

Effects of prenatal exposure to different colors on offsprings mood

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ABSTRACT

Objective(s): There is much evidence indicating that depression is influenced by the levels of neurotransmitters such as dopamine, GABA and adrenaline. The current study we designed to investigate the effect of exposure of pregnant rats to different colors on neurotransmitters level, as indicators of mood disorders in off springs.

Materials and Methods: Five groups of pregnant female Wistar rats (eight rats in each group) were enrolled in this study. Dopamine, adrenaline and GABA concentration in sera of rats were measured using ELISA.

Results: The colors black and red elevated the GABA levels in serum and CSF while the colors green and blue decreased the GABA levels. The colors black and red also decreased the sera and CSF levels of dopamine compared to the control group. The concentration of adrenaline was increased following exposure to the colors black, red and blue but decreased only following color green exposure. These results showed serious changes in neurotransmitter levels due to exposure to different colors which can be translated as mood and behavior changes.

Conclusion: It can be concluded that exposure during pregnancy can lead to postpartum behavioral changes even at adulthood and such changes can be made by colors.

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Introduction

It has been proposed that deficiency in some monoamine neurotransmitters such as norepinephrine, serotonin (5-HT) and Dopamine somehow leads to Major Depressive Disorder (MDD) (1). The mood lowering properties of monoamine depletion in MDD has been shown (2, 3). γ -aminobutyric acid (GABA) has been proposed as the main neurotransmitter with inhibitory properties in brain so that about 30-50% of all central synapses are GABA-ergic (4). It can be mentioned that GABA has an inhibitory control on noradrenergic, dopaminergic and serotonergic neurons (4). It has been reported that changing GABA level may modify several behavioral responses such as sleep, eating, sexual behavior, learning and memory and therefore any variation in central GABA activity may contribute to mood disorders (4). Some association between the increase in monoaminergic function of the brain and decrease in cerebral metabolism has been documented by Niklasson and Agren (5). Many researchers reported that dopamine dysfunction can be involved in mania and some types of depression

such as bipolar and retarded depressions.

It is not yet clear that whether dopamine dysfunction is primary to the pathogenesis of these mood disorders or a secondary phenomenon but it is evidenced that dopamine-active treatments can cause some therapeutic effects on mood disorders (6). Norepinephrine (NE) is the primary sympathetic neurotransmitter of the nervous system in the periphery and the brain. High levels of NE have been associated with aggression in animals, healthy adults and patients with depression and mania. NE has been proved to enhance aggressive behaviors and also β -adrenoreceptor blockers decreased aggression in some animal models (7).

Anxiety could be diagnosed with high cortisol levels; while over arousal is indicated with high concentrations of adrenaline, noradrenaline, dopamine and 5-HT. Such reactions might have an inhibitory control on mood and cognition (8). Noradrenaline as a neurotransmitter plays an important role in working memory performance in the central nervous system (CNS) and also controls the emotions hence involved in mood and arousal.

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High concentrations of 5-HT suppress the synthesis of dopamine, which in turn controls the performance of tasks involving basal ganglia activity, e.g., reaction time(9). Hormonal changes which occur during pregnancy in rodents influence several aspects of behavior and neural function such as sexual and maternal behavior or general activity, pain sensitivity, nutritional behavior, aggressiveness (10-13), seizure susceptibility (14), intracranial self-stimulation (15), and body temperature (8) and such changes vary depending on the stage of estrus and pregnancy. Amongst such variations, the behavioral changes induced following exposure to environmental stressors are particularly influenced by the stage of estrus. O'Connor *et al* investigated the prenatal depression and its effects on the fetus and infant (16). Their model explained that maternal anxiety in pregnancy had resulted in higher cortisol and changed the childhood stress response in fetus by changing the metabolism and uptake of catecholamines in cerebral of infants. The study revealed that maternal conditions during pregnancy and the mood disorder in postpartum are highly associated with infant outcomes as well as the emotional and behavioral problems noted in infants of mothers with depression. Duman investigated the effect of exercise, diet and metabolism on regulation of the mood and reported that exercise and enriched environment could inhibit the effects of stress and depression by inducing the neurotrophic and neurogenic effects (17). The aim of the current study was to examine the levels of such neurotransmitters in offspring of female rats exposed to different colors as stimulators of depression.

Color is a complex of visible wavelengths between 400 to 700 nanometers. It has been revealed that the greatest impact of vibratory activity appears to occur within the visible range of light. It seems that all living things on earth possess some systems designed to operate within these visible lights. This may justify the natural potency of the visible spectrum as a therapeutic agent for humans (18). New breakthroughs in the use of color as a healing agent for rapid recovery of trauma, depression, physical pain and spiritual blockages have increased the power of color for healing (18).

There are numerous excellent studies and reviews focused on GABA, DA and NE functions in mood disorders, but most of them focused on depression and antidepressant processes. On the basis of our best knowledge, none of such studies has focused on variations of neurotransmitters levels due to color exposure during growth. At the same time there are several evidences indicating the higher prevalence of depression during pregnancy and as such depression is influenced by variation of neurotransmitters levels we designed the current study to investigate the effect of exposure of

pregnant rats to different colors on variations of neurotransmitters level, as indicators of mood disorders, in offspring.

Materials and Methods

Animals

Five groups of pregnant female Wistar rats (eight rats in each group) were purchased from the animal house department of Ilam University, Ilam, Iran. All procedures were approved by the division of animal house, and also the ethical committee of Ilam University of Medical Sciences. Rats were healthy and free of respiratory infection and disease at all times.

Color wavelengths

Color groups including green, red, blue, black and white were analyzed with thin-layer chromatography (TLC) for quantitative wavelengths determinations. The colors black and white were considered as the control groups.

Chamber design and exposure of rats to different colors

The chamber was constructed of clear Plexiglas covered by different colors including green, red, blue, black and white (16 inches × 20 inches with 8-inch-high walls). The floor was layered with wood chips of different colors according to the classification of working groups. The floor was separated from rats by a 1-inch-high wire mesh. For each chamber the surrounding walls and roofs were all painted with the same color as the chamber color. Immediately after the mating of male and females, the female rats were transported to their specific chambers in order to be exposed to their given color during pregnancy for a total of about 3-4 weeks. Each rat experienced a mean of 12 hr light exposure time per 24 hr. After the delivery the offspring, male rats were transported to the clear chambers (i.e. transparent chambers) until they were matured (week 9-10) and then the rat CSF was extracted according to the animal handling rules (19).

Neurotransmitters analysis

Dopamine, adrenaline and GABA concentration in sera of rats were measured using ELISA kits (20) as follows:

GABA ELISA (Serum/Plasma)

Primarily, 25 µl of the derivatized standards, controls and samples were pipetted into the appropriate wells of the GABA Microtiter Strips. Then, 50 µl of the GABA antiserum was pipetted into all wells and mixed shortly. Plates were covered with adhesive foil and incubated for 15-20 hr (overnight) at 2-8 °C. The foils and the contents of the wells were discarded and each well was washed three times thoroughly with 300 µl of wash buffer.

Table 1. Descriptive analysis of mean levels of GABA, dopamine and adrenaline concentrations (nmol/l) among offspring of different color-exposed groups

Groups	CSF GABA	Serum GABA	CSF dopamine	Serum dopamine	CSF adrenaline	Serum adrenaline
Green	0.76	1.62	0.25	0.1	1.42	3.77
Blue	0.91	1.36	1.4	0.91	1.1	2.25
Red	5.59	18.11	0.02	0.09	1.26	3.59
Black	6.83	20.68	0.02	0.03	1.29	4.03
Control	6.25	10.69	0.22	0.09	2.78	2.55

The figures are mean concentration of each neurotransmitter

wells were dried by tapping the inverted plate on absorbent materials. Next, 100 μ l of the enzyme conjugate was added to all wells and the plates were covered with adhesive foil and incubated for 30 min at room temperature (20-25°C) on a shaker (approx. 600 rpm). After removing the foils, the contents were aspirated followed by three times washing using 300 μ l washing buffer. The wells were dried by tapping the inverted plate on towel. Then, 100 μ l substrate solution was added and the plate was incubated for 20-30 min at room temperature (20-25°C) on a shaker (approx. 600 rpm). Subsequently, 100 μ l of the stop solution was added to each well and microtiter plates were shaken to ensure a homogeneous distribution of the solution. The absorbance of the solution was read after 10 min at 450 nm.

Adrenaline ELISA

Briefly, for the adrenaline 20 μ l of controls and the standards and 500 μ l of serum samples were added to the respected wells of the extraction plates. Then, 500 μ l of distilled water was added to all wells except for the sera to correct the differences of the volumes. Next, 1000 μ l of extraction buffer was added to each well. Plates were covered with foil and kept at room temperature for 30 min on an orbital shaker.

Dopamine ELISA

First, 25 μ l of the enzyme solution was pipetted into all wells of the Dopamine Microtiter Strips. Then, 100 μ l of the standards, controls and samples was added into the appropriate wells and incubated for 30 min at room temperature on a shaker (approx. 600 rpm). Dopamine antiserum (50 μ l) was added to the wells and the plate was covered with adhesive foil and incubated for 2 hr at room temperature on a shaker. Solution in wells was discarded three-time washing was applied and enzyme conjugate was added (100 μ l) into each well. Then, plate was incubated for 30 min at room temperature. After washing, 100 μ l of the substrate was added into each well and incubated for 20-30 min at room temperature followed by adding 100 μ l of the stop solution to each well and reading the absorbance of the solution in the wells within 10 min, using a micro plate reader set at 450 nm.

Statistical analysis

Sample size was calculated using the following formula where $\alpha=0.05$ and $1-\beta=90\%$ based on which, five groups of rats each of 8 rats were used. Descriptive statistics was employed to analyze three neurotransmitters in five groups exposed to different colors. Then, one way ANOVA test was conducted to compare the effect of exposure to the colors green, black, red

Table 2. ANOVA analysis of mean concentrations (nmol/l) of different neurotransmitters among and within the groups

		Sum of squares	df	Mean square	F	Sig.
CSF GABA	Between groups	2.51	4.00	0.63	24.57	0.00
	Within groups	0.26	10.00	0.03		
Ser GABA	Between groups	22.78	4.00	5.70	3309.73	0.00
	Within groups	0.02	10.00	0.00		
CSF Dop	Between groups	0.09	4.00	0.02	0.89	0.51
	Within groups	0.27	10.00	0.03		
Ser Dop	Between groups	0.52	4.00	0.13	1.23	0.36
	Within groups	1.05	10.00	0.11		
CSF Adr	Between groups	0.13	4.00	0.03	2.60	0.10
	Within groups	0.13	10.00	0.01		
Ser Adr	Between groups	0.18	4.00	0.04	3.07	0.07
	Within groups	0.14	10.00	0.01		

Dop: dopamine, Adr: adrenaline; Ser: serum; Df: degree of freedom

and blue on three neurotransmitters in offspring. Finally, the correlation between CSF and serum neurotransmitters was conducted. In this study, $P < 0.05$ was considered as statistically significant (21, 22).

$$n = \frac{26^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2}$$

Results

Mean and standard deviation of GABA, dopamine and adrenaline of offspring that were prenatally exposed to five colors are shown in Table 1.

GABA

A one-way between subjects ANOVA (Table 2) was conducted to compare the effect of prenatal color exposure on the CSF and serum GABA concentration of offspring in green, black, red, blue and control groups. Color exposure had a significant effect ($P < 0.001$) on CSF GABA of the offspring in the five conditions. *Post hoc* comparison using HSD test indicated that the mean score for the green (M=0.76) and blue (M=0.91) was significantly different from the control groups (M=6.25). In other words, exposure of pregnant rats to the colors green and blue reduced the CSF GABA in their offspring.

This study also showed that there was a significant effect of color exposure on serum GABA of the offspring at the $P < 0.001$ level for different groups ($P \leq 0.001$). *Post hoc* comparison using HSD test indicated that there was a difference of mean score among the black (M=20.7), red (M=18.1) and the control groups (M=10.7). These results indicate that the level of serum GABA in offspring exposed to the color black is two times higher than that in control group. These results show that exposure of pregnant rats to the colors black and red increase the serum concentration of GABA in their offspring.

Dopamine

A one-way between groups analysis of variance was conducted to explore the effect of prenatal color exposure on the CSF and serum dopamine concentration of offspring in green, black, red, blue and control groups. There was no significant effect of color exposure on both CSF and sera dopamine in the offspring in five groups. However, observing the descriptive statistics among five groups revealed that mean level of CSF dopamine was strongly increased among the offspring that have prenatal exposure to the color blue (M= 1.4) and slightly increased following prenatal exposure to the color green (M=0.24) while it was reduced following prenatal exposure to the colors black (M=0.02) and red (M=0.02) compared to the control group (M=0.22)

(Table 1). It is worth noting that the mean sera level of dopamine decreased in black (M= 0.03) and green (M= 0.10) while increased in groups exposed to the color blue (M= 0.91).

Adrenaline

The results of one-way ANOVA among subjects showed that there is no significant effect of color exposure on both CSF and sera adrenaline in the offspring for the five conditions. However, considering descriptive statistics summarized in Table 1, it is obvious that the mean CSF level of adrenaline was decreased in all groups compared to the control group (M=2.78). In contrast, the mean sera level of adrenaline was increased in green (M= 3.76), red (M=3.59) and black (M=4.3) exposed groups while slightly decreased in color blue-exposed group (M=2.25).

Pearson correlation was conducted to assess the relationship of neurotransmitters concentration in CSF and serum of offspring and mothers. The finding shows that there is a statistically significant correlation between the GABA concentrations in serum and CSF of offspring ($P < 0.008$) and mothers ($P < 0.013$). The concentration of dopamine in sera and CSF of the mothers was statistically significant ($P < 0.004$).

Furthermore, we ran a series of correlation analysis to find the association between neurotransmitters of mother and offspring. Our findings showed that there is a significant correlation between GABA ($P < 0.001$) and dopamine ($P < 0.005$) in the sera of mothers with that of their offspring.

Finally, we examined the inter-correlation of neurotransmitters in mothers and offspring by using Pearson correlation. Our results indicated that there is a significant association between adrenaline concentrations in sera of mothers with the concentration of GABA in sera ($P < 0.031$) and CSF ($P < 0.001$) of their offspring. The correlation between the concentration of serum dopamine with CSF GABA of mothers was significant ($P < 0.003$). The correlation of adrenaline concentrations in CSF of mothers was statistically significant where it was compared with their concentration of dopamine in the sera and CSF ($P < 0.006$ and $P < 0.001$, respectively).

Discussion

Exposure to environmental stress during pregnancy is of great importance because exposures in early developmental stage can affect the maturation of the offspring in pregnant women (23, 24). Prenatal stress results in learning, behavioral and emotional changes in later life though the mechanisms underlying such effects are not fully understood (25). Mental and physical health care of pregnant women might affect normal development of the fetus. Some kinds of mild stress such as crowding, saline injection or heat stress, applied in

the critical period of synaptogenesis, were reported to make changes in the behavior and learning ability of the offspring accompanied by impairment of synapse formation (26). It was demonstrated that a combination of noise stress and forced swimming clearly affected both the emotional behavior and the learning ability of the offspring in a sex-dependent manner (i.e. observed only in male offspring) (23, 27). The finding in rodents that prenatal stress can cause deficiencies in some early indices of physical maturation and also that these deficiencies can be continued into adulthood are consistent with those in humans. Matter of time of maternal stress is critical in determination of the outcomes. This model helped us to analyze the association between prenatal stimuli like exposure to different colors and changes in neurotransmitters concentrations of serum and CSF of both mothers and offspring rats that can be considered as the indicators of behavioral mood.

The result of our study showed an increase in the CSF GABA concentration among groups of offspring whose mothers were exposed to black and decrease in mothers exposed to the other three colors. Therefore, it is to say that the color black can be considered as a stimulator of GABA secretion in CSF which has a potential effect in mood disorders. In order to clarify the intensity of the variations of neurotransmitters concentration among the groups, a ratio of concentration of each neurotransmitter was calculated by dividing the mean concentration of that neurotransmitter in each group by the concentration of it in the control group. CSF GABA ratio in groups exposed to the colors green, blue, red and black was 0.11, 0.14, 0.95 and 1.1, respectively showing an increase in concentration of CSF GABA in color black-exposed group. At the same time, the concentration of serum GABA was increased in groups of offspring whose mothers were exposed to the colors black and red and decreased in groups of offspring whose mothers had exposure to the colors green and blue. This means that blue and green colors lowered the concentration of GABA in serum of pups during pregnancy and continued till the adulthood. This was more confirmed by the ratio of GABA concentration in serum that was 0.15, 0.13, 1.7 and 2 in the colors green, blue, red and black groups, respectively. It was revealed that low GABA levels may be specific for a subgroup of patients with mood disorder, perhaps those with a family history of mood disorder (28).

The hypothesis of reduced GABAergic activity in mood disorders may complement the monoaminergic and serotonergic theories, proposing that the balance between multiple neurotransmitter systems may be altered in these disorders (28). Thus, the hypothesis of a GABA deficit in mood disorder is also suggested by the mechanism of available

treatments for these disorders (28). Surprisingly, it can be said that the colors red and black are some environmental factors that if pregnant mothers get exposed to during pregnancy, enhanced the concentration of GABA in serum and CSF of the offspring. This finding is similar to the finding of other scientists who reported that the exposure to colors red and white changed the bird behavior more than exposure to the colors green and blue as more activity was seen in them though their weight was reduced (29). Birds exposed to different colors showed a preference for the blue and green light, because they kept the birds calmer (29). Environmental factors, including stress elevate GABA and provoke symptoms of depression or mania (30). Therefore, our results are in agreement with previous finding by showing that the colors blue and green can decrease GABA and therefore reduce the depression.

In contrast, the concentration of CSF dopamine was increased in groups of offspring which had been prenatally exposed to the colors blue and green and decreased with the exposure to the colors red and black. The concentration ratio of CSF dopamine in groups exposed to colors green, blue, red and black was 1.14, 6.61, 0.1, and 0.1, respectively. This finding shows that colors green and blue are on top of the list of all colors for enhancing the concentration of dopamine. The concentration ratio of serum dopamine in offspring which had been prenatally exposed to the colors green, blue, red and black was 1.1, 1, 0.88 and 0.33, respectively. This finding is in accordance with the monoamine hypothesis examined by Lambert *et al* (31) who indicated that a deficit in brain norepinephrine and dopamine existed in patients with depressive illness. There are some studies demonstrating that depressive symptoms in schizophrenic patients are related to a reduction in dopamine synthesis and that antidepressant treatment leads to an increase in striatal dopamine release. These results suggest that dopaminergic neurotransmission is lessened during the periods of depression (9, 32, 33). The psychopharmacological studies suggest a wide range of behavioral functions for ascending midbrain dopaminergic systems. The dopamine-mediated changes during specific behavioral tasks indicated substantial elevation in dopamine-mediated activities that are related to rewards and reward-predicting stimuli.

This scenario was applied for the adrenaline by which the concentration of CSF adrenaline was decreased in all groups of offspring whose mothers had exposure to the colors green, blue, red and black. The concentration ratio of CSF adrenaline in groups exposed to the colors green, blue, red and black were 0.54, 0.4, 0.46 and 0.47, respectively showing a decrease in all color groups though to a higher extent for the blue one. The concentration of serum

Adrenaline increased in all groups of offspring whose mothers were exposed to the colors black, green and red, while it was decreased among the offspring whose mothers had exposure to blue color. The concentration ratio for serum adrenaline following exposure to the colors green, blue, red and black was 1.50, 0.89, 1.43 and 1.60, respectively again indicating a decrease only in the blue group. It has been suggested that heat stress can cause deterioration of the performance of central executive tasks and perceptions of mood state. Such an effect can be predicted by changes in body mass loss and plasma concentrations of the hormones cortisol and adrenaline (34). Similarly, the decrease in sera and CSF levels of adrenaline due to exposure to the color blue can be translated as an indicator of mood status in rats offspring. In order to expand the findings of this study it is recommended to conduct further behavioral examinations in future.

Conclusion

It can be said that though most of the performed studies so far have been designed to evaluate the effects of light as a behavioral stressor in a short time mode, our study that was conducted in a long time fashion, indicated that changes of dopamine, adrenaline and the GABA levels either in CSF or serum affect offspring behavior. Colors black and red elevated the GABA levels in either serum or CSF, provoking symptoms of depression or mania while exposure to the colors green and blue during pregnancy decreased the GABA levels indicating the role of the colors black and red in depression and the color green or blue in positive mood behaviors. At the same time, these colors decreased the sera and CSF levels of dopamine compared to the control group while the green and blue increased such levels for dopamine again confirming the role of colors black and red in depression and green or blue in positive mood behavior. Our finding showed that the concentration of adrenaline was increased in all black, red and blue but decreased only in green-exposed offspring indicating the latter color as a stimulator of a calmer condition while the others as a stimulator of aggressiveness.

These results showed serious changes of neurotransmitters due to exposure to different colors which can be translated as mood behavior changes. We can document that stimulation during pregnancy can lead to behavioral changes postpartum even at adulthood and such changes can be made by colors. Accordingly, designing the color of pregnant women can simply be applied for desired changing in mood and behavior from the childhood till the adolescence.

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Conflict of Interests

Authors have no conflict of interests in this study and ethical committee approved the study.

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