Opium Effect in Pregnancy on the Dynamics of Maternal Behavior: Testing a Neurochemical Model

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Abstract
Background: Investigations into neurochemical mechanisms of opioid addiction are difficult due to the complexity of behavior and multiplicity of involved neurotransmitter and hormonal systems. The aim of this study was to examine the benefits of structured analysis of these mechanisms using the framework of the neurochemical model Functional Ensemble of Temperament (FET) and the example of maternal behavior under the condition of opium consumption in pregnancy. The FET differentiates between (a) endurance, (b) speed of integration, and (c) emotionality aspects of behavior suggesting that these systems are differentially regulated by (a) serotonin-neuropeptides-brain-derived neurotrophic factor (BDNF), (b) dopamine-GABA, and (c) opioid receptor systems, correspondingly. The FET also suggests that mu-opioid receptors (MORs) binding the endorphines (including opium's ingredient morphine) have a stronger association with regulation endurance, whereas delta-OR have a stronger association with integration of behavior and kappa-OR – with the perceptual mobilization seen in anxiety. To test the predictions of this model, we compared the impact of massive MOR dysregulation on 3 behavioral aspects of behavior and on serotonin, BDNF, and corticosterone levels. *Methods:* The study used 24 female white Wistar rats which were randomly divided into (1) control group: pregnant rats without any intervention; (2) opium-exposed group: animals that were exposed to opium during pregnancy and after the delivery until the end of the study. At the end of the study, the levels of BDNF, serotonin (5-HT) in the hippocampus of the mother's brain, and serum corticosterone, as well as 12 aspects of the maternal behavior were evaluated. The differences between control and experimental groups were assessed using the *t* test for independent samples. *Results:* The BDNF and serotonin concentrations in the hippocampus of the mother rats which were exposed to opium were lower than in the control group; the mean corticosterone in exposed mothers was higher than in the control group. Behaviorally, opium-consuming mothers showed lower endurance in 4 distinct behavioral categories (nesting, feeding, grooming, and retrieval) than the mothers in the control group. Ease of integration of behavior was affected to a lesser degree, showing a significant effect only in 1 out of 5 ap-

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plied measures. Self-grooming, seen as an emotionality-related aspect of behavior, was not affected. ***Conclusion:*** Opium exposure during pregnancy in our experiment primarily reduced the endurance of rat's maternal behavior, but the speed of integration of behavioral acts was less affected. This negative impact of opium on endurance was associated with a decrease of BDNF and serotonin levels in the hippocampus and an increase in corticosterone level in opium-consuming mothers. There is no effect of opium exposure on self-grooming behavior. This pattern supports the FET hypothesis about the role of 5-HT and BDNF in endurance, differential regulation of endurance, integrative and emotionality aspects of behavior, and differential association of the MOR system with endurance aspects, in comparison with kappa- and delta opioid receptors.

Introduction

Analysis of the neurochemical nature of behavioral changes in individuals affected by opiate use can help to choose directions in psychopharmacological and psychological interventions and as well could highlight the fundamental interactions between neurochemical systems. One of the oldest psychostimulants in the world is opium, which contains >40 alkaloids (including morphine, thebaine, codeine) and >70 other components, such as sugars and organic acids [1, 2]. Opioid drug use is illegal in Iran, but has a long history, and still is very much prevalent among males and females [3, 4], especially in southern Iran [5]. Currently, opium dependency among the Iranian women has become a public health problem [4, 6, 7] as it is illegally used for leisure or to treat pain, diarrhea, and insomnia [1, 2].In a study that examined the pattern of drug abuse in rural communities in Iran, it was reported that 8.3% of rural Iranian women consumed opium daily [8]. The abuse of opiate substances results in consistent changes in nerve physiology and its connectivity leading to psychopathology [4, 9, 10].

One of the most sensitive forms of drug abuse is during pregnancy, which may cause compromised cognitive and social functions, increased pain sensitivity and anxiety in mothers, altered pain and drugs sensitivity in children [4, 11], and changes in maternal behavior. Maternal behavior is complex and multifaceted, so measuring it even in healthy individuals is a challenge. In studies of maternal behavior in rats, it has been shown that morphine (i.e., opium component), inhibits and disturbs the maternal behavior in rats leading to a reduction of mothers' postpartum cleanup of infants, pup retrieval, crouching over the pups for feeding, etc. [12, 13]. Šlamberová and colleagues [14] showed that morphine-treated mothers were less often in contact with their litter in the nest and had less licking and grooming their pups.

This study examined the analytical benefits of the structure proposed within the neurochemical model Functional Ensemble of Temperament (FET) [15–18]. The FET highlights the 12 formal-dynamical aspects of behavior that are universal in the behavior of animals and humans and are proven to be neurochemically based. These 12 behavioral aspects are classified into 3 formal-dynamical categories (energetic, or endurance; speed of integration of actions; orientation) and emotionality. Our study analyzed the benefits of differentiation between 3 aspects of maternal behavior: physical endurance related, speed of integration of actions, and emotionality.

The role of 5-HT and brain-derived neurotrophic factor (BDNF) in physical endurance (later – endurance) relates to the ability of an individual to sustain prolonged physical activities. As summarized in the FET, endurance is regulated by multiple endocrinal and neuropeptide factors, and, out of all monoamine systems, serotonin has the strongest links to it [15, 17, 18]. Serotonergic (5-HT) regulation of the endocrine system depends on many factors, 2 of which were the focus of this study: BDNF and mu-opioid receptors (MORs). It has been shown that 5-HT deficiency plays an important role in the dysregulation of maternal behavior. Reduction in 5-HT release weakens the maternal behavior and reduces the chance of survival of infants in laboratory animals [19]. BDNF interacts with the 5-HT transporter in the regulation of RNA synthesis within the cells of the nervous system [20] and plays an important role in the neuroplasticity of synaptic transmission and maintenance of neural circuits [21]. Increased BDNF mRNA levels in the hippocampus in animals with a deficiency of 5-HT transporter were shown to increase susceptibility to the environment [22]. Reduced BDNF levels reportedly result in neuronal atrophy and cell death in the hippocampus as observed during stress, whereas enhanced BDNF levels are associated with neurogenesis, cell survival, and dendritic arborization [22]. Animal studies show that methylation of the BDNF gene (which leads to reduced production of BDNF) is associated with depression, in which low endurance (fatigue) and psychomotor retardation are the main symptoms [23]. Previous studies have shown that a low level of BDNF (BDNF methylation) disrupts maternal behavior [24].

Ease of Integration of Actions

Behavioral integration requires a strong of the most relevant elements of actions and suppression of a large number of irrelevant elements. The FET model highlights the key role in this selection process of dopaminergic-GABA systems that facilitate the speed of integration of actions. The dopaminergic systems also interact with BDNF and MOR systems, and therefore MOR over-activation by an excess of morphine potentially could compromise the integrative aspects of behavior. According to the FET model, and as commonly reported [16, 25], the MOR activates the release of dopamine, facilitating behavioral integration (speed of actions, ease of putting together a program of actions). Recent animal studies have shown that chronic morphine consumption suppresses the expression of the BDNF gene in the part of the rat brain causing an increase in dopamine release in response to morphine [26]. Addiction to morphine that binds to MOR, therefore, can deregulate the speed of integration of actions in maternal behavior, such as plasticity and tempo of mothers in performing their parenting routines.

Opium use is likely to affect all areas of the brain [4], and serotonin systems, which project to all areas of the brain, are good targets for research on the effects of this substance. Since we could not examine all the areas of brain affected by opium, we chose the measurement of serotonin in the hippocampus since this brain structure plays a role in the homeostatic behavior (i.e., supporting well-learned behavioral actions by memory schemata). This functionality of the hippocampus is supported by direct projections of serotonergic neurons from the median Raphe Nucleus (RN) to hippocampus and then cortex, which are separate from the serotonergic projections of the dorsal RN to other brain structures. Our study analyzed a differential pattern for the endurance of actions versus number of integration of actions, and, therefore, we felt that the potential changes in endurance might be associated with the changes in 5-HT release in hippocampus.

Entanglement of MOR System with 5-HT Systems and Differences in Functionality between Kappa Opioid Receptors and Delta Opioid Receptors

MORs are a part of the G-protein-coupled receptor family that also include kappa opioid receptors (KORs) and delta opioid receptors (DORs). MORs also regulate the release of serotonin, and this regulation depends on the type of 5-HT receptors and their location [27–29]. It has been shown that administration of morphine, a key component of opium-activating MOR, induces a short-term increase followed by a long-term decrease in brain serotonin release [30]. Therefore, addiction to opium might cause changes in serotonin, BDNF, and MOR systems, impacting physical endurance required to maintain maternal behavior. The FET model underlines the important role of the DOR system in the speed of integration of actions rather than in supporting emotional, energetic, or orientational processes [15, 16, 18]. The DOR and MOR systems often use each other's binding peptides, that is, enkephalins and endorphins, but morphine, as an endorphin, preferentially binds to MOR. This means that when pregnant rats are exposed to opium that contains morphine and codeine, it might affect the MOR (and therefore endurance) rather than the DOR (i.e., speed of integration) processes, and 5-HT rather than DA release. From this perspective, we would expect that opium use compromises endurance aspects of behavior more severely than integration aspects of behavior. However, due to MOR-DOR and 5-HT-DA frequent coactivation, we should expect that speed of integration aspects should be also affected to some degree.

HPA-Based Arousal and Anxiety

The morphine component of opium binds to MOR, activation of which was linked to the induction of positive mood, relaxation, and suppression of an acute stress response within the hypothalamus-pituitary-adrenal axis (HPA) [31]. Responses to stress involve a set of physiological changes, including neuromuscular changes and neurochemical processes within the HPA [31]. Activation of the HPA axis is associated with the release of stress hormones, such as corticosterone. *cAMP* response element-binding protein plays an important role in the interaction between 5-HT and BDNF, with all 3 agents changing the basic chemistry of hippocampal cells when the reprogramming regulatory system is overwhelmed during prolonged stress [32, 33]. Anxious dispositions (over reactivity to stressors and dispositional behavioral alertness in the absence of specific triggers) are described in the FET model as neuroticism, in line with the common terminology in differential psychology [15, 16]. The FET model highlights research into the contribution of KORs in anxious dispositions (seen in chronic anxiety) and KOR entanglement with noradrenalergic and stress-hormone systems.

Two opioid receptor systems, KORs and MORs, often mutually suppress each other and work in opposite directions in suppressing or facilitating the release of

monoamine neurotransmitters and their action on the HPA axis. MOR activation leads to the release of dopamine, selective release of 5-HT, suppression of the release of noradrenaline, and suppression of the stress-response associated with HPA arousal. KOR activation is associated with the opposite pattern. However, it is important to note that KOR activation is linked more with behavioral and sensory mobilization rather than with negative emotions [16]. This mobilization is a key component of chronic anxiety, but also an important component of the alertness needed in normal behavior. When it comes to the impact of morphine (i.e., the key component of opium), animal studies showed that it had paradoxical effects on HPA axis activity. Some studies have reported an increase in activity of the HPA axis, but others reported a decreased activity following the use of morphine [31, 34]. Klausz and colleagues [31] used adult female Wistar rats in their study of morphine exposure during pregnancy and observed the rats' performance on the elevated plus maze (EPM) (provoking anxiety) and the forced swimming test (provoking initiation of actions). Despite of anxiety-provoking task, there was a hypoactivity of adrenocorticotropin and corticosterone measured postpartum even though the morphine exposure in these animals was associated with spending less time in the open arms of the EPM and longer time with floating (i.e., hesitation) and shorter time with climbing during the forced swimming test [31]. This suggests that morphine might not have a direct impact on stress hormones. The decrease in the activation of the HPA axis and a reduction in corticosterone release were found to reduce the quality of maternal behaviors [35]. In fact, Graham et al. showed that removal of the adrenal gland leads to reduced maternal behaviors and corticosterone injections enhance maternal behaviors [36]. There are, therefore, complex relations between the activation of MOR-KOR systems (triggered by the use of opium), corticosterone release, and behavioral mobilization, including anxiety symptoms, which should be investigated.

Considering a possible effect of opium on serotonin levels, corticosterone, and BDNF during pregnancy, the goals of the current study were (1) to investigate the effect of opium on these neurochemical systems; (2) to analyze the effect of these neurochemical changes on maternal behavior using the structure of the FET framework, differentiating between the aspects of (a) endurance, (b) easy of integration, and (c) emotional regulation of behavior.

The *hypothesis* of the study was based on the current literature and the proposed mechanisms of the interaction between specific neurochemical systems (opioid receptor systems, BDNF, and serotonin) and behavioral patterns. According to this hypothesis, the compromised endorphin activity (administration of morphine) would:

A. Decrease the endurance aspect of maternal behaviour, serotonin release and BDNF presence, possibly entangled with the MOR system.
B. Possibly slow down the integration of behavioural acts, due to the impact of morphine on dopaminergic receptors and the MOR system.
C. The effect of opium might be less profound on emotionality-related aspects of behaviour, such as self-grooming, however it might increase corticosterone as part of behavioural mobilization compensating for the 5-HT deficiency.

Materials and Methods

Sample
In total, 24 female Wistar rats (weighing 200–220 g) were used for the present study. The rats were kept under controlled environmental conditions at the Animal Research Center for Laboratory Animals of Zahedan University of Medical Sciences with free access to water and food, a 12-h cycle of darkness-lighting and a temperature of 22 ± 2°C. Animals were randomly divided into 2 groups: (1) Control group: animals, which were pregnant without any other intervention; (2) Opium exposed: animals, which were pregnant and were exposed to opium, continuing opium consumption till the end of the study.

Materials and Procedures
To induce pregnancy, every 3 female rats were kept in a cage with 2 male rats. A sperm smear was taken from the vagina of the female animals every morning, and those animals whose test was positive were transferred to another cage [37].

In the experimental group, opium dependence began 10 days before the mating process and continued until the end of the study. To enforce the opium exposure, opium was dissolved in the drinking water with a gradual increase of dosage [2, 38]. The schedule was as follows: in the first 48 h, a dose of 0.1 mg/mL; the second 48 h – a dose of 0.2 mg/mL; the third 48 h – a dose of 0.3 mg/mL; the fourth 48 h – a dose of 0.4 mg/mL. After that, the dose of 0.4 mg/mL continued till the end of the study [2, 39].

Opium was provided by the Iranian police's narcotics division and was documented as having originated from Afghanistan. The opium composition was analyzed by GC-mass spectrometry. Over 35% of the opium used in this study consisted of alkaloids, the most abundant of them were morphine 18%, codeine 7%, thebaine 5%, and papaverine 5%. Other materials were organic compounds and nonorganic ones. Available water (moisture) was 15%. This alkaloid content was similar to the study by Remberga et al. [40] on Afghanistan's alkaloid opium content.
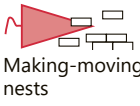
	Endurance (A): energetic aspect, how long or how efficient a behavioural act could be performed.	Ease of integration, switching (B) as a function of the number of actions.
Making-moving nests	A1) Duration (in seconds) of nesting: moving the nest with the mouths and claws and building the nest.	B5) Number of nestings, i.e. number of moving the nest with the mouths and claws and building the nest
Breast-feeding	A2) Duration (in seconds) of breastfeeding (nursing), when the mother is hanging on the pups or lying next to the pups.	B6) Number of breastfeedings (nursing), when the mother is hanging on the pups or lying next to the pups.
Grooming	A3) Duration (in seconds) of pup grooming, with a separate observation of licking the body and licking the genital area of the pups.	B7) Number of pup groomings that involve licking the bodies of pups
Retrieval	A4) Efficiency (speed, in seconds) of pup retrieval. Retrieval means the mother takes the pups to their nest or to a new place where the new nest is made. The longer it takes the mother to complete the retrieval, the less efficient it was considered.	B8) Number of pup retrievals (finding a pup and bringing it to the nest). B9) Latency to onset pup retrieval (in seconds): These behaviours appear more often after childbirth and when the pups tend to disperse. In fact, this behaviour reflects the level of care and protection of children.

Fig. 1. Matrix of behavioral measurements of 2 dynamical aspects, endurance and ease of switching of actions in maternal behavior.

At the end of the study, blood samples were taken from the heart of the animals for measurement of the level of corticosterone using the ELISA kit. Brain hippocampal tissue was analyzed for levels of BDNF and serotonin using the ELISA kits.

Measurement of Maternal Behavior

The rat's maternal behavior includes the following actions that require endurance and plasticity of behavior, and elements of attachment behavior: building the nest, moving the nest, retrieval of pups scattered around the area and moving them into the nest, and pup grooming and pup nursing. On the second day after childbirth, the dams were removed from their cages. Then, the pups were immediately placed inside the cage (opposite to the nest and scattered around the cage), and the mother was returned to the cage.

Maternal behavior observations were performed between 9:30 and 11:30 a.m. The following maternal behaviors were observed and recorded [41] for 60 min:

A. Related to endurance behavior (duration of actions)
A1. Duration (in seconds) of nesting: moving the nest with the mouths and claws and building the nest.
A2. Duration (in seconds) of breastfeeding (nursing): when the mother is hanging on the pups or lying next to the pups. The purpose of this position is to allow the pups to access the nipple and breast milk to maintain their body temperature, and support environmental factors.
A3. Duration (in seconds) of pup grooming, with a separate observation of licking the body, and licking the genital area of the pups.
A4. Efficiency (speed, in seconds) of pup retrieval. Retrieval means the mother takes the pups to their nest or to a new place where a new nest is made. The longer it takes the mother to complete the retrieval, the less efficient it was considered.

B. Related to the ease of integration of behavior (speed of actions and latency of actions)
B5. Number of nestings, that is, number of moves of the nest with the mouths and claws, and building of the nest.
B6. Number of breastfeedings (nursing): when the mother is hanging on the pups or lying next to the pups. The purpose of this position is to allow the pups to access the nipple and breast milk, to maintain their body temperature, and to support environmental factors.
B7. Number of pup groomings that involve licking the genital areas of pups.
B8. Number of pup retrievals (finding a pup and bringing it to the nest).
B9. Latency to the onset of pup retrieval (in seconds): these behaviors appear more often after childbirth and when the pups tend to disperse. In fact, this behavior reflects the level of care and protection of children.

C. Behavior related to emotionality (self-calming or anxiety)
C10. Duration (in seconds) of self-grooming.
C11. Number of self-groomings (Fig. 1).

Data Analysis

GraphPad Prism Ver. 7 was used to analyze the data. At first, the normality of the distribution of data was assessed by the Kolmogorov-Smirnov test. The result of the Kolmogorov-Smirnov test showed that all the variables of the control and opium-exposed groups had normal distributions ($p > 0.05$). To assess the differences between the control and experimental groups, we used the independent samples T tests, with $p < 0.05$.
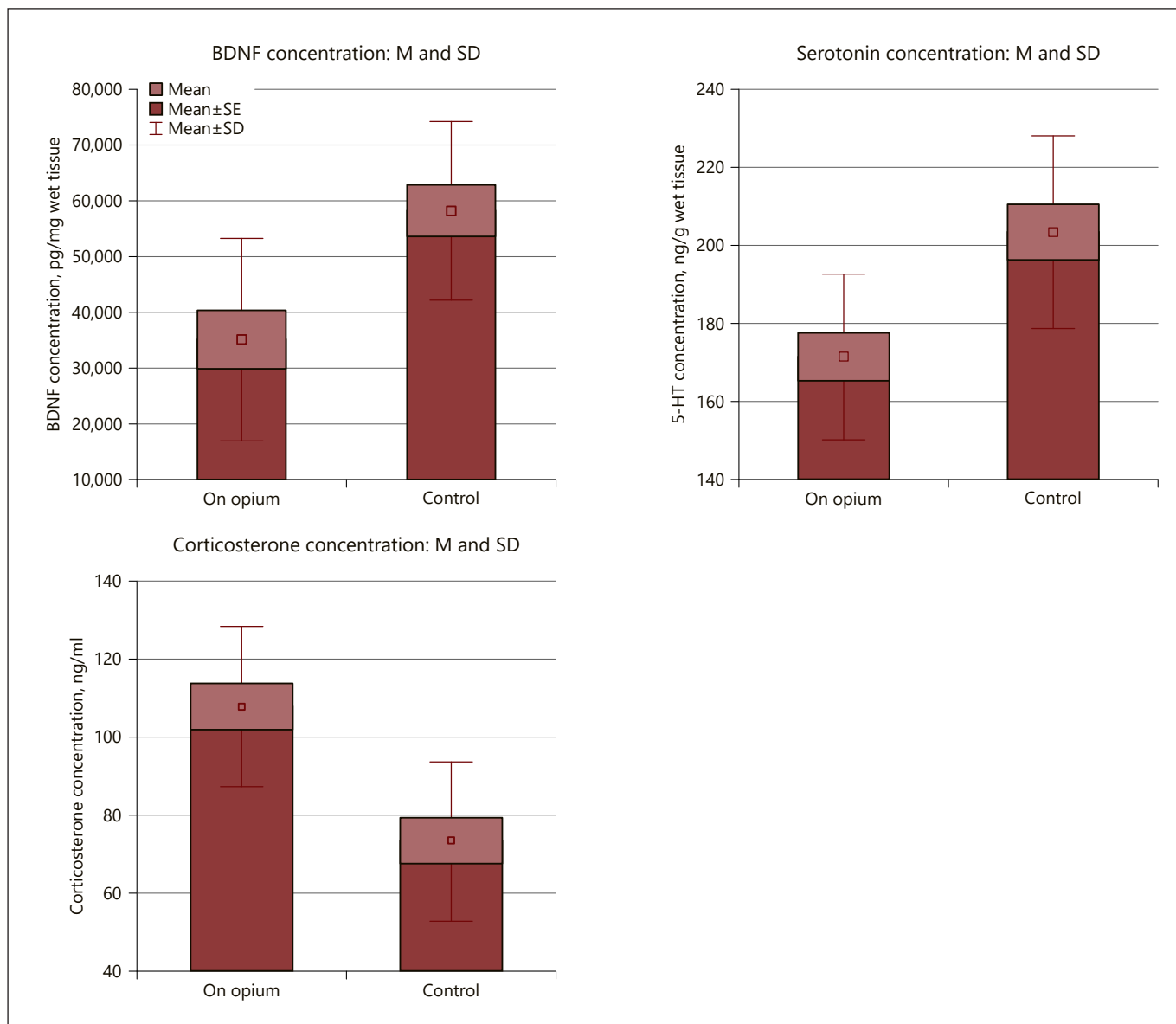
Fig. 2. Results of neurochemical measures: BDNF, serotonin (5-HT), and corticosterone. BDNF, brain-derived neurotrophic factor.

Results

The results of the study are presented in Table 1 and Figures 2–4.

Biochemical Analysis

The results of biochemical analysis showed that the mean concentration of BDNF in the hippocampal tissue of opium-consuming mothers was significantly lower than in nonconsuming mothers. The mean blood serum concentration of corticosterone in opium-exposed dams was significantly higher than in nonexposed dams. The mean serotonin concentration of the hippocampus in the opium-exposed dams was significantly lower than the serotonin concentration of the hippocampus tissue of the nonexposed dams (Fig. 2).

Behavioral Analysis

A. The results related to the endurance of maternal behavior showed that the mean durations of nesting, pup
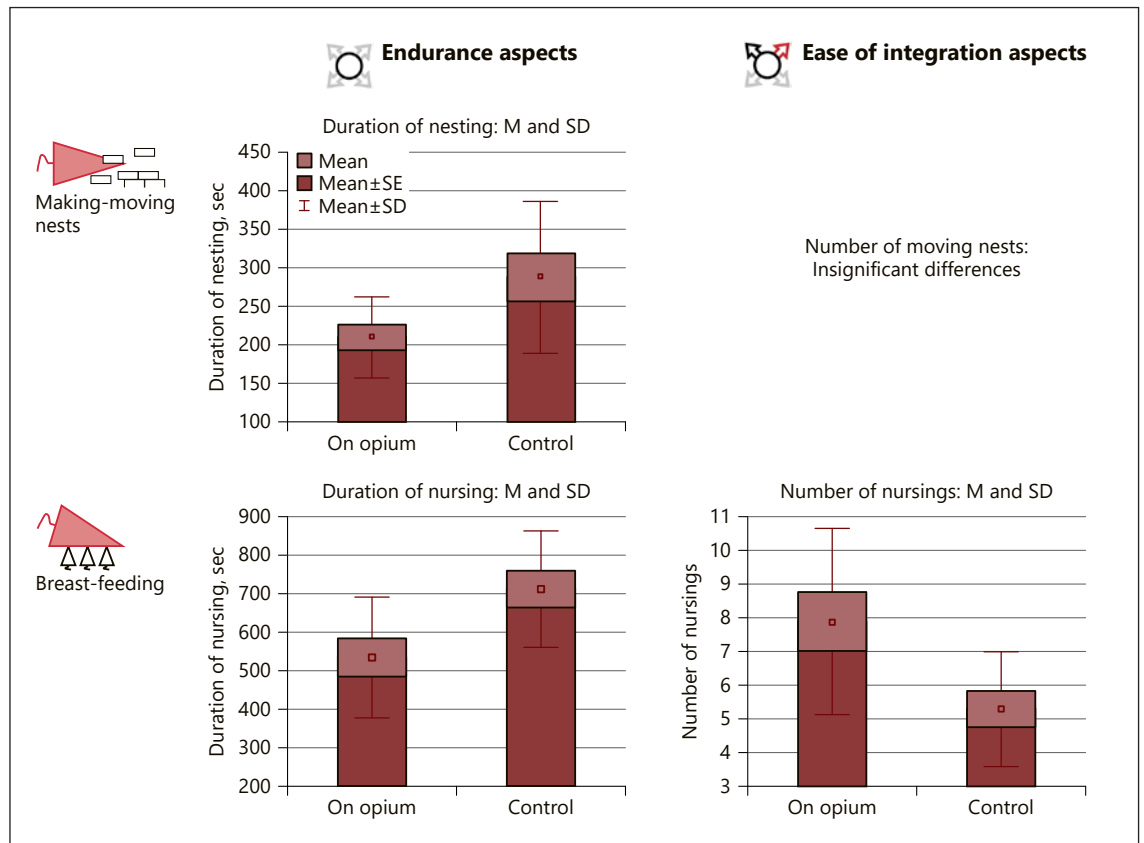
Fig. 3. Results of behavioral measures (means, SE and SD): nesting and breast-feeding.

Table 1. Mean, standard deviation, confidence intervals, and effect sizes of the measured behavioral and biochemical variables in experimental and control groups

Variables	Experim. group Mean (SD)	Confidence intervals	Control group Mean (SD)	Confidence intervals	t	p value	Effect size (Cohen's d)
Related to endurance of behavior							
Duration of nesting, s	212.4 (17.03)	173.9–250.9	292.3 (100.93)	220.1–364	2.21	0.040	3.12
Duration of breastfeeding, s	534.8 (49.73)	422.3–647.3	712.7 (151.59)	604.3–821.1	2.57	0.019	3.64
Duration of pup grooming, s	163.1 (53.78)	124.6–201.6	238.8 (67.08)	190–286	2.75	0.013	3.89
Number of pup grooming	22.6 (6.63)	17.85–27.35	27.9 (8.08)	22.11–33	1.60	0.126	2.27
Related to the ease of integration of behavior							
Speed of pup retrieval (duration, s)	714.41 (173.1)	604.4–824.4	480.83 (176.89)	368.4–593	3.27	0.003	4.62
Latency in onset pup retrieval, s	264.5 (95.39)	196.3–332.7	199.3 (55.77)	159.4–239	1.86	0.078	2.63
Number of moving the nest	1.1 (0.31)	0.87–1.32	1.3 (0.48)	0.95–1.64	1.09	0.287	1.56
Number of pup retrievals	7 (1.15)	6.00–7.99	7 (0.94)	6.23–7.77	0	0.999	0
Number of breastfeeding	7.9 (2.76)	5.92–9.87	5.3 (1.7)	4.08–6.51	2.53	0.021	3.66
Related to emotionality (self-calming or anxiety)							
Duration of self-grooming, s	73.3 (25.55)	55.02–91.58	61.3 (31.97)	38.43–84.1	0.92	0.366	1.31
Number of self-grooming	6.66 (2.73)	4.56–8.77	5.33 (1.73)	4–6.66	1.23	0.234	1.75
Biochemical variables							
BDNF concentration, pg/mg wet tissue	35,119.8 (18,161.94)	23,580–446,659	58,224.4 (16,018.1)	48,047–68,402	3.31	0.003	1.34
Corticosterone concentration, ng/mL	107.8 (20.55)	94.77–120.9	73.42 (20.43)	60.43–86.4	4.11	0.000	1.65
Concentration of serotonin, ng/g wet tissue	171.42 (21.3)	157.9–185	203.42 (24.72)	187.7–219.1	3.39	0.002	1.45

Zeros are omitted for p values. BDNF, brain-derived neurotrophic factor; s, seconds.
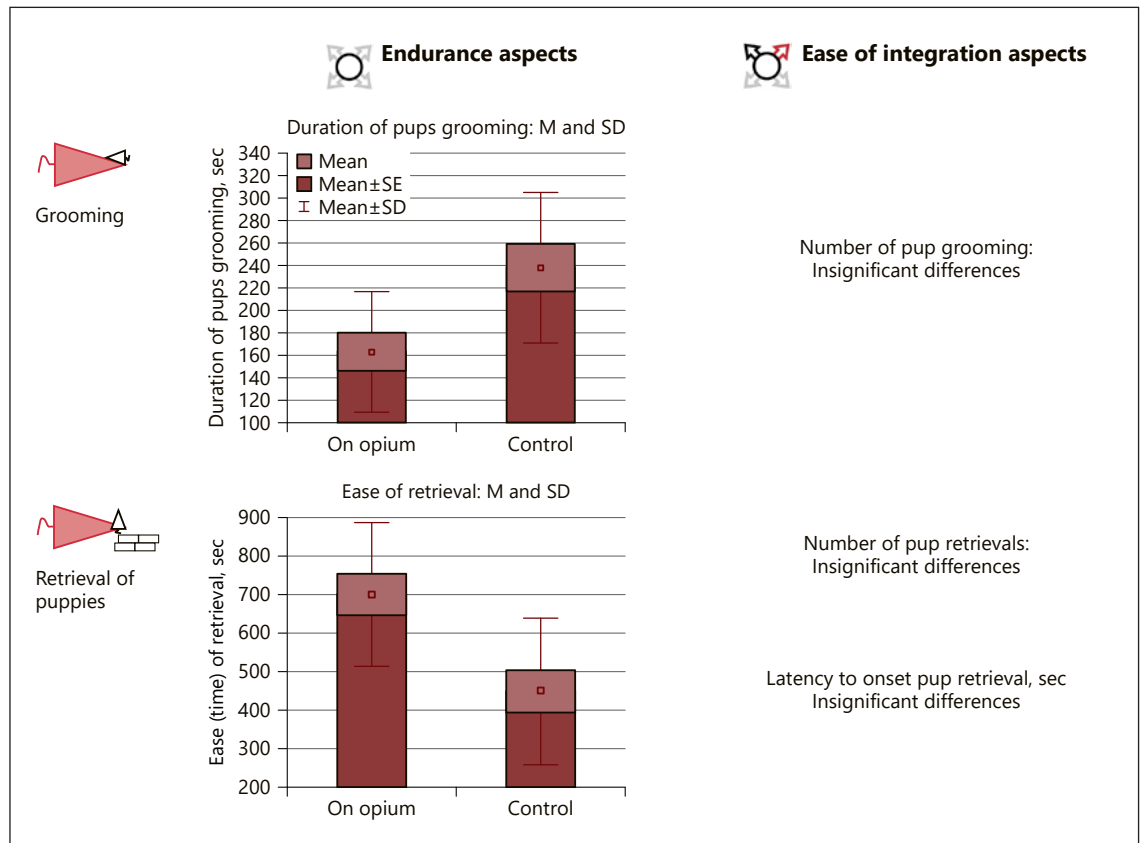
Fig. 4. Results of behavioral measures (means, SE and SD): grooming and retrieval.

grooming, and breastfeeding (nursing) were significantly lower (shorter) in opium consuming mothers than the same scores in nonconsuming mothers (Fig. 3, 4). The duration of pup retrieval was much longer in opium-consuming mothers, indicative of lower efficiency in these elements of behavior, in comparison to controls.

B. The results showed a tendency for a slower speed of integration, however, not in all aspects of maternal behavior in opium-exposed rats, in comparison to the control group. The number of nursing attempts in the exposed group was significantly higher than in controls, likely due to the significantly short duration of nursing requiring more initiations for breastfeeding (Fig. 3). However, the numbers of nestings, pup groomings, and pup retrievals were not significantly different between 2 group (Fig. 3, 4). The mean latency to the onset of pup retrieval in opium-consuming mothers was also longer than in the control group; however, it did not reach the level of statistical significance ($p = 0.078$), likely due to the small sample.

C. Number and duration of self-groomings did not show statistically significant differences between experimental and control groups.

Discussion

Previous studies on the impact of opiate exposure on maternal behavior showed a detrimental effect of this exposure. However, many neurochemical systems are involved in this process, and maternal behavior itself is a multifaceted phenomenon. It is, therefore, challenging to decouple the various neurochemical and behavioral factors in analyzing the mechanisms of opioid addiction. Unlike previous investigations, our study approached this analysis in a more systematic way, classifying the behavioral aspects in line with the functional specificity of neurochemical systems, as proposed in the neurochemical FET model. When behavioral aspects of maternal behavior were grouped ac-

cording to the FET framework, it became evident that the endurance aspects of maternal behavior were affected by opium consumption, and therefore, part A of our hypothesis was supported by the results. As noted above, opium, as an opioid substance acting on MORs, might impact endurance due to MOR regulation of the release of serotonin and BDNF. Indeed, in all 4 elements of maternal behavior studied (nesting, feeding, grooming, and retrieval), energetic aspects (i.e., endurance [duration of actions]) and efficiency were significantly compromised in opium-consuming mothers, in comparison with controls. This was associated with lower concentrations of 5-HT and BDNF in hippocampal tissue. Our results support the findings from other studies [41–43] showing that morphine treatment during pregnancy reduces the number of pup groomings and nursings in rats [42, 43] or increases the latency time to nursing of pups [44]. The low level of BDNF (methylation of BDNF) reduces and disrupts maternal care behaviors. These results support the FET assignment of the regulation of endurance to 5-HT-neuropeptide systems and the BDNF, and the stronger links between these systems and MOR, in comparison to DOR and KOR systems. Our results showed that the ease of switching between actions (i.e., the ease of integration of maternal behavioral acts) was significantly less affected than its endurance aspects.

We can suggest that an abnormal influx of exogenous endorphins (administered as opium) lead to the downregulation of MOR receptors – a mechanism that is well documented in the literature on opioid receptors [1, 28, 45, 46]. This is also in line with results reported elsewhere. For example, Šlamberová et al. [14] showed that the treatment of pregnant dams with morphine leads to a slower return of pups into the nest than the control group. Yim and colleagues [44] showed that the dopamine concentration in the morphine-treated group was significantly lower when compared to the control group.

With regards to part C of our hypothesis, related to possible anxiety and changes in self-soothing, it was supported in terms of the neurochemical component. Our results showed that the serum corticosterone concentration in the opium-consuming group was higher than in the control group. However, in the opium-consuming group, the duration and number of self-groomings showed no difference from the control group. There were, therefore, no differences in aspects of behavior related to emotionality, even though the corticosterone level was increased in opium exposed. Pos-

sible explanations of these results could be that (1) physical touch of mothers to pups might suppress the activation of the HPA axis associated with an emotional arousal [47]; (2) corticosterone might be associated with behavioral arousal but not always with anxiety, especially when there is an ongoing influx of endorphins suppressing HPA axis activation This complexity could explain the conflicting reports in the literature. Nock et al. [48] showed that chronic morphine treatment increased serum corticosterone of juvenile rats that were exposed to mild stress, and this might be associated with an increased corticosteroid-binding globulin [49]. Yet, Yim et al. showed that morphine treatment in female rats before mating increased corticosterone levels but had no effect on corticosterone concentration in late pregnancy [44]. This highlights the challenges in the analysis of anxiety symptoms of behavior that likely have multiple aspects and neurochemical mechanisms. Our results also suggest that the MOR system is likely not directly associated with anxiety, behavioral arousal or its suppression, and despite suppressing KOR (which was linked to chronic anxiety), it has relative independence from KOP in behavioral regulation.

This study was limited by administering opium with drinking water and not using more common ways of opium administration employed by the general public. Moreover, animals had individual differences in how much water they drank, and so likely there were variations in the doses received. However, the standard deviations in our plots showed a trend that supported our results. Opium distribution in the brain was measured only in hippocampus and, perhaps, studies using other brain areas could be a promising line of future research.

Conclusion

Since maternal behavior consists of multiple components, and its neurochemical regulation has multiple entangled neurotransmitter and hormonal systems, a novel structured and a formal approach was needed. This study was structured in line with the neurochemical framework of the FET model, highlighting the role of 5-HT and BDNF in the endurance aspects of behavior, the role of DA in speed of integration of behavioral acts, and the potential for dysregulated MOR systems to negatively impact the release of 5-HT and DA. To test the predictions of the FET model, the present study investigated how dys-

regulation in multiple neurochemical systems caused by the opium consumption can impact maternal behavior in rats. The results confirmed the benefits of this structured framework in differentiating between energetic, integrative, and emotionality-related aspects of behavior. The results showed that the following:

1. The most profound negative effects of opium consumption in pregnancy and maternal behavior related to endurance (ability to sustain prolonged actions), and these effect were significant in all 4 types of behavior under study (nesting, feeding, grooming, and pups' retrieval).
2. These changes were associated with significantly lower BDNF and serotonin concentrations and higher corticosterone in the experimental group, in comparison with controls. This suggests that there are negative effects of opium associated with MOR action on hippocampal 5-HT and BDNF systems.
3. In contrast to endurance, to the ease of integration measured as numbers of various maternal actions was affected to a much lesser degree. Only the frequency of breastfeeding was much higher, likely as a compensation for dramatically low duration of breastfeeding in exposed rats, in comparison to controls.
4. There were, however, no statistically significant differences between experimental and control groups in behavior related to the symptoms of anxiety, such as number or duration of self-grooming actions. This suggests that the MOR system might act relatively independent from the DOR and KOR systems.
5. This study also provided evidence that the quality of maternal behavior dramatically decreased in opium exposed animals, which lead to negative psychological and developmental impact on the newborns.

Author Contributions

Study concept and design: Bakhshani N.M., Rezaei S., and Fanaei H. Data gathering and data analysis: Bakhshani N.M., Rezaei S., and Fanaei H. Manuscript first draft: Bakhshani N.M., Rezaei S., and Fanaei H. Major editing and critical revision of the manuscript: Bakhshani N.M., Fanaei H and Trofimova I.N. Preparation of graphs: Trofimova I.N.

References

1 Ghazavi A, Mosayebi G, Solhi H, Rafiei M, Moazzeni SM. Serum markers of inflammation and oxidative stress in chronic opium (Taryak) smokers. Immunol Lett. 2013; 153(1–2):22–6.

2 Yarahmadzehi S, Fanaei H, Mirshekar MA, Atashpanjeh AR. Opium consumption exerts protective effect against cerebral ischemia through reducing inflammation and enhancing antioxidant defense in male rats. Neurol Psychiat Br Res. 2020;37:15–20.

3 Fodor A, Zelena D. The effect of maternal stress activation on the offspring during lactation in light of vasopressin. ScientificWorldJournal. 2014;2014:265394.

4 Fanaei H, Riki, F. Khayat, S. Bornavard, M.. Brain-derived neurotrophic factor and nerve growth factor concentrations in maternal and umbilical cord blood of opium-addicted mothers. Int J Dev Neurosci. 2020;80(7):594–600.

5 Akbari M, Naghibzadeh-Tahami A, Khanjani N, Baneshi MR, Kamali E, Hesampour M, et al. Opium as a risk factor for bladder cancer: a population-based case-control study in Iran. Arch Iran Med. 2015;18(9):567–71.

6 Ghaffari Nejad A, Ziaadini H, Banazadeh N. Comparative evaluation of psychiatric disorders in opium and heroin dependent patients†This article has been published in the Journal of Rafsanjan University of Medical Sciences in Persian language. Addict Health. 2009;1(1):20–3.

7 Saberi Zafarghandi MB, Jadidi M, Khalili N. Iran's activities on prevention, treatment and harm reduction of drug abuse. Int J High Risk Behav Addict. 2015;4(4):e22863.

8 Ziaaddini H, Ziaaddini T, Nakhaee N. Pattern and trend of substance abuse in eastern rural iran: a household survey in a rural community. J Addict. 2013;2013:297378.

9 Martins SS, Fenton MC, Keyes KM, Blanco C, Zhu H, Storr CL. Mood and anxiety disorders and their association with non-medical prescription opioid use and prescription opioid-use disorder: longitudinal evidence from the National Epidemiologic Study on alcohol and related conditions. Psychol Med. 2012;42(6): 1261–72.

10 Feltenstein MW, See RE. Systems level neuroplasticity in drug addiction. Cold Spring Harb Perspect Med. 2013;3(5):a011916.

11 Jiang J, He X, Wang MY, Sui N. Early prenatal morphine exposure impairs performance of learning tasks and attenuates in vitro heterosynaptic long-term potentiation of intermediate medial mesopallium in day-old chicks. Behav Brain Res. 2011;219(2):363–6.

12 Mayer AD, Faris PL, Komisaruk BR, Rosenblatt JS. Opiate antagonism reduces placentophagia and pup cleaning by parturient rats. Pharmacol Biochem Behav. 1985;22(6):1035–44.

13 Mann PE, Kinsley CH, Bridges RS. Opioid receptor subtype involvement in maternal behavior in lactating rats. Neuroendocrinology. 1991;53(5):487–92.

14 Šlamberová R, Szilágyi B, Vathy I. Repeated morphine administration during pregnancy attenuates maternal behavior. Psychoneuroendocrinology. 2001;26(6):565–76.

15 Trofimova I. The interlocking between functional aspects of activities and a neurochemical model of adult temperament. In: Arnold MC, editor. Temperaments: individual differences, social and environmental influences and impact on quality of life. New York, NY: Nova Science Publishers; 2016. p. 77–147.

16 Trofimova I. Functionality versus dimensionality in psychological taxonomies, and a puzzle of emotional valence. Philos Trans R Soc Lond B Biol Sci. 2018;373(1744):20170167.

17 Trofimova I, Robbins TW. Temperament and arousal systems: a new synthesis of differential psychology and functional neurochemistry. Neurosci Biobehav Rev. 2016;64:382–402.

18 Trofimova I. Functional constructivism approach to multilevel nature of biobehavioural diversity. Front Psychiatry. 2020 Forthcoming.

19 Angoa Pérez M, Kane MJ, Sykes CE, Perrine SA, Church MW, Kuhn DM. Brain serotonin determines maternal behavior and offspring survival. Genes Brain Behav. 2014;13(7):579–91.

20 Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, et al. Molecular cloning and expression of brain-derived neurotrophic factor. Nature. 1989;341(6238):149–52.

21 Park H, Poo MM. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci. 2013;14(1):7.

22 Thakker-Varia S, Alder J. Neuropeptides in depression: role of VGF. Behav Brain Res. 2009;197(2):262–78.

23 Xu X, Ji H, Liu G, Wang Q, Liu H, Shen W, et al. A significant association between BDNF promoter methylation and the risk of drug addiction. Gene. 2016;584(1):54–9.

24 Unternaehrer E, Meyer AH, Burkhardt SC, Dempster E, Staehli S, Theill N, et al. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women. Stress. 2015;18(4):451–61.

25 Bodnar RJ. Endogenous opiates and behavior: 2017. Peptides. 2020;124:170223.

26 Koo JW, Mazei-Robison MS, Chaudhury D, Juarez B, LaPlant Q, Ferguson D, et al. BDNF is a negative modulator of morphine action. Science. 2012;338(6103):124–8.

27 Tao R, Auerbach SB. Opioid receptor subtypes differentially modulate serotonin efflux in the rat central nervous system. J Pharmacol Exp Ther. 2002;303(2):549–56.

28 Lutz PE, Kieffer BL. Opioid receptors: distinct roles in mood disorders. Trends Neurosci. 2013;36(3):195–206.

29 Zhao ZQ, Gao YJ, Sun YG, Zhao CS, Gereau RW, Chen ZF. Central serotonergic neurons are differentially required for opioid analgesia but not for morphine tolerance or morphine reward. Proc Natl Acad Sci U S A. 2007;104(36):14519–24.

30 Tao R, Ma Z, Auerbach SB. Alteration in regulation of serotonin release in rat dorsal raphe nucleus after prolonged exposure to morphine. J Pharmacol Exp Ther. 1998;286(1):481–8.

31 Klausz B, Pintér O, Sobor M, Gyarmati Z, Fürst Z, Tímár J, et al. Changes in adaptability following perinatal morphine exposure in juvenile and adult rats. Eur J Pharmacol. 2011;654(2):166–72.

32 Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci. 1996;16(7):2365–72.

33 Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA. cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. J Neurosci. 2002;22(8):3262–8.

34 Domokos A, Mergl Z, Barna I, Makara GB, Zelena D. Congenital vasopressin deficiency and acute and chronic opiate effects on hypothalamo-pituitary-adrenal axis activity in Brattleboro rats. J Endocrinol. 2008;196(1):113–21.

35 Rees SL, Panesar S, Steiner M, Fleming AS. The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat. Horm Behav. 2004;46(4):411–9.

36 Graham MD, Rees SL, Steiner M, Fleming AS. The effects of adrenalectomy and corticosterone replacement on maternal memory in postpartum rats. Horm Behav. 2006;49(3):353–61.

37 Bornavard M, Fanaei H, Mirshekar MA, Farajian Mashhadi F, Atashpanjeh A. Morphine consumption during pregnancy exacerbates neonatal hypoxia-ischemia injury in rats. Int J Dev Neurosci. 2020;80(2):96–105.

38 Zimmerberg B, Gray MS. The effects of cocaine on maternal behaviors in the rat. Physiol Behav. 1992;52(2):379–84.

39 Vahedian J, Mirshekari TR, Nabavizadeh F. Effect of opium dependency on secondary intention wound healing in a rat model: an experimental study. Int Wound J. 2013;10(3):351–5.

40 Remberg B, Sterrantino AF, Artner R, Janitsch C, Krenn L. Science in drug control: the alkaloid content of afghan opium. Chem Biodivers. 2008;5(9):1770–9.

41 Carini LM, Nephew BC. Effects of early life social stress on endocrinology, maternal behavior, and lactation in rats. Horm Behav. 2013;64(4):634–41.

42 Cruz Ade M, Maiorka PC, Canteras NS, Sukikara MH, Felicio LF. Morphine treatment during pregnancy modulates behavioral selection in lactating rats. Physiol Behav. 2010;101(1):40–4.

43 Sukikara MH, Platero MD, Canteras NS, Felicio LF. Opiate regulation of behavioral selection during lactation. Pharmacol Biochem Behav. 2007;87(3):315–20.

44 Yim AJ, Miranda-Paiva CM, Flório JC, Oliveira CA, Nasello AG, Felicio LF. A comparative study of morphine treatment regimen prior to mating and during late pregnancy. Brain Res Bull. 2006;68(5):384–91.

45 Nagi K, Piñeyro G. Regulation of opioid receptor signalling: implications for the development of analgesic tolerance. Mol Brain. 2011;4(1):25.

46 Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem. 2004;73(1):953–90.

47 van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. Front Cell Neurosci. 2017;11:87.

48 Nock B, Cicero TJ, Wich M. Chronic morphine increases the pituitary-adrenocortical response of juvenile rats to mild stress. Pharmacol Biochem Behav. 2005;80(1):77–85.

49 Nock B, Wich M, Cicero TJ. Chronic exposure to morphine increases corticosteroid-binding globulin. J Pharmacol Exp Ther. 1997;282(3):1262–8.