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Neural basis of major depressive disorder: Beyond monoamine hypothesis

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The monoamine hypothesis has been accepted as the most common hypothesis of major depressive disorder (MDD) for a long period because of its simplicity and understandability. Actually, most currently used antidepressants have been considered to act based on the monoamine hypothesis. However, an important problem of the monoamine hypothesis has been pointed out as follows: it fails to explain the latency of response to antidepressants. In addition, many patients with MDD have remained refractory to currently used antidepressants. Therefore, monoamine-alternate hypotheses are required to explain the latency of response to antidepressants. Such hypotheses have been expected to contribute to identifying hopeful new therapeutic targets for MDD. Past studies have revealed that the volume of the hippocampus is decreased in patients with MDD, which is likely caused by the failure of the hypothalamic–pituitary–adrenal axis and following elevation of glucocorticoids. Two hypotheses have been proposed to explain the volume of the hippocampus: (i) the neuroplasticity hypothesis; and (ii) the neurogenesis hypothesis. The neuroplasticity hypothesis explains how the hippocampal volume is decreased by the morphological changes of hippocampal neurons, such as the shortening length of dendrites and the decreased number and density of spines. The neurogenesis hypothesis explains how the hippocampal volume is decreased by the decrease of neurogenesis in the hippocampal dentate gyrus. These hypotheses are able to explain the latency of response to antidepressants. In this review, we first overview how the neuroplasticity and neurogenesis hypotheses have been developed. We then describe the details of these hypotheses.

Key words: antidepressant, hippocampus, neurogenesis, neuroplasticity, major depression.

ALTHOUGH MAJOR DEPRESSIVE disorder (MDD) is a common psychiatric disease,[1] the pathophysiology of MDD remains unclear in spite of vast amounts of past researches. Therefore, various hypotheses have been proposed to explain the pathophysiology of MDD. The monoamine hypothesis is the most common of such hypotheses of the pathophysiology of MDD. This hypothesis is quite simple and easily understandable; the concentrations of monoamines, such as serotonin, noradrenaline, and dopamine, in synaptic gaps are decreased in the depressive state.[2] Therefore, most antidepressants have been developed according to the monoamine hypothesis and have been commonly used worldwide. On the other hand, the

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monoamine hypothesis is not without problems. In particular, the most serious problem of the monoamine hypothesis is that it fails to explain why antidepressants have the latency of response; if antidepressants work based on the monoamine hypothesis, they are considered to be rapidly effective.[3] However, antidepressants generally need 2–4 weeks to take their therapeutic effects on depressive symptoms. Moreover, it is well known that up to 30% of patients with MDD are refractory to currently used antidepressants.[4] This suggests that the monoamine hypothesis is inadequate as the pathophysiological theory of MDD. Therefore, the development of a monoamine-alternate hypothesis is ardently desired to elucidate the pathophysiology of mood disorders. It is expected to lead to the development of new drugs to be effective to treatment-refractory MDD.

Neuroplasticity and neurogenesis hypotheses are the most noticeable and valid monoamine-alternate hypotheses of MDD. This article will provide a critical review of these hypotheses of MDD and try to explain the action mechanisms of antidepressants for MDD based on these hypotheses.

DEVELOPMENT OF NEUROPLASTICITY AND NEUROGENESIS HYPOTHESES OF MDD

Stress elevates glucocorticoids via hypothalamic–pituitary–adrenal axis in MDD

Stress is the most significant causal agent of MDD.[5] Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, which plays a pivotal role in stress responses in mammalians. The details of the HPA axis are as follows: Stress promotes the secretion of corticotrophin-releasing factor (CRF) from the hypothalamus. CRF promotes the secretion of adrenocorticotropic hormone from the anterior pituitary. Adrenocorticotropic hormone promotes the secretion of glucocorticoids (GC) from the adrenal gland, which leads to the elevation of GC in the blood and cerebrospinal fluid. Elevated GC suppress the secretion of CRF via GC receptors in the hippocampus and are reversed to normal level, which is known as the negative feedback of the HPA axis. This means that GC are major mediators of stress. Interestingly, MDD causes the failure of the negative feedback of the HPA axis, which leads to the continuation of the elevated levels of GC,[6] which suggests that the failure of the negative feedback of the HPA axis and following continuous elevation of GC may be strongly associated with MDD.

Stress decreases hippocampal volume via GC in MDD

It is well established that the hippocampus plays an important role in the negative feedback of the HPA axis.[7] Meta-analyses over many MRI studies have shown that the volume of the hippocampus is significantly decreased in patients with MDD compared to healthy subjects.[8] In addition, the volume of the hippocampus of patients with Cushing's disease, in which the levels of GC are strongly elevated, is also significantly decreased compared to healthy subjects.[9] It is also well known that patients with Cushing's disease often have depressive symptoms.[10] Taken together, this information suggests that stress, HPA axis, GC, and hippocampal volume seem to constitute a negative spiral in patients with MDD: Stress elevates the levels of GC, the elevated GC decrease the volume of the hippocampus, the decreased volume of the hippocampus may induce the failure of the negative feedback of the HPA axis, and subsequent further elevation of the levels of GC and aggravation of the failure of the HPA axis result in MDD. Therefore, this negative spiral may play a pivotal role in the pathophysiology of MDD.

How do GC decrease hippocampal volume? Development of neuroplasticity and neurogenesis hypotheses

The above discussion suggests that the hippocampus may play an important role in the pathogenesis of MDD; stress/GC-induced decrease of the volume of the hippocampus causes the failure of the negative feedback of the HPA axis and subsequent further elevation of GC. Therefore, elucidating the mechanism underlying stress/GC-induced decreases of the volume of the hippocampus may lead to further understanding of the pathophysiology of MDD and is expected to lead to the development of new therapeutic targets of antidepressants. As such mechanisms, two hypotheses have been proposed: the neuroplasticity and neurogenesis hypotheses (Fig. 1). The neuroplasticity hypothesis suggests that stress/GC induces the atrophy of mature neurons in the hippocampus. The neurogenesis hypothesis

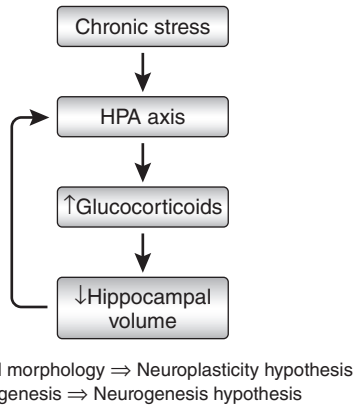

Figure 1. Development of neuroplasticity/neurogenesis hypotheses of major depressive disorder. Chronic stress activates hypothalamic–pituitary–adrenal (HPA) axis and increases glucocorticoids. Elevated glucocorticoids decrease the hippocampal volume via the negative effects of neural morphology (neuroplasticity hypothesis) in hippocampal neurons and/or neurogenesis in the dentate gyrus (neurogenesis hypothesis). Such negative effects of glucocorticoids on the hippocampus cause the failure of the negative feedback of the HPA axis, which leads to the further elevation of glucocorticoids.

suggests that stress/GC decreases the number of newborn neurons and neural precursor cells in the dentate gyrus (DG) of the hippocampus. In these hypotheses, such structural changes in the hippocampus are considered to lead to the decrease of the volume of the hippocampus in patients with MDD. We discuss these hypotheses in detail below.

NEUROPLASTICITY HYPOTHESIS OF MDD

Effects of stress and antidepressants on hippocampal neurons

Stress is divided into two types: acute stress and chronic stress. Chronic stress is considered to recapitulate the behavioral characteristics of patients with MDD rather than acute stress.[11] Watanabe *et al.* showed that repeated restraint stress, a model of chronic stress, induced the atrophy of neurons (shortening of the length of dendrites and decreasing of the density of spines) in the hippocampus.[12] In addition, they showed that repeated administration of GC also induced the atrophy of neurons in the hippocampus[13] and that tianeptine, an antidepressant, recovered stress-induced atrophy of hippocampal neurons in a few weeks.[14] These studies suggest that stress/GC induce the atrophy of hippocampal neurons and that antidepressants can recover it in a few weeks, which is the base of the neuroplasticity hypothesis and explains the latency of response to antidepressants.

Brain-derived neurotrophic factor as a major player of neuroplasticity hypothesis

Repeated restraint stress decreases mRNA expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is recovered by tianeptine.[15] BDNF has positive effects to the morphology of neurons[16] and antidepressant-like effects.[17,18] In addition, various types of antidepressants recover repeated restraint stress-induced decrease of mRNA expression of BDNF in the hippocampus.[19] These findings suggest that BDNF is a major player in the neuroplasticity hypothesis. Chronic stress decreases the expression of BDNF, which leads to negative morphological changes of neurons in the hippocampus. Antidepressants can recover stress-induced morphological changes by increasing the expression of BDNF. Numerous basic and clinical studies have been performed based on the BDNF–neuroplasticity hypothesis. Clinical studies have shown that BDNF levels in serum are decreased in patients with MDD compared to healthy subjects[20] and that the DNA methylation profiles of the BDNF gene are different between MDD patients and healthy subjects.[21] Therefore, BDNF is expected to be a valuable biomarker for MDD.

Ketamine: A new antidepressant based on the neuroplasticity hypothesis

Ketamine is an antagonist of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors and generally used as an anesthetic. It has been shown that antagonists of NMDA receptor have antidepressant-like effects in animal models of MDD[22,23] and that antidepressants reduce NMDA-induced behavioral changes.[24] Based on these findings, the clinical effects of ketamine on MDD have been tested. In general, antidepressants need 2–4 weeks to improve depressive symptoms. However, surprisingly, single intravenous administration of ketamine rapidly improves depressive symptoms in 1–3 days.[25] The rapid efficacy of ketamine on MDD has been reproduced by a number of

studies.[26,27] Therefore, unlike traditional antidepressants, such as monoamine reuptake inhibitors, ketamine may work for MDD based on a brand new action mechanism. Elucidating this mechanism is expected to lead to the development of more rapid and effective new antidepressants.

In 2010, Li *et al.* achieved a breakthrough finding that ketamine elicits the rapid efficacy for depressive symptoms by increasing the number and function of neural spines in the prefrontal cortex via activating the mammalian target of rapamycin (mTOR) pathway,[28] which is well known to mediate cell proliferation, survival, and metabolism. Following this breakthrough finding, it was shown that ketamine activates the mTOR via stimulating the a-amino-3-hydroxy-5-methyl-isoxazolepropionic acid receptor and following increase of BDNF mRNA expression both in the prefrontal cortex and the hippocampus.[29] These findings seem to elucidate how stress causes the negative effects on neural morphology and ketamine rapidly recovers it. Although ketamine is expected as a new antidepressant, it has some problems for clinical use: inducing psychotic symptoms and abuse. Therefore, developing new antidepressants based on the BDNF–mTOR pathway is considered to be preferable rather than promoting clinical use of ketamine itself.

NEUROGENESIS HYPOTHESIS OF MDD

Overview of adult hippocampal neurogenesis

There was once a widespread belief that neurogenesis was limited to prenatal development. In the 1960s, Altman and Das first discovered that new neurons were created in the DG of the adult rat hippocampus by a series of excellent studies using tritiated thymidine.[30] However, unfortunately, these great findings were largely ignored for a long period. In the 1990s, adult hippocampal neurogenesis was rediscovered by Gould and associates.[31,32] Finally, Gage and colleagues developed and established adult hippocampal neurogenesis as a stand-alone field in neuroscience by their three great contributions to this field: (i) the development of the bromodeoxyuridine labeling method[33] (ii) the retrovirus-mediated green fluorescent protein labeling method[34] and (iii) adult hippocampal neurogenesis in humans.[35] Today, adult hippocampal neurogenesis is one of the most active fields in neuroscience.

Adult hippocampal neurogenesis is a series of differentiation processes from neural stem cells to mature neurons in the DG of the adult hippocampus. These differentiation processes consist of two phases: the mitotic phase and the post-mitotic phase. The mitotic phase occurs in the subgranular zone, the border between the granular cell layer (GCL) and the hilus in DG. This phase is divided into four distinct stages (stem cells [type-1 cells]; transient amplifying cells [type-2a and type-2b cells]; and progenitor cells [type-3 cells]) based on the expression patterns of various marker proteins, such as nestin, glia fibrillary acidic protein, and doublecortin.[36,37] The cell fate to neuron is determined between type-2a cells and type-2b cells because type-2a cells are more proliferative than type-2b cells. In addition, type-2b cells, but not type-2a cells, express some marker proteins of immature neurons, such as doublecortin, NeuroD, and PSA-NCAM.[38] Interestingly, it is shown that type-2a cells are the majority in the mitotic phase; type-2a cells are much more proliferative than type-1 cells and type-2b cells.[38] In the post-mitotic phase, cells begin to migrate to the lower third of the GCL. Following this migration process, the development of dendrites and the expression of neuronal markers, such as NeuN, are initiated.[39,40] Then, cells differentiate into immature neurons. Surprisingly, the great mass of these newborn immature neurons is eliminated within a few days by apoptosis.[40–42] Following this elimination process, surviving immature neurons migrate to the upper two-thirds of the GCL,[43,44] where these surviving immature neurons mature into granular cells – fully matured neurons – in 2 months.[34,45]

Effects of stress and GC on adult hippocampal neurogenesis

It is well established that chronic stress, an animal model of MDD, decreases adult hippocampal neurogenesis[46,47] Stress-induced elevation of GC also decreases adult hippocampal neurogenesis.[48] Both GC and dexamethasone (DEX), an agonist of GC receptors, decrease adult hippocampal neurogenesis.[31,32,49] In addition, Snyder *et al.* showed that the reduction of adult hippocampal neurogenesis causes the failure of the HPA axis,[50] which means that adult hippocampal neurogenesis mediates the negative feedback of the HPA axis. This suggests that stress, GC, and adult hippocampal neurogenesis

seem to constitute a negative spiral: stress decreases adult hippocampal neurogenesis via GC, which leads to the failure of the negative feedback of the HPA axis and the subsequent continuous elevation of GC. This elevated GC decreases adult hippocampal neurogenesis again. This negative spiral may play a pivotal role in the pathophysiology of MDD.

Effects of antidepressants on adult hippocampal neurogenesis

The neurogenesis hypothesis of MDD easily leads to the idea that antidepressants may increase adult hippocampal neurogenesis. First, Malberg *et al.* showed that various classes of antidepressants, such as selective serotonin reuptake inhibitors (SSRI), noradrenaline reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitor, increase adult hippocampal neurogenesis.[51] Next, the same group showed that SSRI reversed the negative effects of inescapable stress on adult hippocampal neurogenesis.[52] This suggests that antidepressants have positive effects on adult hippocampal neurogenesis. However, it remains unclear whether these positive effects of antidepressants on adult hippocampal neurogenesis were actually required for the therapeutic effects of antidepressants on depressive symptoms. Santarelli *et al.* showed that radiation-induced inhibition of adult hippocampal neurogenesis canceled the positive effects of antidepressants on depressive-like behaviors.[53] This study is the first study to show the requirement of adult hippocampal neurogenesis for the behavioral effects of antidepressants. Moreover, antidepressants generally need 2–4 weeks to take their positive effects on adult hippocampal neurogenesis, which suggests that the neurogenesis hypothesis seems to overcome the problem of the latency of response in the monoamine hypothesis. In addition to antidepressants, non-pharmacological and effective intervention of MDD, such as electroconvulsive therapy, vagus nerve stimulation, and short-term sleep deprivation, also increase adult hippocampal neurogenesis.[54–56] Therefore, the neurogenesis hypothesis of MDD has reached consensus as one of the most noticeable and valid monoamine-alternate hypotheses of MDD.

HOW DO ANTIDEPRESSANTS AFFECT ADULT HIPPOCAMPAL NEUROGENESIS?

Development of *in vitro* culture system of adult DG-derived neural precursor cells

The positive effects of antidepressants on adult hippocampal neurogenesis have been confirmed by many past studies. However, such studies are performed *in vivo* with the administration of antidepressants to the individual animal organism. In principle, it is impossible for such *in vivo* studies to determine whether administered drugs and substances affect target cells directly or indirectly; antidepressants may affect neural precursor cells directly or indirectly via other cells, such as neurons and astrocytes. To elucidate the presence or absence of direct effects of antidepressants on neural precursor cells in adult DG, an *in vitro* culture system of adult DG-derived neural precursor cells is required. Therefore, we developed the *in vitro* culture system of adult rat DG-derived neural precursor cells (ADP).[57] As mentioned above, the mitotic phase in adult DG has four distinct stages: type-1 cells, type-2a cells, type-2b cells, and type-3 cells.[36,37] In these stages, type-2a cells are the majority in adult DG.[38] In addition, ADP correspond to type-2a cells.[57] Therefore, ADP are considered to serve as a good *in vitro* model for the investigation of direct effects of drugs and substances on neural precursor cells in adult DG.

Effects of antidepressants on adult DG-derived neural precursor cells

Neurogenesis is constituted of three phenomena: proliferation, survival, and differentiation. Most *in vivo* studies of the effects of antidepressants on adult hippocampal neurogenesis have focused on the proliferation of neural precursor cells. In addition, Encinas *et al.* showed that fluoxetine, an SSRI, mainly increases that of early progenitor cells (type-2 cells) in adult DG.[58] Therefore, we have focused on the proliferation of neural precursor cells to investigate the direct effects of antidepressants on neural precursor cells on adult DG.

First, the direct effects of antidepressants on the proliferation of ADP were examined. Surprisingly, none of the various types of antidepressants, such as SSRI, noradrenaline reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors, had a direct effect on the proliferation of ADP,[59] which suggests that antidepressants affect the

proliferation of neural precursor cells in adult DG by indirect mechanisms via neurons and/or astrocytes surrounding neural precursor cells in adult DG. Next, we discuss the possibility of such indirect mechanisms of the positive effects of antidepressants on adult hippocampal neurogenesis in more depth.

Serotonin and noradrenaline: Neurons-dependent action mechanisms of antidepressants

Most common antidepressants are reuptake inhibitors of serotonin and noradrenaline. In addition, it is well established that there are both serotoninergic and noradrenergic inputs to the hippocampus.[60,61] Depletion of serotonin decreases the proliferation of neural precursor cells in adult DG.[62] Depletion of noradrenaline also decreases the proliferation of neural precursor cells in adult DG.[63] This suggests the possibility that reuptake-inhibitors-mediated elevation of serotonin and/or noradrenaline concentration at synaptic terminals of serotonergic and/or noradrenergic neurons projected into adult DG may directly increase the proliferation of neural precursor cells in adult DG. In addition, ADP express noradrenaline receptors (α1A, α1B, α1D, α2C, β1, β2) and serotonin receptors (2A and 2B).[59] Therefore, we examined direct effects of serotonin and noradrenaline on the proliferation of ADP. Although serotonin had no direct effect on the proliferation of ADP, noradrenaline directly increased the proliferation of ADP.[59]

We also showed this direct increasing effect of noradrenaline on the proliferation of ADP via β2 noradrenaline receptor.[59] The activation of β2 receptor elevates the intracellular concentration of cyclic adenosine monophosphate (cAMP).[64] The elevation of the intracellular concentration of cAMP increases the proliferation of neural precursor cells in adult DG via increasing the phosphorylation of cAMP response element-binding protein (CREB).[65] This suggests that noradrenaline directly increases the proliferation of neural precursor cells in adult DG via the cAMP-CREB pathway, which may be a part of the neuron-dependent action mechanisms of antidepressants.

In contrast to noradrenaline, serotonin had no direct effect on the proliferation of ADP. However, serotonin surely increases the proliferation of neural precursor cells in adult DG *in vivo*.[66] In addition, the positive effects of fluoxetine on adult hippocampal neurogenesis are canceled in a knockout mouse for serotonin 1A receptor.[53] Serotonin 1A receptor is expressed in neurons and astrocytes,[67] but not ADP.[59] This suggests that serotonin may affect adult hippocampal neurogenesis via unknown indirect mechanisms, for example, astrocyte-dependent action mechanisms.

Neurotrophic/growth factors: Astrocytes-dependent action mechanisms of antidepressants

Astrocytes play a pivotal role in the neurogenic niche in adult DG[68] and the proliferation of adult hippocampus-derived neural precursor cells are increased in co-culture with adult hippocampus-derived astrocytes.[69] Therefore, astrocytes are considered to promote adult hippocampal neurogenesis. In addition, neurotrophic/growth factors, such as BDNF, fibroblast growth factor 2 (FGF2), glial cell-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF), increase adult hippocampal neurogenesis.[70–73] These factors are secreted from not only neurons but also astrocytes.[74] Amitriptyline, a tricyclic antidepressant, induces the expression and secretion of BDNF, FGF2, GDNF, and VEGF in primary cultured astrocytes.[75] This suggests the possibility that antidepressants indirectly increase the proliferation of neural precursor cells in adult DG via inducing the secretion of neurotrophic/growth factors from astrocytes. Hence, we examined whether antidepressants-induced neurotrophic/growth factors can increase the proliferation of ADP or not. Amitriptyline-treated conditioned medium from primary cultured astrocytes increased the proliferation of ADP. FGF2, but not BDNF, VEGF, or GDNF, directly increased the proliferation of ADP. Both anti-FGF2 antibody and a specific inhibitor of FGF receptor canceled the positive effects of amitriptyline-treated conditioned medium from primary cultured astrocytes on the proliferation of ADP.[76] In addition, astrocytes are the predominant source of FGF2.[77] This suggests that antidepressants indirectly increase the proliferation of neural precursor cells in adult DG via inducing the secretion of FGF2 from astrocytes. Although this 'astrocytes-FGF2 hypothesis' is potentially attractive as an explanation of the indirect positive effects of antidepressants on the proliferation of neural precursor cells in adult DG, it was based on only

in vitro experiments. Therefore, *in vivo* experiments are required for confirming this hypothesis.

Antidepressants inhibit the reuptake of monoamines, which easily leads to the idea that monoamines mediate antidepressants-induced mRNA expression of FGF2 in astrocytes. However, interestingly, antidepressants-induced mRNA expression of FGF2 in astrocytes is monoamines-independent.[75] It suggests the possibility that antidepressants induce mRNA expression of FGF2 in astrocytes via unknown target molecules other than monoamine transporters. Although Kajitani *et al.* recently showed that antidepressants-induced mRNA expression of FGF2 are mediated by the matrix metalloproteinases/receptor tyrosine kinases-extracellular signal-regulated kinases-early growth response 1 pathway,[78] it remains unclear to which proteins antidepressants directly bind in this pathway. Therefore, elucidating the detailed molecular mechanisms underlying antidepressants-induced mRNA expression of FGF2 in astrocytes is expected to contribute to finding new therapeutic targets of MDD and further understanding of the pathophysiology of MDD.

CONCLUDING REMARKS

Numerous studies of biological aspects of MDD, such as the failure of the negative feedback of the HPA axis and the decrease of hippocampal volume, have led to two monoamine-alternate hypotheses of MDD, such as the neuroplasticity hypothesis and the neurogenesis hypothesis. The neuroplasticity hypothesis indicates that GC have negative effects on neural morphology, which is attenuated by antidepressants and BDNF. In addition, ketamine has rapid efficacy on MDD by improving neural morphology via the BDNF–mTOR pathway. The neurogenesis hypothesis indicates that GC have negative effects on the proliferation of neural precursor cells, which is attenuated by antidepressants. In addition, antidepressants recover the negative effects of GC on neural precursor cells indirectly via the neuron–noradrenaline–CREB pathway and/or the astrocytes–FGF2 pathway (Fig. 2). Interestingly, although common antidepressants work as inhibitors of monoamine transporters, the BDNF–mTOR pathway and the astrocytes–FGF2 pathway may be monoamine-independent. This suggests the possibility that antidepressants may have unknown target proteins other than monoamine transporters in their action mechanisms. The elucidation of such unknown target proteins of antidepressants is expected to lead to the discovery of new therapeutic targets of MDD as well as further understanding of the pathophysiology of MDD.

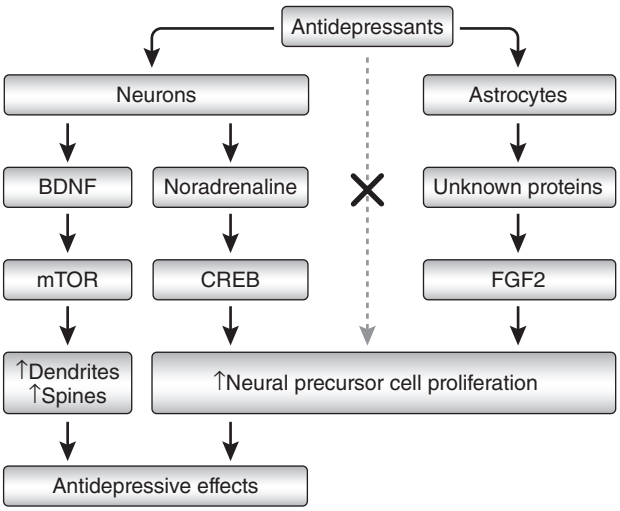

Figure 2. Putative action mechanism of antidepressants based on neuroplasticity/neurogenesis hypotheses. Antidepressants have the positive effects on neural dendrites and spines via brain-derived neurotrophic factor (BDNF)–mammalian target of rapamycin (mTOR) pathway and neural precursor cells via neuron–noradrenaline–cAMP response element-binding protein (CREB) pathway and/or astrocytes–fibroblast growth factor 2 (FGF2) pathway, but not the direct action to the neural precursor cells. Finally, these positive effects lead to antidepressive effects.

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DISCLOSURE STATEMENT

All of the authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

S.B. drafted the manuscript and all authors edited it.

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