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Investigating the role of the central melanocortin system in stress and stress-related disorders

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ABSTRACT

The melanocortinergic neural circuit, known for its influence on energy expenditure and feeding behavior, also plays a role in stress and stress-induced psychiatric disorders, including anxiety and depression. The major contribution is given by the melanocortin-4 receptor (MC4R) subtype, highly expressed in brain regions involved in the control of stress responses. Furthermore, the MC4R appears to profoundly affect the activity of the hypothalamic-pituitary-adrenal (HPA) axis, and it has been also highlighted a functional and anatomical interaction with the corticotropin-releasing factor (CRF), an important mediator of stress and stress-related behaviors. The MC4R agonists seem to exacerbate stress-inducing anxiety- and depressive-like behavior, while MC4R antagonists have been demonstrated to mitigate such disorders, as shown in several preclinical behavioral tests. The evidence collected in the present review suggests that the melanocortin system, through the MC4R, could possibly modulate behavioral responses to stress, suggesting the use of MC4R antagonists as a possible novel treatment for anxiety and depression induced by stress.

Abbreviations: HPA, hypothalamic-pituitary-adrenal; CRF, corticotropin-releasing factor; AVP, vasopressin; PVN, paraventricular nucleus of the hypothalamus; MC4R, melanocortin-4 receptor; ACTH, adrenocorticotropic hormone; POMC, pro-opiomelanocortin; α-MSH, α-melanocyte-stimulating hormone; MCRs, melanocortin receptors; CNS, central nervous system; ARC, arcuate nucleus of the hypothalamus; AgRP, agouti-related protein; NPY, neuropeptide Y; CSDS, chronic social defeat stress; MTII, melanotan-II; EPM, elevated plus maze; FST, forced swim test; ICV, intracerebroventricular; MeA, medial amygdala; NAc, nucleus accumbens; VTA, ventral tegmental area; MSN, medium spiny neurons; PRCP, prolylcarboxypeptidase; BNST, bed nucleus of the stria terminalis; OF, open field; SPS, single prolonged stress; DRN, dorsal raphe nucleus.

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1. Introduction

1.1. Stress

Stress, a primary risk factor for many neuropsychiatric diseases, including depression, mood, and anxiety is characterized by a physiological reaction to environmental changes, and can be positive and pro-adaptive or, under certain circumstances, negative and maladaptive [1–4]. The activation of the autonomic nervous system and of the hypothalamic-pituitary-adrenal (HPA) axis represents the hallmark of the stress response, and the "fight or flight" reaction is typically recognized as a behavioral and physiological feature in response to a threat, resulting from dangerous conditions [1,2,5]. During stress, the activation of the sympathetic nervous system leads to noradrenaline secretion from the highly distributed synapses and adrenaline release from the adrenal medulla [1,2,5]. Under situations perceived as stressful, different neuronal circuits are activated in the brain to respond to the requests, and specifically, the corticotropin-releasing factor (CRF) and vasopressin (AVP) represent the two neuropeptides essentially involved in both the behavioral and the metabolic components of stress. These peptides are produced in the parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus and activate the HPA axis, resulting in the release of corticosteroid hormones from the adrenal cortex [1].

A normal functioning of the stress response is required for survival of the organism, but an aberrant or excessive adrenocortical or autonomic activity might be harmful for the health of individuals. Indeed, during exposure to stressful experiences, the organism responds releasing chemical mediators such as catecholamines, which lead to several effects including an enhanced blood pressure, heart and respiratory rate, core temperature and intermediate metabolism, and promote adaptation to the stressor, maintaining the homeostasis. However, a chronic elevation of the same mediators might lead to pathophysiological conditions, increasing the risk of cardiovascular adverse events [2,5].

Generally, the processes that characterize an effective stress response (rapidly activated when needed and appropriately extinguished in the absence of the stressor) are collectively defined as "allostasis", while if the response is dysregulated, excessive or prolonged, there is the establishment of a condition known as "allostatic load or overload" [1,5,6].

A great interest of the research in stress and stress-related psychiatric disorders has been directed towards the neurobiological determinants of resilience, the ability of individuals to avoid the negative social, psychological, and biological consequences of excessive stressful events, that would compromise their psychological or physical well-being. Resilience is based on several factors, including the genetic background, environmental influences, and adverse events during life, which may induce epigenetic changes and consequently affect the individual response to stress [2,3,6,7].

As will be discussed in the next sections, among the central mediators of the stress response, the melanocortin system, mainly through the melanocortin-4 receptor (MC4R), has been demonstrated to play an important role in the regulation of the HPA axis activity, with potential implications in the treatment of stress and stress-related disorders [8,9]. Thus, the development of MC4R antagonists could represents a useful pharmacological strategy for the treatment of these pathological conditions.

1.2. The melanocortin system

The central melanocortin system is known for its implications in homeostatic and/or non-homeostatic aspect of food consumption [10,11], as highlighted by the recent approval of setmelanotide, an MC4R agonist, for the treatment of obesity caused by rare genetic disorders [12]. However, this system is also highly involved in neuropsychiatric disorders, especially those in which stress plays a key role, such as anxiety and depression, characterized by pathological alterations of emotional processes commonly accompanied by cognitive impairment, altered memory and reduced social interaction in humans and animals, significantly impairing the quality of life [13–15].

The stress response system is a complex network, dependent on feedback regulation and, particularly, on the functioning of the HPA axis. Anxiety and depression are very intricate conditions with a still unclear pathogenesis, with a possible mutual involvement on HPA axis response.

The activation of HPA axis consists in the secretion of the CRF from the PVN into the circulation, which in turn stimulates the release of adrenocorticotropic hormone (ACTH) by the pituitary, whose precursor is Pro-opiomelanocortin (POMC) [13,16,17].

POMC is the same precursor of α-melanocyte-stimulating hormone (α-MSH), endogenous agonist of melanocortin receptors (MCRs). Localization of POMC neurons in the central nervous system (CNS) is in the nucleus of the tractus solitarius and in the arcuate nucleus of the hypothalamus (ARC). In the ARC, close to POMC cells, agouti-related protein (AgRP) and the neuropeptide Y (NPY) neurons are localized and produce AgRP (endogenous antagonist of MCRs) and NPY (α-MSH inhibits NPY-induced feeding and NPY inhibits the α-MSH production), respectively [17–20].

The melanocortin system comprises five receptors, all members of the superfamily of G-protein-coupled receptors, discovered since the 1990 s [21–23], and, precisely, the MC1R, MC2R and MC5R are mainly found in the periphery, while MC3R and MC4R are particularly abundant in the CNS [17,24].

Notably, the MC4R has a more widespread expression in the CNS compared to the MC3R, and is predominantly expressed in several hypothalamic areas, indicating a principal role in energy homeostasis. The MC4R is also strongly expressed in the brainstem and moderately in the cortex, amygdala, striatum, hippocampus, thalamus, and spinal cord. In less quantity, the MC4R is present in the peripheral nervous system, suggesting that more effects are mediated by this receptor beyond the control of energy expenditure and feeding behavior [17,21,24–26].

The melanocortin pathway interacts in the hypothalamus with other crucial neuropeptides and hormones that promote the processing of POMC and are strictly involved in stress mechanisms [27–31]. Mice exposed to chronic social defeat stress (CSDS), a protocol of chronic stress able to repeatedly activate HPA axis and to induce a wide range of responses like anxiety, depression-like behavior and social avoidance [32], demonstrated that the combination of decreased leptin levels plus central leptin resistance, along with decreased melanocortin signaling, attenuated depressive and anxiety symptoms [31]. In this context, intraperitoneal administration of the MC4R agonist Melanotan-II (MTII), has led to an increase in social avoidance and depressive like-behavior in mice exposed to CSDS compared to control animals [31]. Conversely, MC4R-null mice showed an improvement in mood symptoms, assessed by several tests to evaluate anxiety-like (exploration of the open arm of the elevated plus maze (EPM) [33,34]) and depressive-like behavior (immobility time in the forced swim test (FST) [35,36]) and less social avoidance [31]. Therefore, these results suggest how the melanocortin signaling could possibly regulate behavioral responses to chronic stress through the MC4R.

The aim of this review is to summarise the literature regarding the influence of the melanocortin system in stress response and in the susceptibility of developing psychiatric disorders. Studies and hypothesis related to the role of the MC4R on stress, anxiety and depression will be evaluated, highlighting its impact in animals and in humans and in the future development of possible new anxiolytic and antidepressant therapies targeting the MC4R.

1.3. Search strategy

For the design and development of this narrative review, preclinical studies up to year 2022, focusing on the role of the melanocortin system

(mostly of the MC4R) on stress and stress-related psychiatric conditions, such as anxiety and depression, were selected and carefully reviewed. Additionally, clinical studies relevant for the same topic were considered to be included, even though a limited number of publications were found.

Only studies that were published in English were considered for inclusion. The selection of peer-reviewed publications was conducted in two electronic databases, PubMed and Scopus.

The following search terms (Keywords) were used in the databases:

- *Melanocortin System* (melanocortin system* OR MC4R* OR α-MSH* OR AgRP* OR POMC* OR MC4R antagonists*) AND
- *Stress* (stress* OR CRF* OR anxiety* OR depression*)

Initially, the titles and abstracts of the total number of articles identified through the databases' search were screened by the authors, who selected the studies concerned with the role of the MC4R in stress, anxiety and depression, principally conducted in rodents and humans. Secondly, if the articles were considered eligible to be included, their full-text was obtained and carefully examined by the authors.

In total, 142 articles were selected as references.

2. The role of melanocortin pathway in stress

2.1. The melanocortin system affects stress response selectively via the MC4Rs

The MC4R has been recognized as the main mediator of the melanocortin system influence on the stress response and negative emotional states, including anxiety and depression [8,9], identifying the MC4R as a potential target for the treatment of these psychiatric conditions. The MC4Rs are expressed in the limbic system and, particularly, in several nuclei of the amygdala, such as central and basolateral nuclei, lateral septal nucleus, hippocampus and entorhinal cortex [26]. The localization of the MC4R in the brain suggests an important participation of this receptor in promoting negative emotional states [8,9]. Moreover, the MC4R, in contrast to the MC3R, has been detected in the PVN, where it is supposed to regulate the activity of the HPA axis, via AVP and CRF neurons [8,26].

Initial evidence linking the MC4R to the regulation of HPA axis activity and stress response came from preclinical studies that revealed a stimulatory activity on grooming behavior, following administration of α-MSH and ACTH [37–41]. Grooming represents a rodent behavioral response to stressful conditions, characterized by activities directed to the animal body surface, like face washing, licking, scratching, body and genital grooming [42].

Grooming stimulated by intracerebroventricular (ICV) injections of MCR agonists can be attenuated by the non-selective MC3R/MC4R antagonist SHU9119, which also blocks grooming evoked by exposure to a novel environment [37].

The influence of α-MSH on grooming is principally due to its agonist activity on the MC4Rs, as demonstrated in the study of Adan et al., in which grooming behavior induced by MCRs agonists, was positively correlated with a greater affinity and potency for the MC4R, rather than for the MC3R [37]. Subsequent studies supported the selective involvement of the MC4R in this behavioral effect, excluding the participation of the MC3R. In fact, the rise of ACTH and corticosterone concentrations in plasma, and the excessive grooming elicited by ICV administrations of the non-selective MC3R/MC4R agonist $ACTH_{1-24}$ were inhibited by its co-injection with the MC3R/MC4R antagonist SHU9119 and with the selective MC4R antagonist [D-Arg^8]$ACTH_{4-10}$, while no effect on HPA axis activity and on grooming was observed after the administration of the selective MC3R agonist Lys-γ2-MSH [43]. Furthermore, the MC4R agonist MTII increased grooming in wild type rats, but had no robust activity in rats with a $MC4R^{K314X}$ mutation, which results in a loss of function of this receptor, supporting that grooming behavior is principally mediated by the MC4Rs, and not by the MC3R subtype [44].

2.2. The impact of stress on MC4R expression in the brain

Stress procedures have been demonstrated to affect the expression and activity of the central melanocortin system, under different experimental conditions.

Rats exposed to the electric foot shock stress paradigm, able to consistently activate the HPA axis of these animals, revealed a significant increase in POMC and MC4R mRNA in the hypothalamus and in the amygdala [45], brain regions both involved in the regulation of emotional- and fear-related behaviors [46].

Activation of the central melanocortin pathway was also investigated analyzing c-Fos mRNA expression (a marker of neuronal activation) in the rats' brain, in response to restraint and FST, which are classified as psychological/emotional stressors. After exposure to 30 min of restraint and 10 min of FST, c-Fos expression was robustly induced in the ARC, PVN and medial amygdala (MeA), one of the principal extra-hypothalamic targets of POMC and AgRP projections, which expresses high levels of MCRs [18,26,47,48]. Double-labeling in situ hybridization analysis revealed that the greater amount of c-Fos-positive cells were predominantly POMC-expressing neurons, in comparison to the number of activated AgRP neurons, after exposure to the same stressors [48]. Considering that POMC neurons in the ARC provide melanocortin inputs to the amygdala [18] and that the MeA abundantly contains α-MSH immunoreactive fibers and MC4Rs [18,26,47], Liu et al. extended the previously described study. They investigated the responsiveness of MC4Rs-expressing neurons in the MeA of acutely restrained rats, observing that this stressor promotes the activation of MC4R-positive cells in this brain region, as indicated by c-Fos mRNA induction [47].

These results show that POMC neurons in the ARC are rapidly recruited after exposure to acute stressors and then might act targeting the MC4Rs expressed in the MeA, which represents a key component in mediating the stress excitatory responses activated by the central melanocortin system [47,48].

Accordingly, it was also demonstrated that both stress-induced anorexia and corticosterone release, in response to the acute restraint stress, can be prevented by administration of the MC4R antagonist SHU9119 directly infused in the MeA [47,48].

The effect of stress on MC4Rs expression and on the activity of MC4Rs-expressing neurons might be also dependent on the intensity and duration of the stressor, as reported by Chagra et al. [49] using a dual-label immunohistochemical analysis, to investigate the co-localization of c-Fos with the MC4R, in several rat hypothalamic regions, after exposure to acute and repeated restraint stress. The animals were placed in a plastic restrainer, where only limited movements were allowed. The results revealed an upregulation in the number of c-Fos- and MC4R-expressing cells in the lateral hypothalamic area of acutely stressed rats, while a significant decrease in c-Fos- and MC4R-positive neurons was detected in the ARC, compared to both control and acutely stressed groups [49], probably indicating a desensitization of the MC4R pathway after repeated stress conditions.

However, a later study reported a different result: the rats exposed to chronic restraint stress for 12 days showed an increase in the MC4R mRNA expression in the ARC, compared to control rats (not exposed to stress). In the same experiment, MC4R expression was not influenced by treatment with RU486, an antagonist of glucocorticoid receptors, 1 h before the stressful procedure, suggesting that a chronic stressful condition might promote an upregulation of MC4Rs, that is not correlated with the activation of glucocorticoid receptors [50].

2.3. The interaction of MC4R with CRF neurotransmission

The melanocortin (via MC4R) and the CRF systems were revealed to display functional similarities and anatomical overlapping with

important implications in feeding and HPA axis regulation. The expression of MC4R mRNA is observed in the medial-parvocellular subdivision of the PVN, where CRF-positive neurons are also present [51–53]. More specifically, the MC4R is expressed in a subset of CRF-positive neurons, particularly concentrated in the ventromedial part of the parvocellular PVN, as demonstrated by double-labeling in situ hybridization [53], in accordance with studies evidencing α-MSH neuronal projections in this subdivision of the PVN [18,54]. The anatomical co-expression of MC4R and CRF is accompanied by a functional interaction of these two systems. The phosphorylated cAMP response element-binding protein (a marker of neuronal activation) is induced in more than 50 % of the CRF neurons in the PVN after central administration of α-MSH [55], and ICV administration of the MCR agonist MTII leads to a rapid and dramatic increase of CRF gene expression in the PVN [53].

The ICV injection of MTII also produces a significant rise in corticosterone plasma levels, which is inhibited by pretreatment with both the MC4R antagonist HS014 and the CRF receptor antagonist α-helical-CRH9–41 [53]. These in vivo findings are supported by in vitro analyses, in which the application of α-MSH on rats' hypothalamic explants results in a significantly enhanced release of CRF and AVP [56].

The interplay of melanocortin and CRF is not limited to the influence on stress response, but has additional implications on stress-induced anorexia, as highlighted by the study of Kawashima et al. [57]. The authors observed that the anorectic effect, elicited by central administration of NDP-MSH (α-MSH synthetic analogue), was accompanied by increased CRF mRNA hypothalamic levels. The NDP-MSH-induced suppression of food intake was then abolished by pretreatment with astressin, a non-selective CRF receptor antagonist, and was also absent in CRF knockout mice [57]. Overall, these studies highlight that the activation of the CRF system might represent a mechanism by which the melanocortin pathway, predominantly through the MC4R, stimulates the HPA axis and participates in the stress response.

3. The role of the melanocortin system in anxiety

Anxiety is a negative emotional state, known to be controlled by several distributed brain pathways. However, the beginning, sustainment and perseverance of this neuropsychiatric condition remain poorly understood.

The excessive HPA activity has been implicated in several stress-related conditions, including depression and anxiety. Indeed, as previously stated, elevated self-grooming behavior was reported to be induced in rats by α-MSH, a response correlated to anxiety-like behavior and modulated by the limbic circuitry. This system includes the hypothalamus and the amygdala, brain regions involved in the manifestation of anxiety disorders, fear, aggression and in which the MC4R is highly expressed [37,38,43,58–60]. In these two brain regions there is an increase in the MC4R mRNA after exposure to stress [45].

In 1996, Gonzales et al. found that ICV injection of α-MSH in the ventromedial hypothalamus and medial preoptic area, both involved in social and emotional behaviors [61,62], increases aggressive behavior, reduces exploration and the time spent in the open arms of the EPM, indicating anxiogenic outcomes [63]. Similarly, Kokare et al., using the EPM test, observed that rats exhibited a reduction in both time and number of entries in the open arms when treated with α-MSH, directly injected in the amygdala. α-MSH was also able to reverse the anxiolytic effect of NPY, highlighting how these two neuropeptides not only act as functional antagonists in the context of food intake, but also interact in the regulation of anxiety behaviors in the amygdala [64]. These opposite behavioral effects are consistent with the existence of an extensive anatomical association between NPY nerve fibers and POMC-producing neurons [65]. Specifically, NPY fibers are in synaptic contact with POMC cell bodies [66], and NPY appears to negatively regulates both α-MSH content and POMC mRNA levels in the hypothalamus [20,66]. Interestingly, the amygdala shows a dense population of α-MSH as well as NPY-positive fibers [67–69], and expression of both MC4R and NPY-Y1 receptor is observed in this brain region [59,70,71].

Considering the known anxiolytic effect associated to the activation of the NPY-Y1 receptor in the amygdala [70,72–74], and that this effect is attenuated by a prior intra-amygdala α-MSH administration [64], these behavioral and neurobiological considerations suggest that the endogenous NPY and α-MSH neurotransmissions interact in the amygdaloid complex in order to antagonistically modulate anxiety-like behaviors in rodents.

Besides NPY, even the activation of a subpopulations of hypothalamic AgRP neurons, that release the AgRP neuropeptide (MCR antagonist), promotes a reduction in anxiety levels in mice, as assessed performing several behavioral tests. This response might be related to an influence on midbrain dopamine neuronal activity [75–77], since both α-MSH and AgRP are involved in the mesocorticolimbic and mesostriatal dopamine transmission and consequently affect behavior [78]. Indeed, α-MSH generally appears to stimulate dopaminergic neurotransmission, considering that its injection in the ventral tegmental area (VTA) increases dopamine turnover [79] and that the ablation of AgRP neurons results in enhanced extracellular dopamine in the Nucleus Accumbens (NAc) [75]. However, contrasting data exist evidencing that central injection of AgRP increases c-Fos immunoreactivity in VTA-dopamine neurons and dopamine turnover in the medial prefrontal cortex [80], and that α-MSH decreases the strength of excitatory synapses on D1 dopamine receptor-expressing NAc medium spiny neurons (MSN) [81]. Thus, the influence of α-MSH and AgRP on the mesocorticolimbic dopamine system is complex with divergent findings obtained throug different studies (see review [78]), and additional investigations are necessary to clarify how these neuropeptides affects dopamine neurotransmission and related-behaviors.

Even though not completely understood, the neural mechanisms underlying the anxiogenic effects of α-MSH might involve the GABAergic neurotransmission. Indeed, diazepam and muscimol, drugs that promote the activity of $GABA_A$ receptors, reduced the anxiety-like behaviors induced by ICV injections of α-MSH, while bicuculline, a $GABA_A$ receptor antagonist, enhanced α-MSH-induced anxiety [82]. Moreover, GABAergic neurons express the prolylcarboxypeptidase (PRCP), the enzyme responsible for the inactivation α-MSH [83].

The involvement of GABAergic neurotransmission in α-MSH-induced anxiety-like behaviors was also highlighted by a recent work, in which it was demonstrated a critical role for GABAergic projections from AgRP neurons to the MC4Rs-expressing neurons in the dorsal bed nucleus of the stria terminalis (BNST), in the modulation of high fat diet-related anxiety- and depressive-like responses [84]. Specifically, it was observed that a prolonged exposure to high fat diet led to a decrease in both basal activity and hyperexcitability of AgRP neurons, and that the desensitization of this populations of neurons was correlated to the manifestation of anxiety-depressive responses. To deeply explore the neuronal circuitries implicated in these behavioral phenotypes, the authors genetically inactivated GABAergic signaling in AgRP neurons of mice (AgRP-GABA knockout mice), observing an increased anxiety and depression in these animals, which were prevented by chronic infusion of bretazenil (a $GABA_A$-receptor partial agonist) in the dorsal BNST [84]. Interestingly, AgRP neurons were demonstrated to establish monosynaptic connections with the downstream MC4Rs-expressing neurons of the dorsal BNST, and these neurons also post-synaptically express the $GABA_A$-receptor α5 subunit, which is significantly decreased after 5 weeks of high fat diet. Altogether, these findings support that GABAergic signaling within MC4R-dorsal BNST neurons modulates high fat diet-associated anxiety and depression through $GABA_A$-receptor-α5 subunits, and that the manipulation of the $AgRP^{ARC} \rightarrow MC4R^{dorsal\ BNST}$ neuronal circuitry might be associated to beneficial behavioral outcomes in obesity-related comorbidities [84].

Additionally, the injection of α-MSH or RO27–3225 (selective MC4R agonist), into the central nucleus of the amygdala, potentiated the caffeine anxiety-like activity, suggesting also a contribution of

adenosine receptors blockade for anxiety induced by melanocortin system [85]. The possible interaction is also reported by [86], in which adenosine A_{2A} receptor knockout mice presented a markedly increase in α-MSH concentration in the amygdala (and not in hypothalamus), signaling a loss of regulation of the normal secretion, due to the disruption of the A_{2A} receptor gene.

Consistently with the role of the melanocortin system and in particular of the MC4R in the amygdala, subsequent studies investigated if the administration of the MC4R antagonists in this brain region was able to exert anxiolytic activities. Liu et al. observed that intra-MeA microinjection of the highly selective MC4R agonist, the Cyclo (β-Ala-His-D-Phe-Arg-Trp-Glu)-NH2, elicited anxiogenic-like responses, while administration of the MC4R antagonist SHU9119, before exposure to the acute restraint stress, significantly attenuated stress-induced anxiety evaluated by the EPM [47,48]. Accordingly, Iemolo et al. found that injection of the same compound into the central nucleus of the amygdala prevented the anxiety states elicited by the pituitary adenylate cyclase-activating polypeptide, known to promote stress and anxiety-related behaviors via PAC1 receptors [87].

SHU9119 is a MC4R antagonist, but it also binds the MC3R and MC5R. However, it is believed that its anxiolytic effect, when injected in the central nucleus of the amygdala, is predominantly mediated by the MC4Rs, considering the low expression of the MC3Rs in this brain region [23,47,87].

Conversely, HS014 and HS024 are two selective MC4R antagonists [88], firstly tested by Kack et al. [88,89], whose ICV injection in rats did not influence any parameters in the EPM and in the open field (OF) test [88], a paradigm used to analyze locomotor activity and anxiety-like behavior [90], when compared to control rats [64,88]. Meanwhile the intra-amygdala co-administration of HS014 with NPY or the NPY Y1 and Y5 receptor agonist [Leu31, Pro34]-NPY elicited significant anxiolytic effects [64].

The same MC4R antagonist demonstrated to potentiate the anxiolytic effect of ethanol and to prevent the ethanol withdrawal anxiety in the EPM [91], and to reverse the anxiety-related phenotypes in rats socially isolated for 6 weeks, by increasing social interaction and the time spent in the open arms in the EPM, after the isolation, without influencing these parameters in group-housed rats [92]. In addition to the central injection, HS014 has been tested by nasal infusion in an animal model of post-traumatic stress disorder induced by exposure to single prolonged stress (SPS), since excessive and/or prolonged activation of the HPA axis affects the development of this disorder [93]. In this study HS014, prior to SPS, was able to significantly increase the time and the entries in open arms of EPM, to enhance frequency of unprotected head dips, to extend duration of risk assessment and, accordingly, the rodents spent more time in central zone of the OF compared to rat treated with vehicle [93]. Furthermore, HS014 proved to be effective when infused 30 min prior to SPS, meanwhile, when the compound is infused immediately after SPS procedure, it failed to produce any anxiolytic-like effects, in contrast to NPY. This suggests that the activation and the involvement of MC4R could be only at the first phase of traumatic stress and the MC4R might need to be antagonized prior to the beginning of the stress [94].

The acute treatment with the antagonist HS014 demonstrated to reduce mRNA levels of tyrosine hydroxylase in the locus coeruleus. This enzyme is involved in norepinephrine biosynthesis [94] and stressful events lead to a marked increase in norepinephrine release in several rat brain areas. Among them, there is the locus coeruleus, evidencing as this event may be closely associated with anxiety and fear [95].

Evidence of the melanocortinergic neural circuit in exerting anxiogenic-like effects was also showed by ICV injection of α-MSH in rats performing the Vogel conflict test [96], an experimental method to determine anxiolytic characteristics of compounds [97]. The injection in thirsty rats decreased the number of licking periods addressing to α-MSH anxiogenic properties [96].

The same test was used by Chaki et al., obtaining the same results: ICV administration of α-MSH and MTII, a peptidomimetic MC4R agonist, reduced the number of punished licking periods, indicating their anxiogenic-like activity in rats [98]. In the same work, it was explored the possibility that the peptidomimetic MC4R selective antagonist MCL0020 might reveal an anxiolytic effect. Indeed, this compound significantly attenuated the swim stress-induced anxiogenic-like behavior in light/dark exploration task, by increasing the time spent in the light area after the stressor, and was also able to revert the stress-induced anorexia, while MCL0020 alone did not affect food consumption [98]. Therefore, this novel MC4R antagonist showed high affinity and selectivity for this receptor compared to the other MCR subtypes [88,98,99] and it was able to attenuate aberrant behaviors, such as anxiety and anorexia induced by stress [98].

In the same laboratories, MCL0129, a potent and selective MC4R non-peptide antagonist was synthesized and, likewise MCL0020, it ameliorated the anxiogenic-like behavior caused by swim-stress in the EPM and in light/dark exploration task. Thus, the animals prolonged the time spent in the open arms and in the light area in the considered procedures, contrasting the aversion of rodents to open spaces [100]. The compound was also tested in the marble-burying paradigm, model of anxiety and obsessive - compulsive disorder [101], proving to decrease the numbers of marbles buried [100]. MCL0129 did not affect rotarod performance in rats, a test used to detect motor coordination and balance in rodents [102], while MCL0129 only at the highest doses (100 mg/kg) inhibited spontaneous locomotor activity [100].

Accordingly, in the social interaction test, an animal test of anxiety [103] performed inside an OF apparatus, the central injection of MCL0129 stimulated social interaction between rats reducing the anxiogenic-like behavior (promoted instead by the injection of MTII), but only if administered for 1 week, while no effects were produced with acute treatment, and the locomotor activity was no affected at the dosage used in the experiment [104].

Among the MC4R antagonists, compound MCL0042 exhibits along with the capacity to block the MC4R, also inhibitory activity toward serotonin transporter due to the high affinity for this target. In fact, subcutaneous administration of MCL0042 markedly and significantly increased the number of licks in the Vogel conflict test and increased the time spent in open arms of EPM, following the exposure to the swim-stress procedure, showing an impressive anxiolytic-like potential without affecting spontaneous locomotor activity [105].

The behavioral effect could also be explained by a potential interaction between MC4R and the serotonergic neurons in the dorsal raphe nucleus (DRN). Indeed, the long-term blocking of the MC4R due to MCL0042 could up-regulate the serotonin transmission, exerting anxiolytic result, in light of the additional ability of this compound to inhibit the serotonin transporter [106]. Notably, DRN is implicated in a complex mood regulation and is rich of serotonergic inputs to limbic system [107]. In this brain region it also projects α-MSH hyperpolarizing MC4R neurons. Bruschetta et al., have recently revealed that infusion of α-MSH induced a decreased neuronal activity of DRN, and that generating mice lacking PRCP selectively in this cerebral area, the elevated α-MSH levels reduced neuronal activation and the serotonin concentration. Moreover, mice developed anxiety- and depressive-like behavior. Afterwards, when PRCP was re-expressed in DRN, the behaviors were completely reverse, highlighting a key role of DRN and melanocortin signaling in the regulation of these neuropsychiatric disorders and an influence in the serotonin system [108].

All together, these investigations support the anxiogenic effects induced by α-MSH through the activation of MC4R, and that pharmacological antagonism of this receptor has the potential to reverse anxiety states in rodents (Table 1).

4. The role of the melanocortin system in depression

Depression is a common and complex medical illness whose pathogenesis involves multiple genetic and biological mechanisms, that

Table 1
Studies performed in rodents to investigate the potential effect on anxiety-like behaviors caused by activation or antagonism of the MC4R.

Subjects	Compound	Site of injection	Behavioral test	Behavioral outcome	Ref.
Female rats	α-MSH	ICV	EPM	↑ aggressive behavior ↓ exploration ↓ time in the open arms	[63]
Male rats	α-MSH	Amygdala	EPM	↓ time and number of entries in the open arms	[64]
Male rats	α-MSH + NPY (or [Leu^{31}, Pro^{34}]-NPY)	Amygdala	EPM	α-MSH ↓ anxiolytic effect induced by NPY (or [Leu^{31}, Pro^{34}]-NPY)	[64]
Male rats	HS014 + NPY (or [Leu^{31}, Pro^{34}]-NPY)	Amygdala	EPM	HS014 ↑ anxiolytic effect induced by NPY (or [Leu^{31}, Pro^{34}]-NPY)	[64]
Male mice	α-MSH or RO27–3225 (selective MC4R agonist)	Central nucleus of the Amygdala	Social interaction	↑ anxiety-like behavior induced by caffeine	[85]
Male rats	Cyclo (β-Ala-His-D-Phe-Arg-Trp-Glu)-NH2 (selective MC4R agonist)	Medial Amygdala	EPM	↓ open arm entries ↓ time spent in the open arms	[47]
Male rats	SHU9119 (MC3R/ MC4R antagonist)	Medial Amygdala	Acute restraint stress + EPM	↓ stress-induced anxiety ↑ open arm entries ↑ time spent in the open arms	[47]
Male rats	SHU9119	ICV	Acute restraint stress or FST + EPM	↓ acute stress-induced anxiety ↑ open arm entries ↑ time spent in the open arms	[48]
Male rats	SHU9119 + PACAP	Central Amygdala	EPM	SHU9119 ↓ anxiogenic effect of intra-CeA PACAP	[87]
Male rats	HS014 (MC4R antagonist) + ethanol	ICV	EPM	HS014 ↑ ethanol induced anxiolytic action. Prevented the ethanol withdrawal anxiety	[91]
Male rats	α-MSH + ethanol	ICV	EPM	α-MSH ↓ ethanol induced anxiolytic action	[91]
Male rats	HS014	ICV	Social isolation and EPM	↑ time spent by the rats in the social interaction ↑ open arm entries ↑ time spent in the open arms	[92]
Male rats	HS014	Intranasal	SPS + EPM	↑ open arm entries ↑ time spent in the open arms	[93, 94]
Male rats	HS014	Intranasal	SPS + OF	↑ time spent in the center of the OF ↓ defecation	[93]
Male rats	α-MSH	ICV	Vogel conflict test	↓ punished licking	[96]
Male mice	MCL0020 (MC4R antagonist)	ICV	Swim stress + light/dark exploration	↑ time spent in the light area after the swim stress	[98]
Male mice	α-MSH	ICV	Vogel conflict test	↓ punished licking	[98]
Male mice	MTII (MC4R agonist)	ICV	Vogel conflict test	↓ punished licking	[98]
Male mice	MCL0129 (MC4R antagonist)	Subcutaneous/oral	Swim stress +light/dark task	↑ time spent in the light area after the swim stress	[100]
Male mice	MCL0129	Subcutaneous	Marble-Burying	↓ marble-burying behavior	[100]
Male rats	MCL0129	Oral	Swim stress + EPM	↑ time spent and entries in the open arms after the swim stress	[100]
Male rats	MCL0129	Oral	Social interaction (in OF)	↑ time spent in social interaction	[104]
Male rats	MTII	ICV	Social interaction (in OF)	↓ time spent in social interaction	[104]
Male rats	MCL0042 (MC4R antagonist)	Subcutaneous	Vogel conflict test	↑ number of licks	[105]
Male rats	MCL0042	Subcutaneous	Swim stress + EPM	↑ time spent and entries in the open arms after the swim stress	[105]

↓: decrease; ↑: increase; α-MSH: α-Melanocyte-Stimulating Hormone; NPY: Neuropeptide Y; ICV: intracerebroventricular; EPM: elevated plus maze; CeA: Central Amygdala; OF: open field; SPS: single prolonged stress; MTII: melanotan-II.

negatively impacts the quality of life [109]. Symptoms of this disorder include markedly diminished interest, extremely sadness, anhedonia, change in appetite, sleeping alteration, psychomotor agitation, loss of energy, guilty, difficulty in concentration and suicidal ideation [110].

Pharmacological blockade of the MC4R appears to influence not only anxiety- but also depression-like behaviors and the involvement of the melanocortin system in depression has been investigated in several preclinical studies, pointing out the capability of MC4R antagonists to exert antidepressant-like activities.

The weakening of the melanocortin system appears to have an antidepressant effect and the localization of MC4R in the limbic area, and the synaptic interconnection of POMC neurons with serotonergic, catecholaminergic, and GABAergic systems in the ARC emphasize a correlation between melanocortin network and depression [31,59,111–113].

ICV injection of α-MSH markedly increased the immobility time, whereas the MC4R antagonist HS014 reduced the duration of immobility in the FST [114], a behavioral despair model used to predict the efficacy of prospective antidepressant drugs, firstly described by Porsolt [36]. Co-administration of α-MSH, at doses inactive per se, was able to alter the anti-immobility response of NPY or [Leu^{31}, Pro^{34}]-NPY, meanwhile combined injection of HS014 significantly potentiated their anti-immobility effect [114]. This is an interesting result considering that NPY is co-localized with serotonin and norepinephrine system, primarily involved in depression [115].

Furthermore, HS014 potentiated the anti-immobility response elicited by acute administration of ethanol and attenuated the development of tolerance and withdrawal induced by chronic ethanol treatment, emphasizing the role of MC4R in ethanol withdrawal-generated symptoms, including depression [91,116]. Moreover, Kokare et al. found that social isolation (six weeks) in rats promoted depressive-like symptoms, assessed by the FST, and that ICV administration of HS014 reversed the behavioral responses, decreasing the immobility time in both group-housed and isolated rats. The effect was more marked in social isolated rats compared to the non-isolated ones, confirming that this experimental procedure may upregulate the endogenous melanocortin system in response to the depletion of α-MSH in several brain areas such as the PVN, ARC and central amygdala [92]. These observations are in accordance with the study in which HS014 was administered through intranasal infusion, demonstrating once again the ability of this antagonist to reduce the immobility time in FST compared to the vehicle-treated rats along with the promising result in the SPS procedures [93].

The MC4R antagonists MCL0129, MCL0020 and MCL0042 significantly decreased the immobility time [9,100,105]. In the experiments, the considered parameters were immobility, swimming and climbing, and the MC4R antagonists increased swimming, without influencing climbing behavior, effect that is similarly observed with SSRIs, such as fluvoxamine and fluoxetine [117,118], evidencing the involvement of serotonergic transmission in the antidepressant effects of MCL0129 and in the inhibition of serotonin transporter induced by MCL0042 [100,105]. It was also evaluated the effect of MCL0129 in the learned helplessness test in rats, in which the compound proved to be able to ameliorate the escape deficit, further indicating an antidepressant activity [100]. This was observed after an acute administration of this MC4R antagonist, differently from imipramine or fluvoxamine which require a chronic treatment to perform their activity under the same experimental conditions [100,119]. In a subsequent study, a chronic treatment with MCL0042 for 14 days attenuated olfactory bulbectomy-induced hyperactivity in rats measured in the OF [105]. The removal of bilateral olfactory bulbs produces many behavioral changes, including hyperactivity in the OF, which can be treated using antidepressants [120]. Thus, the reduction of this kind of hyperactivity by MCL0042 is another evidence of the antidepressant potential of MC4R antagonists, considering that also SSRIs are known to have a similar effect. This suggests that the inhibitory activity of MCL0042 at serotonin transporter might be deeply associated to its antidepressant properties [105].

The involvement of MC4Rs in mood regulation is related to their interaction with serotonin and dopamine transmission and, additionally, a role of the NAc melanocortin signaling was shown in depression [81]. During a chronic stress protocol, which elicits depression-associated behaviors in rodents as anhedonia, the activation of MC4Rs in the NAc triggered stress-induced synaptic adaptations. Blockade of MC4R signaling in this brain area reversed stress-elicited anhedonia in mice, supporting a link between dysphoria and the melanocortin system [81].

Fang et al. investigated the possible activity of AgRP neurons in the context of depression in both male and female mice [121], through the chronic unpredictable stress for 10 days, one of the most commonly used animal models of depression [122]. This model consists of constantly exposing animals to several type of stressors (mechanical, psychological or environmental), thus developing behavioural changes, resembling the symptoms of human depression.

Using this animal model, in the above mentioned study [121], the authors decided to exclude water and food deprivation during the chronic unpredictable stress (considering the direct effect on AgRP neuron activity), without changes in inducing anhedonia and hypercortisolism, core features found in individuals with major depression [123,124]. This study reported that the chronic stress, but not short-term exposure (i.e. 3 days), led to AgRP neurons dysfunction in the ARC in both sexes, with enhanced inhibitory synaptic transmission and diminished intrinsic neuronal excitability [121]. AgRP neural chemogenetic inhibition increased susceptibility to unpredictable stress, while the activation reversed the stress-induced anhedonia and despair behavior highlighting how chronic stress causes maladaptive synaptic and intrinsic plasticity of AgRP neurons [121]. It is noteworthy that AgRP is the endogenous antagonist of the MC4R, and it was suggested that AgRP may interact with the mesolimbic pathway, circuit implicated in depression [125,126]. At the same time, mice subjected to chronic restraint stress displayed anhedonia in a sucrose preference test and increased immobility time in FST and tail suspension test, indicating depression-like behaviors with a hyperactivity of POMC in the ARC capable to decrease dopamine VTA neuronal activity via direct or indirect pathway [127]. Conversely, selective inhibition of the $POMC^{ARC}$→VTA circuit reduces the depression-like behaviors in mice exposed to chronic restraint stress, suggesting an involvement of POMC in mood dysregulations [127], considering also that α-MSH increases dopamine turnover mediated principally by the MC4R [79]. Indeed, MTII injected into the VTA decreases sucrose preference in rats, while SHU9119 reverses this effect [128].

Finally, depression as well as anxiety are associated with a heightened perception of the severity of pain, and the prolonged duration of pain increases mood dysregulation [129]. Antidepressants are used to treat chronic pain, such as neuropathic pain [130] and the use of MCR antagonists seems to be effective against allodynia and hyperalgesia associated with neuropathic pain, having also a role in the nociception modulation [131–134].

Therefore, given the promising results observed in preclinical studies (as summarized in Fig. 1 and Table 2), the antagonism of MC4R could be a potential functional strategy for the modulation of anxious or depressive states and treatment of these disorders.

5. Clinical evidence

The principal research in humans was conducted to assess the role of melanocortin system on aberrant feeding behaviors, while only few studies were performed to investigate the possible influence of MC4R signaling or MC4R polymorphism in affective disorders. In particular, Yilmaz et al. were interested in studying the link between MC4R genetic variants and depressed mood, and if this connection was able to affect also eating behavior. The authors hypothesized that the MC4R polymorphism could predispose individuals to weight gain in two possible ways: by overeating and by a depressive status, observing that the presence of the rs17782313 polymorphism was associated with a depressed mood [135]. Recently, an inverse association has been found between adiposity and psychological distress in subjects with MC4R rs17782313, considered this latter as a key element of obesity for the dysfunction of MC4R activity, even though, conventionally, obesity and stress are positively correlated [136].

These findings overall highlight a possible association between MC4R signaling with distress and depression in humans, even though, to the best of our knowledge, no studies investigated directly MC4R and psychiatric conditions without the context of obesity in clinical research.

6. Conclusion

The melanocortin system, principally involved in the food intake and energy expenditure, has received more and more interest in the stress regulation and stress-induced psychiatric conditions, such as anxiety and depression, affective disorders mainly explored in rodents through appropriate behavioral tests or genetic ablation. The activation of the melanocortin system by the endogenous ligand α-MSH (predominantly via the MC4R) was highlighted as an important neurobiological mechanism underlying the stress response and the associated psychiatric conditions. These effects involve different neuronal circuitries and multiple neurotransmitters, which are summarized in Fig. 2.

Through the studies and experiments involving MC4R receptor ligands it emerged that MC4R antagonists attenuate grooming, a rodent behavioral response to stressful conditions and mediate anxiolytic- and antidepressant-like responses compared to agonists of this receptor. Moreover, endogenous antagonists, such as AgRP and NPY also demonstrated behavioral effects opposite to α-MSH, modulating anxiety status and depressive symptoms in preclinical models, underlying mechanisms involved in the mood regulation.

Taking into account all the previously discussed findings, it has been clearly demonstrated the existence of an interaction between the melanocortin system (via MC4Rs), stress responses, anxiety and depression, and that this relationship and the weakening of the melanocortin system may have an important impact on the possible treatment of psychiatric disorders. Nonetheless, it needs to be mentioned that animal experiments revealed an increase in food consumption linked to the use of MC4R antagonists, for example following the ICV administration of SHU9119 [137], acute/chronic treatment with HS014 [89,138] and intrathecal administration of AgRP [139]. The increased appetite and weight gain could, in fact, limit their use, especially in people who are

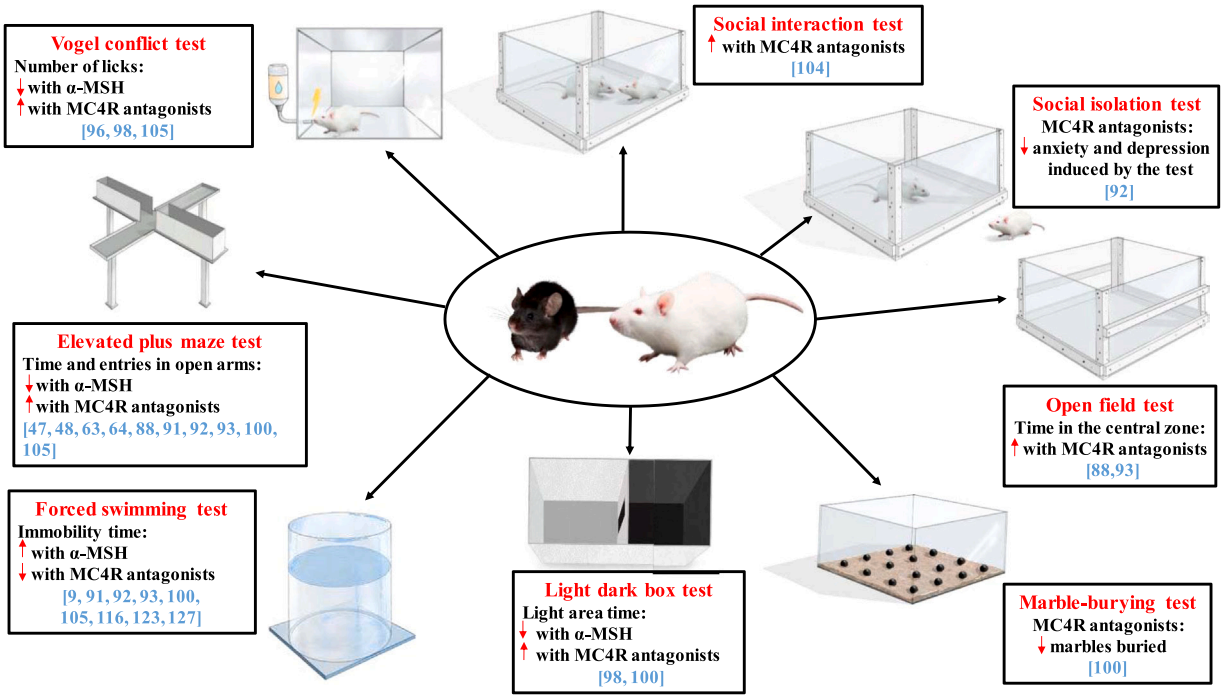

Fig. 1. Principal tests performed in rodents to evaluate the anxiolytic and antidepressant-like effect of MC4R antagonists. The figure summarises the behavioral effects induced by the injection of MC4R antagonists in rodents' studies. The behavioral tests used revealed the anxiolytic and/or antidepressant activies of the MC4R antagonists, meanwhile α-MSH administration (a MC4R endogenous agonist), showed the opposite effects (anxiety- and depressive-like behaviors). ↓: decrease; ↑: increase; α-MSH: α-Melanocyte-Stimulating Hormone; MC4R: Melanocortin-4 Receptor.

Table 2
Studies performed in rodents to investigate the potential effect on depressive-like behaviors caused by activation or antagonism of the MC4R.

Subjects	Compound	Injection	Behavioral test	Behavioral outcome	REF.
Male rats	HS014	ICV	Social interaction+ FST	↑ time spent by the rats in the social interaction ↓ immobility time	[92]
Male rats	HS014	Intranasal	FST	↑ swimming ↓ immobility time	[93]
Male rats	HS014	Intranasal	SPS + FST	↓ immobility time	[93, 94]
Male rats	HS014 + NPY	Intranasal	SPS + FST	↓ immobility time	[94]
Male rats	MCL0129	Subcutaneous	FST	↓ immobility time	[100]
Male rats	MCL0129	Subcutaneous	Learned Helplessness	↓ number of escape failures	[100]
Male rats	MCL0042	Subcutaneous	Olfactory bulbectomy	↓ Olfactory bulbectomy-induced hyperactivity	[105]
Male rats	α-MSH	ICV	FST	↑ immobility time	[114]
Male rats	NPY (or [Leu^{31}, Pro^{34}]-NPY)	ICV	FST	↓ immobility time	[114]
Male rats	HS014	ICV	FST	↓ immobility time	[114]
Male rats	α-MSH + NPY (or [Leu^{31}, Pro^{34}]-NPY)	ICV	FST	α-MSH ↓ the anti-immobility response induced by NPY (or [Leu^{31}, Pro^{34}]-NPY)	[114]
Male rats	HS014 + NPY (or [Leu^{31}, Pro^{34}]-NPY)	ICV	FST	HS014 ↑ the anti-immobility response induced by NPY (or [Leu^{31}, Pro^{34}]-NPY)	[114]

↓: decrease; ↑: increase; α-MSH: α-Melanocyte-Stimulating Hormone; ICV: intracerebroventricular; FST: forced swim test; SPS: single prolonged stress; NPY: Neuropeptide Y.

obese or suffering from binge eating disorder. On the other hand, this stimulatory effect on food consumption may be useful for the treatment of cachectic conditions [140–142].

CRediT authorship contribution statement

Emanuela Micioni Di Bonaventura: Conceptualization, Writing – original draft, Writing – review & editing, Visualization (contributed equally to this review). **Luca Botticelli:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization (contributed equally to this review). **Fabio Del Bello:** Writing – review & editing, Visualization. **Gianfabio Giorgioni:** Writing – review & editing, Visualization. **Alessandro Piergentili:** Writing – review & editing, Visualization. **Wilma Quaglia:** Writing – review & editing, Visualization. **Adele Romano:** Writing – review & editing, Visualization. **Silvana Gaetani:** Writing – review & editing, Visualization. **Maria Vittoria Micioni Di Bonaventura:** Writing – review & editing, Supervision, Visualization. **Carlo Cifani:** Writing – review & editing, Supervision, Visualization. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

All authors declare that there are no conflicts of interest associated with the content of this paper.

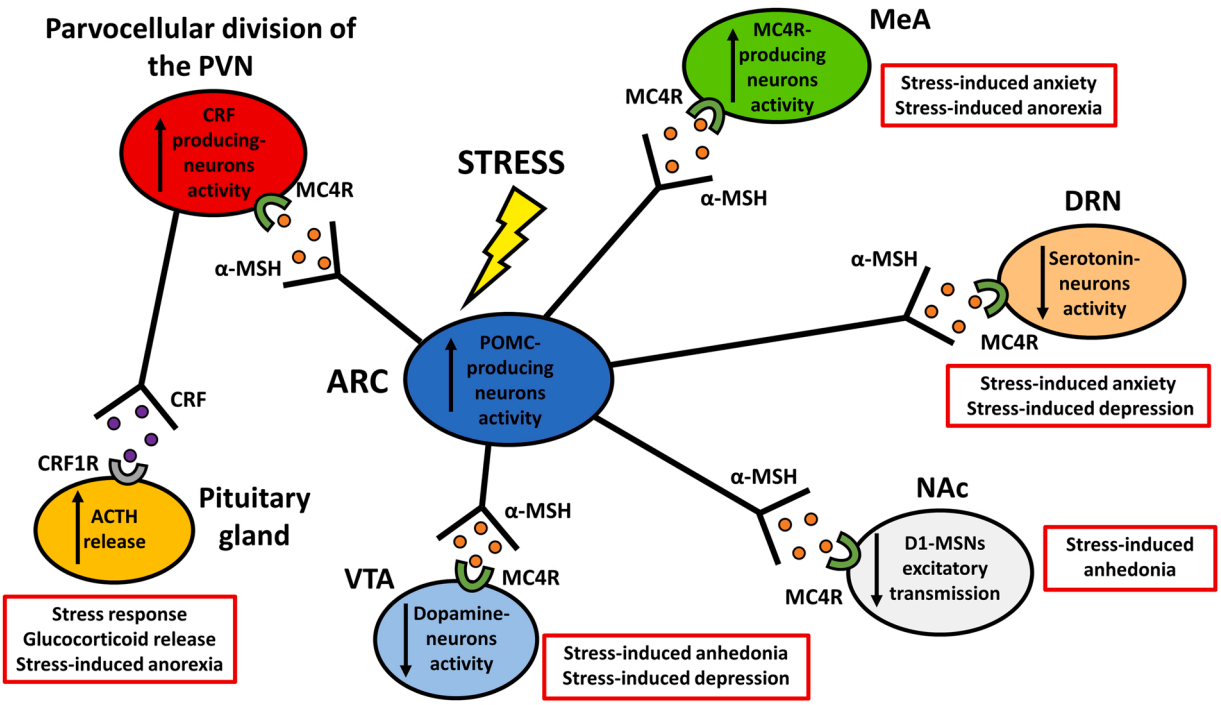

Fig. 2. Schematic illustration representing the neuronal circuitries implicated in the melanocortin influence on stress and stress-related psychiatric conditions. POMC-expressing neurons in the ARC are activated by several stressors. These neurons send projections to different brain regions, including the PVN, MeA, DRN, NAc and VTA, which express the MC4Rs. The activation of the MC4Rs by the ligand α-MSH influences the activity of specific sub-populations of neurons in these brain regions, resulting in a posi-tive modulation of stress and stress-related behaviors, such as anxiety, anhedonia, and depression. ↓: decrease; ↑: increase; α-MSH: α-melanocyte-stimulating hormone; ACTH: Adrenocorticotropic Hormone; ARC: Arcuate Nucleus of the Hypothalamus; CRF: Corticotropin-Releasing factor; CRF1R: CRF Type 1 Receptor; DRN: Dorsal Raphe Nucleus; MC4R: Melanocortin-4 Receptor; MeA: Medial Amygdala; MSNs: Medium-Spiny Neurons; NAc: Nucleus Accumbens; POMC: Pro-opiomelanocortin; PVN: Paraventricular Nucleus of the Hypothalamus; VTA: Ventral Tegmental Area.

Data availability

No data was used for the research described in the article.

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