 130(6), pp. 3378–3386. doi: 10.1210/endo.130.6.1597149.

Stephan, H. and Manolescu, J. (1980) 'Comparative investigations on hippocampus in insectivores and primates.', *Zeitschrift fur mikroskopisch-anatomische Forschung*. Germany, 94(6), pp. 1025–1050.

Stiles, J. (2008) *The Fundamentals of Brain Development: Integrating Nature and Nurture*.

Stiles, J. and Jernigan, T. L. (2010) 'The basics of brain development.', *Neuropsychology review*, 20(4), pp. 327–48. doi: 10.1007/s11065-010-9148-4.

Strange, B. A. *et al.* (2014) 'Functional organization of the hippocampal longitudinal axis', *Nature Reviews Neuroscience*. Nature Publishing Group, 15(10), pp. 655–669. doi: 10.1038/nrn3785.

Swanson, L. W. *et al.* (1983) 'Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study.', *Neuroendocrinology*. Switzerland, 36(3), pp. 165–186. doi: 10.1159/000123454.

Sweatt, J. D. (2004) 'Mitogen-activated protein kinases in synaptic plasticity and memory', *Current Opinion in Neurobiology*, 14(3), pp. 311–317. doi: https://doi.org/10.1016/j.conb.2004.04.001.

Szymanski, S. *et al.* (1995) 'Gender differences in onset of illness, treatment

response, course, and biologic indexes in first-episode schizophrenic patients.', *The American journal of psychiatry*. United States, 152(5), pp. 698–703. doi: 10.1176/ajp.152.5.698.

Taber, K. H., Hurley, R. A. and Yudofsky, S. C. (2010) 'Diagnosis and Treatment of Neuropsychiatric Disorders', *Annual Review of Medicine*, 61(1), pp. 121–133. doi: 10.1146/annurev.med.051408.105018.

Takeuchi, T., Duszkiewicz, A. J. and Morris, R. G. M. (2013) 'The synaptic plasticity and memory hypothesis: encoding, storage and persistence', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1633), pp. 20130288–20130288. doi: 10.1098/rstb.2013.0288.

Tamás, K., Hitoshi, Y. and Akira, A. (1998) 'Distribution of urocortin-like immunoreactivity in the central nervous system of the rat', *Journal of Comparative Neurology*. Wiley-Blackwell, 391(1), pp. 1–10. doi: 10.1002/(SICI)1096-9861(19980202)391:1<1::AID-CNE1>3.0.CO;2-6.

Timpl, P. *et al.* (1998) 'Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1.', *Nature genetics*. United States, 19(2), pp. 162–166. doi: 10.1038/520.

Torrey, E. F. *et al.* (2005) 'Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains', *Biological Psychiatry*, 57(3), pp. 252–260. doi: 10.1016/j.biopsych.2004.10.019.

Trimmer, P. C. *et al.* (2015) 'Adaptive learning can result in a failure to profit from good conditions: implications for understanding depression', *Evolution, Medicine, and Public Health*, 2015(1), pp. 123–135. doi: 10.1093/emph/eov009.

Turgeon, S. M., Anderson, N. and O'Loughlin, K. (2010) 'Phencyclidine (PCP) produces sexually dimorphic effects on voluntary sucrose consumption and elevated plus maze behavior', *Pharmacology Biochemistry and Behavior*, 95(2), pp. 173–178. doi: https://doi.org/10.1016/j.pbb.2010.01.001.

Uribe-Mariño, A. *et al.* (2016) 'Prefrontal Cortex Corticotropin-Releasing Factor Receptor 1 Conveys Acute Stress-Induced Executive Dysfunction', *Biological Psychiatry*. Elsevier, 80(10), pp. 743–753. doi: 10.1016/j.biopsych.2016.03.2106.

Vale, W. *et al.* (1981) 'Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin.', *Science (New York, N.Y.)*. United States, 213(4514), pp. 1394–1397.

Valenti, O. *et al.* (2011) 'Antipsychotic Drugs Rapidly Induce Dopamine Neuron Depolarization Block in a Developmental Rat Model of Schizophrenia', *Journal of Neuroscience*, 31(34), pp. 12330–12338. doi: 10.1523/JNEUROSCI.2808-11.2011.

Vamvakopoulos, N. C. and Chrousos, G. P. (1993) 'Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimophism of the stress response and immune/inflammatory reaction.', *The Journal of Clinical Investigation*. The American Society for Clinical Investigation, 92(4), pp. 1896–1902. doi: 10.1172/JCI116782.

Vaughan, J. *et al.* (1995) 'Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor.', *Nature*. England, 378(6554), pp. 287–292. doi: 10.1038/378287a0.

Verma, R., Balhara, Y. and Gupta, C. (2011) 'Gender differences in stress response: Role of developmental and biological determinants', *Industrial Psychiatry Journal*, 20(1), pp. 4–10. doi: 10.4103/0972-6748.98407.

Vertes, R. P. (2004) 'Differential projections of the infralimbic and prelimbic cortex in the rat.', *Synapse (New York, N.Y.)*, 51(1), pp. 32–58. doi: 10.1002/syn.10279.

Viau, V. *et al.* (2005) 'Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat', *Endocrinology*, 146(1), pp. 137–146. doi: 10.1210/en.2004-0846.

Viau, V. and Meaney, M. J. (1991) 'Variations in the Hypothalamic-Pituitary-Adrenal Response to Stress during the Estrous Cycle in the Rat', *Endocrinology*, 129(5), pp. 2503–2511. Available at: http://dx.doi.org/10.1210/endo-129-5-2503.

Vicentini, E. *et al.* (2009) 'Transient forebrain over-expression of CRF induces plasma corticosterone and mild behavioural changes in adult conditional CRF transgenic mice', *Pharmacology Biochemistry and Behavior*, 93(1), pp. 17–24. doi: https://doi.org/10.1016/j.pbb.2009.03.015.

Vogt, B. A. *et al.* (2005) 'Architecture and neurocytology of monkey cingulate gyrus.', *The Journal of comparative neurology*. United States, 485(3), pp. 218–239. doi: 10.1002/cne.20512.

Waltereit, R. and Weller, M. (2003) 'Signaling from cAMP/PKA to MAPK and synaptic plasticity.', *Molecular neurobiology*. United States, 27(1), pp. 99–106. doi: 10.1385/MN:27:1:99.

Wang, A. Y. *et al.* (2011) 'Bipolar disorder type 1 and schizophrenia are accompanied by decreased density of parvalbumin- and somatostatin-positive interneurons in the parahippocampal region', *Acta Neuropathologica*, 122(5), pp. 615–626. doi: 10.1007/s00401-011-0881-4.

Wang, H. L. *et al.* (1998) 'Corticotrophin-releasing factor produces a long-lasting enhancement of synaptic efficacy in the hippocampus.', *The European journal of neuroscience*, 10(11), pp. 3428–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9824456.

Wang, J. *et al.* (2007) 'Gender difference in neural response to psychological stress', *Social Cognitive and Affective Neuroscience*, 2(3), pp. 227–239. Available at: http://dx.doi.org/10.1093/scan/nsm018.

Wang, X.-D. *et al.* (2011) 'Forebrain CRF1 Modulates Early-Life Stress-Programmed Cognitive Deficits', *Journal of Neuroscience*, 31(38), pp. 13625–13634. doi: 10.1523/JNEUROSCI.2259-11.2011.

Wang, X.-D. *et al.* (2011) 'Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling.', *Neurobiology of disease*. United States, 42(3), pp. 300–310. doi: 10.1016/j.nbd.2011.01.020.

Wang, X.-D. *et al.* (2013) 'Nectin-3 links CRHR1 signaling to stress-induced memory deficits and spine loss.', *Nature neuroscience*. United States, 16(6), pp. 706–713. doi: 10.1038/nn.3395.

Watanabe, Y., Gould, E. and McEwen, B. S. (1992) 'Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons', *Brain Research*, 588(2), pp. 341–345. doi: https://doi.org/10.1016/0006-8993(92)91597-8.

Weathington, J. M., Hamki, A. and Cooke, B. M. (2014) 'Sex- and region-specific pubertal maturation of the corticotropin-releasing factor receptor system in the rat.', *The Journal of comparative neurology*, 522(6), pp. 1284–98. doi: 10.1002/cne.23475.

Weathington, J. M., Hamki, A. and Cooke, B. M. (2014) 'Sex- and region-specific pubertal maturation of the corticotropin-releasing factor receptor system in the rat', *Journal of Comparative Neurology*, 522(6), pp. 1284–1298. doi: 10.1002/cne.23475.

Webster, E. L. *et al.* (1996) 'In vivo and in vitro characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation.', *Endocrinology*. United States, 137(12), pp. 5747–5750. doi: 10.1210/endo.137.12.8940412.

Wei, J. *et al.* (2014) 'Estrogen protects against the detrimental effects of repeated stress on glutamatergic transmission and cognition.', *Molecular psychiatry*. England, 19(5), pp. 588–598. doi: 10.1038/mp.2013.83.

Weinberger, D. R. (1987) 'Implications of normal brain development for the pathogenesis of schizophrenia.', *Archives of general psychiatry*. United States, 44(7), pp. 660–669.

Weitemier, A. Z., Tsivkovskaia, N. O. and Ryabinin, A. E. (2005) 'Urocortin 1 distribution in mouse brain is strain-dependent', *Neuroscience*, 132(3), pp. 729–740. doi: https://doi.org/10.1016/j.neuroscience.2004.12.047.

Van De Werd, H. J. J. M. *et al.* (2010) 'Cytoarchitectonic and chemoarchitectonic characterization of the prefrontal cortical areas in the mouse', *Brain Structure and Function*, 214(4), pp. 339–353. doi: 10.1007/s00429-010-0247-z.

Wierońska, J. *et al.* (2003) *Involvement of CRF but not NPY in the anxiety regulation via NMDA receptors*, *Polish journal of pharmacology*.

Wilson, C. A., Schade, R. and Terry, A. V (2012) 'Variable prenatal stress results in impairments of sustained attention and inhibitory response control in a 5-choice serial reaction time task in rats', *Neuroscience*, 218, pp. 126–137. doi: https://doi.org/10.1016/j.neuroscience.2012.05.040.

Wilson, M. A. and Biscardi, R. (1994) 'Sex-Differences in Gaba Benzodiazepine Receptor Changes and Corticosterone Release after Acute Stress in Rats', *Experimental Brain Research*, 101(2), pp. 297–306.

Wodarz, A. and Huttner, W. B. (2003) 'Asymmetric cell division during neurogenesis in Drosophila and vertebrates.', *Mechanisms of development*. Ireland, 120(11), pp. 1297–1309.

von Wolff, G. *et al.* (2011) ‘Voltage-sensitive dye imaging demonstrates an enhancing effect of corticotropin-releasing hormone on neuronal activity propagation through the hippocampal formation.’, *Journal of psychiatric research*. Elsevier Ltd, 45(2), pp. 256–61. doi: 10.1016/j.jpsychires.2010.06.007.

Wong, M. L. *et al.* (1999) ‘Chronic administration of the non-peptide CRH type 1 receptor antagonist antalarmin does not blunt hypothalamic-pituitary-adrenal axis responses to acute immobilization stress.’, *Life sciences*. Netherlands, 65(4), pp. PL53-8.

Wood, G. E. and Shors, T. J. (1998) ‘Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones’, *Proceedings of the National Academy of Sciences*, 95(7), p. 4066 LP-4071. Available at: http://www.pnas.org/content/95/7/4066.abstract.

Workman, A. D. *et al.* (2013) ‘Modeling transformations of neurodevelopmental sequences across mammalian species.’, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 33(17), pp. 7368–83. doi: 10.1523/JNEUROSCI.5746-12.2013.

Yang, X.-D. *et al.* (2015) ‘Stress during a Critical Postnatal Period Induces Region-Specific Structural Abnormalities and Dysfunction of the Prefrontal Cortex via CRF1’, *Neuropsychopharmacology*, pp. 1203–1215. doi: 10.1038/npp.2014.304.

Young, E. A. *et al.* (2001) ‘Effects of Estrogen Antagonists and Agonists on the ACTH Response to Restraint Stress in Female Rats’, *Neuropsychopharmacology*, 25(6), pp. 881–891. doi: https://doi.org/10.1016/S0893-133X(01)00301-3.

Yu, J. *et al.* (2018) ‘Department of Neurobiology , Key Laboratory of Medical Neurobiology of Ministry of Health of’. doi: 10.1016/j.neuropharm.2018.04.002.This.

Yuen, E. Y. *et al.* (2009) ‘Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory’, *Proceedings of the National Academy of Sciences*, 106(33), pp. 14075–14079. doi: 10.1073/pnas.0906791106.

Yuen, E. Y. *et al.* (2012) ‘Repeated Stress Causes Cognitive Impairment by Suppressing Glutamate Receptor Expression and Function in Prefrontal Cortex’, *Neuron*. Elsevier Inc., 73(5), pp. 962–977. doi: 10.1016/j.neuron.2011.12.033.

Yuen, E. Y., Wei, J. and Yan, Z. (2016) ‘Estrogen in prefrontal cortex blocks stress-induced cognitive impairments in female rats’, *Journal of Steroid Biochemistry and Molecular Biology*. Elsevier Ltd, 160, pp. 221–226. doi: 10.1016/j.jsbmb.2015.08.028.

Zhang, Z. J. and Reynolds, G. P. (2002) ‘A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia’, *Schizophrenia Research*, 55(1–2), pp. 1–10. doi: 10.1016/S0920-9964(01)00188-8.

Zhu, J. J. *et al.* (2002) ‘Ras and Rap control AMPA receptor trafficking during synaptic plasticity.’, *Cell*. United States, 110(4), pp. 443–455.

Zieba, B. *et al.* (2008) ‘The behavioural and electrophysiological effects of CRF in rat frontal cortex’, *Neuropeptides*, 42(5–6), pp. 513–523. doi: 10.1016/j.npep.2008.05.004.

Zimmerman, E. C. *et al.* (2013) ‘Abnormal stress responsivity in a rodent developmental disruption model of schizophrenia.’, *Neuropsychopharmacology*, 38(11), pp. 2131–9. doi: 10.1038/npp.2013.110.

Zorrilla, E. P. *et al.* (2002) ‘Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat.’, *Brain research*. Netherlands, 952(2), pp. 188–199.