# Essential elements in depression and anxiety. Part I

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Review article

# Essential elements in depression and anxiety. Part I

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ABSTRACT

Essential elements are very important for the proper functioning of the human body. They are required for fundamental life processes such as cell division and differentiation and protein synthesis. Thus a deficiency of these essential elements is associated with an enormous health risk that can ultimately lead to death. In recent years, studies have provided valuable information on the involvement of essential elements in psychiatric disorders, in particular depression and anxiety.

There is strong evidence indicating that deficiency of essential elements can lead to the development of depressive and/or anxiogenic behaviour and supplementation can enhance therapeutic effect of antidepressants and anxiolytics. This review presents the most important results from preclinical and clinical studies showing involvement of essential elements such as zinc, magnesium, lithium, iron, calcium and chromium in depression and anxiety. From these studies it is evident that different types of depression and anxiety respond to treatment at different receptors indicating that the underlying mechanisms are slightly different. Furthermore, administration of low dose antidepressants supplemented with an element is effective and can reduce unwanted side effects in different types of depression/anxiety.



**Contents**



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## Introduction

Essential elements are required for the proper functioning of living organisms (e.g. [1]) and are classed as essential if they are required for a specific biochemical function, or if dietary deprivation in animal experiments causes defects in biological function, which can be restored or prevented by administration of the element. These elements are involved in a diversity of mechanisms such as (i) regulation of cellular function, (ii) growth and maintenance, (iii) neuromodulation, and are either synthesised by the body or obtained through diet. A lack of essential elements may result in severe consequences including alterations in immune function, altered cognition and growth and developmental changes [1].

Data from preclinical and clinical studies have shown the importance of many elements in the pathophysiology of affective disorders [2–7]. There is an abundance of evidence regarding the involvement of elements in depression and anxiety, particularly alterations of metal element homeostasis, however the results have been slightly conflicting. Many elements have an influence on the neural transmission involved in emotional processes, such as the serotonergic, noradrenergic, dopaminergic, glutamatergic and GABAergic systems [8–11].

Deficiency of essential elements can arise through low dietary intake. A Western-style diet high in processed foods (including processed meat, white bread, sugar) is associated with a higher incidence of affective disorders compared to a traditional diet (including fruit, vegetables, wholegrain foods) [12]. Based on preclinical studies, deficiency of elements can lead to neurodegenerative processes and, by association, learning and memory impairments [13]. Patients suffering from depression and/or anxiety also show disturbances of cognition [14,15]. Reversing the deficit of the essential element concerned improves memory function [16]. Supplementation with the deficient element improves the therapeutic effect of commonly used antidepressants, and enhances antidepressant therapy in refractory depression [17]. In addition, supplements of essential nutrients have been shown to be beneficial in susceptible patients, and furthermore, amino acid supplements, such as tryptophan, are also effective as they can be converted into neurotransmitters [18]. Some elements may have similar functions (i.e. iron and manganese) and could compensate for a deficiency of another element [1].

This review will analyse preclinical and clinical evidence regarding essential elements and their relationship with the pathophysiology and treatment of depression and anxiety. We will discuss the most important data from preclinical and clinical studies indicating involvement of essential elements including zinc, magnesium, lithium, iron, calcium and chromium in depression and anxiety.

## Zinc

Zinc is widely recognised as one of the most common trace elements involved in the pathophysiology of depression and anxiety. Zinc is essential for numerous bodily functions, including replication, transcription and protein synthesis, and can thus influence cell division and differentiation [32]. According to the Institute of Medicine of the National Academies the daily requirement for zinc varies depending on age and gender [33]. Adequate daily zinc allowance is 3–8 mg for children, increasing for teenagers and adults to 11 mg (for males) and 8–9 mg (for females). During pregnancy and lactation zinc requirements increase to 11–13 mg. The main sources of zinc include red meat, seafood (in particular oysters which contain 493% of the daily recommended allowance), nuts, beans and whole grains [33].

### *Zinc deficiency*

Early clinical studies reported lower zinc serum in depressed patients compared to healthy patients [4,34,35] and later was proposed as a state marker of depressive disorder [17,34,36]. Thus numerous studies have sought to determine whether low zinc levels contribute to the development of depressive symptoms, or whether low zinc levels are a consequence of the mechanisms that lead to depression. Behavioural paradigms such as the forced swim test and tail suspension test are used in pre-clinical studies to determine the cause of depression and to screen for potential antidepressant treatments.

Animals fed a zinc deficient diet exhibited increased immobility time in FST [2,37–41] or TST [42], indicating that zinc deficiency contributes to the development of depressive-like behaviour. Moreover, zinc deficiency impaired the efficacy of numerous antidepressants which have different mechanisms of action [2,39,41,42].

Together these results indicate that zinc deficiency plays an important role in the development of depression, and the subsequent restoration of zinc reverses behavioural signs of depression in animal models.

### *Antidepressant effects of zinc*

Subsequently the antidepressant effects of zinc were investigated in pre-clinical and clinical studies – zinc was active in the screening tests and models of depression indicating the antidepressant properties of zinc (Table 1 describes commonly used animal tests and models of depression). Different zinc salts such as zinc sulphate, zinc hydroaspartate or zinc chloride showed antidepressant properties in the forced swim test (FST) [7,43–46], tail suspension test (TST) [46,47], chronic mild stress (CMS) [48], chronic unpredictable stress [49] and olfactory bulbectomy (OB) [7] models of depression. Moreover, joint administration of zinc and antidepressants (both ineffective doses) with diverse mechanisms of action was active in the FST [44,45,50,51], TST [47] and CMS [49]. This suggests that zinc may enhance antidepressant action and reduce side effects of commonly used antidepressants. Zinc supplementation seems also to reduce the time required to achieve a therapeutic effect.

These findings were repeated in clinical studies. In 1997, Maes and colleagues demonstrated that serum zinc levels were significantly lower in treatment resistant depression (TRD) patients than in healthy patients [34]. Siwek et al. [17] showed that the serum zinc concentration in treatment resistant patients was 14% lower compared to controls. In this study, 60 patients fulfilling criteria for major depression received imipramine

**Table 1**
Animal tests and models of depression.

| Test/model | Description | References |
|---|---|---|
| Forced swim test (FST) | Rodents are placed in a restricted space filled with water from which they cannot escape and are forced to swim. Initially the rodents attempt to swim, however as the test progresses they show apathy and float on the water (immobility). This is a behavioural sign of despair and represents hopelessness, reflecting negative mood in humans. This behaviour can be reduced by the use of antidepressants which have a different of mechanisms. | [19,20] |
| Tail suspension test (TST) | Mice are subjected to short-term, inescapable stress of being suspended by their tail. Mice become immobile after a period of unsuccessful attempts to escape. The observed immobility is interpreted as behavioural despair in rodents, and may reflect depressive disorders in humans. Various clinically effective antidepressants can reverse the immobility time and increase the time of escape-related behaviours. | [21] |
| Learned helplessness in rats | Rats are placed in a box with a grid floor which has an escape platform that can be inserted through one of the side walls. Learned helplessness is produced in rodents that have no access to the platform by exposure to electric shock on the floor for 1 h. After an appropriate drug treatment, trained rats are tested in the same apparatus with the platform inserted. Rats that were exposed to inescapable and unavoidable electric shock fail to escape shock when escape platform is present. This phenomenon was evaluated as a potential animal model of depression. Antidepressant drugs tend to reduce learned helplessness in rats by decreasing the number of failures to escape. | [22,23] |
| Chronic mild stress model of depression (chronic unpredictable, variable or intermittent stress, CMS) | Chronic mild stress is a widely used rodent model of depression, developed to simulate anhedonia, a major symptom of depression. CMS paradigms consist of repeated exposure to different and unpredictable mild stressors such as placing animals in small cages with bells ringing every few minutes, overnight illumination, food and water deprivation, or group housing in a damp cage, over a sustained period of time (ranging from 10 days to 8 weeks). Chronic exposure to mild stress leads to significant reduction of sucrose consumption in animals, resembling the anhedonic changes observed during episodes of human depression. Decreased sucrose intake, and anhedonia, can be reversed by chronic treatment with antidepressants. | [24,25] |
| Hypermotility in olfactory-bulbectomised rats | The olfactory-bulbectomised rat is used as an animal model of depression to show disruption of the limbic-hypothaliac axis, which leads to behavioural and neurochemical changes, resembling the changes occurring in depressed humans. It has been shown that rodents develop behavioural changes following bilateral olfactory bulbectomy, including hyperactivity in the open field test, enhanced nocturnal activity, deficits in memory, increased open-arm entries in the elevated-plus maze, and changes in food-motivated and conditioned taste aversion behaviour. Moreover alterations in noradrenergic, serotonergic, cholinergic, GABAergic, and glutamatergic neurotransmitter systems are observed after olfactory bulbectomy. Changes in exploratory behaviour in rats after bilateral olfactory bulbectomy are reversed by chronic treatment with antidepressant drugs. | [26,27] |
| Chronic administration of LPS | Repeated injections of LPS once daily for 5 days and repeated monthly for 4 consecutive months induces chronic anhedonia in female mice. Behavioural symptoms are observed up to 7 weeks post injections, whilst administration of fluoxetine for 3 weeks abolished the neurobehavioral effects of LPS. | [28] |
| The Flinder Sensitive Line (FSL) | FSL is a rat strain that displays distinct behavioural and neurochemical features of major depression, such as psychomotor retardation (reduced mobility in the forced swim test, lower activity in novel open-field tests), decreased appetite and weight compared to controls, memory impairments, and several abnormalities in the serotonergic system. Treatment with selective serotonin reuptake inhibitors (SSRIs) reverses these symptoms in FSL rats. | [29] |
| Chronic administration of corticosterone | Chronic administration of corticosterone in mice and rats increases immobility behaviour in the forced-swim test and tail suspension test in a time-dependent manner. | [30,31] |

(~140 mg/day) and once daily either placebo or zinc supplementation (25 mg/day) for 12 weeks. Following treatment the depression scores of TRD patients receiving zinc supplements were significantly reduced. The results show that zinc supplements augment the efficacy of imipramine, suggesting a link between drug resistance and disturbances in glutamatergic transmission [17].

The antidepressant properties of zinc may be explained by attenuation of the glutamatergic system via inactivation of the glutamatergic NMDA (N-methyl-D-aspartate) receptor [52]. Studies in mice that were fed with a zinc deficient diet indicated that depressive behaviour could be explained by hyperactivity of the glutamatergic system. In stressed animals higher levels of glutamate accumulated due to corticosterone-mediated blockade of glutamate transporter activity under abnormal levels of corticosterone secretion [53] (Fig. 2). Based on the antidepressant-like activity of drugs that impair glutamatergic transmission, a link was observed between the hyperactivity of the glutamate system and depression [54–57]. Zinc inhibits excessive activation of the NMDA receptor via its binding site (Fig. 1), whilst intraperitoneal administration of NMDA (75 mg/kg) antagonised the antidepressant action of zinc. Additionally, ineffective doses of NMDA antagonists (CGP 37849, L-701,324, D-cycloserine, and MK-801) administered with ineffective doses of zinc exhibited a significant reduction of immobility time in the FST [58].

Furthermore hyperactivity of the hypothalamus–pituitary–adrenal (HPA) axis seems to be specifically important in depressive and stress related behaviours. Animals deficient in zinc demonstrated higher corticosterone levels indicating hyperactivity of the main stress axis [37,38,40,42]. In our previous study we observed increased corticosterone levels in mice after 4 and 10 weeks of zinc deficient diet, correlating with pro-depressive behaviour in FST. Yang et al. [59] indicated that increased glucocorticoid secretion contributes to the blockade of glutamate uptake. This impairment was prevented by administering a glucocorticoid receptor antagonist (RU38486) before inducing stress.

Previous studies have shown interesting results which may explain the involvement of zinc in the pathophysiology of depression. Holst et al. [60] showed that zinc is a natural agonist of the GPR39 receptor. GPR39 belongs to the ghrelin receptor family, and is widely expressed in the central nervous system. GPR39 is thought to inhibit apoptosis and mediate neural synaptic signalling [61]. In our recent studies we found decreased expression of cortical GPR39 receptor in mice given a zinc-deficient diet [62]. Moreover, administration of selective antidepressants caused an increase in expression of cortical GPR39-$Zn^{2+}$-sensing receptor [63]. In our latest study we found GPR39 was down regulated in the hippocampus and frontal cortex of suicide victims [64]. Overall these results indicate the significant

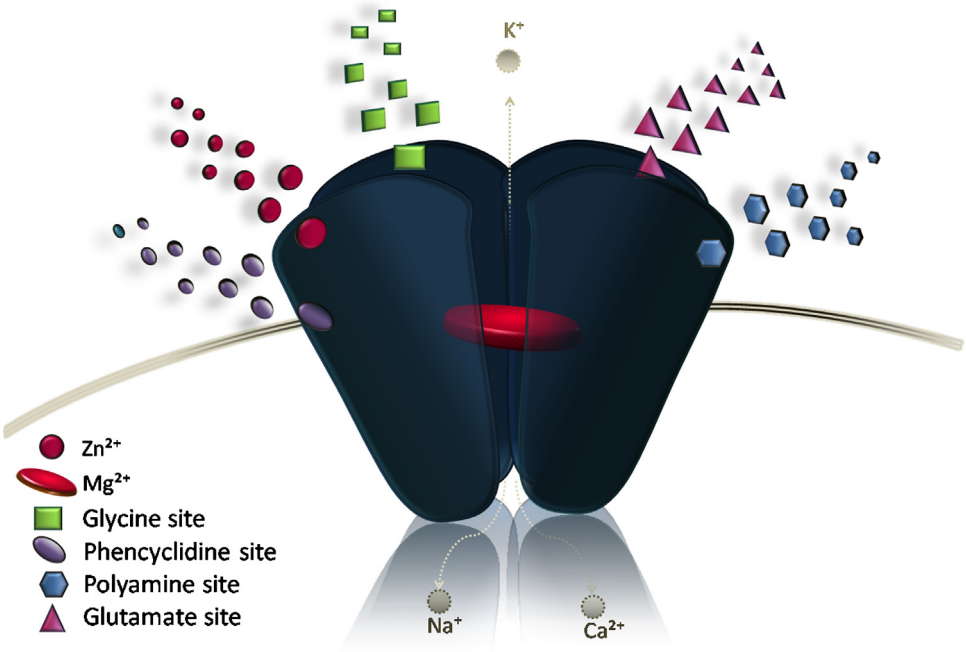


**Fig. 1.** The NMDA receptor is composed of four subunits with glutamate binding sites. Magnesium can regulate the influx of calcium into cells by binding to its binding site. Calcium channel blockers also prevent calcium from entering cells and can be used as an antidepressant.

involvement of the GPR39 receptor in pathophysiology and therapy of depression.

*Zinc and anxiety*

Data from preclinical studies implicates zinc deficiency as a possible cause of anxiogenic-like behaviour. Zinc deficient diet caused an anxiogenic effect in the novelty suppressed feeding test in mice, and was prevented by chronic desipramine administration [41]. The increased anxiety-like behaviour due to zinc deficiency was observed in rats as well – the frequency of line crossing and the time spent grooming in the open field test were decreased after 2 weeks of zinc deficient diet. In the plus-maze test, rats fed with zinc deficient diet spent less time in the open arms [65].

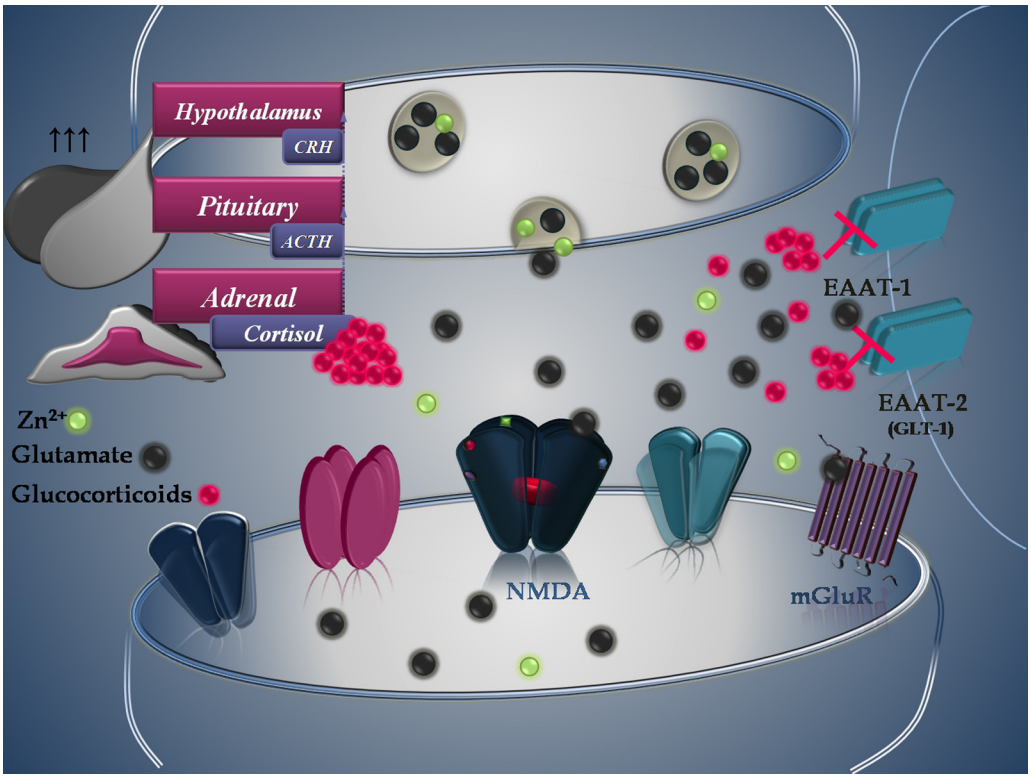


**Fig. 2.** Glutamate accumulation may occur through cortisol/corticosterone-mediated blockade of glutamate transporter activity under stress conditions. In zinc deficiency the hyperactivation of the glutamatergic system is observed. High levels of glutamate are accumulated through corticosterone-mediated blockade of glutamate transporter activity under abnormal corticosterone release in stressed or zinc-deficient animals.

**Table 2**
Animal tests of anxiety.

| Test/model | Description | References |
|---|---|---|
| Four plate test | Mice are placed in the test box with the floor covered with four identical rectangular metal plates separated from one another by a gap. The plates are connected to a source of continuous current. Every time the animal crosses from one plate to another it receives a mild foot shock suppressing exploratory behaviour of the novel environment. Classic anxiolytic compounds such as benzodiazepines reduce shock-induced anxiety and increase the number of plate crossings. | [91,92] |
| Elevated plus maze | The model is based on rodents' aversion to open spaces. Rats or mice are placed in a plus-shaped apparatus with two open and two closed arms, elevated 40–70 cm from the floor. The animals' aversion of open spaces leads to the avoidance of open areas. Known anxiolytics increase the time spent in the open arms and the number of entries into the open arms. | [93,94] |
| Vogel test | In this procedure, naive rats are punished with mild electrical shock when drinking water. This leads to a significant reduction of water consumption in animals. Drugs with anxiolytic properties increase the number of shocks and reestablish drinking responses. | [95] |
| Light–dark model | Rats and mice tend to explore a novel environment but have an innate aversion to brightly illuminated areas. The test apparatus consists of a small dark safe area and a large brightly-lit compartment. Compounds with anxiolytic properties increase the numer of crossings between two chambers and locomotor activity of rodents. Classic anxiolytics as well as the newer anxiolytic-like compounds (e.g. serotonergic drugs) can be detected using this paradigm. | [96,97] |
| Fear potentiated startle (FPS) | The FSP consists of two sessions. In the initial step a cue (e.g. light) is paired with aversive unconditioned stimulus (foot shock). In the next step the startle response is elicited in the presence or absence of the cue. It has been shown that startle activity can be reduced by anxiolytic drugs such as benzodiazepines. | [98–100] |
| Contextual fear conditioning | Rats are placed individually in a novel environment where an aversive stimulus is present. Animals learn to associate the aversive stimulus in context to the environment. This elicits the fear response and animals freeze (the absence of all movements except for respiration), indicating fear. This can be reversed by benzodiazepines. | [100,101] |
| Cued fear conditioning | Cued fear conditioning is conceptually similar to contextual conditioning. In this test animals are given a pre-exposure trial without an aversive stimulus. In this case the fear response is due to a cue (a tone paired with footshock). It is suggested that the context (environment) is not as accurate as a shock to provoke the fear response. In this test freezing in animals (the absence of all movements except for respiration) is considered as an indicator of fear. | [100,101] |

There is strong evidence that zinc also exhibits anxiolytic-like activity. Acute administration of zinc hydroaspartate showed anxiolytic-like effects in the elevated plus maze test in rats and mice, the four-plate test in mice and the stress induced hyperthermia test in mice [66] (Table 2 describes commonly used animal tests of anxiety). In addition Abdel-Maksoud et al. [67] showed anxiolytic-like effects of chronic zinc chloride administration in the Vogel conflict test (VCT). Further, intraperitoneal administration of zinc for 7 days showed anxiolytic-like effects in the elevated plus maze test. An increase in the number of open arm entries and time spent in the open arms was observed with different doses of Zn (15 and 20 mg/kg) [68].

In summary, (i) zinc deficiency plays an important role in the development of depression, (ii) reversal of depressive like symptoms can achieved using zinc, and (iii) zinc is likely to exert its antidepressant effects on the NMDA receptor pathway.

## Magnesium

Magnesium is an essential element required for normal physiological homeostasis, is required as a cofactor by in excess of 300 enzymes and is involved in production of ATP and nucleic acids [69]. Magnesium deficiency can have direct consequences on reactions that require ATP, such as muscle contraction, glucose utilisation and protein synthesis. Bone provides a large store of this element, and a third of this can be used to balance changes in the serum magnesium levels. This pool of magnesium is essential for maintaining homeostasis and can undergo active transport between the blood and cerebrospinal fluid – a vital mechanism that ensures the magnesium level in the brain is constant.

Magnesium is acquired through food and drink and the daily recommended intake of magnesium for adults is 0.30–0.35 mEq/kg [70]. Magnesium deficiency can arise though low dietary intake and is common in the developed world as consumption of refined and processed food has increased [71] which have reduced amounts of essential elements [72]. Furthermore, prolonged periods of magnesium deficiency can lead to a reduction in the levels of magnesium in the brain. Consequently, affective disorders, cardiac arrhythmias and neuromuscular hyper-excitability can occur [73,74]. Previous studies have shown that low magnesium levels are related to depression, anxiety and apathy [75], however magnesium has not been implicated in panic disorder which often manifests alongside depression [76].

### *Mechanisms of magnesium in depression and anxiety*

The monoamine neurotransmitter system is considered to be one of the central pathways previously implicated in depression theory [77]. Magnesium can block the NMDA receptor (Fig. 1) in a voltage dependent manner [78–80], however if insufficient magnesium is present there is an abnormal influx of calcium into cells causing the release of intracellular glutamate, the excitatory neurotransmitter. Glutamate causes further depolarisation starting the cycle over and can lead to neuronal dysfunction and depression in severe cases (Fig. 3).

The involvement of the glycineB site of the NMDA receptor was demonstrated by antagonising the anxiolytic effects of magnesium (20 mg/kg) with D-serine (100 nmol/mouse) in the elevated plus maze test [81]. Furthermore NMDA administration (75 mg/kg) antagonised the effects anti-depressant effects of magnesium hydroaspartate (20 and 30 mg/kg) in the FST [10]. These studies concluded that the NMDA/glutamate pathway is an important mechanism is the anxiolytic and anti-depressant effects of magnesium.

In addition, the limbic–hypothalamus–pituitary–adrenocortical (HPA) axis is thought to be implicated in magnesium deficiency [6]. Alterations to the HPA axis often occur in affective disorders, such as up-regulation of the HPA axis following magnesium deficiency [3].

### *Magnesium deficiency*

Clinical data has shown that patients deficient in magnesium are more susceptible to affective disorders and accompanying

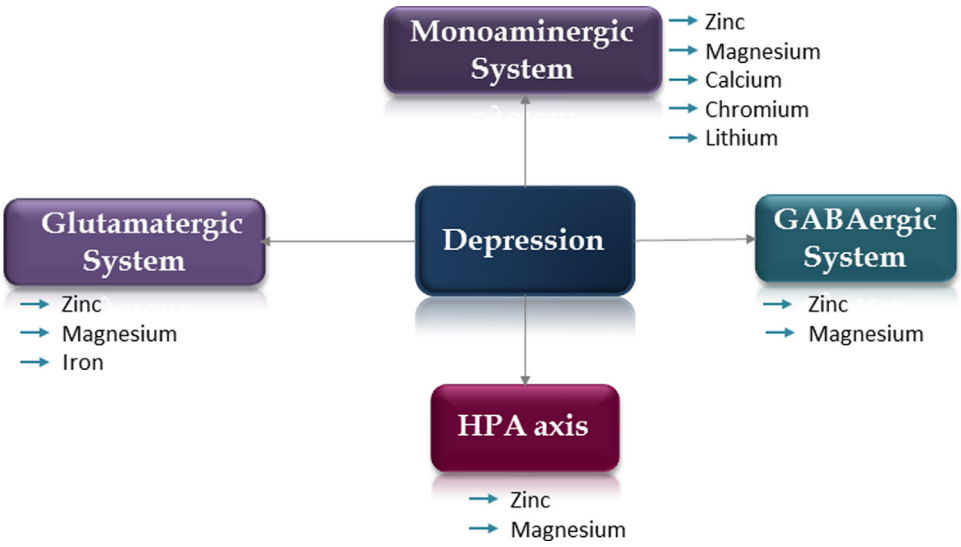


**Fig. 3.** Neurotranmitter systems involved in the pathophysiology of depression. Depression has a multitude of effects on different neurotransmitter systems, which may be due to the aetiology of the various affective disorders. Many elements have an effect on the monoaminergic system, in particular dopaminergic, noradrenergic and serotonergic pathways. Elements such as zinc and magnesium are important modulators of the NMDA receptor. Appropriate levels of these elements regulate the HPA axis and prevent the hyperactivity seen due to deficiency of zinc or magnesium.

health problems. Major and suicidal depression are considered to be a consequence of magnesium deficiency and a recent clinical study used magnesium to treat depression that arose through different circumstances [6]. Following treatment with magnesium taurinate or glycinate (125–300 mg) all depressive- and anxiety-like symptoms were alleviated, as well as any mental deficits. Lower serum levels of magnesium have been linked to patients with major depression [6], and depressed patients with suicidal tendencies had lower cerebrospinal fluid levels of magnesium [82]. Total magnesium was also found to be lower in depressed patients [83], which was restored following lithium treatment.

Animals subjected to a magnesium diet deficient have shown behavioural deficits associated with depression and anxiety. A recent study tested different strains of mice (C57Bl/6N and Balb/c) in stress related paradigms following a magnesium deficient diet [84]. Some of these tests were previously supported in different studies using C57Bl/6J and reported similar results [3,85]. Significant results include reduced time in the centre of the open field test, slower entry into the bright side of the light/dark test and increased latency to eat the food in the centre of the arena during the hyponeophagia test by the magnesium deficient animals compared to control. In all the tests prolonged treatment with the SSRI paroxetine had no effect on the anxiety levels of the mice, however in the hyponeophagia test mice given desipramine had a reduced latency to eat the food. Desipramine had no effect on anxiety levels in the light/dark test and open field test [3]. These studies indicate that a diet deficient in magnesium resulted in anxiety, and that desipramine was effective at lowering stress in these animals in some of the behavioural paradigms [84]. Furthermore the changes in behaviour following drug treatment were complimented by changes in the HPA axis, restoring this system to normal.

*Antidepressant and anxiolytic effects of magnesium*

Previous studies have shown that magnesium has antidepressant and anxiolytic properties [3,6,81,82,86]. Magnesium chloride (15–50 mg/kg) showed antidepressant activity by reducing the immobility time in the FST in rats, however chronic administration (14 days) of magnesium was not effective [87]. In a similar study tolerance to magnesium hydroaspartate (30 mg/kg) was not observed in the FST and elevated plus maze [86]. This study also showed that plasma magnesium levels are increased following acute and chronic magnesium treatment.

Many classical antidepressants are effective at treating depression by reducing or altering NMDA function and Poleszak and colleagues [10,81] showed that magnesium administered with low dose NMDA antagonists caused a reduction in anxiety-related behaviours using the elevated plus maze and a reduction in depressive-like behaviours in the FST. NMDA antagonists were unable to reduce anxiety or depression alone at low doses: 0.3 mg/kg CGP 37849 (competitive antagonist), 1 mg/kg L-701,324 (glycineB antagonist), 2.5 mg/kg D-cycloserine (partial glycineB agonist) and 0.05 mg/kg MK-801 (non-competitive antagonist). Low dose magnesium hydroaspartate (10 mg/kg) in combination with these low dose NMDA antagonists was effective in reducing the anxiety or depressive related behaviour in both behavioural paradigms.

Preclinical studies that investigated magnesium deficiency showed enhanced depressive-like behaviour in the forced swim test (FST) and increased anxiety-like behaviour in the light/dark and open field tests in mice [3,84]. Desipramine and *Hypericum perforatum* extract improved the immobility time in the forced swim test, whilst only *H. perforatum* extract was able to reduce anxiolytic effects in the open field study [3]. Similar results were demonstrated by Decollogne et al. [88] in which rats were treated with a single dose of magnesium organic salt. This treatment reduced immobility time in the FST (at doses between 30 and 100 mg/kg), and in addition increased plasma levels of magnesium. Furthermore magnesium chloride (15–50 mg/kg) was administered to rats and was able to reduce immobility time in the FST, suggesting antidepressant-like actions of magnesium [89]. These results indicate that magnesium induces an antidepressant effect and suggests potential antidepressant activity of magnesium in depression. Moreover, ineffective doses of magnesium (5 and 10 mg/kg) given jointly with ineffective doses of imipramine reduced immobility time in FST [87]. These results provide evidence that lower doses of antidepressants could be used in combination with magnesium to treat depression and avoid the side effects observed with higher doses of antidepressant treatment.

The effectiveness of combining anti-depressants and anxiolytics with magnesium in patients was also observed in clinical studies. Combination of magnesium oxide and verapamil was able to reduce the BPRS scores of manic patients compared to control [90].

These studies indicate that magnesium deficiency is (i) a cause of depressive disorder and anxiety, (ii) is an element with antidepressant and anxiolytic activity at the NMDA receptor, and (iii) that magnesium can be used as an adjunct to antidepressant treatment.

## Lithium

Lithium is a trace element that is considered to be essential. The biological effects of lithium involve interactions with numerous enzymes, hormones and vitamins, whilst animal studies have also shown that lithium is involved in expansion of the pluripotent stem cell population to mature progenitor cells and components of the blood [102]. The recommended daily intake in adults (70 kg) is 650–3100 μg [102] and baseline levels of lithium in adult humans range between 7 and 28 g/L and is obtained through diet, particularly in grains, vegetables and, in certain areas, drinking water. Lithium deficiency can arise due to low dietary intake and also due to certain diseases, including kidney disease, although no human diseases (except behavioural deficits) have been observed as a result of lithium deficiency [102].

Lithium is used widely for treatment of manic depression and most patients are relieved from their symptoms and are able to integrate into society. At therapeutic doses chronic lithium administration probably has neuroprotective/neurotrophic properties [103,104]. However, despite its effectiveness there are still many negative reports regarding lithium tolerance and cases of toxicity (doses used therapeutically [0.6–1.2 mmol/L] are slightly lower than toxicity levels [>1.5 mmol/L]) [105,106].

Previous studies have concluded that lithium may exert its effects through the serotonergic system in depression [107], in particular the 5-$HT_{1B}$ receptors [108], although actions of lithium on other neurotransmitter systems in different affective disorders cannot be ruled out [109].

### *Lithium as treatment for anxiety and depression*

An early study showed that 13 out of 22 patients with either unipolar or bipolar depression responded to lithium treatment, and 8 of these became symptom free [5]. Patients with bipolar depression responded better to lithium treatment (1800 mg/70 kg) than those with unipolar depression. The effects of lithium treatment were observed about 9 days after the start of treatment and treatment was well tolerated even though side effects, such as ataxia, were common in 73% of patients. This study has shown that unipolar and bipolar depression respond differently to lithium treatment indicating that the underlying mechanisms may be different. A similar study used a dose of 1800 mg/70 kg of lithium carbonate for the effective treatment of bipolar disorder with minimal side effects [110]. The response to lithium treatment in patients with unipolar depression was much lower. The study recognised that this may be due to the age, sex and the onset of symptoms, thus no conclusion can be drawn from this.

Many antidepressants have a delayed onset of action and lithium has been used to accelerate the response. Furthermore there has been conflicting reports on whether lithium has a synergistic effect when used in addition to selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs).

Administration of lithium at doses of 2, 4 and 8 mg/kg 30 min before testing was able to reduce the immobility time in FST [111], although lithium (0.5–8.0 mEq/kg) was not effective when given 45 min before testing [107]. In the study by Nixon et al. [107] administration of low dose lithium (1 mEq/kg) enhanced the actions of antidepressants which act at serontonergic receptors, including imipramine and citalopram, when given at sub-effective doses. Addition of lithium with antidepressants that mediate their effects via the noradrenergic (e.g. desipramine) and dopaminergic (e.g. bupropion) did not decrease the immobility time in the FST. This study concluded that the effects of lithium are mediated via the serotoninic system.

The effectiveness of lithium in combination with antidepressant treatment has been replicated in some clinical studies. In a meta-analysis of placebo-controlled double-blind study of patients with unipolar or bipolar disorder, it was found that lithium slightly accelerated the effects of antidepressants but did not reach significance [112]. Another meta-analysis of placebo-controlled double-blind controlled study of patients with refractory depression who did not respond to initial antidepressant treatment [113] identified that lithium augmentation (minimum 800 mg/day to achieve serum levels of ≥0.5 mEq/L) for a duration of at least 2 weeks is effective for depressed patients who do not respond to antidepressant therapy. This finding was not observed in mild to moderately depressed patients in a study by Bloch et al. [114]. In this study patients received the tricyclic antidepressant desipramine alone (100–300 mg/day), or in combination with lithium carbonate (300–1200 mg/day). Joint administration of lithium with desipramine resulted in more side effects (including tremor and dizziness) and did not accelerate or enhance the actions of desipramine.

These studies indicate that (i) lithium dose needs to be sufficient to reach serum levels of ≥0.5 mEq/day and (ii) lithium augmentation is not effective in all types of depression.

## Iron

Iron is essential trace element required for normal cellular function, and DNA and neurotransmitter synthesis [115]. Iron plays an essential role in the oxygenation of cells and tissues, and is taken up into haemoglobin by a complex mechanism. Furthermore, iron provides oxygen to the brain parenchyma, and allows oxygenation of neurotransmitters and enzymes.

The main source of iron is red meat [116], and vegetarians often suffer from iron deficiency [117]. Iron deficiency is a common disorder which can occur with or without anaemia – studies have indicated that a deficiency in this element occurs in 11% of women and 4% of men. Abnormal iron levels can alter mood and behaviour with similar symptoms to those observed in depressed subjects, even if anaemia is not established.

### *Iron in depressive disorder*

Depression is a multifactorial disease, thus iron can have a positive or negative effect on depression and other affective disorders. Studies have investigated the relationship between iron, nitric oxide and the NMDA receptor to determine the mechanisms and their association with depression. Jaffrey et al. [118] concluded that activation of the NMDA receptor with nitric oxide (NO) produces post-transcriptional activation, which occurs when the iron responsive-element binding protein (IRE-BP) binds to RNA. All IRE-BP structures are located in areas with NMDA receptors, and this study provided the fundamental theory that connects iron and NMDA receptors. More recently another group developed a similar experiment explaining the mechanism more clearly and proposed the chain reaction. Using this chain reaction they suggest that nitric oxide synthetase (NOS) inhibitors prevent NMDA toxicity and stroke damage [119]. Moreover, it was proven that

psychological stress can cause iron deposits in the brain and this brain oxidative stress decreases superoxide dismutase (SOD) activity, reduces glutathione (GSH) and can damage antioxidant capacity. Iron could alter NMDA levels in all brain tissues [120].

Children over 10 years old who had severe and chronic iron deficiency in infancy developed behavioural and long term problems compared to their peers. Despite reversal of iron deficiency, these children still had mental problems even when iron was in excess or within normal iron parameters. Children who had severe, chronic iron deficiency in early infancy have low mental and motor function; they showed symptoms of anxiety and depression, and had social problems. This highlights an important association between iron and development, especially the development of mental and motor function; however causality and the underlying mechanisms are difficult to clarify [121]. Other studies have demonstrated that treatment with iron leads to decreased depression in anaemic mothers and control patients [122].

A contradicting study based on the Minnesota Multiphasic Personality Inventory (MMPI) questionnaire showed that levels of serum iron, ferritin and haemoglobin are not associated with depression [123]. Patients with inflammatory diseases, thalassemia or taking multivitamin supplements were excluded from their study as these can give false results. Comparing this with a previous study in which an inflammatory response was observed, the conclusions suggest that alterations in iron metabolism in major depression could also be a possible response in depression. This hypothesis is supported by another study which showed a reduction in the release of iron during the inflammatory process [124].

Moreover, it is believed that a low red blood cell count and haemoglobin could be major markers of depression. Patients receiving haemodialysis were studied to look for an association between major depression and high ferritin levels [125]. They observed a significant decrease in albumin and a significant increase in ferritin levels in depressive patients compared with non-depressed patients treated with haemodialysis. Stratifying by sex, a study of 312 men and 216 women reported that there is a higher prevalence of depression associated with low serum ferritin in men, but this is not seen in women.

## Calcium

Calcium is an abundant cation present in the human body which is required for many cell functions, especially regulation of neurotransmitter homeostasis [126], muscle contraction, cofactor for enzymes including the blood clotting cascade. Ninety-nine percent of calcium is stored in bones with the other 1% present in the blood and tissues. A mineral store of calcium is present in bone and this can be released into the blood when required. A deficiency of this element can increase the risk of osteoporosis. The recommended daily allowance of calcium is 1000 mg or adults, which is obtained through diet. Milk and dairy products are good sources of calcium, although many foods are now fortified with calcium to ensure this requirement is met in full.

### *Calcium in affective disorders*

An influx of calcium has been suggested as a possible cause of affective disorders. Calcium influx into cells may be explained by the fact magnesium and calcium compete for the same membrane binding sites [69], and a deficiency in magnesium may allow calcium to enter into the cytosol unregulated. This may also occur if there is a high intake of calcium and a low intake of magnesium. As the actions of the two elements antagonise each other, an abundance of calcium will prevent absorption of magnesium in the intestine.

Disturbances in calcium metabolism have been reported in different types of affect disorders. A study showed that intracellular calcium concentrations are increased in platelets following application of 10 μM 5-HT to platelets taken from patients with bipolar disorder and depression but not control, suggesting that patients with these mood disorders have an increased calcium signalling system via 5-HT 2A receptors [127]. A study by Bowden and colleagues [128] investigated extracellular and intracellular calcium in patients with unipolar, bipolar or manic patients. They found that plasma calcium levels were decreased in unipolar and manic patients compared to controls, unipolar patients had lower plasma calcium concentrations compared to bipolar patients, and red blood cell calcium ATPase (an enzyme that is responsible for the removal of calcium from the cytosol) was lower in patients with unipolar depression. These studies highlight the differences in the underlying effects that are observed in affective disorders, and could help explain the variations symptomatology.

### *Regulation of intracellular calcium in depression*

Excessive influx of calcium may be part of the mechanism that leads to affective disorders, thus preventing the influx of calcium into cells pharmacologically may improve depressive-like symptoms. In a double-blind, crossover study, the authors found that the calcium channel blocker verapamil was effective in the treatment of acute mania [129]. Verapamil improved patients scores in the Manic State Rating Scale (MSRS) and Brief Psychiatric Rating Scale (BPRS), compared to placebo.

These results were also evident in animal models of depression and anxiety. Galeotti et al. [130] showed that administration of TMB-8 (which prevents release of intracellular calcium) decreased immobility time in the forced swim test, and produced a similar response compared to the tricyclic antidepressants amitryptyline and clomipramine. The opposite effect was seen when calcium uptake was prevented to the endoplasmic reticulum using the selective inhibitor thapsigargin. Administration of substances which prevent the release of calcium from intracellular stores such as xestospongin C (InsP3 receptor antagonist) and 4-chloro-m-cresol (ryanodine receptor agonist) increased immobility time, indicating a depressant-like effect. Furthermore, selective antagonism of the ryanodine receptor using ryanodine also caused an antidepressant effect.

A study investigating the effect of calcium channel blockers for the treatment of anxiety in rats showed that verapamil, diltiazem and flunarizine were effective at reducing anxiety related behaviours [131]. These drugs were able to increase the number of entries, time and rearing in the light compartment of the light–dark test. In the elevated plus maze paradigm only verapamil and diltiazem were able to increase number of entries to the open arms and the percentage of time spent in the open arms. These results are comparable to the anxiolytic effects of diazepam.

Taken together, the data strongly suggest that (i) there is a disturbance in intracellular calcium dynamics in some affective disorders and (ii) regulating calcium levels with calcium channel blockers can be effective at reducing depressive-like symptoms in both animal and human studies.

## Chromium

Chromium is an essential microelement which exhibits potential antidepressant properties in pre-clinical and clinical studies. Daily chromium requirement varies depending on age and gender. The adequate daily requirement for chromium is 30–35 μg in males and 20–25 μg in females [33]. The main sources of chromium are broccoli, brewer's yeast, beef, eggs, liver, oysters and chicken [132]. Deficiency of this element is implicated in the

development of diabetes [133], which is often linked with depressive symptoms. The co-existence of diabetes and depression is associated with significant morbidity, mortality, and increased healthcare costs [134,135].

Several preclinical studies showed antidepressant properties of different chromium salts, including chromium picolinate and chromium chloride. Khanam and Pillai [136] showed increased swimming behaviour in the modified forced swim test in rats after chromium picolinate. Based on this result they suggested the involvement of serotonergic pathways in the antidepressant action of chromium. The study of Attenburrow et al. [137] showed increased tryptophan levels and elevated brain 5-HT content after chromium picolinate treatment. The authors correlated the changes in serotonergic transmission with the sensitivity of central 5-$HT_{2A}$ receptors. Similar results were observed by Franklin and Odontiadis [138], which demonstrated an increase in serotonin level and metabolism, and a reduced sensitivity of the serotonin 5-$HT_{2A}$ receptors following chromium picolinate administration.

Piotrowska et al. [11] showed antidepressant-like activity of chromium chloride in the FST in mice and rats. Additionally in these studies the effects of chromium on the monoaminergic system were demonstrated using ritanserin (an antagonist of the 5-$HT_{2A/C}$ receptor), WAY 1006335 (an antagonist of the 5-$HT_{1A}$ receptor), propranolol (β-adrenoreceptor antagonist), prazosin (α1-adrenoreceptor antagonist), yohimbine (α2-adrenoreceptor antagonist), SCH 23390 (antagonist of dopamine D1 receptor), and sulpiryd (dopamine D2/D3 receptor antagonist). Moreover, antidepressants, like imipramine, fluoxetine and reboxetine, but not bupropion, enhanced the antidepressant activity of chromium chloride in the FST [11]. Our previous results have shown that chromium(III) chloride can reduce the immobility time in the FST by 20% [139]. To determine the effects of chromium on the glutamate system in the FST, animals were pre-treated with NBQX (AMPA receptor antagonist) and NMDA (NMDA receptor agonist) before chromium(III) chloride treatment [139]. NBQX completely antagonised chromium(III) chloride whilst NMDA partially antagonised the effects of the element. These studies indicate the involvement of the monoaminergic and glutamatergic systems in the potential antidepressant activity of chromium.

Clinical studies confirmed the antidepressant properties of chromium in mood disorders. As reviewed by Iovieno et al. [140] chromium picolinate was effective in major depressive patients in single- and double-blind clinical trials. Moreover, in one clinical placebo-controlled, double-blind study, chromium picolinate treatment caused antidepressant effects in atypical depression, which, according to the authors, was related to down regulation of the 5HT2A receptors [141]. Brownley et al. [142] observed reduced mood symptoms in premenstrual dysphoric disorder patients supplemented with chromium. Potentiation of antidepressants by chromium was also found in dysthymic disorder [143].

Thus chromium displays antidepressant activity via the monoaminergic and glutamatergic systems and is likely to exert its effect through the serotonergic system.

## Conclusion

This review presents data on some of the important findings regarding essential elements and their involvement in depression and anxiety. In conclusion it seems evident that depression is a multifactorial disorder which can arise through an imbalance of numerous trace elements and vitamins (not reviewed here). Our findings show that the underlying mechanisms in these different disorders are not always the same and imbalances in different proportions may manifest as different types of affective disorder, such as unipolar, bipolar, or anxiety. Thus as there are differences underlying these conditions optimal treatment options may vary slightly from patient to patient. Many of the studies discussed have shown that supplementation with the deficient element in combination with common antidepressants can help to reduce unwanted side effects of antidepressants. With the advent of personalised medicine and new techniques that can lead to better understanding of disease, patients can be given treatment tailored to their symptoms.

## Conflict of interest

None declared.

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