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MODELLING DEPRESSION IN ANIMALS AND THE POTENTIAL ANTIDEPRESSANT EFFECT OF HISTAMINERGIC MODULATION

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Modelling depression in animals and the potential antidepressant effect of histaminergic modulation
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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A Veronica,

alla quale ho richiesto tanto,

and for my friends:

"Once, in my father's bookshop, I heard a regular customer say that few things leave a deeper mark on a reader than the first book that finds its way into his heart. Those first images, the echo of words we think we have left behind, accompany us throughout our lives and sculpt a palace in our memory to which, sooner or later - no matter how many books we read, how many worlds we discover, or how much we learn or forget - we will return. For me those enchanted pages will always be the ones..." written during these years by my friends Giada, Laura, Caitlin and Rosaria.

(From "The shadow of the wind" by Carlos Ruiz Zafón)

ABSTRACT

Depression is at the top position for "years lived with disability" (Smith, 2014). Its aetiology is unknown, but the pathogenesis implicates changes in glutamatergic neuronal plasticity. Glutamatergic plasticity likely mediates the effects of antidepressants acting through monoamines. Histamine is a monoaminergic neuromodulator able to regulate glutamatergic plasticity and synaptic transmission.

The Flinders sensitive line (FSL) rat has face and predictive validity as model of depression when using traditional behavioural tests. However, these tests are usually missing complex explorative strategies that the animal performs in novel situations and that may be a relevant feature for a model of depression. We aimed to profile the FSL rat in terms of explorative strategies and coping styles displayed in a novel environment. The multivariate concentric square field™ (MCSF) consists of zones with different degrees of aversion. In the MCSF test, FSL rats showed lower exploratory drive, less recurrence to the shelter, and more risk assessment compared to Sprague Dawley rats, but no difference in risk-taking behaviours. In the novel cage test (consisting in a new bare environment) and in the home cage change test (to measure social behaviours), the FSL rat displayed a reactive coping style, described by immobility and lower aggression compared to Sprague Dawley rats. This profile shows similarities with temperaments and coping styles related to depression.

Depression is linked to alteration of glutamatergic plasticity and similar alterations have been found in the hippocampus of FSL rats. Histamine H3 receptor (H3R) antagonists have displayed antidepressant properties in preclinical studies. We assessed the antidepressant properties of the H3R antagonist, clobenpropit, and its effect on hippocampal glutamatergic transmission in FSL rats. In the forced swim test, both systemic and hippocampal injections of clobenpropit reduced the immobility time. Clobenpropit improved memory in the novel object recognition and passive avoidance tests, with no effect on anxiety-related tests. Clobenpropit applied on hippocampal slices enhanced long-term synaptic potentiation (LTP), and, accordingly, in vivo administration increased the hippocampal levels of the NMDA receptor subunit GluN2A. Clobenpropit's effects both in the forced swim test and on LTP were prevented by blocking the hippocampal H1 and H2 receptors. In summary, clobenpropit exhibits antidepressant properties and regulates hippocampal glutamatergic plasticity, likely by an increase of histamine release and subsequent activation of the H1 and H2 receptors.

Histamine receptors trigger intracellular signalling involved in the regulation of glutamatergic synaptic receptors, a mechanism that can affect synaptic strength. We assessed the histaminergic modulation of glutamatergic synaptic strength by recording miniature excitatory postsynaptic currents (mEPSCs) from CA1 pyramidal neurons in hippocampal slices from Sprague Dawley rats. The H1R, but not the H2R, agonist reduced mEPSC frequency, with no change of amplitude, suggesting a reduction of vesicle release probability. However, the paired-pulse facilitation (a measure of presynaptic release probability) was not altered by either the H1R or the H2R agonist, possibly due to a differential modulation of evoked versus spontaneous vesicle release. However, a postsynaptic origin of mEPSC frequency reduction cannot be excluded.

LIST OF SCIENTIFIC PAPERS

I. **Magara S.**, Holst S., Lundberg S., Roman E., Lindskog M.
Altered explorative strategies and reactive coping style in the FSL rat model of depression.
Front Behav Neurosci. 21 April 2015. doi: 10.3389/fnbeh.2015.00089

II. *Femenia T., ***Magara S.**, DuPont C.M., Lindskog M.
Hippocampal-dependent antidepressant action of the H3 receptor antagonist clobenpropit in a rat model of depression.
Int J Neuropsychopharmacol. 2015. doi: 10.1093/ijnp/pyv032

III. **Magara S.**, Lindskog M.
Histamine H1 and H2 receptor-mediated modulation of glutamatergic synaptic strength in the hippocampus.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AMPAR	AMPA receptor (glutamate receptor)
CA1, CA3	Cornu ammonis 1 and 3 (hippocampal subfields)
cAMP	cyclic 3'-5' adenosine monophosphate
EAAT	Excitatory amino acid transporter (glutamate transporter)
FRL rats	Flinders resistant line rats
FSL rats	Flinders sensitive line rats
GLAST	Glutamate aspartate transporter (glutamate transporter)
GLT-1	Glial glutamate transporter-1 (glutamate transporter)
GluN1, GluN2A, GluN2B	NMDA receptor subunits
GluR1, GluR2	AMPA receptor subunits
H1R, H2R, H3R, H4R	Histamine receptor subtypes
IP3	Inositol trisphosphate
LAL/SAL mice	Long attack latency/Short attack latency mice
LTP	Long term potentiation
MCSF	Multivariate concentric square field
mEPSC	Miniature excitatory postsynaptic currents
NMDAR	N-methyl-D-aspartate receptor (glutamate receptor)
PI3K	Phosphoinositide 3-kinase
PKA	Phosphokinase A
PKB	Phosphokinase B
PKC	Phosphokinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
Ras-MEK-ERK	Mitogen-activated protein kinases (MAPKs) cascade
RDoC	Research domain criteria
SD rats	Sprague Dawley rats

CHAPTER 1

DEPRESSION IN CLINICAL PSYCHIATRY AND IN NEUROSCIENCE: towards a dimensional approach

Diagnostic criteria for psychiatric diseases are codified in the Diagnostic and Statistical Manual of Mental Disorders that has been recently revised into the fifth edition (APA, 2013). Because of the lack of knowledge about aetiology, psychiatric diseases are still defined according to a categorical approach, i.e., the diagnosis is based on the presence of a number of signs and symptoms. As a direct consequence of such approach, psychiatric symptoms are often common to more than one categorical diagnosis and the borders among the different disorders are unclear. A classical example is represented by the depressive symptoms (loss of interest, mood deflection, sleep alterations and concentration problems) that are features of major depressive disorder, but also common in other psychiatric conditions (bereavement, bipolar disorder, schizophrenia, personality disorders). An analogous overlap also involves the treatment of choice: antidepressants are useful to treat depression as well as many anxiety disorders, while antipsychotics treat both schizophrenia and bipolar disorder (Stahl, 2013). Ultimately, the categorization of symptoms does not correspond to the biological processes and mechanisms underlying the disease; rather, drugs seem to affect mechanisms implicated transversally in multiple diseases. The underpinnings of mental disorders are the targets of investigation of neuroscience research. However, can neuroscience look for the cause of a disease that has been identified by lumping symptoms together? Are individual disorders really different if they commonly present overlapping symptoms? For instance, major depressive disorder and anxiety disorders share several symptoms (ruminative thoughts, concentration deficits, irritability and sleep problems), but they also overlap in aspects related to genetics, environmental risk factors, biological alterations, and pharmacological response (Hyman, 2007; Hettema, 2008). Such evidences have induced to reconsider the use of a categorical diagnosis in neuroscience research, in favour of a dimensional approach. According to this approach, mental disorders are not separated in distinct categories; instead disease groups are disposed along a continuum. Examples are: the continuum of healthy mental state - subtle cognitive deficits - schizotypal personality disorder - schizophrenia, or pure depressive disorder - mixed depressive/anxiety states - anxiety disorders (Hyman, 2007). Such dimensional approach has inspired the Research Domain Criteria (RDoC) stated by the National Institute of Mental Health in 2008 (Cuthbert and Insel, 2013). The aim was "to develop, for research purposes, new ways of classifying mental disorders based on dimensions of observable behaviours and neurobiological measures" (NIMH, 2008). Brain functions can be conceptually isolated and studied separately, each of them carried out by a specific brain circuitry, such as fear and defence, reward and appetitive behaviours. The RDoC system promotes the study of these single domains, instead of studying domains clustered in a complex syndrome/pathology as it was proposed by the categorical approach (Cuthbert and Insel, 2013).

The dualism "categorical/dimensional approach" has marked animal research. Animal models are far from ideally representing all the features of human disease, but the definition of validity criteria helps to estimate in which aspects the model recapitulates the human pathology. Following are the most commonly used validity criteria (Nestler and Hyman, 2010):

- Face validity: the animal model displays behavioural, anatomical, biochemical or neuropathological features of the human disease;
- Predictive validity: the response of the animal model to treatments predicts the effect of those treatments in humans;
- Construct validity: the model has been constructed by controlling or manipulating factors that are relevant (or thought to be relevant) for the aetiopathogenesis of the human pathology.

Less commonly estimated are (Stewart and Kalueff, 2015):

- Evolutionary validity: the model targets aspects of the disorder that are preserved across species;
- Inter-relational validity: the features displayed by the model and resembling the human symptoms are part of inter-related domains, i.e., these domains are often altered together within the modelled disorder and may share pathophysiological overlaps;
- Population validity: the model displays features that follow variability similar to humans. It derives from the genetic and environmental heterogeneity.

Evidently, the validity criteria are inspired by the categorical approach that base psychiatric diagnoses: the more are the similarities with the human disease, the stronger is the validity of the model. However, modelling psychiatric diseases in animals has encountered the immediate problem that most of the symptoms in humans pertain to emotional and cognitive processes (e.g., negative thoughts, lack of interest and energy, difficulty of concentration, memory deficits). These symptoms are not unequivocally reproduced in animals: the experimenter's observation can only derive interpretations from animal behaviour (Nestler and Hyman, 2010). A dimensional approach that focuses on single domains across different pathologies may relatively bypass the "categorical problem", but still cognitive and emotional domains remain hardly accessible in animals. From here comes the need of finding new domains that may help in profiling animal models (Stewart and Kalueff, 2015). The construct of behavioural testing develops from the pre-constituted viewpoint of evoking an either-or response, in order to help the interpretation of the animal behaviour. These paradigms compel the animal responses into a restricted range of behaviours, and cut off a wide part of behaviours that may still be of importance for both understanding the patterns of responses and modelling the human pathology.

FITTING THE CONCEPTS OF DEPRESSION INTO ANIMAL MODELS

Major depressive disorder is characterized by emotional symptoms (e.g., depressed or irritable mood, diminished interest or pleasure (anhedonia), feelings of guilt and worthlessness, and suicidal ideas), cognitive symptoms (lack of concentration, negative ruminative thoughts), homeostatic or neurovegetative symptoms (sleep abnormalities, loss of energy, appetite and weight), and psychomotor agitation or retardation. Among those, only anhedonia, homeostatic features and psychomotor behaviour can be assessed in rodent models, and represent the elements characterizing the model face validity (Nestler and Hyman, 2010). Some tests (forced swim test, tail suspension test and learned helplessness test) measure the avoiding/passive behavioural response of rodents to brief stressful situations (Duman, 2010). These responses are ascribed in the negative valence domains of the RDoC system and have predictive validity for screening antidepressants, which reduce the animal's negative response. When modelling depression in animals, an aspect to consider is the comorbidity of depression with anxiety disorders. Anxiety-like behaviour is evaluated by tests where the rodent can choose between aversive and secure zones: the avoidance of the aversive in favour of the secure compartment is considered as anxiety-like behaviour (Nestler and Hyman, 2010). Behavioural tests for animal models have been conceived to elicit animal behaviours that may represent an analogue of human symptoms. However, this approach leaves broad space to approximate anthropomorphic interpretation since depression in humans is associated to complex psychic experiences that are completely different and inaccessible in animals. Examples are represented by the sucrose preference test and the elevated zero-maze test. In the sucrose preference test, the lack of preference for sweet water over normal water is indicative of anhedonia, one of the features of depression. Although the paradigm construct seems to accurately target the anhedonic domain, the test has important limitations due to reproducibility and accuracy (Strekalova et al., 2011). It may be argued that anhedonia is represented by an increase in the threshold for pleasure; therefore anhedonic animals may paradoxically consume more sucrose in order to get satisfaction. In the elevated zero-maze test the preference to stay in the closed versus the open areas of the setup is interpreted as anxiety-like behaviour. Some studies have suggested that behaviours related to the assessment of the risk (stretched attend postures) can be affected by anxiolytics at doses lower than the doses required to change the recurrence to the open areas (Bickerdike et al., 1994; Shepherd et al., 1994), thus bringing up the problem of specificity and sensitivity of the behavioural measurements. The tests are traditionally designed to derive variables concerning an either-or response (e.g., immobility versus mobility in the forced swim test, sucrose versus water in the sucrose consumption test, closed versus open areas in the traditional anxiety-related tests). An alternative behavioural approach derives from ethology: it is based on the evaluation of a broad repertoire of explorative strategies that include exploratory behaviours, the use of shelter areas, risk-assessment and risk-taking behaviours, while coping with a novel situation (Steimer et al., 1997a; Meyerson et al., 2006; Marques et al., 2008). This approach aims to minimise interpretation and to give the free choice of exploration among

several options. Are behaviours captured by this approach relevant to better understand animal models? Are they relevant for modelling depression in animals?

EXPLORATIVE STRATEGIES AND COPING STYLES IN DEPRESSION

The exploration of a novel environment derives from the balance between risk/benefit evaluation and novelty-seeking drive (Hughes, 1997). When exposed to a novel situation, the animal response is described by certain variability within two extreme patterns. These patterns can be identified with trait characteristics, i.e., patterns of response that are stable over time and across different situations (Koolhaas et al., 2007). The individual variability could be described by the tier model: two independent dimensions identified with coping styles and emotional reactivity (Steimer et al., 1997b; Koolhaas et al., 2007). The coping style concepts derived from the individuation of two different patterns of response to stressful situations: the proactive response of fight/flight, characterized by aggression, territorial control, exploration of the environment, and active resolution of the conflicts; the reactive response of conservational/withdrawal, characterized by low aggression, immobility, and avoiding/passive resolution of conflicts. Coping styles have also been related to neuroendocrine responses to stress: proactive coping is associated with high adrenergic and low corticosteroid hormones, while reactive coping is associated with the opposite trends (Koolhaas, 2008). However, when analysed with a principal component analysis, the neuroendocrine responses to novelty exploration loaded with emotionality-related factors and not with coping styles (Van Reenen et al., 2005), suggesting that coping styles and emotional reactivity constitute two different dimensions. These concepts can be translated to humans.

In the Cloninger's model for human temperaments and characters, the temperaments are largely determined by genetic factors, while the characters are built mainly on learning from life experiences and regard the development of self. Coping styles and the elements characterizing the response to novel situations can be identified in the temperaments of novel-seeking and harm avoidance (Cloninger et al., 1993; Celikel et al., 2009). Notably, temperament and character constitute the individual personality, which has been related in different ways to psychiatric disorders. Depressed patients have reduced exploratory drive for novelty, and novelty seeking inversely correlates with the severity of depressive symptoms (Hansenne et al., 1999). Likewise, reactive coping style and harm avoidance are associated with increased risk for depression or higher scores of depressive symptoms (Celikel et al., 2009; Nagase et al., 2009; Cairns et al., 2014; Roohafza et al., 2014). Despite the evidence that temperamental traits are linked to mood disorders, the nature of this association is largely speculative: temperamental traits may either predispose to the development of the disorder, represent early markers, or relate to the clinical manifestation of the disorder. The study of coping responses in animals identifies separate subgroups of individuals with distinct temperamental traits, which may be relevant for the development of models of depression (Koolhaas, 2008). These traits (such as exploratory activity and coping styles) are associated with certain behavioural and biological patterns, and are maintained over time. For instance,

low exploratory mice (identified on the base of their object-directed exploratory activity in a novel environment) are characterized by more anxiety-like behaviours, lower locomotion, and worse performance at the passive avoidance. Moreover, they were less aggressive or submissive (Kazlauckas et al., 2005). These features can be ascribed in the reactive coping style with high emotionality (Koolhaas, 2008). The selective breeding of animals with extreme temperament traits has confirmed that the trait persists across generations and is associated to specific behavioural patterns (Steimer et al., 1997a; Veenema et al., 2003). Indeed, selective breeding for aggression distinguished two lines with different coping responses: long attack latency/low aggressive (LAL) mice displayed reactive responses and higher immobility in the forced swim test compared to short attack latency/high aggressive (SAL) mice (Veenema et al., 2003). The Roman lines selected for low avoidance profile have reduced novelty seeking and high emotional reactivity (Steimer et al., 1997a), while they were surprisingly more aggressive in the resident-intruder test (Coppens et al., 2013). This reinforces the hypothesis that emotional reactivity and coping styles are separate, but interacting, dimensions. Notably, coping styles and exploratory activity may predict the sensitivity to stress and drug response (Taghzouti et al., 1999; Veenema et al., 2003; Veenema et al., 2005). In summary, coping style and exploratory profile are crucial factors to understand the individual variability in animal testing and to interpret the behaviour expressed in different paradigms. Above all, they resemble depression-related temperamental traits, hence may help the translation between animal behavioural models and human depression.

With this perspective, we looked at the exploratory behaviours and coping styles in an animal model of depression, the Flinders sensitive line (FSL) rat. The breeding selection of the FSL line targeted the sensitivity to cholinergic agents, with no intentional selectivity for behavioural profiles. However, the FSL rat was found to exhibit features modelling human depression (Overstreet and Wegener, 2013). We found that the FSL rat exhibits altered explorative strategies and reactive coping style that reinforce the FSL behavioural profiling as an animal model of depression.

THE FLINDERS SENSITIVE LINE RATS

The FSL was originally generated from Sprague Dawley (SD) rats by selective breeding on the basis of their sensitivity to the anticholinesterase diisopropyl fluorophosphate (Overstreet et al., 1979). The intention was to create a line that was resistant to the agent. Instead, the breeding generated a line that was more sensitive (the FSL) and a line (Flinders resistant line, FRL) with a response of the same extent than the control SD rats. It was then observed that FSL sensitivity was accounted for by an increase of cholinergic muscarinic receptors, with normal levels of acetilcholinesterase (Overstreet et al., 1984). Given the similarities of cholinergic abnormalities with depressed patients, FSL rats were further investigated as an animal model of depression. FSL rats display increased REM sleep, passive response to the forced swim test that is reverted by antidepressant treatments, and reduced weight and

appetite (Overstreet et al., 2005; Overstreet and Wegener, 2013). Cognitive symptoms of depression include impairment of concentration and of declarative and spatial memories (Austin et al., 2001; Bremner et al., 2004; Gould et al., 2007), which are common residual symptoms in depressed patients (McClintock et al., 2011). These symptoms have been related to hippocampal dysfunctions (Bremner et al., 2004; Deckersbach et al., 2006). Recent studies have found emotional and recognition memory impairments in the FSL rats (Eriksson et al., 2012; Gomez-Galan et al., 2013). One of the core symptoms of depression, anhedonia, is not observed in FSL rats when tested for sweet intake and sucrose preference. However, after exposure to the chronic mild stress (a validated paradigm to induce depressive-like behaviours in animals), FSL rats exhibit anhedonia at a larger extent than control rats (Pucilowski et al., 1993). Therefore, the FSL rat shows face and construct validity in modelling many aspects of human depression and displays a good predictive validity for antidepressant response (Overstreet and Wegener, 2013). Anxiety dimension is often associated with depression and has been repeatedly assessed in FSL rats, with no univocal conclusion. In our study (see paper II) FSL rats exhibited increased anxiety-like behaviours in a number of traditional paradigms (Femenia et al., 2015). Instead, other groups have reported that FSL rats display less (Abildgaard et al., 2011) or no (Schiller et al., 1991) anxiety-related behaviours. This discordance leads to the possibility that different tests evoke different explorative strategies that influence the final behavioural outcome. Biochemical alterations in the serotonergic, cholinergic, and glutamatergic systems of FSL rats resemble analogous alterations found in depressed patients, and confer further validity to the animal model (Overstreet et al., 2005). The alterations of glutamatergic transmission in FSL rats will be described in chapter 2 in the context of the neuroplasticity hypothesis of depression.

ALTERED EXPLORATIVE STRATEGIES AND REACTIVE COPING STYLE IN THE FSL RATS - (PAPER I)

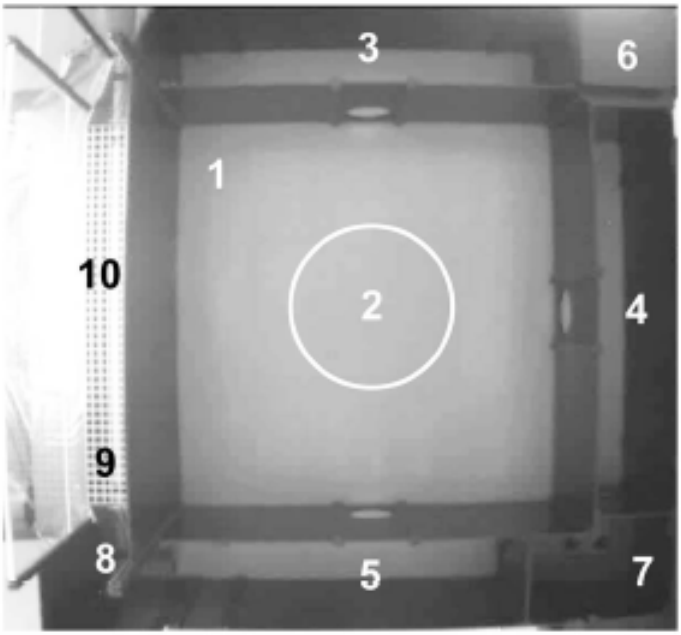
Summary

Translational neuroscience is in need of alternative behavioural tests to validate animal models. Many aspects of human depression and anxiety symptoms are modelled in animals by interpreting the animal response in tests evoking an either-or response. An alternative approach derives from ethology: explorative strategies and coping styles are observed in an environment that allows the free expression of the animal's behavioural repertoire. We evaluated these aspects in the FSL rat, an animal model of depression, in comparison to SD rats, i.e., the rat strain that the FSL was derived from. For many years, FSL rats have been compared with the inbred resistant line, the FRL rat (Overstreet and Wegener, 2013). However, for our purpose, the use of the SD rats as control strain was more appropriate than the FRL rat. The choice of an outbred strain preserves individual variability in behavioural response to stress and in resilience/vulnerability to stressors within a group of animals. Moreover, FSL and FRL selective breeding was based on the responsiveness to cholinergic agents and may have consequently forced the segregation of genetic features related to

exploratory behaviours and coping styles. Since the relationship between behaviour and the cholinergic alterations is out of our scope, an outbred strain was preferred.

The behavioural methodology consisted of three different tests: multivariate concentric square field™ (MCSF), novel cage, and home cage change tests.

The MCSF test is constituted by an arena that includes shelter areas, elevated illuminated passages, enriched zones, open areas, and different light conditions. This multivariate context is able to evoke diverse strategies that include exploratory behaviours, risk assessment, risk taking, and shelter seeking (Figure 1). The test has been behaviourally validated with regard to the identification of risky and safe areas (Meyerson et al., 2006).



(From Magara et al., 2015)

Figure 1: picture of the MCSF arena with the defined zones. 1. center of the arena; 2-10. central circle and bridge (aversive zones); 3-5. corridors; 6. dark corner room (sheltered zone); 7. hurdle with the elevated hole board (to stimulate exploration); 8-9. slope and bridge entrance (to allow risk assessment towards the bridge.

The MCSF test was sensitive in identifying subgroups that differed for particular behaviours in other tests. For instance, subgroups identified by the risk-assessment levels in the MCSF test showed differences in voluntary alcohol intake, with high risk assessment associated with higher alcohol intake (Momeni et al., 2014). The MCSF has also been used for behavioural profiling of selectively bred animals, where it evidenced different explorative strategies between different lines. For instance, the Alko alcohol-preferring/non-alcohol-preferring (AA/ANA) rat lines differed for many explorative strategies, with the AA line expressing lower general activity, exploratory activity and shelter seeking, and higher risk assessment and risk taking (Roman et al., 2012).

The novel cage test is based on scoring exploratory behaviours in a non-aversive environment (Marques et al., 2008). Marques et al. (2008) measured the free exploratory behaviours in the novel cage test (a novel home-cage without cage-mates) and in the concentric squared field test (similar to our MCSF arena). The exploratory behaviours included exploring and avoiding/passive behaviours that can be considered indicative of emotional reactivity and coping styles (proactive versus reactive) (Steimer et al., 1997a; Koolhaas et al., 1999; Marques et al., 2008). They characterized mouse-strain differences in both the novel cage and the concentric square field tests, with a good correspondence between tests. Steimer et al. (1997) measured exploratory activity (as an indirect measure of proactive coping) and emotional reactivity in a "small open field". It consisted in an illuminated box in order to create a mild aversive environment. The test showed differences between rat lines selectively bred on the basis of their active avoidance response (Roman high/low avoidance lines) (Steimer et al., 1997a). In our study, the aim was to expose the animal to a novel environment with minimal incentives or aversive elements, so that exploratory behaviours were mainly driven by the internal motivational state (Hughes, 1997) and reflected the expression of coping styles. Therefore, for our novel cage test, the apparatus consisted in a symmetrical small open field similar to the one used by Steimer et al. (1997), but with dimmed lights, and tall, opaque walls to allow for rearing and to protect from external visual cues.

The home cage change test aimed to score social behaviours elicited by the change of cage. A new home cage is free of all "familiar" odours that the rat uses to mark and establish dominance (Burn et al., 2006). Therefore, the change of cage intensifies the expression of dominant-subordinate behaviours (Craig, 1986). The type of social behaviours displayed by the animals were scored and classified according to previously defined categories (Koolhaas et al., 1980; Fernandez-Espejo and Mir, 1990).

In the MCSF test, the exploration of FSL rats was slightly altered compared to SD rats. FSL rats exhibited reduced exploratory drive and a strategy of exploration with more risk-assessment behaviour and less recurrence to the shelter. When the rats were re-exposed to the MCSF test, the exploration strategies of FSL rats were similar to SD rats. Indeed, FSL rats increased the exploratory activity and the shelter seeking in the second compared to the first trial, thus reaching the SD rat's level. FSL rats used more stretched attend postures as risk assessment behaviour, but they explored the risk areas of the arena to the same extent of SD rats. Therefore, the behaviour exhibited by the FSL rat in the MCSF test indicates a slower explorative strategy, but it does not describe an avoiding, anxiety-related profile. FSL rats demonstrated behavioural flexibility by learning from the first exposure to the arena and consequently increasing their exploratory behaviour. In the novel cage test, FSL rats exhibited reduced exploratory activity (characterized by less investigative behaviour) and a reactive coping style compared to SD rats. The reactive coping style of FSL rats was also expressed by reduced aggressive behaviours, cage-mate avoidance, and burrowing in the home cage change test. Burrowing is considered a proactive defensive behaviour acted in anticipation of a potential threat (Dudek et al., 1983). Instead, stretched attend postures are risk-assessment strategies related to high emotionality and are used to search for and

acknowledge potential threats (Blanchard and Blanchard, 1989). Burrowing behaviour in the home cage change test and stretched attend postures in the MCSF test were inversely correlated. In conclusion, the FSL rat is characterized by low exploratory drive and a reactive coping style. During exposure to novelty, the reactive coping style is expressed by higher immobility, less proactive strategies (such as recurrence to a home base, investigative behaviour, anticipatory defensive burrowing), and high emotional reactivity during the evaluation of risk/benefit balance. In social contexts, reactive coping is expressed by avoidance and less aggressive behaviours.

Implications

The FSL rat's behavioural profile has previously evidenced low novelty-induced and goal-directed exploration (Overstreet, 1986; Gomez-Galan et al., 2013). In agreement, we found that FSL rats display low exploratory drive and a reactive coping style during exploration of a novel environment and in social dominant-subordination behaviours. These features may be related with the FSL sensitivity to antidepressants. Indeed, in a previous study (Taghzouti et al., 1999), SD rats were grouped by exploratory levels exhibited in a novel environment. Low exploration was associated with a passive response (high immobility) in the forced swim test. The response in the forced swim test can be considered as an expression of coping style, and a drug-induced reduction of immobility time could be due to a switch in coping response (Koolhaas et al., 2007). Indeed, fluoxetine reduced the immobility of low-exploring rats in the forced swim test, while it increased the immobility of high-exploring rats (Taghzouti et al., 1999). The serotonin 5HT1A receptor agonist 8-OH-DPAT exerted a similar differential effect: it reduced the immobility time of LAL mice (that exhibit a reactive coping style in the active avoidance test and in the defensive burying test (Benus et al., 1991; Sluyter et al., 1996)), but not of SAL mice (that exhibit a proactive coping style) (Veenema et al., 2005). In agreement, the FSL rat, which is characterized by a reactive coping style and low exploratory drive, has good predictive validity for the antidepressant action of drugs (Overstreet and Wegener, 2013). The reactive coping style of LAL mice is associated with reduced expression of the 5HT1A receptor in the hippocampus and prefrontal cortex compared to SAL mice (Korte et al., 1996), which may explain the different behavioural responses to serotonergic drugs (Veenema et al., 2005). Notably, both FSL rats and LAL mice differed from their respective control lines in the hypothermic response to 8-OH-DPAT, with the former more sensitive (Overstreet, 2002) and the latter less sensitive (van der Vegt et al., 2001). As earlier described, a reactive coping style is associated to higher levels of corticosterone and lower levels of adrenaline in response to stress (Koolhaas et al., 1999), and to a different behavioural response to stress compared to animals with a proactive coping style (Veenema et al., 2003). It seems that coping style may be associated with a different resilience/vulnerability balance for the development of some stress-related diseases (Koolhaas et al., 1999; Veenema et al., 2003). Taken together, these results indicate that coping styles are associated with different levels of activation of neurotransmitter systems,

and both may explain animal responses to experimental testing, exposure to stress and drugs. These patterns of responses may be relevant both for the FSL rat and for other depression models.

We have evidenced that the FSL rat performs risk-taking behaviour to the same extent as SD rats in the MCSF test, but with greater risk assessment. In the next chapter, we will discuss that FSL rats have displayed anxiety-like behaviours in the novelty suppressed feeding, social interaction, and light/dark box tests (paper II: Femenia et al., 2015). A possible explanation may derive from differences in the explorative strategies expressed in these tests. Exploratory responses to novelty are executed through sequences of behavioural strategies, such as choice of a home base for exploration, evaluation risk/benefits, and risk-assessment behaviours (Blanchard and Blanchard, 1988; Eilam and Golani, 1989; Drai et al., 2001). Laboratory behavioural tests may interfere with these sequences by curtaining the expression of animal behaviour repertoire to few responses. The significance of either-or responses may not be generalized to other contexts where the animal has a wider choice to freely express its exploratory behavioural strategies. This hypothesis is discussed in the paper I in the paragraph "Risk-assessment strategies and coping styles influence the interpretation of anxiety-related tests" (Magara et al., 2015).

The FSL rat's explorative strategies and reactive coping style may represent targets for evaluating antidepressant drugs and may also help to interpret FSL behaviour expressed in traditional tests. Explorative strategies and coping styles may be considered as further domains for the validation of animal models of depression and of antidepressant drugs. Although not reported in the categorical definition of diagnostic criteria for human diseases, explorative strategies and coping styles represent evolutionary-preserved features. They may be potentially relevant for the resilience/vulnerability to stressors.

CHAPTER 2

THE BIOCHEMISTRY OF DEPRESSION: monoamines and glutamatergic neuroplasticity

For many years the pathogenesis of depression has been explained by the so-called "monoamine hypothesis", for which depression originates from depletion of the monoamines, i.e., serotonin and the catecholamines noradrenaline and dopamine (Baumeister et al., 2003; Racagni and Popoli, 2008). The monoamine hypothesis of depression was derived by two serendipitous discoveries in the 1950s: reserpine, used as antihypertensive drug, could cause depressive symptoms and depletes serotonin and catecholamine; iproniazid, used as antituberculosis drug, treated depressive symptoms (apathy, anorexia, feeling of well-being) and inhibits the monoamine oxidase, the enzyme that catabolises monoamines. Accordingly, the major drugs currently available for the treatment of depression are inhibitors of monoamine reuptake (for instance, selective serotonin reuptake inhibitors, SSRI; serotonin/noradrenaline reuptake inhibitors, SNRI). However, alteration in monoamine levels in depression has not yet been confirmed and the increase of monoamines is not sufficient to elicit an antidepressant effect. Indeed, while antidepressant drugs acutely increase monoamine levels, the antidepressant effect has a latency of several weeks. This evidence indicates that the actual therapeutic effect is underlain by neuroplasticity mechanisms, i.e., slow changes occurring within days in response to the perturbation created by the antidepressant drug. These changes may involve postsynaptic receptor adaptation, transcriptional and epigenetic modifications, neurotrophic factors and neurogenesis, morphological changes of neuronal spines and dendrites, and synaptic transmission (Racagni and Popoli, 2008). The neuroplasticity hypothesis accounts both for maladaptive plasticity (at the origin of the pathogenesis of depression) and for compensatory/beneficial plasticity (generated by antidepressants), which likely involve the glutamatergic transmission (Sanacora et al., 2012). The first suggestions of the involvement of the glutamatergic system in depression came from the observation that the NMDA receptor (NMDAR) is implicated in antidepressant responses. NMDAR antagonists were efficacious in animal tests predictive of an antidepressant activity (Trullas and Skolnick, 1990). Moreover, adaptation of the NMDAR complex consistently occurred after chronic, but not acute, administration of antidepressants (Paul et al., 1994). These discoveries were later strengthened by clinical evidences: in depressed patients, ketamine (NMDAR non-competitive antagonist) exerts a fast antidepressant effect that lasts long after the drug has been eliminated (Berman et al., 2000; Zarate et al., 2006). The brief pharmacological manipulation of glutamatergic transmission by ketamine triggers rapid mechanisms of synaptic plasticity (Autry et al., 2011; Nosyreva et al., 2013) that may persist for long time underling the antidepressant effect (Li et al., 2010; Duman et al., 2012; Kavalali and Monteggia, 2015). The involvement of glutamatergic transmission in depression is further supported by the morphological neural alterations found in patients. In patients with depression, brain areas related to emotional/cognitive processing

and stress-responses (i.e., frontal areas and hippocampus) show volumetric reduction, glial loss and neuronal atrophy (Rajkowska, 2000; Rajkowska and Miguel-Hidalgo, 2007; Koolschijn et al., 2009). These alterations are likely to involve glutamate transmission, since the vast majority of synaptic connections in these areas are glutamatergic (Orrego and Villanueva, 1993; Douglas and Martin, 2007). Indeed, glial loss in depressed patients has been associated with reduction of the glial glutamate transporters (EAAT1 and EAAT2, also called GLAST and GLT-1, respectively) that provide for the reuptake of extrasynaptic glutamate (Rajkowska and Miguel-Hidalgo, 2007). Excessive levels of extrasynaptic glutamate trigger excitotoxicity mechanisms and neurodegeneration, likely underlying the development of the depressive disorder and ultimately leading to neuronal atrophy and a reduction of glutamatergic transmission (Sanacora et al., 2012). The causal relation among glial loss, reduced glutamate reuptake, increased glutamate transmission, and excitotoxicity is missing, but it is likely that some factors (genetic susceptibility or environmental stress) may produce adaptive plasticity mechanisms that, if challenged repeatedly, will result in maladaptive plasticity (Popoli et al., 2012). According to the neuroplasticity hypothesis, changes in glutamatergic neuronal plasticity may mediate the therapeutic effects of classical antidepressants that target the monoaminergic systems of serotonin and noradrenaline (Berton and Nestler, 2006). Histamine is a monoaminergic neuromodulator that has been far less studied in regard to depression, but has relevant effects on glutamatergic plasticity (Haas et al., 2008).

HISTAMINE IN THE BRAIN

Histaminergic neurons are localized in the tuberomammillary nucleus of the hypothalamus and project widely throughout the brain, including to the hippocampus (Purves et al., 2001). Histamine receptors are G-protein coupled receptors classified in 4 subtypes (Brown et al., 2001). The H1 and H2 receptors (Gq- and Gs- coupled, respectively) are classically considered postsynaptic receptors. They are diffusely expressed in the brain, with some local differences: in comparison with the H2 receptors, the H1 receptor is more expressed in the cortex (layer IV) and the thalamus, but less expressed in the superficial layers of the cortex and in the striatum. The H4 receptor is coupled with a Gi-protein. It is mainly expressed in peripheral immunological tissues and cells, while its expression in the central nervous system is mainly limited to the dentate gyrus, thalamus and layer IV of the cortex (Connelly et al., 2009). The H3 receptor is a presynaptic auto-receptor (localized on histamine-releasing varicosities) and hetero-receptor (localized on axons releasing other neurotransmitters, such as glutamate, acetylcholine, and GABA) (Haas et al., 2008). It is also localized postsynaptically on the somata and dendrites of histaminergic tuberomammillary neurons and possibly on other neurons (for instance, the striatal medium spiny neurons (Pillot et al., 2002)). The H3 receptor is coupled with a Gi-protein that, when stimulated, inhibits N- and P-type calcium channels (Takeshita et al., 1998) and reduces the activity of the adenylate cyclase (Torrent et al., 2005). Through these mechanisms, the H3 receptors reduce

neurotransmitter release from histaminergic and non-histaminergic terminals, and reduce cell firing and histamine synthesis in histaminergic neurons (Haas et al., 2008). The H3 receptor has constitutive activity, i.e., the Gi-protein coupled to the receptor is tonically active and partially inhibits the production of cAMP by adenylate cyclase in absence of the receptor agonist (Morisset et al., 2000; Moreno-Delgado et al., 2006). The H3 receptor can activate phosphokinase B (PKB, also called Akt, resulting in inhibition of the glycogen synthase kinase GSK3) (Bongers et al., 2007), phospholipase PLA2 (with the production of arachidonic acid) (Morisset et al., 2000), and the Ras/MEK/ERK cascade (Giovannini et al., 2003). These pathways have a prominent role in plasticity mechanisms (Thomas and Huganir, 2004; Richter and Klann, 2009; Allyson et al., 2012), thus the H3 receptor may represent a target to modulate neuronal plasticity.

Histamine neurotransmission regulates cognitive functions (motivation, arousal, learning and memory processes) and autonomic functions (sleep/wake cycle, food intake, thermoregulation) (Brown et al., 2001). Notably, many of these processes regulated by histamine are altered in depression, which is characterized by lack of motivational drive, difficulty of concentration, deficit in episodic memory, insomnia or hypersomnia, reduced body weight or increased food intake (APA, 2013). Acting on the histaminergic transmission may either directly impact the symptoms of depression or indirectly modulate glutamatergic plasticity mechanisms that are relevant for the antidepressant response.

THE H3 RECEPTOR AS PHARMACOLOGICAL TARGET FOR DEPRESSION

The H3 receptor is widely expressed throughout the brain. It inhibits the release of histamine as well as of many other neurotransmitters (dopamine, serotonin, acetylcholine, glutamate) (Esbenshade et al., 2008). Deficit of these neurotransmitters are linked to cognitive symptoms in many mental disorders. Due to its constitutive activity, the H3 receptor can be pharmacologically targeted to either antagonize the binding of endogenous histamine (by H3R neutral antagonists) or reduce the intrinsic activity of the receptor (by H3R inverse agonists) (Moreno-Delgado et al., 2006). The H3R antagonists/inverse agonists have been studied in the hypothesis that they could improve cognitive symptoms by disinhibition of neurotransmitter release. Tests in animal models have demonstrated that H3R antagonists improve performances related to several cognitive domains, i.e., recognition memory, working memory, and spatial orientation (Esbenshade et al., 2006). Given the promising results from experimental studies, the H3R antagonists have been tested in clinical trials to evaluate the cognitive effects in Alzheimer disease and schizophrenia (Tiligada et al., 2011) (www.clinicaltrials.gov). Moreover, some studies have tested the antidepressant effect of the H3R antagonists in animals. Clobenpropit and thioperamide (H3R antagonists/inverse agonists) reduced the immobility time of mice in the forced swim test (Lamberti et al., 1998; Pérez-García et al., 1999). New H3R antagonists have been reported to exert antidepressant-like effects (Gao et al., 2013) and reduce anxiety-like behaviours in the open field, elevated plus maze and novelty suppressed feeding tests (Bahi et al., 2014). Many aspects of the

therapeutic mechanisms of the H3R antagonists are still not clear: would they ameliorate the behavioural phenotype and glutamatergic transmission/plasticity alterations of animal models of depression? Would their effect be mediated by modulating the release of histamine or other neurotransmitters?

THE NEUROPLASTICITY HYPOTHESIS OF DEPRESSION IN REGARD TO THE FSL RATS

The FSL rat model presents glutamatergic alterations of synaptic transmission and plasticity in the hippocampus (Gomez-Galan et al., 2013), resembling aspects that have been connected with depression pathogenesis. Reduced hippocampal volume has been associated with depression (Campbell et al., 2004; Stockmeier et al., 2004), and the hippocampus shows atrophic signs and alterations of glutamatergic transmission and plasticity in animal models of depression (Dranovsky and Hen, 2006; Qiao et al., 2014). FSL rats have increased frequency of hippocampal synaptic inputs onto CA1 pyramidal neurons and impaired long-term synaptic plasticity (LTP) at CA3-CA1 connections. These alterations in FSL rats have been associated with astrocyte reactivity, with consequent reduction of D-serine release by astrocytes (necessary for LTP) and decreased expression of the glutamate transporter GLAST (responsible for the increase of the excitatory inputs) (Gomez-Galan et al., 2013). In agreement with the impaired hippocampal plasticity, FSL rats have shown deficits in recognition memory in the novel object recognition test and reduced emotional memory in the passive avoidance test (Eriksson et al., 2012; Gomez-Galan et al., 2013). Our study focused on the potential antidepressant properties of the histamine H3 receptor antagonist, clobenpropit, and its modulation on glutamatergic transmission and plasticity in the FSL rat model.

HIPPOCAMPAL-DEPENDENT ANTIDEPRESSANT ACTION OF THE H3 RECEPTOR ANTAGONIST CLOBENPROPIT IN A RAT MODEL OF DEPRESSION (PAPER II)

Summary

Depression may arise from the dysregulation of glutamatergic transmission and plasticity (Sanacora et al., 2012). The hippocampus presents important alterations both in depressed patients and in animal models of depression (Stockmeier et al., 2004; Dranovsky and Hen, 2006), and is a key region for the integration of emotional and cognitive domains, both of which are affected in depression (Femenía et al., 2012). Several evidences suggest that the effect of monoaminergic antidepressants is mediated by the modulation of glutamatergic plasticity (Sanacora et al., 2012). Histamine H3R antagonists can regulate the release of several neurotransmitters and have displayed both antidepressant and pro-cognitive effects in animal experiments (Lamberti et al., 1998; Pérez-García et al., 1999; Esbenshade et al.,

2006). We aim to study the effect of the H3R antagonist clobenpropit in the FSL rat. The focus of the investigation will be on the antidepressant properties of clobenpropit tested by the forced swim test, and the involvement of the hippocampus. We will explore the ability of clobenpropit to revert biochemical and glutamatergic alterations found in the hippocampus of FSL rats, and will identify the targets of clobenpropit's modulation.

In FSL rats, clobenpropit acutely reduced the immobility time in the forced swim test, both after systemic and after hippocampal local administrations, indicating an antidepressant-like effect. Moreover, it improved memory performances in the passive avoidance and novel object recognition tests, with no alteration of the locomotor activity. FSL rats displayed anxiety-like behaviours in the novelty suppressed feeding, social interaction, and light/dark box tests, which were only minimally affected by the administration of clobenpropit. Therefore, clobenpropit's effects seem to be limited to cognitive and some emotional aspects, i.e., increased learning of a passive avoidance response to stress and reduced immobility time in the forced swim test, but no effect on anxiety-like behaviours. Notably, the action of clobenpropit in the hippocampus (by bilateral hippocampal injections) was sufficient for the antidepressant-like effect. Hippocampal protein levels after systemic injection of clobenpropit were measured by western blot. In the hippocampus, the levels of GLAST, which are reduced in FSL rats, were not affected by clobenpropit. GLAST reduction has been linked to increased excitatory inputs onto CA1 pyramidal neurons in FSL rats (Gomez-Galan et al., 2013). Since clobenpropit exerted an effect in the forced swim test also when locally administered in the hippocampus, we tested whether direct application of clobenpropit on hippocampal slices could reduce the excitatory inputs. In agreement with the absence of effect on GLAST levels, clobenpropit did not change the frequency of excitatory postsynaptic currents measured by whole-cell patch clamp in FSL hippocampal slices. Systemic clobenpropit reverted the low levels of the GluN2A subunit of the NMDA receptor, with no changes of the other NMDA- and AMPA- receptor subunits. The GluN2A subunit mostly characterizes synaptic NMDA receptors and is crucial for LTP (Bartlett et al., 2007; Papouin et al., 2012). Since the FSL rat has impaired hippocampal LTP at CA3-CA1 connections (Gomez-Galan et al., 2013), we measured the effect of clobenpropit (applied on hippocampal slices) on LTP by recording CA1 field excitatory postsynaptic potentials evoked by Schaffer collateral stimulation. In accordance to the increase of the GluN2A subunit, clobenpropit enhanced the LTP. Both the LTP enhancement in slice and the antidepressant effect of clobenpropit in the forced swim test were prevented by the co-administration of the H1R and H2R antagonists. We hypothesise that the effect of clobenpropit could be mediated by disinhibition of histamine release, and consequent activation of postsynaptic H1 and H2 receptors (Figure 2). The block of either the H1 or H2 receptor in the hippocampus did not increase the immobility in clobenpropit-treated rats. This indicates that the activation of either H1 or H2 receptor in the hippocampus was sufficient to mediate the antidepressant effect of clobenpropit.

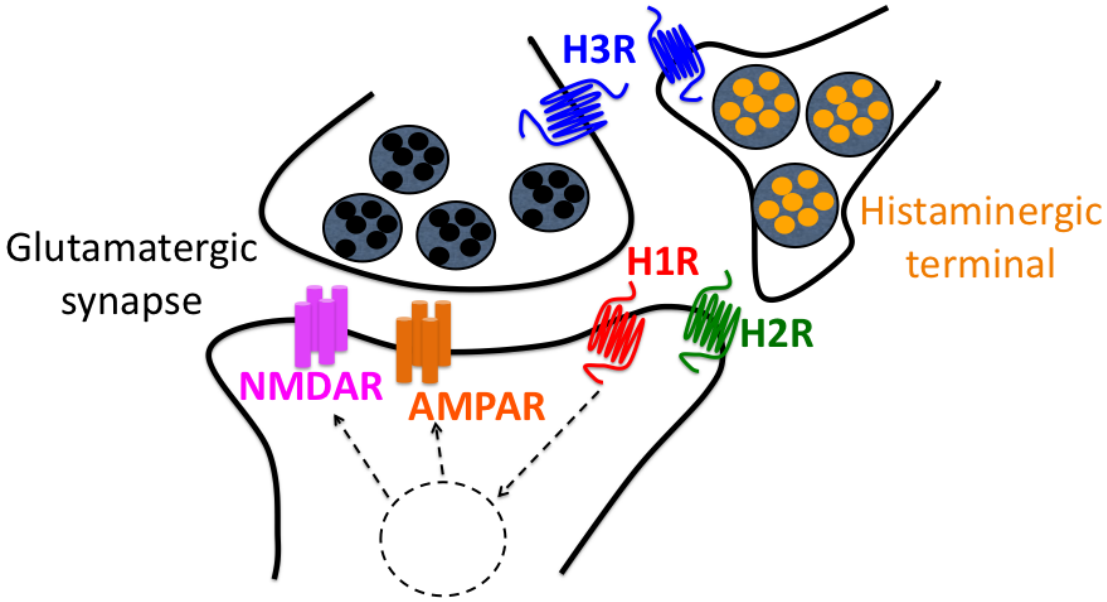


Figure 2: schematic drawing of the glutamatergic synapse reached by the histaminergic terminal. In the presynaptic side, big circles containing small circles represent vesicles and neurotransmitter molecules, respectively. In the postsynaptic side, the dashed lines represent intracellular signalling pathways converging on the postsynaptic AMPA- and NMDA- receptors.

Implications

So far, the action of antipsychotics and antidepressant drugs on histamine receptors has been considered mainly in regard to the origin of their side-effects (as for sedation and weight gain induced by the H1R antagonism of olanzapine and tricyclic antidepressants) (Stahl, 2013). The H3R antagonism and inverse agonism have been explored for their potential therapeutic effects in Alzheimer disease, narcolepsy, schizophrenia, obesity, and neuropathic pain (Tiligada et al., 2011). Our results, in agreement with previous studies, encourage further investigation of the H3R-mediated modulation to exert antidepressant effects. Future experimentation should consider the use of the H3R antagonists in validated animal models of depression and focus on the long-term effects of the drug after chronic and acute clobenpropit administrations. An advantage of the H3R antagonists in depression may derive from their pro-cognitive properties. Depression includes cognitive symptoms (Austin et al., 2001), and many antidepressant drugs produce cognitive side-effects (Fava et al., 2006). The H3R antagonists may be explored in augmentation of classical antidepressants, in order to obtain a synergistic effect on neurotransmitter release and neuronal plasticity, to potentiate the effect on mood, reduce the drug doses and increase tolerability. A possible approach is the synthesis of molecules that contain active fragments of H3R antagonists and reuptake inhibitor antidepressants (Sander et al., 2008). Unfortunately, some issues make the H3 receptor a difficult pharmacological target. The drug affinity of H3R antagonists shows species-related differences, which may explain the differences in the drug effect in rat versus

humans. The gene coding for the H3 receptor has many splice variants that result in a large number of receptor isoforms with different distributions and pharmacological properties. Despite the progresses of the H3R pharmacology and the promising results of the H3R antagonists in clinical and preclinical studies, the contribution of specific isoforms of the receptor in the action of the H3R antagonists is largely unknown (Esbenshade et al., 2006; Sander et al., 2008). Moreover, the H3 receptor has constitutive activity. As a consequence, inverse agonists and antagonists at the H3 receptors may have different effects depending from the surrounding levels of endogenous histamine. The inverse agonist and the antagonist would produce the same effect in the presence of endogenous histamine, for instance they will disinhibit neurotransmitter release. Instead, in the absence of histamine, only the inverse agonist would be able to exert an effect (Moreno-Delgado et al., 2006). Hence the effect of the H3R antagonists may be strongly dependent from the histaminergic tone, and therefore from the actual mental state. In conclusion, the use of H3R antagonists in clinic and animal research represents a difficult issue due to its species-specific pharmacology, presence of different H3R isoforms with different pharmacological properties, and the receptor constitutive activity. However, the H3R modulation is not the only target to explore for a therapeutic perspective. Our results indicated that clobenpropit's effect was mediated by the activation of the H1 and H2 receptors. In a previous study, the antidepressant effect of the H1 receptor activation and increased endogenous histamine was evidenced (Lamberti et al., 1998). In the paper III, we will illustrate how H1 and H2 receptors differentially modulate glutamatergic synaptic strength in the hippocampus. These data encourage the exploration of strategies to modulate the histaminergic transmission in order to promote neuronal plasticity for a therapeutic effect.

CHAPTER 3

HISTAMINE RECEPTOR SIGNALS

Although the hippocampus receives moderate histaminergic innervations (Panula et al., 1989), hippocampal neurons are highly responsive to histamine (Haas et al., 2008). We will discuss the intracellular signalling downstream of the H1 and H2 receptors and relevant for the modulation of neuronal properties.

H1 receptors

The H1 receptor is coupled to a Gq-protein and activates an intracellular pathway mediated by phospholipase C (PLC) and phosphokinase C (PKC). The PLC signalling induces mobilization of calcium from intracellular storages (Haas et al., 2008). In many neuronal cells, H1R activation leads to depolarization by block of leak potassium conductance (Reiner and Kamondi, 1994). Instead, in the hippocampal pyramidal neurons H1 receptors can hyperpolarize the membrane potential by the opening of calcium-activated potassium (K_{Ca}) channels, causing a consequent decrease of neuronal excitability (Selbach et al., 1997; Weiger et al., 1997). This effect on neuronal excitability may explain how H1R antagonists are epileptogenic (Yokoyama and Iinuma, 1996). Moreover, H1 receptors can target neuronal electrical properties by PKC-mediated facilitation of voltage-gated calcium channels and of sodium/calcium exchanger (Haas et al., 2008). The H1 receptor increases NMDAR-mediated currents by reducing the magnesium block (Payne and Neuman, 1997), likely through PKC phosphorylation (Chen and Huang, 1992). The H1 receptor is also able to trigger the production of arachidonic acid by PLA2 (Leurs et al., 1994), and of nitric oxide by calcium-dependent nitric oxide synthase (Yang and Hatton, 2002), which can potentially act as retrograde messengers that modulate synaptic transmission. The H1 receptor activation in the hypothalamus in vivo increases noradrenaline release (Bealer, 1993), suggesting that H1 receptors may either be localized presynaptically or stimulate the release of transmitters that in turn regulate the presynaptic release of noradrenaline.

H2 receptors

The H2 receptor is coupled to a Gs-protein and activates an intracellular pathway mediated by cAMP and phosphokinase A (PKA) (Haas et al., 2008). H2R activation inhibits K_{Ca} channels responsible for afterhyperpolarization, thus blocking the accommodation of neuronal firing, an effect mediated by PKA-mediated phosphorylation (Haas and Konnerth, 1983). The H2 receptor also induces a depolarizing current (I_h) by shifting the activation threshold of the hyperpolarization-activated cation channel (McCormick and Williamson, 1991). Therefore, the major effect of the H2 receptor is the depolarization of pyramidal

neurons and the increase in firing. However, early studies have pointed out that histamine, in several brain areas, reduces neuronal excitability when locally administered, likely by activation of H2 receptors on inhibitory interneurons (Haas and Greene, 1986; Brown et al., 2001). A presynaptic localization of the H2 receptor has not been demonstrated, however some biochemical studies have reported that the H2 receptor can increase noradrenaline release from membrane homogenates (Timm et al., 1998), and H2 receptors may be localized on striatonigral endings (Vizuete et al., 1997). The intracellular signalling of the H2 receptor is very similar to the dopamine D1-like and the adrenergic β1 receptors, which are localized on presynaptic terminals (as well as postsynaptically) and mediate neurotransmitter release (Brown et al., 2001).

HISTAMINERGIC MODULATION OF SYNAPTIC PLASTICITY

The intracellular signalling pathways activated by histamine (PKC, intracellular calcium signal, cAMP/PKA pathway) are involved in neuronal plasticity. Histamine produces a long-lasting increase of neuronal excitability in hippocampal pyramidal neurons, with an increase of cell firing (Selbach et al., 1997) by H2 receptor activation. However, the H1 receptor strongly potentiates the effect of the H2 receptor, likely by activation of intracellular calcium signalling and calcium/calmodulin subunit that potentiates the H2R-activated adenylate cyclase (Garbarg and Schwartz, 1988; Bakker et al., 2004). Another coincidence detector for synaptic plasticity is the NMDA receptor. The NMDA receptor plays a major role in several forms of synaptic plasticity, by increasing intracellular calcium levels and activating calcium-dependent kinases (Luscher and Malenka, 2012). The H1 receptor both increases NMDAR-mediated currents by reducing the magnesium block (Payne and Neuman, 1997) (likely through PKC phosphorylation (Chen and Huang, 1992)) and increases intracellular calcium levels, playing a potential role for long-term plasticity. The H2 receptor activates the cAMP/PKA pathway (moreover synergistically amplified by the H1 receptor) that can alone induce NMDAR-independent synaptic potentiation in hippocampal slices (Frey et al., 1993). Potentiation of the NMDA receptor can also occur by direct action of histamine on the polyamine-binding site of NMDA receptors (Bekkers, 1993; Brown et al., 1995b) (or, as recently suggested, by a different binding site (Burban et al., 2010)). However, this action is restricted to GluN1/N2B-containing receptors (Williams, 1994) and occurs only in conditions of low tissue pH (7.2), like during high firing frequency, epileptic attacks, ischemia, hypoglycemia, and tetanic stimulation in slice (Brown et al., 1995a; Saybasili et al., 1995; Yanovsky et al., 1995). AMPA receptor (AMPAR) trafficking regulates receptor expression on the synaptic membranes. It is involved in synaptic plasticity processes regulated by calcium/calmodulin kinases and PKA (Esteban et al., 2003; Luscher and Malenka, 2012). Histamine increases the synaptic insertion of particular AMPAR subunits (GluR2L and GluR1) (Qin et al., 2005). AMPAR subunits are differentially recruited during activity-dependent plasticity: GluR2-lacking AMPA receptors (formed by GluR1 homodimers) underlie the short-term changes of plasticity, whereas the GluR2-containing AMPA receptors

increase in the long-term (Isaac et al., 2007). Notably, histamine-triggered insertion of the GluR2L and GluR1 subunits at synaptic level is mediated by two different pathways: the Ras-MEK-ERK signal and the Ras-Pi3K-PKB signals, the former required for GluR2L synaptic trafficking and both required for GluR1 (Qin et al., 2005). Therefore, histamine may shape the progression of short- and long-term plasticity. The contribution of the specific histamine receptor subtypes has never been explored.

In conclusion, the main effect of histamine concerns the neuromodulation of neuronal properties and plasticity, by facilitation or prevention of activity-dependent plasticity processes. Moreover, histamine affects synaptic glutamate receptors and mechanisms of vesicle release, by which it is likely to modulate synaptic transmission.

HISTAMINE H1 AND H2 RECEPTOR-MEDIATED MODULATION OF GLUTAMATERGIC SYNAPTIC STRENGTH IN THE HIPPOCAMPUS - (PAPER III)

Summary

Synaptic strength can be defined as the product of vesicle release probability, number of release sites, and quantal size (i.e., the postsynaptic response to presynaptic neurotransmitter release of a single vesicle) (Kerchner and Nicoll, 2008). Changes of synaptic strength occur in response to synaptic activity perturbations (for instance, adaptation to synaptic inactivity (Turrigiano et al., 1998) or activity-driven long-term potentiation (Frey and Morris, 1997)). Miniature excitatory postsynaptic currents (mEPSC, or minis) are generated by the spontaneous release of individual synaptic vesicles and provide a good measure of synaptic strength (Kerchner and Nicoll, 2008). Neuromodulators, such as dopamine, have been found to change mEPSCs. In hippocampal cultured neurons, the D1 receptor activation increases mEPSC frequency, likely by the recruitment of AMPA GluR1 subunits in silent synapses (Smith et al., 2005). As previously described, histamine can modulate activity-dependent plasticity. Our aim is to study the neuromodulatory effect of histamine on hippocampal synaptic strength. We recorded mEPSCs by whole-cell patch clamp of CA1 pyramidal neurons in slices from Sprague Dawley rats.

We found that the activation of the H1 receptor reduced the frequency, but not amplitude, of mEPSCs recorded from CA1 pyramidal neurons. Instead, the H2R agonist did not change mEPSC frequency or amplitude. The selective change in frequency with no effect on the amplitude of mEPSCs suggests two possible mechanisms of modulation (Kerchner and Nicoll, 2008): reduction of presynaptic vesicle release probability; internalization of postsynaptic AMPA receptors with consequent silencing of the synapses. The paired-pulse facilitation at CA3-CA1 synaptic connections (which measures the release probability of action-potential evoked transmission) was not affected by the H1R agonist. Although this suggests to exclude a presynaptic modulation of mEPSC frequency, the H1 receptor may

selectively modulate the probability of release of spontaneous, but not evoked, glutamatergic transmission. For instance, spontaneous mEPSC frequency can be selectively modulated by the activation of apolipoprotein E receptor2 (ApoER2) signalling and release of calcium from presynaptic intracellular storages, with no effect on evoked transmission (Bal et al., 2013). Similarly to the ApoER2, the H1 receptor leads to an increase of intracellular calcium via the PLC/IP3 cascade, possibly explaining the presynaptic H1R modulation of spontaneous vesicle release. Notably, it has been found that the H1 receptor modulates the vesicle release machinery. Indeed, in chromaffin cells, H1R activation increases the neurotransmitter release by PLC activation, priming of the synaptic vesicles, and increase of the readily releasable pool (Bauer et al., 2007). We should note that the mechanisms described lead to an increase of vesicle release, while we observed that the H1R agonist decreased mEPSC frequency, suggesting a reduction of release probability. The H1 receptor is known to be localized on the postsynaptic side, however some data suggest that histamine can affect presynaptic release of noradrenaline in the hypothalamus by H1 receptors (Bealer, 1993). To date, the demonstration of a presynaptic localization of the H1 receptor is lacking. Alternatively, postsynaptic H1 receptors may trigger a retrograde signal mediated by endocannabinoids (Leurs et al., 1994) that would reduce presynaptic release probability. However, preliminary data from our lab seem to exclude that the effect of the H1R agonist could be blocked by the endocannabinoid CB1 receptor antagonist AM-251. The H1R-mediated reduction of mEPSC frequency may also be explained by internalization of postsynaptic AMPA receptors and silencing of synapses. AMPAR trafficking is likely to be involved in the modulation of mEPSCs by dopamine D1 receptors (Smith et al., 2005). Currently, we are working to test the hypothesis that the change of mEPSC frequency has a postsynaptic origin: in this case, the block of postsynaptic intracellular signalling triggered by the H1 receptor (for instance, by using a PKC inhibitor in the patch pipette) would prevent the reduction of mEPSC frequency induced by the H1R agonist. The AMPAR internalization will be tested by isolating the postsynaptic density from tissue exposed to the H1R agonist: we hypothesise that the H1R agonist will induce a reduction of the AMPAR levels in the postsynaptic density fraction.

Implications

The H1 receptor, but not the H2 receptor, can affect glutamatergic synaptic strength. The differential modulatory effect of the histamine receptor subtypes has been already observed on neuronal properties and activity-dependent plasticity. As previously described, the main effect of histamine involves neuronal firing properties (Haas et al., 2008). Here we evidenced that the H1 receptor can modulate glutamatergic synaptic strength with no clear changes of evoked transmission, potentially predisposing the synapse to activity-dependent plasticity. This phenomenon is called metaplasticity (MacDonald et al., 2007). An example of metaplasticity is the facilitation of the LTP observed by the H3R antagonist clobenpropit (paper II: Femenia et al., 2015) or by histamine (Brown et al., 1995b). Changes of spontaneous neurotransmission (like the mEPSC modulation by the H1 receptor) may be an

important mechanism of metaplasticity. NMDAR-mediated minis suppress local dendritic protein synthesis and participate to mechanisms of synaptic homeostasis (Sutton et al., 2006; Sutton et al., 2007). Those mechanisms are also involved in the fast antidepressant response of ketamine, by disinhibition of dendritic protein synthesis of BDNF in the hippocampus (Autry et al., 2011). We show that the H1 receptor reduces mEPSC frequency in the hippocampus, but it has not been explored whether the H1R-mediated modulation is relevant for synaptic homeostasis in the long term. However, testing the antidepressant properties of the H3R antagonists has indicated that hippocampal H1 receptors mediate the antidepressant response in the FSL rats (paper II: Femenia et al., 2015). Moreover, the H1 receptor agonist induced an antidepressant response in mice (Lamberti et al., 1998). The histaminergic modulation of homeostatic plasticity may represent a novel frontier to interfere with mechanisms relevant for a therapeutic perspective.

CONCLUSIONS

The identification of altered explorative strategies and reactive coping style in the FSL rat contributes to the understanding of the FSL behavioural profile and supports its face validity as model of depression. Differences in explorative strategies and coping styles may explain the behavioural variability in animal testing and drug response, and may constitute the basis to understand the resilience/vulnerability to stress.

The high emotional reactivity and reactive coping style of the FSL rat are characterized by risk-assessment behaviours and avoidance/immobility. This exploratory profile matches with the anxiety-like behaviours (increased latency to reach the food, preference for the dark compartment, less social interaction) that we observed in traditional tests (i.e., novelty suppressed feeding, light/dark box, and social interaction tests). However, in the MCSF test, FSL rats performed risk taking to the same extent as SD rats (or even more when re-exposed to the MCSF arena). This suggests that the setting and duration of the test may allow the animal to adopt adequate explorative strategies and reach the level of SD rats' performance.

To our knowledge, the MCSF, novel cage and home cage change tests (used for the evaluation of explorative strategies and coping styles) have not been explored with regard to their predictive validity for drug antidepressant effects. However, the immobility rate in the forced swim test could be considered as an expression of coping style, suggesting that the assessment of coping styles may be useful to evaluate antidepressant properties. Given the positive results of clobenpropit in the forced swim test, it may be worthwhile to study the effects of both clobenpropit and classical antidepressants on the performances measured in the MCSF and novel cage tests.

Clobenpropit's antidepressant properties and its impact on plasticity should be followed up by studies investigating its long-term effects. We provide evidence that the antidepressant action is mediated by activation of hippocampal H1 and H2 receptors, likely through an increase of histamine release. This mechanism is likely to trigger neuronal plasticity in FSL rats, and, accordingly, we found an increase in GluN2A expression and enhancement of hippocampal plasticity induced by clobenpropit. However, we do not know whether the effect of clobenpropit is dependent on new protein synthesis. It is relevant to note that the crucial step for clobenpropit's antidepressant mechanism seems to be the activation of the H1 and H2 receptors, but not the H3R antagonism by itself. We showed that the H1 receptor is involved in the modulation of synaptic strength in Sprague Dawley rats; potentially a similar modulation is maintained in FSL rats. The mechanisms and relevance of such modulation are still unclear. The H1 receptor may affect synaptic homeostasis, which could represent a new aspect of the histaminergic neuromodulation.

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