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ANIMAL CARE

THE PHYSIOLOGY OF EMOTIONS

An insight in the psycho-physiological world behind Major Depressive Disorder

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INDEX

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Abstract

Emotions represent a fundamental aspect of welfare, significantly influencing the well-being of beings close to us. Animals, to many, are indeed included in the circle of the ones we care for.
Why is it important to study the emotional states in animals? Because it is essential for assessing welfare conditions, as understanding emotional experiences provides insight into whether or not an individual is living in optimal conditions. Furthermore, examining animal physiology is critical, as the body operates like a complex machine; understanding its mechanisms allows for informed interventions aimed at improving or maintaining health and welfare when possible and necessary.
This thesis will explore the consequences of negative emotions on the body by examining their associated physiological reactions. It will begin by defining both negative and positive mental states, as well as their relationship to animal welfare. Following this, the physiological processes underlying the targeted emotional states will be described, with comparisons drawn to existing knowledge in humans. The final section will focus on interventions aimed at mitigating or reversing the effects of these negative emotional states.
One question remains open for exploration: can the concept of mental health be appropriately applied to animals?

chapter 1
Negative and positive welfare and mental states: a definition

The concept of "animal welfare" remains an inherently abstract and complex construct, defying straightforward definition. Given the intrinsic limitations of verbal communication and the challenges of interpreting body language, the study of animal welfare can only be grounded in our understanding of animals at biological, physiological, and psychological levels. Despite extensive scholarly efforts, a universally accepted definition remains elusive. This difficulty is compounded by the fact that the notion of "positive welfare" has emerged as a conceptual evolution from "negative welfare," highlighting their distinct, yet interconnected, natures. As noted by Rault et al. (2020), attempts to define and study positive welfare are deeply influenced by

underlying ethical perspectives, further complicating the development of a single, cohesive definition.

1.1 Positive Welfare: A Spectrum of Interpretations

A prevailing consensus among scholars suggests that positive welfare should not be simplistically framed as the absence of suffering; rather, it involves qualitatively distinct constructs. This distinction has given rise to diverse interpretations of what constitutes positive animal welfare. Some scholars associate it with providing opportunities for animals to engage in positive experiences, often informed by the ethical principle that pain should not be inflicted on sentient beings. Others conceptualize positive welfare as emerging from an alignment with animals' likes, wants, and preferences, and its measurement as a balance where positive experiences outweigh negative ones (Yeates & Main, 2008).

A related view posits that animal welfare improves when conditions approximate an animal's natural, wild state (Yeates & Main, 2008). In contrast, another perspective emphasizes that positive welfare reflects an accumulation of positive experiences, recognizing the species-specific and individual-specific nature of these experiences. Importantly, this framework acknowledges that animals are not predisposed to reminisce on positive experiences but are instead influenced by negative ones, which can bias their current and future states.

In seeking an inclusive definition, Fraser's "Three Circles Model" offers a compelling framework. This model integrates diverse values and scientific perspectives that have historically shaped debates around animal welfare. It emphasizes that true welfare encompasses three fundamental dimensions: basic health and functioning, affective states, and natural living. Together, these dimensions advocate for conditions where animals have access to food and water, are free from disease, experience positive emotions, and are spared prolonged negative states. Moreover, it underscores the importance of enabling animals to engage in natural behaviors and interact with their environment in ways that satisfy their instinctual needs (Fraser, 2008).

1.2 Affective States: Exploring the Essence and Perception of Emotions

The concept of emotion, or affective states, in the animal kingdom is a topic of considerable debate, particularly with regard to the cognitive prerequisites for experiencing such states. Traditional perspectives often argue that the recognition of emotions necessitates a level of cognitive sophistication, casting doubt on whether many animals possess the complexity required to recognize and, consequently, feel emotions. This assumption raises fundamental questions: must an organism possess "higher

intelligence" to experience emotions, or is emotional experience independent of cognitive recognition?

As humans, we have an intrinsic tendency to categorize and define both tangible and intangible phenomena. This inclination extends to emotions, where we attempt to assign names and explanations to subjective experiences; several scholars as a matter of fact have contributed significant perspectives on the role of emotions in understanding animal welfare. For instance, Marc Bekoff and Jaak Panksepp, in their seminal work *"Animal Emotions: Exploring Passionate Natures,"* characterize animal emotions as evolutionary adaptations that facilitate not only survival but also social bonding. They argue that animals experience emotions such as joy, empathy, and playfulness, emphasizing that these affective states are both individual and social in nature. Moreover, they highlight that these emotions are rooted in neural structures analogous to those found in humans. Similarly, Boissy et al.(2007), in *"Emotion and Cognition: A New Approach to Animal Welfare,"* define positive emotions in animals as critical components for evaluating welfare. They describe positive emotional states as reflective of an animal's sense of well-being, encompassing feelings of safety, pleasure, and satisfaction derived from the fulfillment of their needs. Webb and Veenhoven(2019), in *"What is Animal Happiness?"* propose a framework for understanding animal happiness through the lens of positive psychology. This perspective suggests that positive emotions in animals arise from a combination of physical well-being and psychological satisfaction, which are intricately linked to the quality of their environment and social interactions. These contributions collectively underscore the importance of affective states in shaping and assessing animal welfare.

While certain emotions may be effectively articulated, their essence resides in their experiential nature, which resists full encapsulation by language. An emotion, fundamentally, is a response to external stimuli that elicits physiological and psychological changes within the organism. This intrinsic quality of emotions exists independently of our attempts to define or classify them. Recognition or labeling of an emotion does not alter the way it is experienced in a given moment.

If we set aside the necessity of formal definitions, it becomes plausible to propose that animals are capable of experiencing emotions, or affective states, irrespective of their cognitive abilities to recognize or interpret them. This hypothesis aligns with the classical understanding of emotions as multidimensional experiences comprising three core components:

1. Behavioral Component: Observable responses such as posture or activity.
2. Autonomic Component: Physiological changes, including visceral and endocrine reactions.

3. Subjective Component: The internal, experiential quality of the emotion (Boissy et al., 2007).

Within this framework, a positive emotional state is defined as an emotional experience associated with positively reinforcing situations—contexts that enhance well-being or fulfill specific needs (Rault et al., 2020). Such states are observable not only through behavioral indicators but also measurable physiological markers, providing evidence for their existence in animals.

This reconceptualization challenges anthropocentric assumptions about the nature of emotions, emphasizing that the ability to feel is not contingent upon cognitive recognition. Instead, emotions are foundational, embodied experiences shaped by environmental stimuli and intrinsic biological responses. By adopting this perspective, we acknowledge the plausibility of emotional experiences in animals, thereby enriching our understanding of their welfare and affective lives.

The same principle applies to negative emotions, which differ in that they are associated with negatively reinforcing situations and past experiences. These emotions elicit adverse responses to stimuli that evoke memories of previous traumatic events, further reinforcing the negative emotional state.

1.3 Negative Welfare: A Framework for Defining Deficits

The topic of negative welfare has been extensively explored and is characterized by four distinct but interrelated frameworks, each offering a unique perspective on its definition and assessment:

1. The Five Domains Model: This model conceptualizes negative welfare as the result of deficits in key domains, including nutrition, environmental conditions, health, and behavioral interactions. Such deficits give rise to adverse mental states such as fear, distress, or frustration, highlighting the interplay between physical and psychological factors (Johnson et al., 2022; Lanzoni et al., 2023).
2. Negative Indicators in Welfare Protocols: This framework emphasizes the identification of specific conditions indicative of negative welfare, such as lameness, injury, and behavioral suppression. These indicators are often evaluated using physiological markers, including elevated cortisol levels, or through outcomes such as increased mortality rates (Lanzoni et al., 2023; Nestler & Hyman, 2010).
3. Mental State and Negative Welfare: Within this perspective, negative welfare is defined by psychological discomfort arising from unmet needs or adverse environmental conditions. Emotional states such as frustration or fear are

central, with manifestations observable through behavioral changes and physiological stress markers (Delgado & Dantas, 2020; de Waal, 2011).

4. Ethical Considerations and Broader Perspectives: This approach expands the concept of negative welfare to encompass both physical suffering (e.g., disease, malnutrition) and psychological harm (e.g., lack of agency, inability to express natural behaviors). Crucially, it argues that the absence of suffering does not necessarily equate to the presence of positive welfare, thereby underscoring the nuanced nature of animal well-being (Johnson, 2022; de Waal, 2011).

Collectively, these frameworks offer a comprehensive perspective on negative welfare, emphasizing its multifaceted nature and underscoring the necessity of interdisciplinary methodologies to effectively evaluate and address the complexities of animal welfare.

The question is not what emotions *are*—at least, not yet—as the concept is too vast and complex to be adequately captured by a single definition without diminishing its multifaceted nature. Instead, the focus is on how emotions can be recognized. Attempting to define emotions outright may be overly ambitious, but identifying reliable markers that indicate specific emotional states is a feasible and valuable endeavor. Once we establish that particular indicators correspond to distinct emotional experiences, we can then begin to study how these states are expressed in individuals.

With the growing interest in understanding mental states, scientists have turned their attention to unraveling a profound and often hidden phenomenon: depression. Major Depressive Disorder (MDD) is a condition whose effects on humans are well-documented, but what about animals? Do animals experience depression? Or, more precisely, can they comprehend what it means to be depressed?

The following chapters will delve into the physical manifestations of depression, with a focus on identifying biomarkers and drawing behavioral comparisons to better understand how this condition may be expressed across different species.

chapter 2
Physiological mechanisms involved in negative emotions in animals

The study of biomarkers has emerged as a cornerstone for investigating depression in animals, providing critical insights into psycho-physiological mechanisms when direct verbal communication is impossible. Biomarkers offer a potential link to the underlying pathophysiology of depression, yet their identification represents an initial step rather

than a definitive conclusion. These indicators act as valuable hints, guiding researchers toward a deeper understanding of animal psycho-physiology, albeit with certain limitations. While their presence in non-human animals is inferred through analogy to human studies, this inference remains hypothetical. The plausibility of such biomarkers indicating similar physiological states in non-human mammals is supported by the shared biological processes and functional similarities across species.

Notably, not all biomarkers identified in human studies have been confirmed in non-human animals. However, those investigated thus far demonstrate considerable overlap, lending credence to their relevance. The challenge of biomarker identification is compounded in non-laboratory environments, necessitating controlled conditions and animal models to rigorously examine their validity. Experimental protocols have thus relied on inducing depressive-like states in animals through exposure to a range of stressors, including electric shocks, cold water immersion, sleep deprivation, food deprivation, maternal separation, isolation, immobilization, and light/dark cycle reversal. These interventions, typically applied for durations ranging from three weeks to three months, have been designed to emulate "learned helplessness," a hallmark of depression-like behavior in animals (Abelaira, H. M., Réus, G. Z., & Quevedo, J., 2013).

Following the induction of depressive-like states, researchers validate these conditions through behavioral assays, subsequently collecting biological samples to analyze potential biochemical changes. Sampling methodologies include blood draws, cerebrospinal fluid extraction, brain tissue analysis, as well as urine, feces, and gut microbiota profiling. The utility of biomarkers is further assessed based on the responsiveness of these models to established antidepressant therapies, including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), and electroconvulsive therapy. Positive responses in these models reinforce the plausibility of the identified biomarkers, though they do not guarantee absolute validity.

Despite the inherent uncertainties, the findings derived from these experiments underscore the potential of biomarkers as indicators of depressive states in animals. The results reflect a convergence of behavioral, physiological, and biochemical evidence, offering a promising yet cautious avenue for advancing the understanding of depression through animal studies.

The information exposed below are the results of the carried out experimentations.

2.1 Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a well-characterized neurotrophin, extensively studied for its crucial role in the development, maintenance, and modulation

of brain function. BDNF contributes significantly to neuronal survival, synaptic plasticity, and overall brain homeostasis. Its expression has been localized to key brain regions, including the cortex, hippocampus, and amygdala, with quantifiable mRNA expression and immunoreactivity observed in these areas.

BDNF is produced both centrally and peripherally, with the central nervous system serving as the primary site of synthesis, complemented by limited peripheral production. BDNF expression correlates with beneficial behaviors such as playfulness, improved spatial learning, and heightened exploratory activity. These behavioral effects are associated with increased BDNF concentrations in regions such as the medial forebrain, cerebral cortex, hippocampal formation, and hindbrain. However, the utility of peripheral BDNF measures to assess experiential states remains contentious. While studies in pigs and rats suggest a moderate correlation between peripheral (plasma and whole blood) and central (hippocampal) BDNF levels, other investigations report no such relationship. Moreover, saliva BDNF concentrations often fail to reflect plasma BDNF levels. This inconsistency may stem from various factors influencing BDNF production, including sex, metabolic state, and life experiences.

BDNF production is tightly regulated by neuronal activity and sensory stimulation. The process begins with a stimulus—such as learning, sensory input, or memory engagement—that initiates an intracellular signaling cascade. Neuronal firing induces calcium influx, triggering the activation of the cAMP response element-binding protein (CREB). CREB subsequently binds to BDNF gene promoters, enhancing transcription. The resulting BDNF mRNA is translated into proBDNF, the precursor form, which undergoes intracellular or extracellular cleavage to form mature BDNF. Both forms are biologically active but exert distinct effects by binding to different receptors. ProBDNF preferentially binds to the p75 neurotrophin receptor (p75NTR), mediating apoptosis and synaptic pruning, while mature BDNF activates TrkB receptors, promoting neuronal survival and synaptic plasticity.

Mature BDNF is stored in vesicles at presynaptic terminals and released into the synaptic cleft through activity-dependent exocytosis. Calcium influx further facilitates vesicle fusion with the membrane, enabling the release of BDNF into the extracellular space. The released BDNF operates through a positive-feedback mechanism, amplifying its effects on neuronal and glial signaling.

The role of BDNF in the pathophysiology of brain-related disorders, particularly depression, is well-documented. Patients with depression exhibit reduced BDNF expression in brain tissue and lower peripheral levels, findings mirrored in animal studies where diminished serum BDNF correlates with depressive-like behaviors. Importantly, the administration of antidepressants has been shown to elevate BDNF levels in rodent brains, paralleling findings in human studies. Furthermore, direct

hippocampal administration of BDNF produces antidepressant-like effects in animal models, reinforcing its potential role in mood regulation.

Evidence from human and animal studies suggests that central BDNF expression—and possibly peripheral BDNF concentrations—may serve as biomarkers for both positive and negative experiential states. However, the changes in BDNF levels appear to be asymmetrical: positive experiences trigger an increase in BDNF production, while negative experiences fail to activate the molecular pathways necessary for its release. This disparity underscores the role of BDNF as a mediator of plasticity and adaptability to positive stimuli rather than a direct responder to stress.

The evidence supporting BDNF as a biomarker for depression hinges on its responsiveness to positive experiential stimuli and its modulation by antidepressant interventions. The mechanism involves sensory or cognitive stimuli initiating a cascade that culminates in the release of mature BDNF, fostering synaptic plasticity and resilience. The absence of BDNF activation during negative experiences may reflect the lack of stimuli required to engage this neurotrophin system.

While peripheral measures of BDNF offer accessibility, their relationship to central concentrations remains inconclusive due to confounding factors such as sex, metabolic state, and environmental influences. Thus, while BDNF holds promise as a biomarker for depression, further research is necessary to validate its central and peripheral dynamics and their relationship to experiential states.

This paragraph is based on "Finding biomarkers of experience in animals" (Babington S. et at., 2024) and "Neurotrophins and hippocampal synaptic transmission and plasticity" (Lu B., & Chow A., 1999)

2.2 Hypothalamic-pituitary-adrenal axis(HPA)

The hypothalamic-pituitary-adrenal (HPA) axis is a cornerstone of the body's stress response system and a key biomarker for major depressive disorder (MDD). This intricate neuroendocrine network integrates signals from the central nervous and endocrine systems to maintain homeostasis under both basal and stress conditions. Comprising the hypothalamus, pituitary gland, and adrenal glands, the HPA axis orchestrates a cascade of hormonal events in response to perceived stressors, ultimately modulating physiological and behavioral adaptations essential for survival.

The HPA Axis is structured as (Tsigos, C. et al., 2002)

1. **Hypothalamus: The Control Center**

The hypothalamus serves as the regulatory hub of the HPA axis, monitoring environmental and internal stimuli. Upon detecting a potential threat, neurons in the paraventricular nucleus (PVN) release corticotropin-releasing hormone (CRH), initiating the stress response cascade. CRH integrates excitatory signals from the amygdala and inhibitory signals from the hippocampus, reflecting the interplay of emotion and memory in stress regulation.

2. **Pituitary Gland: The Messenger**

   Once CRH reaches the anterior pituitary, it binds to CRH type 1 receptors (CRHR1), triggering the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. Elevated arginine vasopressin (AVP), another key modulator, amplifies this CRH-mediated ACTH release, particularly during prolonged stress exposure.

3. **Adrenal Glands: The Effector Organs**

   ACTH stimulates the adrenal glands, located atop the kidneys, to release corticosteroids—primarily cortisol—along with adrenaline. These hormones prepare the body for acute stress by mobilizing energy reserves, enhancing cardiovascular output, and suppressing non-essential processes such as digestion and immune function. Cortisol also exerts negative feedback on the hypothalamus and pituitary to regulate CRH and ACTH release, ensuring the stress response resolves once the perceived threat is mitigated.

Under chronic stress, the tightly regulated feedback mechanisms of the HPA axis become impaired, leading to persistent activation and elevated cortisol levels. This dysregulation plays a pivotal role in the pathophysiology of MDD, as evidenced by heightened baseline HPA activity and prolonged stress responses in individuals with depression.

Cortisol regulates the HPA axis through feedback at two levels: the hypothalamus, where it inhibits CRH release, and the anterior pituitary, where it suppresses ACTH secretion. This feedback is mediated through mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which regulate gene expression, including that of pro-opiomelanocortin (POMC, the precursor of ACTH), AVP, and CRH. In healthy individuals, this feedback mechanism ensures cortisol levels return to baseline following stress.

In MDD, however, this feedback system is compromised. Based on “Mice with Mutations in the HPA-System as Models for Symptoms of Depression”(Müller, M. B., &

Holsboer, F., 2006), persistent CRH activation—driven by hyperactivity of the amygdala—overwhelms the regulatory capacity of the hippocampus. Concurrently, AVP becomes less responsive to cortisol-mediated suppression, prolonging ACTH and cortisol secretion. This AVP-mediated resistance perpetuates the stress response, contributing to sustained cortisol elevation and associated depressive symptoms.

The chronic overactivation of the HPA axis in MDD reflects a pathological perception of the environment as persistently threatening, leading to continuous stress responses. Elevated levels of CRH, ACTH, cortisol, POMC, and AVP have been consistently observed in depressed individuals, underscoring the axis's role as "always on alert." This state of hyperactivity not only exacerbates depressive symptoms but also contributes to a host of stress-related disorders, including cardiovascular disease, metabolic dysfunction, and immune suppression.

The sustained overactivity of the HPA axis offers a physiological framework for understanding the development and maintenance of MDD. The heightened baseline activity and exaggerated stress responses reflect an intrinsic dysregulation of the stress system, with far-reaching consequences for both psychological and physical health.

The role of the HPA axis in MDD is further validated by its responsiveness to therapeutic interventions. Antidepressant treatments and psychotherapeutic strategies that normalize HPA function correlate with clinical improvements, reinforcing its utility as a biomarker for both diagnosis and treatment evaluation. Monitoring HPA activity could provide valuable insights into the efficacy of interventions and the trajectory of recovery.

2.3 Homocysteine(HCY)

Homocysteine (HCY), a sulfur-containing amino acid generated during the metabolism of the essential amino acid methionine, plays a pivotal role in energy metabolism and neurotransmitter synthesis. Elevated levels of homocysteine, termed hyperhomocysteinemia, have been implicated in various pathological conditions, including depression and cardiovascular diseases. However, the relationship between homocysteine and depression remains contentious, with studies reporting inconsistent findings, potentially due to methodological variations.

Homocysteine serves as a precursor to S-adenosylmethionine (SAMe), a key methyl donor involved in numerous biological processes, including the synthesis of critical neurotransmitters such as dopamine, serotonin, and norepinephrine—molecules intricately linked to mood regulation and depressive states. Elevated homocysteine

levels, combined with reduced concentrations of these neurotransmitters and lower levels of vitamins critical for SAMe synthesis (e.g., folate, B-6, and B-12), suggest an impaired conversion of HCY to SAMe. This disruption in methylation processes could theoretically contribute to the pathophysiology of depression.

The mechanistic link between homocysteine and neurotransmitter dysregulation raises important questions. Is hyperhomocysteinemia a consequence of genetic polymorphisms affecting metabolic pathways, or does it reflect dietary deficiencies in vitamins required for HCY metabolism? Evidence remains equivocal, as dietary interventions aimed at correcting nutritional deficiencies have shown mood improvements in some cases, while others have failed to yield significant results. Similarly, antidepressants targeting neurotransmitter pathways have demonstrated variable efficacy, suggesting that the underlying cause of depressive symptoms may not always reside in homocysteine metabolism.

The precise role of homocysteine in depression remains unclear, with evidence suggesting that hyperhomocysteinemia may be more of a consequence than a primary cause. Studies on laboratory models of stress-induced depression indicate that chronic stress elevates plasma homocysteine concentrations, potentially as a downstream effect of stress-related metabolic alterations (Xu et al., 2014). Notably, only 20–50% of individuals with severe depression exhibit elevated plasma homocysteine levels, highlighting the heterogeneity of depressive disorders and the complexity of their underlying mechanisms (Xu Y. et al., 2014).

In both human and animal studies, hyperhomocysteinemia is associated with depression, as well as comorbid conditions like cardiovascular diseases. Research using twin studies has identified an association between plasma homocysteine concentrations and depressive symptoms, but the causative direction remains elusive (Vaccarino et al., 2021). These findings support the notion that hyperhomocysteinemia may serve as a biomarker of depression or a related condition rather than a universal causative factor.

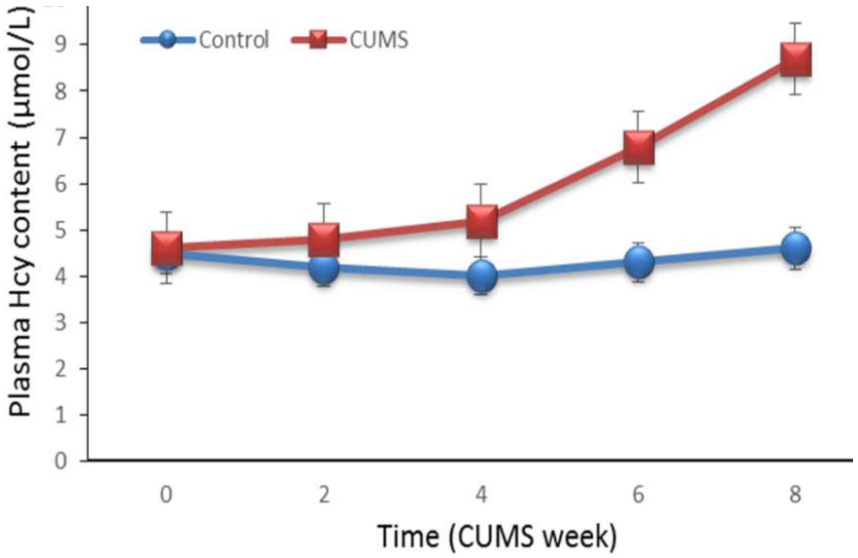

figure 1. Unpredictable chronic mild stress induces hyperhomocysteinemia in rats; The total plasma Hcy values were determined by high-performance liquid chromatography (HPLC) with fluorimetric detection and isocratic elution. Values represent the group mean ± structural equation modeling (SEM) (n = 8 rats per group). *P<0.05 compared with control, repeated measures ANOVA followed by Tukey's multiple comparison tests.

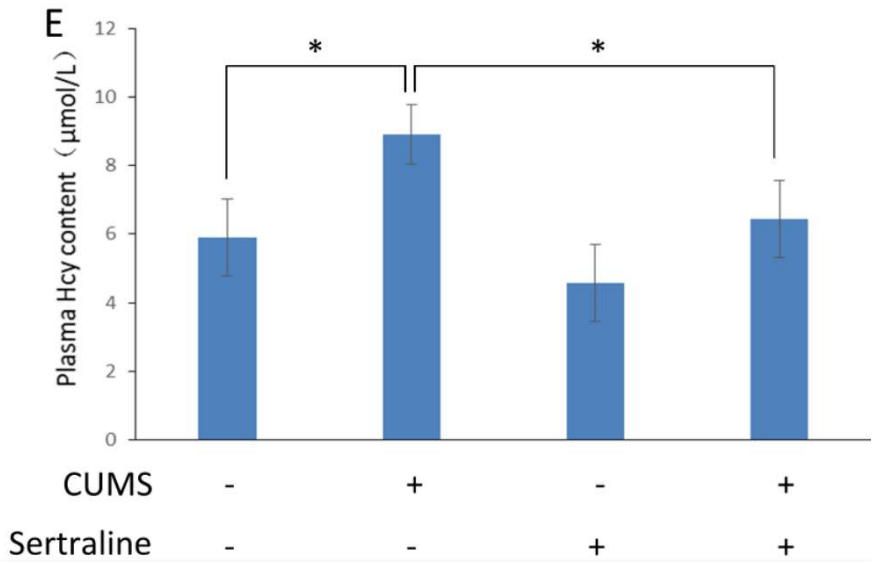

figure 2. Sertraline inhibits plasma Hcy content in CUMS rats; Sertraline inhibits the increase in the plasma Hcy level induced by CUMS. Values represent the group mean ± structural equation modeling (SEM) (n = 8 rats per group). *P<0.05, repeated measures ANOVA followed by Tukey's multiple comparison tests

The pictures above were taken from a Chinese university experiment, which intended to study plasma concentration of HCY as a consequence of depression. The investigation showed a significant difference between the HCY plasma concentration in rats in normal conditions and in depressed rats by the end, with an increase in the latter.

2.4 5-HT - serotonin

Serotonin (5-HT), a well-known neurotransmitter, plays a fundamental role in mood regulation, emotion, sleep, and appetite. Beyond its central nervous system (CNS) functions, serotonin is found in the intestines, blood platelets, and peripheral tissues, where it contributes to cardiovascular regulation, gastrointestinal motility, and pain modulation (Coppen AJ & Doogan DP., 1988). Given its widespread physiological influence and its moniker as the "happiness hormone," serotonin has been a focal point in the study of mood disorders, particularly depression.

Serotonin synthesis is derived from the essential amino acid tryptophan through a two-step enzymatic process:

1. **Hydroxylation:** Tryptophan is converted into 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase, a critical rate-limiting step.
2. **Decarboxylation:** 5-HTP is subsequently decarboxylated into serotonin (5-HT) by aromatic L-amino acid decarboxylase

(Young SN., 2007).

This enzymatic cascade depends on multiple factors, including the availability of tryptophan and the activity of enzymatic cofactors such as tetrahydrobiopterin (BH4). BH4, in turn, is maintained at optimal levels by S-adenosylmethionine (SAMe), which indirectly links serotonin metabolism to homocysteine (HCY). Elevated HCY levels, through SAMe depletion or dysregulation, could impair BH4 availability and, consequently, serotonin biosynthesis (Bottiglieri T., 2002).

While SAMe-mediated regulation plays an important role in serotonin production, the process is also influenced by external and behavioral factors, including:

- **Dietary Intake:** Adequate tryptophan consumption through diet.
- **Sensory Stimuli:** Visual and auditory inputs that influence CNS activity.
- **Physical Activity:** Exercise is known to enhance serotonin synthesis.
- **Emotional and Cognitive States:** Positive emotional and cognitive engagement promotes serotonin production

(Young SN., 2007).

A reduction or absence of these stimuli and nutritional factors may contribute to serotonin deficits, which are often observed in depressive states.

Decreased serotonin concentrations have been consistently observed in animal models exhibiting depressive-like behaviors. This aligns with serotonin's central role in mood regulation and its well-established association with positive affect. Data from 527 metabolomic studies focusing on animal models of depression have identified significant reductions in serotonin levels, further supporting its role as a biomarker for mood disorders.

This decrease in serotonin concentrations may reflect broader disruptions in metabolic pathways rather than isolated neurotransmitter deficits. The integration of metabolomic data with behavioral observations strengthens the hypothesis that serotonin dysregulation contributes to depressive phenotypes.(Vazquez-Matias D. A. et al, 2023 ; Pu J. et al., 2021)

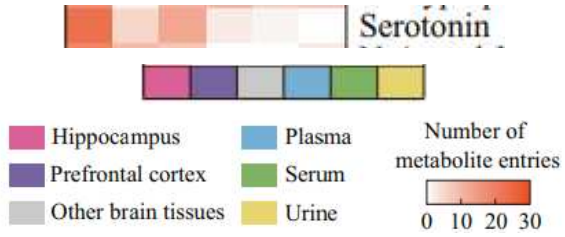

figure 1 Distribution of metabolites in brain, blood, and urine samples of depression models.

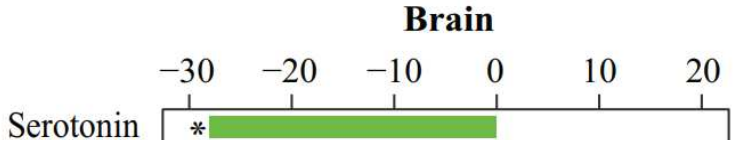

figure 2. brain, serotonin

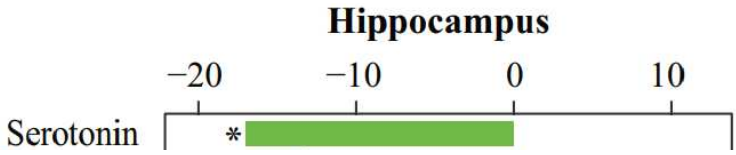

figure 3. hippocampus, serotonin

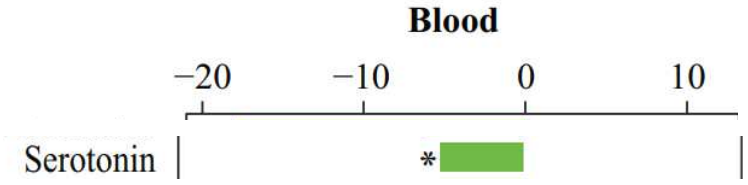

figure 4. blood, serotonin
An asterisk (*) indicates $p < 0.05$, meaning that it is difficult to happen by chance.
(Pu J. et al., 2021)

Data taken from the tables resulted in numbers as

- serotonin (VCS = −28, $p < 0.001$) (fig.2)
- serotonin (VCS = −17, $p < 0.001$) (fig.3)
- serotonin (VCS = −5, $p = 0.031$) (fig.4)

Showing a strong downregulation in the presence of serotonin within the system. There is notable disparity in serotonin (5-HT) concentrations between the bloodstream and brain tissues. While peripheral serotonin levels may be elevated or within normal ranges, central serotonin levels in brain tissue often appear reduced in individuals with depressive symptoms. This observation raises an intriguing hypothesis: the existence of a functional or structural alteration in the blood-brain barrier (BBB), which may impede the proper transport of serotonin into the brain.

The blood-brain barrier plays a critical role in maintaining central nervous system (CNS) homeostasis by regulating the passage of molecules, including neurotransmitters, between the bloodstream and brain tissues. A dysfunction or inefficiency in this barrier could potentially hinder serotonin transport, leading to a central serotonin deficit despite adequate or elevated peripheral levels. This barrier dysfunction may represent a previously underappreciated factor contributing to neurotransmitter imbalances observed in depressive disorders.

A complementary mechanism to the blood-brain barrier hypothesis involves the serotonin transporter (SERT), which facilitates the reuptake of serotonin into neurons, ensuring its proper recycling and availability in synaptic spaces. Positron emission tomography (PET) studies have consistently reported a trend toward reduced SERT availability in key cortical and subcortical regions, including the prefrontal cortex, striatum, and thalamus. The decreased availability of SERT could compound serotonin dysregulation, contributing to both reduced synaptic serotonin and impaired reuptake dynamics.

Together, the reduced availability of SERT and potential blood-brain barrier dysfunction present a compelling framework for understanding central serotonin deficits in depressive disorders. The combined effects could disrupt serotonin's homeostasis in the CNS, despite seemingly sufficient peripheral levels, and further exacerbate mood dysregulation. This interplay underscores the need for further research to elucidate the relationship between peripheral serotonin, the blood-brain barrier, and SERT functionality in depressive pathophysiology.

2.5 Dopamine and Norepinephrine

The interplay of various neurotransmitters is integral to understanding the pathophysiology of major depressive disorder (MDD), with dopamine (DA) and norepinephrine (NE) being of particular significance. Dopamine, a neurotransmitter and hormone, is critically involved in the brain's reward system, where it regulates pleasure, motivation, and motor functions (McLeod, 2023). Its synthesis occurs in neurons located at the base of the brain through a two-step enzymatic process. Initially, the amino acid tyrosine is converted into L-dopa, which is subsequently transformed into dopamine by the action of specific enzymes (Watson, 2024).

Importantly, dopamine serves as a biochemical precursor in the biosynthesis of other catecholamines, including norepinephrine and epinephrine. The conversion of dopamine to norepinephrine is mediated by dopamine β-hydroxylase, an enzyme that operates in the presence of L-ascorbic acid and molecular oxygen (O_2). Norepinephrine, in turn, is further methylated by phenylethanolamine N-methyltransferase, utilizing S-adenosyl-L-methionine (SAMe) as a cofactor, to produce epinephrine (Juárez Olguín et al., 2016).

Deficiencies in dopamine levels could directly reduce the synthesis of norepinephrine, thereby diminishing the availability of epinephrine in the system. Such alterations in catecholamine concentrations are likely to have significant implications for the neurochemical imbalances observed in MDD, further emphasizing the importance of dopamine and its downstream metabolites in the disorder's pathogenesis.

The study previously mentioned(metabolic changes in animal models of depression) reports data regarding the lowering of these metabolites, showing the sequents results:

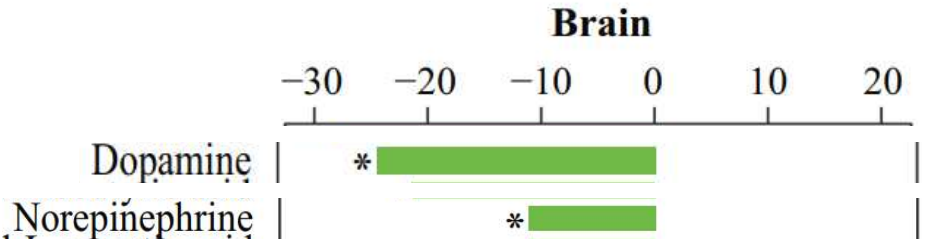

figure 1. brain, dopamine and norepinephrine

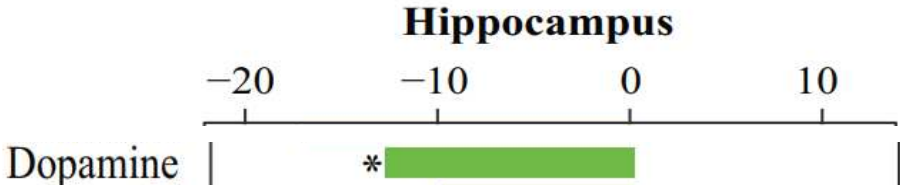

figure 2. hippocampus, dopamine
(Pu J. et al., 2021)

- brain, dopamine (VCS = −24, $p < 0.001$)
- brain, norepinephrine (VCS = −11, $p = 0.017$)
- hippocampus, dopamine (VCS = −13, $p = 0.004$)

A deficit in dopamine (DA) within the system may arise from multiple factors, with one plausible hypothesis being insufficient stimuli or an inadequate supply of essential nutrients necessary for its synthesis. Dopamine production is heavily reliant on the availability of specific precursor molecules, particularly the amino acid tyrosine. Tyrosine can be acquired through dietary intake—commonly from animal-based products, soy, nuts, seeds, and beans—or synthesized endogenously from phenylalanine, another amino acid. Notably, phenylalanine is an essential amino acid, meaning it cannot be synthesized by the human body and must be obtained through diet, primarily from animal or plant-based sources. Therefore, a deficiency in phenylalanine could gradually result in reduced tyrosine levels, leading to impaired dopamine synthesis over time (Nelson & Cox, 2017).

This biochemical cascade has broader implications, as norepinephrine (NE) synthesis is directly dependent on dopamine, which serves as its precursor. A reduction in dopamine availability consequently diminishes norepinephrine levels, potentially exacerbating the neurochemical imbalances observed in various psychiatric disorders, including major depressive disorder (MDD). Understanding the nutritional and metabolic underpinnings of these deficiencies underscores the complex interplay between dietary factors, neurotransmitter biosynthesis, and mental health.

2.6 5-HT receptors

Within the serotonergic system, two receptor subtypes, 5-HT1A and 5-HT2A, have been predominantly investigated in relation to the alterations associated with Major Depressive Disorder (MDD). Other serotonin receptor subtypes have largely been examined in the context of antidepressant efficacy studies. Positron Emission Tomography (PET) imaging studies have consistently demonstrated a marked reduction in both the availability and total density of 5-HT1A receptors in the hippocampus across various depressive-like phenotypes. Notably, these studies also report an increase in the proportion of high-affinity state receptors, which may reflect heightened sensitivity to serotonin (Vazquez-Matias et al., 2023). Conversely, 5-HT2A receptors, implicated in initiating anxious responses, are frequently found to be elevated in the frontal cortex of individuals with MDD. This elevation has been linked to reduced activity of protein kinase A, a key regulator of intracellular signaling (Shelton et al., 2009).

A reduction in receptor quantity and availability, as observed in 5-HT1A receptor profiles, may compel the remaining receptors into a high-affinity state, thereby increasing their responsiveness to serotonin and triggering downstream cellular responses. Activation of 5-HT1A receptors initiates intracellular pathways such as the inhibition of adenylate cyclase, leading to reduced cyclic AMP (cAMP) levels, activation of potassium channels, and decreased calcium influx. Collectively, these processes produce a calming effect on neural activity, contributing to mood stabilization. However, excessive receptor activity can disrupt the normal functioning of mood-regulating neural circuits. Such overactivity dampens neuronal excitability and impairs the brain's capacity to respond appropriately to serotonergic signals, potentially exacerbating the symptoms of depression (Albert PR, Vahid-Ansari F., 2019).

These insights are derived primarily from extensive laboratory studies conducted on animal models. Most experimental paradigms isolate individual biomarkers, confirming their relevance as potential indicators of depressive-like states. However, when analyzed in isolation, these biomarkers may also signify other neuropsychiatric conditions, raising questions about their specificity for MDD. Thus, it is critical to investigate whether "natural" examples exist that exhibit multiple biomarkers concurrently, strengthening the hypothesis that these indicators collectively signify depressive states.

Captive animal populations offer a unique opportunity to examine such phenomena. Evidence suggests that routine husbandry practices, even when devoid of malicious intent, can impose chronic stressors that produce depressive-like phenotypes. These stressors appear to elicit a convergence of biomarkers, mirroring those identified in laboratory models, thereby supporting their validity as indicators of depressive states in non-human animals (Lecorps et al., 2021).

Other studied biomarkers cannot be considered reliable to investigate on this matter due to inconsistent results.

Biomarkers	Found in	Observations	Citations
serotonin	animals & humans	Reduced levels	Vazquez-Matias D. A. et al, 2023 ; Pu J. et al., 2021.
dopamine	animals & humans	Reduced levels	Nelson & Cox, 2017 ; Pu J. et al., 2021.

norepinephrine	animals & humans	Dysregulation in its levels	Nelson & Cox, 2017 ; Pu J. et al., 2021.
BDNF	animals & humans	Reduced expression in brain tissues	Babington S. et at., 2024 ; Lu B., & Chow A., 1999.
HPA axis	animals & humans	Malfunction	Müller, M. B., & Holsboer, F., 2006.
Homocysteine	animals & humans	Increased levels	Vaccarino et al., 2021.
5-HT receptors	animals & humans	Reduced function	Albert PR, Vahid-Ansari F., 2019 ; Vazquez-Matias et al., 2023 ; Shelton et al., 2009 ; Lecorps et al., 2021.
GABA	humans	Reduced levels within the system - discordant information in the animal field.	Yuen, J. et al, 2021 ; Cryan J. F. & Holmes A. 2005.
Inflammatory markers (CRP, IL-6, TNF-α)	humans	Generally high levels, showing systemic inflammation - it is still under studies in the animal field	Roohi E., Jaafari, N. & Hashemian F., 2021. ; Kouba B. R. et al, 2024.

table 1: the first column indicates some of the primary human biomarkers, the second shows which ones have been found also in animals

chapter 3
Validation through comparison

Understanding that certain environmental and social stressors predispose individuals to depressive states provides an opportunity to model these phenomena in animals. Behavioral observations under such conditions, compared with human behavior, offer valuable insights into the alignment between species and reinforce the hypothesis that these models can be valid proxies for depressive states in humans.

One extensively studied stressor is maternal separation (MS), which is known to predispose both mothers and offspring to psychological and psychiatric disorders. A systematic review conducted by the University of Porto examined maternal behaviors in rodents subjected to maternal separation from their litters (Alves et al., 2020). The findings indicated that prolonged separation led to depressive-like behaviors in the dams, including increased immobility in the presence or absence of their offspring and a diminished interest in external stimuli, as assessed by sucrose preference tests (Boccia et al., 2007).

At the molecular level, maternal separation was associated with a reduction in serotonin synthesis and decreased availability of 5-HT1A serotonergic receptors in maternal brains (Stamatakis et al., 2015; Sung et al., 2010). These alterations suggest that maternal separation not only affects observable behaviors but also induces neurochemical changes linked to mood regulation. Moreover, hippocampal studies in dams revealed decreased cell proliferation and increased apoptosis, further supporting the neurobiological underpinnings of depressive states triggered by maternal separation (Sung et al., 2010).

Similarly, the effects of maternal separation on offspring have been extensively investigated. Behavioral adaptations observed in offspring subjected to long-term maternal separation include reduced motility, anhedonia, and dysfunctions in the hypothalamic-pituitary-adrenal (HPA) axis. These alterations are often accompanied by a downregulation of noradrenaline, further highlighting the stress-induced neurochemical disruptions associated with early-life adversity (Aisa et al., 2007).

The convergence of behavioral and molecular evidence across these studies underscores the potential for maternal separation paradigms to serve as a translational model for depressive states. These findings not only elucidate the impact of early-life stress on both maternal and offspring populations but also align with the broader neurobiological and behavioral characteristics observed in human depressive disorders.

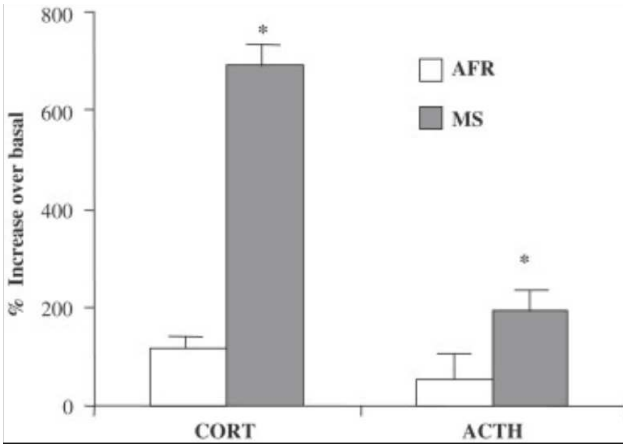

figure 4：Effect of maternal separation (MS) on plasma corticosterone and ACTH responses to an acute stressor (15 min swimming). Data are presented as percentage increase over basal values; *$p<0.001$ vs control (AFR) rats, Student's *t*-test. Basal levels were 68.45±6.30 and 70.19±5.59 ng/ml (corticosterone) and 178.05±28.08 and 165.52±48.28 pg/ml (ACTH) for AFR and MS groups, respectively.

According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5), postpartum depression (PPD) is classified as an episode of major depressive disorder (MDD). Diagnostic criteria include the presence of one or more primary symptoms, such as depressed mood or anhedonia, accompanied by at least five additional symptoms, such as weight changes, sleep and appetite disturbances, psychomotor agitation or retardation, fatigue, feelings of worthlessness or excessive guilt, diminished concentration, and recurrent thoughts of death or suicidal ideation. These symptoms must persist for a minimum of two weeks and may occur during pregnancy or within 4–6 weeks postpartum (American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.)).

Various experimental scenarios have been designed to explore the potential etiologies of PPD, including chronic social stress, hormone withdrawal, gestational stress, pup separation, pre-gestational stress, and high-fat diets. The findings from these studies exhibit striking similarities to those observed in other animal models of MDD. For instance, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been documented both prenatal and postpartum, characterized by fluctuations in corticosterone-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol levels, which rise during pregnancy and sharply decline following delivery (Daigle, 2018). Other neurochemical changes include disrupted serotonin (5-HT) and norepinephrine (NE) levels, reduced oxytocin mRNA expression, and decreased brain-derived neurotrophic factor (BDNF) expression (Mir et al., 2021).

Behavioral studies of PPD models report reduced grooming and general anhedonia, increased latency to eat, diminished food intake, and either reduced or excessive weight gain. These changes may stem from overfeeding or stress-related anorexia. Additionally, maternal behaviors are adversely affected, with reductions in pup grooming, increased immobility, delayed responses to pup calls, restricted access to the udder, and, in some cases, aggressive behaviors toward offspring. Increased anxiety phenotypes have also been observed, further demonstrating the parallels between animal models and human MDD (Mir et al., 2021).

While physiological and behavioral observations align across multiple studies, it is notable that not all outcomes are consistently observed in every individual or in every experimental context. However, corroborating evidence outside laboratory settings further supports these findings. For example, behavioral responses consistent with depressive states have been observed in livestock, such as dairy cows and sows, during and after parturition. These include reduced maternal care, delayed responses to offspring calls, restlessness, or immobility, as well as altered grooming and nursing behaviors (Lecorps et al., 2021).

Social stress in humans represents another critical life event strongly associated with increased depression risk. Correspondingly, several animal studies have sought to model social stress-induced depression, often utilizing paradigms such as the resident-intruder test. In this model, an intruder animal is introduced into the home cage of a resident animal, and their interactions are observed over a 10-minute period before separation. While this paradigm is widely used to evaluate aggression and social stress, its reliability in replicating depressive states has been questioned due to the variability in behavioral and physiological outcomes (Koolhaas et al., 2013).

Biological factors underlying MDD—such as neurotransmitter imbalances, genetic predispositions, structural and functional brain abnormalities, and hormonal changes—are more readily reproducible in controlled experiments. However, psychological and environmental contributors, including early-life adversities, maladaptive cognitive patterns, and chronic life stressors, are more challenging to model in laboratory settings. It is increasingly recognized that MDD arises from a multifactorial interplay of these biological, psychological, and environmental elements, rather than from any single factor. Consequently, studying individual factors in isolation provides only partial insights into the complexity of depressive disorders.

The use of chronic stress paradigms in animal models highlights the cumulative effects of repeated exposure to adverse conditions. Evidence suggests that the additive and potentially multiplicative effects of multiple stressors—particularly when experienced within short time frames—can overwhelm animals' coping mechanisms, leading to persistent negative mood states. This phenomenon is observed in non-human animals

exposed to compounded stressors, such as separation from offspring, restrictive housing, and social mixing with unfamiliar conspecifics during parturition, a known stressor itself. These stressors likely overwhelm animals' adaptive capacities, leading to depressive-like states.

Despite challenges in recreating specific life events in laboratories, behavioral studies of living conditions reveal striking similarities between animals and humans experiencing depressive states. Observable behaviors in animals, such as anhedonia, immobility, and reduced caregiving, align with those reported in humans suffering from MDD. These findings suggest that animals are capable of experiencing depressive states, even though the complete physiological mechanisms underlying these behaviors remain to be fully elucidated.

chapter 4
Approaches to Supporting Individuals with Major Depressive Disorder (MDD)

Having identified key biomarkers and behavioral manifestations of this condition, the focus now shifts toward exploring strategies for addressing and managing this disorder.

One important aspect to be remembered is that, since verbal communication is quite impossible, the way to help an animal who's dealing with depression won't start by talking them through and convincing them to start some sort of therapy but it needs to start with making the individual feel safe.
When an individual is feeling safe, their guard is let down, even for a brief moment, and that allows them to drop the stress, fight or flight, survival mode the body undergoes during this type of disorder.

● Hormonal Changes ● Chronic Illnesses ● Medication Side Effects ● Trauma and Abuse ● Loss and Grief ● Separation from herd/companionship ● Relocation ● Environmental deprivation ● Stressful Life Events	● Neglect ● Maternal deprivation ● Living Conditions ● Lack of Sleep ● Poor nutrition ● Physical Inactivity ● Boredom ● Unmet Instinctual Needs ● Social Isolation

table 3: possible recognised causes of depression in animals

The possible causes of depression are well-known, and it is not attributed to a single factor but rather to the interplay of multiple elements. If these factors remain unaddressed over time, they risk leading to a state of permanent emotional decline.(MacLellan A. et al, 2024)Consequently, the simplest solution may appear to be to "avoid it," yet this is far easier said than done. The starting point must be rooted in respect—respect for other lives that never chose to be subject to human control or manipulation.

Above all, the Five Freedoms shall be followed

- freedom from hunger and thirst
- freedom from discomfort
- freedom from pain, injury and disease
- freedom in expressing normal behaviors
- freedom from pain and distress

These fundamental needs are widely regarded as the foundation for respecting any form of life. Beyond meeting these basic requirements, it is essential to consider the specific needs and individual characteristics of each being to truly enhance their overall welfare.

The next logical step is prevention—ensuring that animals do not develop such conditions in the first place. However, this goal is complicated by the absence of a comprehensive understanding of the factors that may contribute to depression in animals, as well as by the frequent lack of information about the animal's individual history. These challenges often hinder effective and timely intervention when addressing cases of depression.

When faced with an animal already suffering from depression, the focus shifts to intervention. In these cases, therapeutic approaches must be carefully adapted to the animal’s specific needs, offering the best chance for recovery and improved well-being. Some examples of therapies (Neurolaunch, 2024)currently used include:

1. Environmental Enrichment:
    - Goal: Provide mental and physical stimulation to counteract boredom or stress.
2. Companionship:
    - Goal: Address social needs in social animals.
3. Behavioral Therapy:
    - Goal: Encourage normal, healthy behaviors through structured training or modification.

4. Medication:
    - Goal: Address severe symptoms when behavioral or environmental changes are insufficient.
5. Exercise Therapy:
    - Goal: Stimulate endorphin release and physical activity to combat lethargy.
6. Sensory Stimulation:
    - Goal: Provide novel sensory experiences to alleviate stress.

Once therapy has commenced, it is essential to provide consistent and thorough support throughout the process. Observation plays a crucial role in this phase: how is the individual responding to the intervention? A positive outcome indicates progress and suggests that the chosen approach is effective. Conversely, a negative outcome serves as a clear signal to reassess and explore alternative therapies. It is not uncommon for individuals to require several attempts with different therapeutic methods before finding the one that best suits their needs.

Equally important is recognizing the significance of time. Healing is a gradual process, and expecting rapid results is unrealistic. Patience is paramount, as setbacks may necessitate adjustments to the therapy, and in some cases, restarting certain aspects of the process. While it may not always require returning to the very beginning, such setbacks can undo substantial progress.

Consistency, patience, and respect for the individual's need for time and space—both for independent reflection and supported healing—are critical components of effective recovery. Providing this balance ensures the individual can move forward at their own pace while benefiting from the guidance and presence of those offering help.

Some examples of recovery from depression are

1. Elephants Mourning Loss(Carnahan D., 2019):
    - Case: Elephants are known for their strong emotional bonds. After the death of a companion, elephants have been observed to exhibit signs of depression such as refusal to eat or engage in social behavior.
    - Therapy: Allowing the mourning elephant to grieve while providing opportunities to integrate into a supportive group.
    - Outcome: Gradual return to normal behaviors with herd support.
2. Chimpanzee Rehabilitation(Bradshaw G.A. et al, 2008):
    - Case: Chimpanzees rescued from traumatic situations (e.g., circuses or testing facilities) often display withdrawn or self-harming behaviors.
    - Therapy: Environmental enrichment, behavioral training, and forming social bonds with other chimpanzees.

- Outcome: Improved interaction with caregivers and other chimps, and restoration of natural behaviors like grooming and play.

3. Dogs in Shelters(Cussen V. & Pamela J.R., 2019):
    - Case: Dogs in long-term shelter care often exhibit signs of depression such as apathy and refusal to eat.
    - Therapy: Increased human interaction (e.g., regular walks and cuddle sessions), sensory stimulation (toys, music), and adoption into a loving home.
    - Outcome: Notable improvement in activity levels, appetite, and sociability.
4. Parrots with Feather Plucking(VetTimes, 2024):
    - Case: Parrots in isolation or with insufficient stimulation often engage in self-destructive behaviors like feather plucking.
    - Therapy: Increasing mental stimulation with toys, training sessions, or providing a companion parrot.
    - Outcome: Reduced feather plucking and display of more natural behaviors like preening and vocalization.
5. Dolphins in Captivity(Myers J., 2021):
    - Case: Captive dolphins have shown symptoms of depression, such as swimming in repetitive patterns or refusing to eat.
    - Therapy: Interactive sessions with trainers, provision of toys, or introduction to larger, more dynamic groups.
    - Outcome: Renewed playful behaviors and improved appetite.

6. Environmental Enrichment:
    - Goal: Provide mental and physical stimulation to counteract boredom or stress.
    - Example:
        i. Animals: Zoo animals or captive animals.
        ii. Therapy: Introducing interactive toys, puzzles, or social interactions. For example, providing a variety of climbing structures, swings, and foraging puzzles for primates in captivity.
        iii. Outcome: Improved engagement and reduced stereotypic behaviors like pacing.
7. Companionship:
    - Goal: Address social needs in social animals.
    - Example:
        i. Animals: Dogs, cats, elephants, parrots.

        ii. Therapy: Pairing with another companion animal or increasing human-animal interaction. Elephants, for example, thrive in herds, and isolating one often leads to signs of depression.
        iii. Outcome: Enhanced mood and natural behaviors, such as elephants displaying playful or social behavior after reunion with their herd.
8. Behavioral Therapy:
    - Goal: Encourage normal, healthy behaviors through structured training or modification.
    - Example:
        i. Animals: Dogs with separation anxiety or depression after losing an owner.
        ii. Therapy: Positive reinforcement training to rebuild confidence and engagement, along with gradual desensitization to triggers of anxiety.
        iii. Outcome: Dogs showing restored energy levels and reduced anxiety behaviors.
9. Exercise Therapy:
    - Goal: Stimulate endorphin release and physical activity to combat lethargy.
    - Example:
        i. Animals: Horses or dogs.
        ii. Therapy: Regular walks, play sessions, or structured exercise routines. For instance, a depressed horse may benefit from turnout time in a pasture or interactive games.
        iii. Outcome: Increased activity levels and a more alert demeanor.
10. Sensory Stimulation:
    - Goal: Provide novel sensory experiences to alleviate stress.
    - Example:
        i. Animals: Shelter animals or animals in recovery.
        ii. Therapy: Playing soothing music, aromatherapy (lavender or chamomile), or visual stimulation like videos for cats (e.g., bird or fish videos).
        iii. Outcome: Reduced anxiety and improved overall calmness.
11. Medication:
    - Goal: Address severe symptoms when behavioral or environmental changes are insufficient.
    - Example:
        i. Animals: Dogs, cats, or even horses showing persistent depression.
        ii. Therapy: Prescription of antidepressants like fluoxetine (Prozac) or clomipramine under veterinary guidance.

iii. Outcome: Stabilized mood and decreased symptoms such as lethargy or compulsive behaviors.

Examples from 6 to 11 were extracted from “Understanding Animal Depression: Signs, Causes, and Treatment”(Neurolaunch, 2024).

Medication should be regarded as a last resort, reserved for cases where depression is rooted in a genetic predisposition. In most other instances, depressive conditions can be effectively managed through non-pharmacological interventions.

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