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 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 [119] and affective disorders [123]. 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 neoendorphin [3].

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 [98]. 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 adenyl-cyclase 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 [107].

Like opiates, endogenous opioid peptides 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 [22]. In the role of a neuromodulator, endogenous opioid peptides 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 [156].

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 [102] 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. Kline et al. [96] observed that 1.8 to 9.0 mg of intravenous human beta-endorphin improved mood, increased spontaneity, and diminished anxiety of depressed patients; however, the effects disappeared within a few hours. In a study by Angst et al. [6], four bipolar and two unipolar depressive patients showed improvement in
energy, anxiety, and restlessness within 20 to 30 minutes after a 10-mg intravenous injection of beta-endorphin. Four of the six patients showed relapse after two hours, two patients' improvement persisted, and three patients became hypomanic during or soon after the trial. Unfortunately, these trials were not adequately controlled to rule out the roles of expectation, experimental stress, or sleep deprivation. No hypomania was in fact noted in subsequent double-blind, placebo-controlled studies [31, 71], but patients manifested significant improvement in depressive symptoms as reflected in their animated speech, bright facial expression, increased sociability, and psychomotor activation. Negative results have also been reported with beta-endorphin [122] and Met-enkephalin [95]. In the few studies on the effect of subchronic opiate administration, relief of depression has been attained with cyclazocine [61], buprenorphine [55, 56], and methadone [123]. 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 [42, 54, 162] nor in healthy volunteers [80, 86]. Pickar et al. [125] showed that although a range of dysphoric feelings including depressed mood was found in normal subjects after injections of naloxone, effects mainly consisted of non-depressed feelings of irritability and anxiety. The administration of high doses (20-30 mg) of naloxone was reported to cause an observable reduction in manic symptoms [43], but subsequent attempts to replicate this finding were unsuccessful [35, 36, 54, 88, 121]. Using a single-blind design, Cohen et al. [36] observed increases in anxiety with very high doses of naloxone (1-6 mg/kg) in healthy subjects, and
depressive patients manifested a more marked and subjectively more intense worsening compared with control subjects [35]. While the specificity of the antagonist is questionable at this dose, these authors postulate a chronic activation of the opiate system in depression, resulting in a naloxone-sensitive, withdrawal-prone physiological state.

Measurements of cerebrospinal fluid (CSF) opioid levels in manic and depressive patients have also yielded inconclusive results. Initially, Lindstrom et al's work [105] indicated that fraction I 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. Two other groups did not report any difference in total opioid activity among manic, depressive, and euthymic individuals [41, 124], although four bipolar patients in the latter study showed higher levels during the manic phase than in a depressive episode. CSF beta-endorphin immunoreactivity was not different between patients with affective disorders and healthy volunteers [21]. Whereas most studies examined levels of endogenous opioid peptides 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 opioid activity may be associated more with stress and anxiety than with any other psychiatric condition [127], with concordance of the finding by [36] that anxiety increases following opiate blockade.

Although basal plasma beta-endorphin-like immunoreactivity in depression does not seem to show a consistent abnormality (elevated levels: Brambilla et al. [25], Risch [133]; no abnormality: Alexopoulos et al. [4], Cohen et al. [37]), some observations are noteworthy. Dexamethasone failed to suppress plasma beta-endorphin and/or beta-lipotropin values in 50% to 69% of depressive patients [78, 109]. Moreover, thyrotropin-releasing hormone (TRH), luteinizing-hormone-releasing hormone (LHRH) [26] and physostigmine [134] raised plasma beta-endorphin levels in depressed subjects but had less or no effect in control subjects. Early escape of cortisol
in the dexamethasone suppression test (DST) is a measure of a corticotropin-releasing hormone-related hyperactivity of the proopiomelanocortin system [79], and the observed increase in beta-endorphin responsivity may be a proximal sign of the same dysfunction. Convergence is not necessary between cortisol and beta-endorphin non-suppression because of individual differences in adrenal gland autonomy.

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 [141]. 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 [37]. Early resumption of morphine-suppressed cortisol secretion was associated with a diagnosis of major depression and with abnormal DST results [184]. In the interpretations of these data, the cross-tolerance that can develop between opiates and glucocorticoids [59] 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 [64].

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 [33]. On the other hand, tricyclic antidepressants and electroconvulsive shock are synergistic with morphine, and some of the resulting behavioral effects are antagonized by naloxone [48, 87]. Recent results from Tejedor-Real [161] favor optimism for postulating a neurochemical basis of endogenous opiate relationships in the learned helplessness model of depression. The stimulation of the endogenous opiate system either by exogenous administration of Met- or
Leu-enkephalin or morphine raised the levels of endogenous opioid peptides which in turn reversed the escape deficit of rats after inescapable shock treatment. Though these results support the relation of elevated endorphin levels to reverse depressive effects of stress, naloxone treatments gave mixed results. In previous studies [49, 174] naloxone had been found to reverse the learned helplessness in specific experimental conditions. According to a more recent study [19], naloxone should also be able to potentiate the effects of uncontrollable stress. Naloxone prevented predicted learned helplessness in animals unable to escape when administered before exposure to the initial stress, but it did not revert this deficit when administered immediately before the shuttle-box test [83]. These inconclusive results make it difficult to arrive at a conclusion that clearly clarifies the role of endogenous opiates in the learned helplessness model. One has to be cautious drawing any conclusions about how the opiate system might be involved in the behavioral 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 hyporesponsitivity in depression. Early reports indicated that the prolactin response to morphine or methadone might be diminished in depressive patients [57, 90, 135], with some other studies being less supportive [10, 185]. One of the main aims 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

Autistic children appear less sensitive to pain, seldom cry, and seem to have a low desire for social interaction. They are less emotional, show labile affect, fail to express physical affection, have episodes of increased motor activity alternating with quiescence, and evidence an extreme resistance to behavioral extinction [179]. A range of autistic-like symptoms such as elevation of pain threshold, reduction of distress vocalization, diminution of the desire for companionship, poor clinging behavior, and fluctuating responsiveness to stimuli, and learning abnormalities characterized by prolonged extinction can be elicited by enhancement of endogenous opioid function or by administration of opiate alkaloids in infant animals [118]. In addition, both autistic children and children exposed in utero to opiate drugs often are retarded in height and bone development, have feeding problems, and are prone to epileptic seizures. Based on these observations, excessive brain opioid activity has been postulated in the etiology of infantile autism [117]. If this concept is correct, autistic children would have developed tolerance for and dependence on their endogenous opiates, they would manifest symptoms analogous to the behavior of opiate addicts, and they would be sensitive to opiate receptor blockade.

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 [155], and between 8% to 14% of mentally retarded individuals [12]. 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 [2]. 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 [148].

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 naloxone [18, 40, 77, 132, 148, 150] and naltrexone [27, 28, 60, 84, 91, 101, 104, 147, 160, 163, 168, 178]. Despite the fact that relatively low doses of naloxone were administered (usually no more than 1.2 mg) the majority of reports found a palliative effect of these agents. On the other hand Beckwith et al. [14] reported failure of naloxone to reduce self-mutilation in two developmentally disabled female patients and naltrexone failed to reduce self-injurious and autistic behavior in mentally retarded adults in a double-blind placebo-controlled study [175]. Most of these trials had several weaknesses in design, and the results must be regarded as preliminary. Patients treated with opiate antagonists were mostly 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 autistic children or self-abusive patients are addicts of their endogenous opiates, administration of an antagonist should have precipitated withdrawal-like phenomena. Indeed, one study using naloxone reported a transient increase of self-mutilation during naloxone administration [132]. Interestingly, this study used the highest dose (6-hour infusion at 1 and 2 mg per hour), and self-mutilation declined below baseline level in the post-drug period, presumably due to the absence of reward. Initial aggravation of self-injurious behavior was reported in autistic patients receiving naltrexone
Future studies of opiate antagonist treatment of self-injurious behavior have to address this possible biphasic effect and must entail prolonged observation.

There have been reports of the use of naltrexone in treating autistic children [27, 84, 160, 168, 176, 178]. The finding of Szymanski’s group [160] was negative, but the other studies reported improvement in these patients, with reduced self-mutilation. The current consensus is that naltrexone is associated with modest reduction in hyperactivity and restlessness in the group of children with autism. However, the medication did not lead to improvement in learning [101] or communication, a core deficit of autism [60].

Weizman et al. [170, 171] examined plasma concentrations of an endogenous opioid ligand, designated humoral endorphin (a unique opioid peptide that is not cross-reactive with alpha-, beta-, or gamma-endorphin, dynorphin, or enkephalin), in autistic children and, contrary to expectations, observed lowered levels. This deficiency did not appear to be specific to autism, since a similar decrease was found in untreated schizophrenic youths in the same study. However, in the study of Gillberg and his coworkers [75], self-injurious autistic children had higher fractions I and II of endorphin in their CSF than did those patients who had not manifested self-mutilation. There was a trend toward a correlation between high fraction II levels and self-destructiveness and decreased pain sensitivity. The major opioid in fraction II of human CSF is Met-enkephalin. Later on, the same group reported elevated CSF and plasma beta-endorphin in autism [73, 74]. In their early study, Coid et al. [38] reported elevated Met-enkephalin concentrations in the plasma of habitually self-mutilating patients. In a population of 40 developmentally disabled individuals, stereotypic and self-injurious patients had higher levels of plasma beta-endorphin than did control subjects [146]. The plasma beta-endorphin level of the subjects with severe self-injurious behavior proved to be significantly lower than of autistic subjects without severe self-injurious behavior [177]. The results support the idea that severe self-injurious behavior may be related to functional disturbances in the endogenous
opiate system and individuals with severe autism have a heightened response to acute stressors rather than chronic hyperarousal or elevated basal stress response system functioning. Based on these studies, 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 people [139]. This was the leading concept for development of Study Four.

Auto-addiction is a tempting explanation for self-abusiveness. However, increased levels of endogenous opioid peptides are not sufficient to prove the presence of auto-addiction in these patients. They may explain elevated pain threshold, but decreased pain sensitivity alone is not enough to perpetuate self-injury. The missing links have yet to be provided. In conclusion, the opiate paradigm of infantile autism is a promising research area, and there are sufficient data to justify well-designed clinical trials of opiate antagonists in autistic children with a profile of self-injurious behavior.

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 [32, 44, 135, 136, 158, 164, 167, 183]. However, the data on hypothalamic-pituitary-thyroid function are contradictory. Pentazocine, nalorphine, butorphanol, and beta-endorphin do not affect basal thyrotropin levels in humans [45, 131, 137, 138]. After acute morphine administration Tolis et al. [164] and Banki and Arato [10] found only an insignificant increase in serum thyrotropin. At variance with these findings, greater doses of morphine, methadone, DAMME (a long-acting analogue of Met-enkephalin), and dermorphin have been reported to increase thyrotropin serum concentrations [45, 47, 81, 140]. This inconsistency can be explained
by the assumption that only opiate receptors of the mu-type can induce thyrotropin release in humans [45].

Measuring opiate-dependent analgesia, diurnal variation in opiate sensitivity has been demonstrated [67]. In rodents, Kiem et al. [94] found a circadian rhythm in the opiate responsivity of pituitary hormone secretion. 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.

It has been usual to control for time-of-day effects in neuroendocrine studies in psychiatry by undertaking the test at a standard time in the morning. There have been a few attempts to exploit time-of-day effects. Given the diurnal variations of mood found in some types of depression and diurnal variation in hormonal output both in normals and in major depressives, such an approach offers possibilities to explore the relationships between circadian rhythms, neuroendocrine function, mood and the different neurotransmitter systems involved.

Aims: Based on the above, it was reasonable to investigate the prolactin and thyrotropin responses evoked by the selective mu-receptor agonist fentanyl [108] and the diurnal variation in its hormonal and subjective effects.

Methods: Ten informed, consenting, healthy volunteer women, aged 18-55 years (mean±SD = 35±9.4) were investigated. None were receiving medication. One of them was postmenopausal. The others were studied between days 4 and 11 of their menstrual cycle.

Each subject rested supine and had an indwelling heparinized catheter inserted at 8:30 AM. Two basal blood samples were taken at 8:45 and 9:00 AM. At 9:00 AM, 0.1 mg fentanyl was administered intravenously (within one minute. Blood was then sampled after 15, 25, 35, 45, and 60 minutes. All subjects had this procedure at the above hours in the morning and evening in a randomized
order on separate days 36 hours apart. Placebo was also given in single-blind fashion to five of them following an otherwise identical protocol.

Serum prolactin and thyrotropin were assayed in duplicate by commercial radioimmunoassay kits, all having intraassay variances below 7% and interassay variances less than 9%. Assay sensitivities were 1.0 ng/ml for prolactin, and 0.5 µU/ml for thyrotropin.

The occurrence of subjective symptoms such as sedation, sleepiness, euphoria, mental clouding floating sensations, dizziness, nausea, and hot and cold flushes was recorded on a nine-item symptom checklist with a “Yes/No” response format. A baseline assessment of these experiences was also taken before each trial, and only the postdrug symptoms were used in the statistical analysis.

Log-transformed hormonal data (due to skewed distribution) were analyzed using a repeated-measures analysis of variance with multigroup Hotelling and Student's paired t-tests were calculated on subjective symptoms.

Results: In this study the author demonstrated that fentanyl, administered as an intravenous bolus, induces a prompt, clear increase in prolactin and thyrotropin release in normal subjects and that its effect shows a diurnal variation (Figure 1). 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 (Table 1).
Figure 1. Serum prolactin (PRL) and thyrotropin (TSH) (mean ±SE) responses of healthy volunteers following 0.1 mg intravenous fentanyl (N=10) and saline (N=5) in the morning (AM) and in the evening (PM).

<table>
<thead>
<tr>
<th></th>
<th>Morning</th>
<th>Evening</th>
<th>Change</th>
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<tbody>
<tr>
<td>Total number of symptoms</td>
<td>10</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Mean (± SD) number of symptoms per subject</td>
<td>1.0 ± 0.47</td>
<td>1.8 ± 0.92</td>
<td>0.8 ± 0.79</td>
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Table 1. Subjective effects of 0.1 mg intravenous fentanyl in healthy women (N=10).

Conclusions: These results favor the hypothesis that mu-receptors mediate stimulatory effect on thyrotropin release in humans. This is in contrast to rodents, where mu-receptors are supposed to have an inhibitory effect on thyrotropin secretion [130]. The relatively low thyrotropin response in the morning can partly explain the inconsistencies of the literature re-
viewed 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 thyrotropin 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 [59] and the daily rhythm of opiate sensitivity is not demonstrable in adrenalectomized rats [94].

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 [57, 90, 135]. 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 [10, 185].

In addition to alterations of neuroendocrine function, chronobiological shifts and diurnal change of mood are also characteristic of major depression [116, 145]. Circadian variation in the opiate responsivity of prolactin secretion has been shown in rodents [94], 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 against fentanyl in major depression and the diurnal variation in its effect.
Methods: Subjects were ten informed, consenting, newly admitted female inpatients who met DSM-III-R [5] criteria for major depression and ten healthy volunteer women (mean±SD of age: 41±13.5 years and 35±9.4 years, respectively). Premenopausal women were studied between days 4 and 11 of their menstrual cycle.

Patients and controls were drug-free for at least ten days prior to the fentanyl challenge test. They rested supine and had an indwelling heparinized catheter inserted at 8:30 AM. Two basal blood samples were taken at 8:45 and 9:00 AM. At 9:00 AM, 0.1 mg fentanyl was administered intravenously within 1 min. Blood was then sampled at 15, 25, 35, 45, and 60 min. All subjects were investigated with this procedure at the 9:00 AM and 9:00 PM in a randomized order on separate days, 36 hours apart.

Serum prolactin was measured in duplicate by commercial radioimmunoassay kit. Intra- and interassay coefficients of variation were less than 7% and 9%, respectively. Assay sensitivity was 1.0 ng/ml. Log-transformed hormonal data (due to skewed distribution) were analyzed using repeated measures analysis of variance, along with multigroup Hotelling test.

Results: Fentanyl raised plasma prolactin levels in both healthy persons and depressive patients (Figure 2). The multigroup Hotelling test yielded a highly significant test effect, but did not reveal group difference (for numerical details please see publication attached to the dissertation). In this calculation, the morning and evening data were combined. Comparing the evening samples to the morning ones, the chart on the right side of Figure 1 shows that fentanyl exerted much stronger effects in the evening. Separate analysis on the unpooled data of groups provided similar results. While the effect of diagnosis was not significant, the four lowest prolactin responses were found in depressed patients. Three of the four morning low responders also fell into the group of the four evening low responders. Clinical characteristics did not seem to differ, but after a 1-year follow-up, it was discovered that these three consistently low responders committed suicide; no other subject in this study did so (Figure 3).
Figure 2. Prolactin response to 0.1 mg of intravenous fentanyl in healthy volunteers and depressed patients (mean ±SE).

Figure 3. Maximal increase (peak minus the mean of baselines) of serum prolactin in response to 0.1 mg of intravenous fentanyl in healthy volunteers, depressed patients and suicide victims (in boxes).
Conclusions: This result does not correspond to earlier reports [57, 90, 135], but rather agrees with recent studies [10, 185] 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 [24]. Dopamine [82] and serotonin [100] 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 [8, 11].

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.

A synthetic concept is also tempting for the biological theory of depression. It seems that there is a circadian change in opiate responsiveness that is not restricted solely to the secretion of prolactin [66]. The author cautiously proposes that this 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 studies revealed fast and substantial improvement of mood in depressed patients. In their original study, Pflug and Tolle [120] ascertained a remarkable efficacy response rate (69.5%) in depressed patients in the morning hours after the first sleep deprivation. Similar results were published by Rudolf and Tolle [142] and reviewed by Wu and Bunney [182]. Partial SD is less stressful for the patient than total sleep deprivation, and it has been shown to be as effective [151]. It is well documented that if sleep deprivation is applied as a single treatment modality, its positive effect is usually transitory in nature: subsequent sleep tends to reverse the improvement [103].

The original interest in clinical research and applications of sleep deprivation has lessened, possibly due to the focus on pharmacotherapy and the dominance of pharmacological approaches in research on the etiology of psychiatric disorders [180]. Lately, there is a resurgence of interest emphasizing the clinical usefulness of sleep deprivation. First of all, sleep deprivation is the only antidepressant therapy that works within 24 hours. Second, the symptoms favorably influenced by sleep deprivation include those that warrant the most intensive care due to their life-threatening potential: suicidal tendencies and severe psychomotor retardation. Third, it is a valuable augmentation strategy in treatment refractory depression [70]. Apart from its clinical value, another aspect of sleep deprivation is its scientific use. It 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 [76]. Even a limited insight into the neurochemical changes induced by sleep deprivation can provide important information about the recuperation process in successful treatments of depressive illness.
Several abnormalities in neuroendocrine function have been reported in patients with major depression, including disregulation of prolactin secretion, often in response to a specific pharmacological stimulus [116]. Prolactin challenge tests have been considered to provide us with a window to the brain's neurochemical processes. This approach, though indirect, may allow testing hypotheses regarding the biochemical basis of depressive illness.

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. A robust diurnal variation of opiate sensitivity was described [66] with the suggestion that this diurnal change can be superimposed on the fundamental mood disturbance of major depression. The role of endogenous opioids in the mood change following sleep deprivation might be pertinent. In animal models of sleep deprivation the administration of beta-endorphin markedly prolonged the insomnia with the connected psychomotor activation following sleep deprivation. On the contrary, naloxone reduced the latency to sleep in a dose dependent manner [72]. To the author's knowledge, no human data are available on changes in endogenous opiate function after sleep deprivation. Based on their animal studies, Fratta et al. [63] proposed that sleep deprivation induces a hyperactivation of the endogenous opiate system and supposed involvement in the mood change. While their study did not exclude the role of other neurotransmitter a strong mu-opiate/D1-dopamine interaction was suggested.

**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 second objective of the study was to determine the effect of partial sleep deprivation on fentanyl-induced prolactin responses in the same patients.
Methods: Thirty-nine premenopausal female psychiatric inpatients with diagnoses of major depression were evaluated as candidates for the study. Twenty-one of them were not found eligible for the protocol due to high suicide risk, lack of a psychotropic medication-free period lasting for a minimum of seven days prior to the study, improper timing of the menstrual cycle for the fentanyl challenge test, body mass index $< 20$ or $> 25$ kg/m$^2$, smoking and having history of cardiac, endocrine illness and/or allergic reaction to opiates. Subjects with history of illicit drug use and/or alcohol consumption exceeding 50 g/week were also excluded. Enrolled individuals were 18 consenting female inpatients who met DSM-IV [5] criteria for major depression (with the `severe' modifier) and 10 healthy volunteer women as comparison subjects (mean±SD of age: 37.8±11.3 and 35.2±10.2, respectively). Screening entailed physical examination with laboratory work-up (including urine toxicology, total blood cell count, basic metabolic panel and liver function test), and a semistructured interview for current and past history of psychiatric illness.

Fentanyl challenge tests were performed between days 4 and 11 of the participants' menstrual cycle. Subjects were free of psychotropic medications for at least seven days prior to testing. The maximum duration of the lead-in period was ten days, which at times was necessary for optimization of the timing of the test with the menstrual cycle. Participants had a minimum of 6-hour sleep with onset not later than 12 midnight and awakening not earlier than 4 AM on the night prior to the baseline fentanyl challenge test. In the morning hours of the test day, subjects rested supine and had an indwelling catheter inserted at 8:30 AM. Two basal blood samples were taken at 8:45 and 9:00 AM. Following the second blood sampling, 0.1 mg/70 kg fentanyl was administered in a one minute long infusion. Blood samples were obtained for prolactin measurements at baseline (two), 15, 30, 45 and 60 minutes. During the challenge test, the venous access was maintained by using slow saline infusion. Depressive patients were tested with this procedure repeatedly on a separate day after partial, late-night sleep deprivation. The lights-off time was 10 PM. Sleep-
deprived patients were allowed to sleep in their bedrooms until 2 AM where they were monitored with 15-min checks. Sleep deprivation was started at 2 AM. Between 2 AM and the start of the test, sleep deprived patients were staying in an area next to the nursing station and were under constant observation. For the rest of the day and other nights, they were continued on 15-min checks. Patients were randomized regarding the order of the fentanyl challenge test with undisturbed sleep (SL trials) or with partial sleep deprivation (SD trials). One half of them had the SD trial as the first procedure; the other half had the SL trial first. SD and SL trials were timed 72 hours apart for the same individual. One of the patients withdrew her participation before the second challenge test. Since she had fentanyl challenge without partial sleep deprivation, her results were included in the first part of this study. Placebo was also given in a single-blind fashion to five of the comparison subjects following an otherwise identical protocol.

Serum prolactin was measured in duplicate by commercial radioimmunoassay kit. Intra- and inter-assay coefficients of variation were less than 5 and 7%, respectively. Assay sensitivity was 1.0 ng/ml. Data were examined for normality of distribution by using a normality plot and derived correlation coefficient. This analysis suggested that plasma prolactin concentrations were not normally distributed. Therefore, prolactin measurement; were examined by non-parametric methods. Friedman's repeated-measure analysis of variance was used to confirm the prolactin-releasing effect of the test. Illness- and sleep deprivation-related group differences in prolactin levels were analyzed by means of a two stage method using a summary measures procedure. In the first stage, the area under the curve was calculated with correction for the baseline as a summary measure of the net response for each individual from hormone concentrations at time points following the fentanyl challenge. In the second stage, these summary measures were analyzed by means of non-parametric tests. The Mann-Whitney U-test was applied to independent samples and the Wilcoxon matched pairs test was used on the dependent ones.
Results: Serum concentrations of prolactin increased for the first 30 minutes after fentanyl administration and decreased for the remainder of the test procedure. A discernible prolactin peak was observed at approximately 30 minute (Figure 4). There was a significant increase in the mean concentration with time both the comparison and illness groups, with no change in the placebo group. A substantial prolactin response to fentanyl was observed (Figure 5) following both full night sleep and partial sleep deprivation. Prolactin baseline values did not differ between the trials. Statistical analysis revealed significantly increased hormone secretion to fentanyl after partial sleep deprivation. That increase had a normalizing effect, since the prolactin response of depressed patients in their SD trials did not differ significantly from the results of comparison subjects in SL trials. While the study design was lacking an arm, in which comparison subjects had fentanyl challenge after sleep deprivation, the normalizing effect of partial sleep deprivation is suggested by the direction of change. For more details please see publication attached to the dissertation.

Figure 4. Plasma prolactin responses (mean ±SE) to 0.1 mg/70kg intravenous fentanyl after undisturbed sleep in placebo controls (··· 0···; N=5), comparison subjects ( ¦?¦; N=10) and depressive patients ( --¦ --; N=18).
Discussion: There is no consensus in the literature about opiate-induced prolactin responses in depression. The blunted response found in this study is in agreement with reports of Extein et al. [57] and Judd et al. [90], but does not correspond to studies of Zis et al. [185] and Banki and Arato [10]. The previous report (Study Two) represented a middle stance, since decreased opiate sensitivity was found in those patients who later committed suicide. Replicating the study with a larger sample size has certainly improved the statistical power. Empirical human studies in regard to the effect of sleep deprivation on opiate-induced prolactin secretion are missing. The observed augmentation is a unique finding and supports the suggestion of Gehde and Emrich [69], who postulate that sleep deprivation prevents a nightly breakdown of the endogenous opioid tone. In accordance with this concept, the author reported earlier that both healthy and depressed subjects have decreased opiate sensitivity in the AM as compared to the PM hours [66].
The mechanisms involved in the sleep deprivation-induced restoration of the prolactin response to fentanyl in depressed patients warrant a detailed discussion. The way in which sleep deprivation may influence fentanyl--stimulated prolactin release should be discussed in light of the neurotransmitter pathways regulating the lactotrophic system.

A. Regulation of prolactin secretion

The hormone release of pituitary lactotrophic cells is modulated by the hypothalamic paraventricular nucleus [111]. The regulatory inputs to the paraventricular neurons are complex and only partially understood. Multiple neurotransmitters and neuroactive substances are implicated in the control of prolactin release including serotonin, opioid peptides, dopamine, histamine, cholecystokinin and estradiol [15]. The regulation of the lactotrophic system involves the tonic inhibition exerted by tuberoinfundibular dopaminergic (TIDA) neurons. These TIDA neurons represent a common final pathway in regulation of prolactin release. Serotonin exerts its prolactin-releasing properties by inhibiting TIDA neurons [129]. In mammals, the influence of serotonin on prolactin appears to be the result of a synergistic action of 5-HT1A and 5-HT2A/2C receptors [62, 110, 152]. TIDA nerve terminals are subjected to a similar inhibitory control by endogenous opioid peptides. Inhibition of TIDA neurons is responsible for the increased circulating levels of prolactin produced by mu-opiate agonists [112]. The inhibitory action of mu- opiates on TIDA neurons has a dual mechanism [99]. One is a direct action due to their ability to hyperpolarize these neurons by increasing potassium conductance [106]. The other involves the activation of serotonin pathways [46].

Based on the complexity of the prolactin regulation outlined above, a series of questions arises. What kind of opioid mechanism is responsible for the effect of sleep deprivation on fentanyl-induced prolactin secretion? Are opioids involved at all? What if opiate sensitivity is unchanged and the shift
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described reflects an enhanced serotonin effect downstream of the opiate receptors? Due to the limitations of present study (lack of data on treatment response, no comparison subjects after sleep deprivation), the author can address these questions only in the context of other studies on sleep deprivation and will answer them in the framework of the psychostimulant theory of sleep deprivation.

B. Monoamines and sleep deprivation

Unlike what has been observed after chronic treatment with antidepressants, changes in adrenergic receptors after sleep deprivation were modest and contradictory [165]. The evidence for involvement of serotonin mechanisms in the mood change after sleep deprivation is conflicting. On one hand, in laboratory animals, total sleep deprivation enhances the turnover of serotonin [9], increases the firing rate of serotonin neurons in the dorsal raphe nuclei [68] and decreases the sensitivity of 5-HT1A autoreceptors [128]. In bipolar patients, the therapeutic effect of sleep deprivation has been associated with homozygotic long variants of the 5-HT transporter-linked polymorphic region [17]. On the other hand, brain 5-HT levels do not differ from control levels after 24 hours [23] or 72 hours [173] of sleep deprivation, and extracellular serotonin concentrations are not affected [20]. Sleep deprivation does not act exactly as serotonergic antidepressants do, since its effect on the firing rate of septal neurons is different [39], and tryptophan-depletion studies in humans [113] suggest that sleep deprivation does not exert its antidepressant effects by involving brain serotonin systems. A slightly different picture emerges about the role of serotonin in sleep deprivation: it is the prolongation of the sleep deprivation-induced therapeutic changes for which serotonin mechanisms a responsible and not the induction of clinic response per se [114]. The findings of a recent study by Smeraldi et al. [157] are consistent with this notion: pindolol, which augments serotonin release by blocking the 5-HT1A autoreceptor, significantly improved the antidepressant effect of total sleep deprivation, by prevention the short-term
relapse after treatment. These results are markedly different from the interaction observed between sleep deprivation and the dopaminergic amineptine, which has not been shown to lead sustained mood amelioration but augmented the acute effects of total sleep deprivation [16].

C. Prolactin and sleep deprivation

When citalopram-stimulated prolactin concentrations were studied in healthy subjects following sleep and sleep deprivation [153], prolactin responses were blunted following sleep deprivation indicating down-regulation of 5-HT1A or 5-HT2 receptor. This finding goes against the assumption that sleep deprivation-related findings were serotonin-mediated. There is other literature data that fail to support serotonin involvement and point toward alternation hypotheses. For example, after fenfluramine challenge, blunted prolactin levels were found in patients who subsequently responded to sleep deprivation [92]. The same authors (Kasper et al.) [93] indicated that sleep deprivation decreases the nocturnal prolactin release in depressed patients and control subjects. Similarly, a blunted nocturnal increase of prolactin levels under sleep deprivation was reported by Baumgartner et al. [13]. This physiological effect was attributed to a decrease in dopamine release during slow wave sleep [52]. Consistent with the dopamine theory, there is an increased prolactin response to sulpiride after sleep deprivation, possibly attributable to prevention of the physiological decrease of the dopaminergic tone [53].

The present study is far from being conclusive about the exact mechanisms involved in the hormonal and therapeutic effect of sleep deprivation. Combining its findings with previously published results, one can only raise doubt about serotonergic mediation of the observed shift in the prolactin response. At this point, we have no more than indirect evidence supporting an opiate-dopamine mechanism in the behavioral effect of sleep deprivation. Animal studies revealed an active role of opioids, in interaction
with dopamine, in the behavioral syndrome subsequent to sleep deprivation and hyperactivation of the endogenous opiate system was suggested [58, 63]. Single photon-emission computed tomography before and after total sleep deprivation showed significantly different dopamine receptor occupancy in responders, vs. non-responders, suggesting an enhanced dopamine release in responders [51]. Dopaminergic augmentation of sleep deprivation using amineptine was recently reported in humans [16]. Depending on receptor type and brain location, opiates produce diverging response patterns on different dopaminergic neurons. They increase the activity of the major meso-telencephalic dopamine neurons terminating in the striatum and limbic forebrain regions, but inhibit TIDA neurons [112]. The behavioral and physiological effects of sleep deprivation (e.g. euphoria, hyperactivity and hormonal changes) can be attributed to such a opiate-dopamine interaction and are consistent with the psychostimulant theory of antidepressant action [50].

The cingulate gyrus is a likely site of the induced behavioral changes. Brain-imaging studies reported that clinical improvement during sleep deprivation was associated with reduced metabolic activity in the area of the anterior cingulate cortex [166, 181]. Positron emission tomography studies have revealed high levels of opiate receptor binding in the human anterior cingulate region [89], which also receives generous dopaminergic projections from the ventral tegmental area. Anterior cingulate blood flow is increased by anesthetic doses of fentanyl [1, 30], a fact that does not seem to favor the opiate-dopamine model since the direction of change is opposite to the findings within responders after sleep deprivation. It may help with-but will not resolve-the controversy that typically fentanyl causes behavioral effects similar to those induced by sleep deprivation in subanesthetic doses.

At present time, one can only speculate about the neurotransmitter mechanisms involved in the effects of sleep deprivation. One way to explain the apparent divergence in the literature is to suppose that the acute effects of sleep deprivation are based on opiate-dopamine mediation and
serotonergic mechanisms play a more significant role in prolongation of the effect. This hypothesis needs further investigation. A direct crossover comparison of serotonergic and opiate effects in the same subjects would be a powerful experiment in the debate. In conclusion, sleep deprivation has potentials toward better understanding of the biological processes involved in antidepressant action and should be considered in future studies of antidepressant therapy.

**D. Fentanyl and the tragic outcome of the Moscow hostage crisis**

When this study was near to be published, the theater siege in Moscow on October 26, 2002 gave gruesome actuality to fentanyl research in humans. The results presented here and previous findings on robust diurnal change in fentanyl effects [66] indicate that individual responses to fentanyl may vary significantly according to the subjects' sleep pattern, emotional state and the time of the day. Similar findings may reveal some other factors involved in the tragic outcome of the hostage crisis, and show how difficult the dose calculation can be for narcotic opiate use as a non-lethal weapon. Russian authorities stated the fentanyl-derivative they used would not have normally caused death. They argued that hostages had died because they were in a compromised medical condition, such as dehydration, immobilization, food deprivation, lack of oxygen, and were under severe psychological stress due to the conditions under which they had been kept as captives. Indeed, some of the conditions they were referring to are known from animal studies to magnify opiate effects [85, 159] and might have contributed to the fatalities, especially in case of the hostages. What happened in Moscow on that sad morning underlines the importance of collecting more information on intra- and inter-individual variability of fentanyl effects.
3.4. Study Four. Opiate sensitivity test in patients with stereotypic movement disorder and trichotillomania

**Background:** Self-injurious behavior is a devastating clinical phenomenon, which in humans consists of self-biting, head banging, face slapping, skin picking and scratching. It is associated with different psychopathological conditions such as stereotypic movement disorder, psychosis, mental retardation, obsessive-compulsive and personality disorders. In stereotypic movement disorder, they result in significant physical damage to the individual. There is no consistently successful therapy available for this disfiguring and frequently dangerous behavior. Understanding the underlying pathomechanism may facilitate the development of effective treatment strategies. Dopaminergic and serotonergic mechanisms, as well as the endogenous opiate system, have been hypothesized to be involved in the pathogenesis of the self-injurious behavior [115, 126, 179]. The opiate model of self-injurious behavior is especially tempting since patients with self-injurious behavior experience little or no pain [38] and report mood improvement as response to noxious stimuli [144]. Opiate antagonists such as naltrexone and naloxone have shown to diminish repetitive, stereotypic behavior in animals and these compounds have been used in the treatment of repetitive self-injury [40, 84]. Patients with obsessive-compulsive disorder frequently exhibit self-injurious behavior and recent clinical reports have indicated fast symptom relief to opiate administration in subjects with treatment refractory compulsions [154, 169]. However, positive therapeutic responses to the manipulation of the endogenous opiate system have not been reported consistently [175], and doubt has been raised over the endogenous opiate model of self-injurious behavior [143].

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 but not just one manifestation of self-injurious behavior. Incorporation of different forms of self-injurious behavior within the study group and selection of heterogeneous patient population can result in the observed inconsistency of the opiate model and can confuse as well any other theory about self-injurious
behavior. 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 [139]. 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:** In this study, 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 author investigated the possible involvement of the opiate mechanism in stereotypic movement disorder patients, who had severe self-injurious behavior, using subanesthetic doses of fentanyl for prolactin challenge test. Fentanyl is an opiate mu-agonist [108] and it is well established that opiate alkaloids and opiate peptides stimulate the secretion of prolactin [44, 164]. Healthy volunteers were planned to undergo a study for evaluation of dose-effect relationships. The targeted population was a 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 [5]. Therefore, hair-pullers served as patient control subjects in the study.

**Methods:** Forty-six candidates recruited by newspaper advertisement were evaluated for possible participation in the study. Twenty-four of them were not found eligible for the protocol due to lack of repetitive self-mutilation, postmenopausal state, history of psychotropic medication and/or allergic reaction to opiates. Enrolled subjects were 22 premenopausal, psychotropic medication naive, female individuals fulfilling DSM-IV [5] criteria for stereotypic movement disorder (N=10) and trichotillomania (N=12) and 20 healthy volunteer women (mean±SD of age: 35.1±10.1, range: 21-49 years). Subjects of the trichotillomania group had a mean age of 34.5 years (SD: 6.1, range: 26-42
years) and the mean age of the stereotypic movement disorder group was 35.6 years (SD: 10.6, range: 21-50 years). Every patients with stereotypic movement disorder exhibited repetitive skin picking necessitating medical intervention.

After obtaining informed consent, subjects were studied between days 4 and 11 of their menstrual cycle. On the day of the challenge, they rested supine and had an indwelling catheter inserted at 8:45 AM. Basal blood sample was taken at 9:00 AM. Following the blood sampling, 0.1 mg/70 kg fentanyl was given in a slow, 5-min intravenous infusion to ten healthy comparison subjects. A dose of 0.05 mg/70 kg was given to another ten healthy volunteers and to the patients with stereotypic movement disorder and those with trichotillomania. Blood samples were obtained for prolactin measurements at 0, 15, 30, 45 and 60 min. During the challenge test, the intravenous access was maintained by using slow saline infusion. Placebo was also given in a single blind fashion to five of the comparison subjects following an otherwise identical protocol.

Serum prolactin was measured in duplicate by commercial radioimmunoassay kit. Intra- and interassay coefficients of variation were less than 5% and 7%, respectively. Assay sensitivity was 1.0 ng/ml. The occurrence of subjective symptoms, such as sedation, sleepiness, euphoria, mental clouding, floating sensation dizziness, nausea, headache, hot and cold flushes, was recorded on a 10-item symptom checklist with a Yes/No response format. Hormonal data were analyzed using repeated-measures analysis of variance with Tukey’s test for post hoc comparisons. Nonparametric statistics (Mann-Whitney) was applied to the total score of the subjective symptom checklist.

**Results:** Saline injection did not affect prolactin secretion, but fentanyl elevated plasma prolactin concentration in a dose-dependent manner. Results of the fentanyl-induced side effects were in line with the endocrine responses. More subjective symptoms were reported on the 0.1 mg/70 kg dose. For numerical details please see publication attached to the dissertation.

Comparing the data of the healthy controls using 0.05 mg/70 kg fentanyl with the results of patients with stereotypic movement disorder and
trichotillomania, who have received identical challenge, the analysis revealed significant group difference favoring the fentanyl-induced prolactin response. Patients with stereotypic movement disorder, but not with trichotillomania, showed significantly increased opiate sensitivity (Figure 6).

Figure 6. Plasma prolactin responses (mean ±SE) to 0.05 mg/70kg intravenous fentanyl.

Conclusions: Fentanyl in a dose of 0.05 mg/70 kg was well tolerated by subjects and it can be considered as an opiate receptor sensitivity test. Studies hypothesizing decreased opiate receptor responses in a study group may yield better effect using the higher dose (0.1 mg/70 kg) as the author did in previous investigations (Study One to Three).

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, this study is in line with other investigations reporting endogenous opiate involvement in the pathomechanism of severe self-injurious behavior [139]. The presented results support the argument of Sandman et al. [149] that opiate receptors may be up-regulated in the central nervous system of individuals exhibiting self-injurious behavior.
4. Final Discussion

The purpose of presented studies was to explore the involvement of the endogenous opiate system in patients with major depression and self-injurious behavior. Neuroendocrine studies of depressed patients have suggested an abnormal opiate modulation of the hypothalamic-pituitary axis [57, 90, 135], and the author of this dissertation attempted to challenge endocrine function with the opiate agonist fentanyl both in depressed and healthy subjects at different times of the day. Although many studies have previously administered opiate agonists to depressed patients [10, 57, 90, 135, 185], there has been no report of using opiate agonist challenge in depressed patients at two time points during the day.

The findings show that a subanesthetic dose of fentanyl stimulates prolactin and thyrotropin release in normal controls. The effect of fentanyl is modulated by the time of day at which the drug is given, and by preceding sleep deprivation. Present study was the first to show that 0.1 mg fentanyl administration has a significant diurnal change in its outcome with pronounced effects in the evening. Since the effect of prolactin was more robust as compared to thyrotropin, the former was used as index of opiate sensitivity in subsequent studies with clinical subjects and matched controls.

Depressive patients showed blunted response of fentanyl-induced prolactin release which was shifted toward normal levels after a night of partial sleep deprivation. These observations suggest that depressed patients have decreased sensitivity to fentanyl challenge and sleep deprivation has a normalizing effect on the reduced opioidergic tone. According to the literature, the findings of opiate agonist administration in depression are less consistent when the study is based on interindividual design [10, 185]. Nevertheless, there is no satisfactory explanation of the observed effect of sleep deprivation. Review of literature data revealed that dopamine-related mechanisms are more likely to be responsible than the direct involvement of serotonin. This is in line with the renewed interest in the role of dopamine in antidepressant action. Further investigation is definitely required for verification.
While depressed patients may show decreased opiate sensitivity, self-injurious patients with stereotypic movement disorder have significantly increased responses to fentanyl indicating mu-receptor up-regulation. The presented finding is consistent with other studies reporting endogenous opiate involvement in the pathomechanism of severe self-injurious behavior and by the successful use of opiate antagonists in self-mutilation [139, 149].

Even after more than three decades of intensive research, firm conclusions cannot be drawn regarding the role of endogenous opioid peptides in psychiatric disorders. Research of endogenous opiates has led to new, though conflicting findings in the field of mental illness. There is no compelling evidence to link endogenous opioids to schizophrenia, and interest is waning. The initial optimism in affective disorders has also faded. Throughout the years, evidence has existed that links an excess, deficiency, and even static levels of opioid activity to depression. Undoubtedly, as we outlined in the Introduction, a large debate exists in the search for the link between endogenous opiate function and depression, with beta-endorphin being the major endogenous opioid in question. Evidence favors an the opioid link to depression, but there is also disputing testimony showing that changes in endogenous opiate function of depressed patients have not been found in every aspects of clinical research. The most consistent evidence is supporting the notion that endogenous opiate system may contribute to the abnormality of the hypothalamic-pituitary-adrenal axis in depression.

Putting the presented and discussed observations together, it is possible that there is endogenous opiate underactivity in depression. While the evidence is not entirely consistent, where effects have been detected they favor the hypothesis that there is an endogenous opiate hypofunction and a hypersensitivity to opioid receptor blockade in depression [29].

Studies for the involvement of opioids in self-mutilation and eating disorders are on the upsurge. However, most of these works are currently in their early trial phases. Of the eating disorders, not discussed here, bulimia
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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 with the appropriate receptors of the family. Further investigations may yield new opiate receptor subtypes and ligands, a knowledge, which should improve our understanding of the role of endorphins in normal and abnormal physiology. Since monolithic theories are usually short-lived in neurosciences, interactions with other neuroregulators must also be taken into consideration.

5. Clinical Relevance

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.

6. Limitations

The sample size of presented studies was small, but by using well matched comparison subjects, or studying each patient as their own control softens this problem. Finding patients with a major depressive episode free of medication is difficult, which aspect contributes to the size of the sample and may bias the results.

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 [149]. The possible therapeutic implication of presented findings should be explored in further systematic investigations.

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9. List of Personal Publications the Dissertation is Based on


Prolactin response to fentanyl in depression.

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Opiate sensitivity test in patients with stereotypic movement disorder and trichotillomania.
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10. List of Personal Publications Related to the Dissertation

Frecska E.
Abnormal dexamethasone suppression test: Result of tolerance to opioids? Letter to the Editor.

Kulcsar Z, Frecska E, Varga I.
Endogenous opioid functions and personality.

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.
11. Summary

Investigation of Endogenous Opioid Reactivity With Fentanyl Challenge in Major Depression and Self-Injurious Behavior.

Dr. Ede Frecska

Institute of Psychiatry and Neurology, Budapest, 2005.

Semmelweis University, Doctoral School of Neurosciences, Program 6

Some observations support the notion that regulation of the endogenous opiate system is deficient in major depression and sleep deprivation might exert its antidepressant properties via opioid mediation. In another psychiatric condition, namely stereotypic movement disorder, the clinical use of opiate antagonists have shown to diminish self-injurious behavior supporting involvement of opiate mechanisms in this pathological condition. The overall aim of presented studies was to elucidate the role of the endogenous opiate system in the pathomechanism of major depression, self-injurious behavior and in the antidepressant action of partial sleep deprivation. The main method applied was the use of the selective mu-receptor agonist fentanyl, as a challenging agent for testing endogenous opiate sensitivity by monitoring neuroendocrine responses to the drug, particularly measuring fentanyl-induced plasma levels of prolactin. The author had studies focusing on dose-response relationships in healthy volunteers and diurnal changes of opiate sensitivity. His subsequent studies utilized these results and addressed opioid mechanism in depression, self-injurious behavior and after partial sleep deprivation. Based on data obtained by fentanyl challenge tests, it seems possible that endogenous opiates play some role in the conditions investigated. The author of these short publications wishes to avoid oversimplification and is careful about drawing conclusions for treatment strategies from these findings. The role of the endogenous opiate system in psychiatric illnesses and pathological behaviors is not simple and can be best explained in interaction with other neurotransmitter and signal processing mechanisms.