Investigation of endogenous opioid reactivity with fentanyl challenge in major depression and self-injurious behavior

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1. Introduction

Since their discovery in the early 1970s, endogenous opioid peptides (EOP) have been implicated as being either causative or curative agents in a variety of mental disorders. A tremendous effort was made to investigate their possible role in psychopathology and use in psychopharmacology, but initial successes gave way to conflicting results. Most of the early work focused on schizophrenia and affective disorders. Later on, other areas, particularly childhood autism, self-injurious behavior, stereotypic movement disorder, post-traumatic and eating disorders have received increasing attention. While the author had investigations into other areas as well, present dissertation will focus on the possible role of the endogenous opiate system in major depression, in the mediation of the antidepressant effect of sleep deprivation, and the pathomechanism of self-injurious behavior.

1.1. Physiological Role of Endogenous Opiates

During the last thirty years, investigation of the opioid peptides using pharmacological, later on genetic methods, has revealed an intricate system that demonstrates remarkable diversity in terms of the number of endogenous ligands (more than a dozen) yet astonishing convergence at the level of targeted receptors (only three major types). It is out of the scope of the dissertation to address detailed characteristics of the endogenous opioid ligands and their complex receptor binding profiles. Nevertheless, the basic features will be outlined below.

The opioid peptides have precursor molecules encoded by three genes: pre-proopiomelanocortin, pre-proenkephalin, and pre-prodynorphin. Each precursor is subject to elaborate post-translational modifications that result in the synthesis of multiple biologically active peptides. The major endogenous opiate encoded by pre-proopiomelanocortin is beta-endorphin. In addition to beta-endorphin, the proopiomelanocortin is the precursor of adrenocorticotropic hormone (ACTH), alpha-melanocortin (alpha-MSH), and beta-lipotropin. Pre-proenkephalin encodes multiple forms of Met-enkephalin, and a single copy of Leu-enkephalin. Pre-prodynorphin encodes three opioid peptides of various lengths that all begin with the Leu-enkephalin sequence: dynorphin A, dynorphin B, and neoeendorphin.

Three opiate receptor types have been isolated and cloned: the mu-opiate receptors, the delta-opiate receptors, and the kappa-opiate receptor family. All three opiate receptor classes belong to the superfamily of seven transmembrane-spanning G protein-coupled receptors. There is about 60% amino acid identity between opiate receptor types and about 90% identity between a receptor type cloned from different animal species. All opiate receptor types mediate the inhibition of cAMP in response to agonist binding. A high degree of structural similarity exists between the transmembrane domains and intracellular loops of the three opiate receptors. The extracellular loops vary considerably and this divergence may explain differences in ligand selectivity among the opiate receptors.

In general, the selectivity of opiate receptors toward one endogenous ligand is not remarkable. Overall, the kappa-opiate receptor displays the greatest selectivity across the endogenous ligands, with an approximately 1000-fold difference in affinity between the most preferred dynorphin A and least preferred Leu-enkephalin, whereas the mu-opiate receptor and delta-opiate receptor differ only across a 10-fold range.

Like opiates, EOP-s are known most widely for their analgesic properties. But in addition, they also serve role in the organism’s response to stressful stimuli, the induction of euphoria and dream like states, as well as producing a sedating effect. As chemical messengers of the neuropeptide type, endogenous opiates are crucial to the normal functioning of important processes such as motor coordination, memory and learning, gastrointestinal function, seizure control, and the hormonal regulation of the reproductive system. In the role of a neuromodulator, EOP-s act as inhibitory mediators in many excitatory pathways including those like acetylcholine, the catecholamines, serotonin, substance P, and fine-tune neurotransmission across a wide range of neuronal circuits, setting thresholds or upper limits.

Some of the less obvious physiological effects of endogenous opiates include control of thermoregulation, energy expenditure, salt and water balance, as well as involvement in development of tolerance and physical dependence - not only to opiate drugs, but also to other highly abused substances, such as alcohol. Since physiology translates into behavioral patterns, endogenous opiates ultimately affect everyday human behaviors such as feeding and drinking, sexual activity, grooming, locomotor and operant behavior, and learning.

2. Background and Objectives

2.1. Endogenous Opiates in Affective Disorders
Opium and its derivatives have been shown to have antidepressant effects. Indeed, before the introduction of electroconvulsive therapy, back to the 1850’s, opium was widely used for this purpose. The so-called "laudanum cure" was a course of two months treatment with progressive increases in dosage followed by gradual withdrawal. The classical descriptions recommended this treatment mostly for agitated depression and considered it not only palliative but also curative because the whole depressive syndrome disappeared after two months of treatment.

In the 1970s several studies suggested that the endogenous opiate system is deficient in patients with depression, and reported symptom relief following acute intravenous administration of beta-endorphin. Unfortunately, these early trials were not adequately controlled to rule out the roles of expectation, experimental stress, or sleep deprivation. Nevertheless, negative results have also been reported with beta-endorphin and Met-enkephalin with the use of stricter experimental design. In the few studies on the effect of subchronic opiate administration, relief of depression has been attained with cyclazocine, buprenorphine, and methadone. Owing to the risk of addiction, these studies did not approach the duration of the "laudanum cure", and relapse was practically immediate. Despite these clinically disappointing results, the majority of studies on opiate agonist administration in depressive illness support the traditional notion that depressed patients experience symptom reduction following this treatment.

However, the opposite strategy, using opiate antagonists, provided no additional support for the hypothesis that endogenous opiates are involved in the pathophysiology of affective disorders. There is little evidence that these drugs have marked effects on mood. No change of mood was documented after a moderate (10-mg) dose of naloxone, neither in depressed subjects nor in healthy volunteers. Some authors showed that although a range of dysphoric feelings including depressed mood was found in normal subjects after injections of higher doses of naloxone, effects mainly consisted of non-depressed feelings of irritability and anxiety.

Measurements of cerebrospinal fluid (CSF) and plasma opioid levels in manic and depressive patients have also yielded inconclusive results. Initial works indicated that opioid activity was elevated in bipolar patients, with a tendency for manic patients to have higher values. However, many of their subjects were not medication free which fact made the interpretation difficult. Whereas most studies examined levels of EOP-s between diagnostic groups and controls, some evidence is available suggesting that the activity of the opiate system may relate to symptoms that run across psychiatric diagnoses. It now appears that CSF and plasma opioid activity may be associated more with stress and anxiety than with any other psychiatric condition, with concordance of the finding by that anxiety increases following opiate blockade.

Some studies have been successful in relating opioid activity to hypothalamic-pituitary-adrenal axis dysfunction associated with major depression. Urinary free cortisol was increased significantly and correlated positively with CSF opioid activity in depressed subjects but not in healthy individuals. This finding is consistent with the report of a direct relationship between levels of plasma cortisol and plasma beta-endorphin-like activity only in cases of major depression. Early resumption of morphine-suppressed cortisol secretion was associated with a diagnosis of major depression and with abnormal dexamethasone suppression test (DST) results. In the interpretations of these data, the cross-tolerance that can develop between opiates and glucocorticoids is usually overlooked and may shed light on some aspects of these results, for example, the decreased opiate sensitivity of cortisol non-suppressors in the DST.

Animal models of depression and the action of antidepressants indicate that endogenous opiate mechanisms may be operative. Inescapable shock and forced swimming - both of which cause opiate-dependent analgesia - lead to learned helplessness and behavioral despair, respectively. These models of depression involve opioid hyperactivity at onset followed by tolerance to the endogenously released opiates over time. On the other hand, tricyclic antidepressants and electroconvulsive shock are synergistic with morphine, and some of the resulting behavioral effects are antagonized by naloxone. Recent results favor optimism for postulating a neurochemical basis of endogenous opiate relationships in the behavioral models of depression. Nevertheless, one has to be cautious drawing any conclusions about how the opiate system might be involved in the animal models of depression and in the mechanisms of action of antidepressant treatment.

There have been attempts to utilize neuroendocrine challenge by narcotic alkaloids to test opiate hyporesponsivity in depression. Early reports indicated that the prolactin response to morphine or methadone might be diminished in depressive patients, with some other studies being less supportive. One of the main aim of the author’s work was to look into this controversy. The main methodological tool of studies presented in this dissertation was similar: the use of a neuroendocrine challenge for evaluation of endogenous opiate sensitivity in normal states (change according to time of the day, or after sleep deprivation) and pathological conditions (depression, self-mutilation).

2.2. Endogenous Opiates in Childhood Autism and Self-Injurious Behavior
Habitual self-mutilation is a bizarre, sometimes dramatic stereotypy consisting of repetitious and self-inflicted physical harm and tissue damage. Several kinds of stereotypic movements (e.g., rocking, bouncing) with self-injurious behavior, such as banging the head, hitting the body, biting self, and picking sores, are frequent in childhood autism. It has been reported to occur among 40% of institutionalized psychotic children and between 8% to 14% of mentally retarded individuals. In both diagnostic groups, stereotypic movements and self-injurious behavior are extremely resistant to extinction; mere cessation of the behavior seems to be unpleasant for the patients and leads to more violent self-abuse. Chronic administration of opiate alkaloids to rodents results in stereotyped behavior including self-mutilation, which is enhanced by drug withdrawal. Therefore, it has been suggested that these kinds of behavior, especially self-injury that would result in the pain-induced endorphin release, are attempts of the addicted organism to prevent the symptoms of narcotic withdrawal. Thus, self-injurious behavior may be a sign of dependence on endogenous opiates.

Most of the research examining the opiate system in autism and self-injurious behavior relates to these notions. The strongest evidence linking opiate dysfunction to these abnormal conditions is found in case reports of trials with opiate antagonists. Most of these trials had several weaknesses in design, and the results must be regarded as preliminary. Most of the patients treated with opiate antagonists were mentally retarded, and the etiologies of retardation were different among them. Subjects exhibited self-mutilation with considerable variation over time and thus did not have stable baselines. In addition, history of other medication was imperfectly documented in these papers.

If self-abusive patients are addicts of their endogenous opiates, administration of an antagonist should have precipitated withdrawal-like phenomena. Truly, aggravation of self-injurious behavior was reported in autistic patients receiving naltrzxone treatment. Future studies of opiate antagonist treatment of self-injurious behavior have to address this possible biphasic effect and must entail prolonged observation.

Several authors reported elevated CSF and/or plasma opioid activity in groups of self-mutilating patients. The plasma beta-endorphin level of the subjects with severe self-injurious behavior proved to be significantly lower than of subjects without severe self-injurious behavior. Based on these observations, one has to emphasize that it is very important to differentiate between persons with and without severe self-harm in any protocol addressing the opiate function of self-injurious behavior. This was the leading concept for the development of Study Four.

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3. Investigations Performed by the Author

3.1. Study One: Diurnal variation in fentanyl-induced hormone responses and side effects

**Background:** It is well known that opiate alkaloids and opioid peptides stimulate the secretion of prolactin. However, the data on hypothalamic-pituitary-thyroid function are contradictory. Pentazocine, nalorphine, butorphanol, and beta-endorphin do not affect basal thyrotropin (TSH) levels in humans. After acute morphine administration there were found only insignificant increase in serum TSH. At variance with these findings, greater doses of morphine, methadone, and dermorphin have been reported to increase TSH serum concentrations. This inconsistency can be explained by the assumption that only opiate receptors of the mu-type can induce TSH release in humans.

Measuring opiate-dependent analgesia, diurnal variation in opiate sensitivity has been demonstrated in humans and a circadian alteration in the opiate responsivity of pituitary hormone secretion was reported in rodents. However, to the author’s knowledge, there are no data available in human subjects on opiate alkaloid- or opioid peptide-induced pituitary hormone responses taken as a function of the time of day.

**Aims:** Summing up these points, it seemed to be reasonable to investigate the prolactin and TSH responses evoked by the selective mu-receptor agonist fentanyl and the diurnal variation in its hormonal and subjective effects.

**Methods:** The prolactin and TSH secretory response to the opioid agonist fentanyl (0.1 mg IV) was investigated with serial blood sampling in ten healthy women at 9 AM and 9 PM on different days. In five subjects saline control trials were also performed.

**Results:** In this study it was demonstrated that fentanyl, administered as an intravenous bolus, induces a prompt, clear increase in prolactin and TSH release in normal subjects and that its effect shows a diurnal variation. The diurnal change was not accounted for by different baseline levels. Intraindividual examination showed that every subject had higher delta-max values in the evening. Results on fentanyl-induced side effects were in line with the endocrine responses. This mu-receptor agonist caused less hormonal and subjective responses in the morning than in the evening.

**Conclusions:** These results favor the hypothesis that mu-receptors mediate stimulatory effect on TSH release in humans. This is in contrast to rodents, where mu-receptors are supposed to have an inhibitory effect on TSH secretion. The relatively low TSH response in the morning can partly explain the inconsistencies of the literature reviewed in the introduction, because human studies are usually done in the morning. Moreover, most laboratory animals have different
circadian rhythms to humans. Therefore, in addition to diverse receptor functions among species, the opiate effect on
TSH secretion could be a question of the time of investigation. The author supposes that the observed diurnal alternation
may be due to the inverse cycle of the hypothalamic-pituitary-adrenal axis because glucocorticoids and ACTH decrease
opiate responsiveness and the daily rhythm of opiate sensitivity is not demonstrable in adrenalectomized rats.

3.2. Study Two: Prolactin response to fentanyl in depression

**Background:** Neuroendocrine challenge tests by opiate agonists and antagonists are promising in biological
psychiatry because opiate receptors play an important role in the modulation of both endocrine and affective processes. The
dysfunction of opiate mechanisms was suggested in depression and was tested by opiate-induced prolactin secretion.
Although these early reports indicated that the prolactin response to morphine or methadone might be diminished in
depressive patients as compared to healthy controls, later studies were less supporting.

In addition to alterations of neuroendocrine function, chronobiological shifts and diurnal change of mood are also
characteristic of major depression. Circadian variation in the opiate responsivity of prolactin secretion has been shown in
rodents, however, there are no data available in human subjects on opiate alkaloid or opioid peptide-induced prolactin
responses taken as a function of the time of day.

**Aims:** Summing up these points, it seemed to be reasonable to investigate the prolactin response to the selective mu-
receptor agonist against fentanyl in major depression and the diurnal variation in its effect.

**Methods:** Ten unmedicated female inpatients with major depression (DSM-III) and 10 healthy volunteer women were
given an intravenous injection of 0.1 mg fentanyl at 9:00 AM and 9:00 AM on different days. The prolactin secretory response to
this opioid agonist was investigated for one hour with serial blood sampling.

**Results:** In this study it was found that fentanyl, administered as an intravenous bolus, induced a prompt, clear increase in
prolactin release both in normal and depressed subjects without intergroup difference.

**Conclusions:** This result does not correspond to earlier reports, but rather agrees with recent studies on opiate-induced
prolactin responses in depression. This discrepancy may be related to differences in design and to the effect of sex, menopausal
status, and phase of menstrual cycle. The latter studies conclude that with a strict control of these variables, the observed
differences disappear. In Study Two the sample population was homogeneous as to sex, and subjects were studied during the
early follicular phase; however, they were not matched for age and menopausal status.

Regulation of prolactin release is under multiple neurotransmitter control. Dopamine and serotonin are also involved
in its secretion; therefore, a diminished prolactin response to opiates does not necessarily imply an abnormality in the opiate
system. The attenuated prolactin response to fentanyl in later suicide victims can be interpreted on the basis of a deficient
serotonergic transmission, which is postulated to predispose to suicide.

Firm conclusions cannot be made on the basis of these preliminary data; nevertheless, it is also possible that certain
depressed patients, who are vulnerable to suicide, are hyporesponsive to both opiate and serotonin stimuli. Suicide is a final
outcome reached through several different behavioral and neurochemical pathways. Therefore, a complex approach, dealing
with both the opiate and serotonergic mechanisms, would be more appropriate in understanding its psychobiology. Based on a
synthetic concept, it can be cautiously proposed that the diurnal variation of opiate sensitivity can be superimposed on the
fundamental mood disturbance of major depression and may account for the diurnal change of mood in this disorder.

3.3. Study Three: Blunted prolactin response to fentanyl in depression. Normalizing effect of partial sleep
deprivation

**Background:** Sleep deprivation (SD) studies revealed fast and substantial improvement of mood in depressed
patients. Partial SD is less stressful for the patient than total SD, and it has been shown to be as effective. It is well
documented that if SD is applied as a single treatment modality, its positive effect is usually transitory in nature: subsequent
sleep reverses the improvement.

There is an increasing interest emphasizing the clinical and scientific usefulness of SD. Apart from its clinical
value (quick response in severe depression, augmentation of poor treatment response), SD provides a valuable research
model for studying recovery processes in major depression. Patients can be investigated in depressed and non-depressed
states within a short interval and without the need for pharmacological intervention.

In the studies previously presented, the mu-receptor agonist fentanyl as stimulant of prolactin secretion was
found to be a promising challenging agent for estimating the functional state of the central opiate system. According to
animal models, the role of endogenous opioids in the emotional change following SD might be pertinent. To the author’s knowledge, no human data are available on changes in endogenous opiate function after SD.

**Aims:** In the first part of the present study the goal was to repeat and improve the previous investigation (Study Two) when the prolactin secretory response to the mu-opiate agonist fentanyl was measured in patients with major depression. The objective the second part of the study was to determine the effect of partial SD on fentanyl-induced prolactin responses in the same patients.

**Methods:** Medication-free female depressed inpatients (N=18) were participating in two fentanyl challenge tests after partial SD and undisturbed sleep, 3 days apart in random order. Healthy volunteer women (N=10) were enrolled after full night sleep as comparison subjects. Participants were given an intravenous injection of 0.1 mg/70 kg fentanyl in the AM. The prolactin secretory response to the opiate agonist was investigated for 1 hour with serial blood sampling.

**Results:** In this study it was demonstrated that fentanyl, administered as an intravenous bolus after a night of normal sleep, induced a robust and substantial increase in prolactin plasma levels in comparison subjects whereas a blunted response was observed in depressed patients. A night of partial SD elicits increased prolactin secretion to fentanyl, bringing levels in patients close to those in controls after full night of sleep. While the study design was lacking an arm in which comparison subjects had fentanyl challenge after SD, the normalizing effect of partial SD is suggested by the direction of change.

**Discussion:** As it was outlined earlier, there is no consensus in the literature about opiate-induced prolactin responses in depression. In the earlier report (Study Two) a lack of statistical power was supposed. A larger sample size has certainly helped improving the effect size. Empirical human studies in regard to the effect of SD on opiate-induced prolactin secretion are missing. The observed augmentation is a unique finding and supports the suggestion of different authors who postulate that SD prevents a nightly breakdown of the endogenous opioid tone. In accordance with this concept, it was found in Study One and Two that both healthy and depressed subjects have decreased opiate sensitivity in the AM as compared to the PM hours.

The complexity of the prolactin regulation outlined in Study Two makes difficult to defend the conclusions above. The presented study is far from being conclusive about the exact mechanisms involved in the hormonal and therapeutic effect of SD. Combining its findings with results published by other authors, one can only raise doubt about serotonergic mediation of the observed shift in the prolactin response. Animal studies revealed an active role of opioids, in interaction with dopamine, in the behavioral syndrome subsequent to SD and hyperactivation of the endogenous opiate system was suggested. Single photon-emission computed tomography before and after total SD showed significantly different dopamine receptor occupancy in responders, vs. non-responders, suggesting an enhanced dopamine release in responders. Dopaminergic augmentation of SD using amineptine was recently reported in humans. Even with the support of data in the literature, the author has no more than indirect evidence supporting an opiate-dopamine mechanism in the behavioral effect of SD.

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3.4. Study Four. Opiate sensitivity test in patients with stereotypic movement disorder and trichotillomania

**Background:** As it was reviewed in the Introduction the endogenous opiate system have been hypothesized to be involved in the pathogenesis of the self-injurious behavior. Opiate antagonists such as naltrexone and naloxone have been used in the treatment of repetitive self-injury. The opiate model of self-injurious behavior is especially tempting since patients with self-injurious behavior experience little or no pain and report mood improvement as response to noxious stimuli. However, doubt has been raised over the endogenous opiate model of self-injurious behavior.

There is a significant limitation in the interpretation of literature data since most of the studies about self-injurious behavior refer to an array of self-inflicted actions, and use heterogeneous patient population. The majority of studies were performed on mentally retarded patients. This fact raises the possibility that comorbid condition and different etiological process were confounding the findings. Severity of the self-injurious behavior is also a factor to be considered in the interpretation of results. Because self-injurious behavior is only a symptom and not a disorder, the most crucial step in a study on self-injurious behavior would be an appropriate patient selection.

**Aims:** The goal was to target two specific forms of self-inflicted behavior (skin picking and hair pulling) in patients who are separated into two different illness categories and who do not manifest other psychiatric pathology. The plan was to investigate the opiate sensitivity of patients with stereotypic movement disorder - especially those who had severe self-injurious behavior - using subanesthetic doses of fentanyl for prolactin challenge test. The targeted population was the group of patients with stereotypic movement disorder exhibiting skin picking. Patients with trichotillomania exhibit a specific, uncontrollable, repetitive, self-inflicted action but hair pulling is not considered as severe self-injurious behavior and subjects exhibiting that kind of behavior are excluded from stereotypic movement disorder by DSM-IV criteria. Therefore, hair-pullers served as patient control subjects in the study.
**Methods**: Healthy volunteers and trichotillomanic patients were enrolled as comparison subjects. Ten healthy subjects received 0.05 mg/70 kg and another 10 were given 0.1 mg/70 kg dose of fentanyl intravenously in the AM hours. Placebo trials were also completed. A dose of 0.05 mg/70 kg fentanyl was administered to patients with stereotype movement disorder (N=10) and trichotillomania (N=12). Serial blood sampling was performed for prolactin measurements.

**Results**: In this study, the author has demonstrated that a subanesthetic dose of fentanyl administered as an intravenous bolus induces a prompt, clear, dose-dependent increase in prolactin secretion in female subjects. Subjective responses were dose-dependent as well. Patients with stereotypic movement disorder, but not with trichotillomania, showed significantly increased opiate sensitivity.

**Conclusions**: Skin picking involves more pain and self-injurious component than hair pulling - one reason it is classified under stereotypic movement disorder in DSM-IV. In this manner, the presented finding is in line with other studies reporting endogenous opiate involvement in the pathomechanism of severe self-injurious behavior. The presented results support the argument put forward by of others that opiate receptors may be up-regulated in the central nervous system of individuals exhibiting self-injurious behavior.

4. Final Conclusions

This study is among the first ones using fentanyl challenge test in normal subjects at two daily time points and to explore the state of the endogenous opioid system in self-injurious behavior, major depression and following sleep deprivation. In summary, our results suggest that there are differences between the study conditions and the subjects analyzed. These differences are:

1. Normal subjects and depressed patients showed greater opiate sensitivity in the evening. This observation is consistent with previous reports on pain sensitivity and the typical mood changes in melancholic depression.
2. Depressed patients showed a blunted prolactin response to fentanyl administration in comparison with matched controls. This suggests a reduced opioid function in depression.
3. Depressed patients showed a normalized prolactin response to fentanyl administration after partial sleep deprivation. Possibly, sleep deprivation prevents a nightly breakdown of the endogenous opioid tone and has some sort of psychostimulant effect.
4. Within the group of patient with self-injurious behavior, skin-pickers (subjects with severe pain-inducing stereotypic movements) had the increased sensitivity to the opiate agonist as compared to hair-pullers and healthy controls. This finding has clinical relevance for planning treatment modalities of self-mutilation.

The author of these short publications wishes to avoid oversimplification and is careful about not predicting treatment response from these findings. The role of the endogenous opiate system in psychiatric illnesses and pathological behaviors is not simple. Background opioid levels, plasticity of opiate receptors and chronic or intermittent dosing of the opiate antagonist are vital aspects in the prediction of their effects on behavior. The possible therapeutic implication of presented findings should be explored in further systematic investigations.

Even after more than three decades of intensive research, firm conclusions cannot be drawn regarding the role of EOP-s in psychiatric disorders. There is no compelling evidence to link endogenous opioids to schizophrenia, and interest is waning. The initial optimism in affective disorders has also faded. Nonetheless, there is accumulating evidence that the endogenous opiate system may contribute to the abnormality of the hypothalamic-pituitary-adrenal axis in depression. Studies of the roles of opioids in self-mutilation and eating disorders are on the upsurge. However, most of these works are
of their early trial phases. Of the eating disorders, bulimia holds the most promise of yielding straightforward evidence of opioid involvement.

Increasing knowledge of the multiplicity of opioid ligands and receptors highlights the importance of studying opioid peptides individually and the appropriate receptors of the family. Because monolithic theories are usually short-lived in neurosciences, interactions with other neuroregulators must be also taken into consideration.

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6. List of Personal Publications the Dissertation is Based on


7. List of Personal Publications Related to the Dissertation


Frecska E, Davis K.
The opioid model in psychiatric research.

Frecska E, Csokli Z, Nagy A, Kulcsar Z.
Neurophenomenological analysis of the therapeutic relationship in ritual healing.