

NEONATAL TREATMENT WITH CLOMIPRAMINE AND DEPRESSION: A REVIEW OF BEHAVIORAL AND PHYSIOLOGICAL FINDINGS*

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RESUMEN

El presente trabajo describe los principales resultados acerca de un nuevo y probable modelo animal de *depresión*. Este modelo se basa, paradójicamente, en la administración de un antidepresivo, *clomipramina*, a ratas neonatas. Cuando los animales alcanzan la adultez, muestran alteraciones comportamentales que pueden ser interpretadas como depresivas, como hiperactividad, descenso en la búsqueda de placer, anormalidades en el sueño, entre otras. Se analizan los posibles mecanismos neurofisiológicos y neuroendocrinos involucrados. A pesar de las limitaciones que ofrece un modelo animal, es importante cómo logra reproducir algunos síntomas hallados en la depresión y debido a esto las ventajas del mismo son invaluable. Además,

pueden estudiarse los mecanismos cerebrales implicados en la patogénesis y tratamiento del trastorno que por razones éticas son impensables de llevar a cabo en humanos. Por ello, se considera de gran valor el estudio realizado con el modelo de clomipramina con la perspectiva de que se siga trabajando en la validación del mismo.

Palabras clave: Depresión; Clomipramina; Tratamiento neonatal; Ratas.

ABSTRACT

Many times in science, the discovery of a treatment that has certain effect happens accidentally while the scientists are investigating another

phenomenon. This is the case of the discovery of a possible animal model of *depression* by the administration of *clomipramine* (CLI) during neonatal days. Adult animals exposed to CLI in neonatal days showed alterations in REM sleep (for example, the decrease of REM latency); lower weight, disruptions in locomotor activity (the increase in activity depend on the dark / light phase in which the test starts, when the animals were tested in the light phase they found increase in activity, but no changes were observed when the animals were tested in the dark phase); less intracranial self-stimulation, lower saccharin and sucrose consumption, less suppression of the consummatory behavior, sexual alterations in males (for example, expressed as a lower number of mounts and ejaculations; no alterations were found in the activity of the Hypothalamic - Pituitary - Gonadal axis and the level of testosterone was normal), higher alcohol consumption, disruptions in the agonistic response (CLI - treated animals were significantly less aggressive than control groups) and in learning (in the passive avoidance task and 8 radial arm maze) compared to untreated animals (rats that received vehicle during neonatal days). Several of these abnormalities could be reversed with those treatments that are effective for treating depression in humans (antidepressive drugs, nicotine and REM sleep deprivation treatment). These results were obtained in male rats of different strains and in hamsters, and at different months, the majority of them at 3-4 months, and some of them after the sixth, this could be because some changes were caused with the decrement in the age of the animals, although further research is needed to elucidate this issue. Neuroendocrinal alterations analogous to those found in human depression were also discovered in CLI - treated *rats*, although the data is contradictory. These include Hypothalamic - Pituitary - Adrenal axis alterations; while it is true that some experimental results found that CLI - treated rats have a higher basal level of corticosterone than controls, others found that not only do they differ in basal level, also during the stress situation;

circulating corticosterone increases less and returns more rapidly to basal levels than control groups. For this reason, we can conclude that if alterations in the HPA axis indeed exist in CLI - treated animals, it is still unclear in which way the deregulation is manifested. Other results support the hypothesis that alterations found in CLI - treated animals are due to alterations in serotonergic transmission during a critical period of development, such as the neonatal stage; more specifically, a reduction in the hypothalamic concentration of serotonin, like a decrease in the neuronal firing in the dorsal raphe nucleus. An increase in cholinergic activity was also found, although the data in this field is not as vast as that found in relation to the neurotransmission of serotonin. All of these results suggest that rats treated with CLI during neonatal days present alterations in adulthood analogous to human depression, however other findings indicate that is not yet a valid model. Further research is needed, and we have to be cautious with the conclusions because there is some evidence suggesting that this is a promising model but other does not support its validity. If a model like the neonatal administration of CLI achieves the reproduction of some symptoms, neurophysiological and behavioral alterations of depression, the advantages are invaluable. In this sense, *neonatal treatment* with CLI is a very promising animal model for the study of depression.

Key words: Depression; Clomipramine; Neonatal treatment; Rats.

Many times in science, the discovery of a treatment that has a certain effect happens accidentally while the scientists are investigating another phenomenon. This is the case of the discovery of a possible animal model of depression by the administration of clomipramine (CLI) during neonatal days. With the aim of studying the REM sleep function in the

ontogenetic development of the animals, neonatal rats were deprived of REM sleep through the daily administration of a powerful suppressor of it: CLI (Mirmiran, van de Poll, Corner, de Boer, & van Oyen, 1980; Mirmiran, van de Poll, Corner, van Oyen, & Bour, 1981). This drug is a reuptake inhibitor of serotonin and noradrenaline. In these studies, the hypothesis is that the suppression of REM sleep could affect the normal development of the animal. At the moment, other differences in the development of subjects treated with CLI compared to their control groups (animals which received vehicle instead of CLI during the same period of life) were not identified. However, in adulthood CLI - treated rats presented abnormalities in sexual behavior, locomotor activity and other behaviors. The experimental animals showed more activity in the peripheral area of the open field test and a decrease in sexual activity of male rats (lower number of ejaculations and intromissions per mount). In addition, they demonstrated more sleep onset REM periods and more intermittent muscle twitches during REM sleep (Mirmiran et al., 1980, 1981; Mirmiran, Scholtens, van de Poll, Uylings, van der Gusten, & de Boer, 1983).

The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM IV, 1994) specifies that some of the symptoms of Major Depressive Disorder are a sharp decline of interest in or capacity for pleasure, significant gain or loss of weight, alterations in sleep (insomnia or hypersomnia), psychomotor acceleration or retardation, recurrent thoughts of death or suicide, feelings of guilt or uselessness, fatigue or loss of energy. Some of these symptoms can be studied using animal models, while others clearly belong to the human area. From this perspective, different researchers have documented the presence of some of these symptoms in adult CLI - treated rats. For example, CLI - treated rats present sexual abnormalities that could be considered sexual deficiencies, like a decrease in motivation and sexual performance found in depression; the increased locomotor activity found in the open field test could be similar to the motor

agitation of depression and the alterations in REM sleep could be similar to the decrease in the latency for entering REM sleep found in this type of disorder.

These findings could be interpreted as indicators that REM sleep during the neonatal period plays an important role in the development of sleep and of normal behavior in later stages of life (Mirmiran et al., 1980, 1981, 1983), or could be interpreted as marked alterations of a mood disorder, more specifically, endogenous depression, known today as Major Depressive Disorder (Feng, Guan, Yang, & Fang, 2003; Vogel, G. & Vogel, F., 1982), but this later statement could be only justified by clinical evidence. In conclusion, the pattern of these abnormalities suggests that neonatal treatment with CLI could be considered as a possible animal model of depression (Hartley, Neill, Hagler, Kors, & Vogel, 1990; Neill, Vogel, Hagler, Kors, & Hennessey, 1990; Vogel, Neill, Hagler, & Kors, 1990a, 1990b; Vogel, G. & Vogel, F., 1982).

A productive approach to advance the causal knowledge of psychopathology is to develop animal models which reproduce the behavioral and neurophysiological aspects under controlled conditions so as to isolate the causal factors. It is obviously impossible to model all the dimensions of mental alterations, such as depression in humans in rats. The purpose is to model some aspects of neuropsychological alterations that offer a convergent approach with the data provided by clinical and descriptive studies. The aim of this work was to analyze the principal research of this area.

BEHAVIORAL ALTERATIONS

REM SLEEP

As mentioned above, neonatal exposure of CLI results in alterations of REM sleep when the animals reach adulthood. Experimental rats aging from 6 to 11 months, compared to control groups, demonstrate continuous alterations in REM sleep: higher percentage, lesser latency of appearance, and increase in the

frequency of the periods during sleep, and abnormal temporary rebound course REM, in the presence of a total normal rebound (Vogel, Neill, Kors, & Hagler, 1990). In contrast, no differences are found between experimental subjects and the control group in sleep time, the number of times they awake or in the average duration of the periods they remain awake.

The alterations in REM rhythm are those of depression. For example, the decrease of REM latency is an indisputable marker of unipolar depression. Because of this it is one of the most studied in CLI animals.

The changes observed in CLI - treated animals could be the consequence of REM sleep suppression during the neonatal period, by CLI administration (Mirmiran et al., 1981), or could be due to alterations in monoaminergic systems (Frank & Heller, 1997). To test these possibilities, neonatal rats were administered with three types of drugs which suppress REM sleep but with different actions on the monoamine systems: CLI (a serotonin and noradrenaline reuptake inhibitor), zimelidina (ZMI, a selective serotonin reuptake inhibitor) and desipramina (DMI, a selective noradrenaline reuptake inhibitor). The results indicate that it is the serotonergic system disorder in the neonatal stage that provokes the effects of REM sleep alteration during adulthood, since the same results are found with CLI and ZMI, without effects with DMI. This data suggests that the cause of the observed deficit in adulthood depends mainly on the alterations in serotonergic transmission, and not on the suppression of REM sleep, since all the treated groups had experienced REM sleep deprivation in the neonatal stage (Frank & Heller, 1997).

Another hypothesis that was tested in this model was whether other adulthood alterations, such as forced swim and dysfunctions in sexual behavior, are due to REM sleep alterations caused by CLI administration. For this, different drugs were administered to the rats during the neonatal period: CLI, vehicle, scopolamina (a drug which deprives the animal of REM sleep by blocking cholinergic activity) and idazoxan (a drug which increases

adrenergic availability, decreases REM sleep; Velázquez-Moctezuma, Aguilar-García, & Díaz-Ruiz, 1993). In adulthood, only those animals treated with CLI showed greater periods of immobility in the forced swim test and a decrease in different sexual parameters; no differences were found between subjects that had been treated with the other drugs. This result suggests that behavioral abnormalities are not due to the deprivation of REM sleep that occurs during the neonatal period nor are they related to the increased availability of catecholamines at a synaptic level. In the same direction, deprivation of REM sleep in an early stage of life is not sufficient to induce the symptoms of depression that were observed. On the other hand, the increase in the availability of catecholamines at the synaptic level does not induce itself the same depressive syndrome.

IMMOBILITY IN FORCED SWIM TEST

Forced swim test is one of the most widely used procedures to evaluate antidepressive drugs. In this, the immobility of the animal is reduced by those drugs that have an antidepressive action and also by non-pharmacological antidepressive treatments, e.g., REM sleep deprivation and electro-convulsive shocks. Adult rats treated with CLI during postnatal days, compared with those that received vehicle, demonstrated more time of immobility (Bhagya, Srikumar, Raju, & Shankaranarayana Rao, 2008; Bonilla-Jaime, Retana-Márquez, Vázquez-Palacios, & Velázquez-Moctezuma, 2003; Vázquez-Palacios, Bonilla-Jaime, & Velázquez-Moctezuma, 2005; Velázquez-Moctezuma & Díaz-Ruiz, 1992). This effect can be found from the age of three months, reaching a maximum peak at 6-7 months. Then, a spontaneous decline begins from 11 months (Vogel et al., 1990a). The alterations are produced when CLI is administered for a minimum of six days in specific periods; from 14 to 20 days of age is the last period in which this procedure produces abnormalities in adulthood (Feng, Ma, & Vogel, 2001).

Also, Hilakivi, L. and Hilakivi, I. (1987) and Fernández-Pardal and Hilakivi (1989) found that the neonatal administration with desipramine (DMI, a selective noradrenaline reuptake inhibitor) was involved to a subsequent lengthening in immobility in the swim test, same results were found with zimelidine (ZMI, a selective serotonin reuptake inhibitor).

The administration of antidepressive treatments caused a reversal in the immobility in forced swim test observed in CLI rats. The effect of acute, subchronic and chronic treatment of nicotine, fluoxetine, and the combination of both administrations in these animals was studied (Vázquez-Palacios et al., 2005). The acute, subchronic (7 days) and chronic (14 days) treatment with nicotine reversed the immobility of CLI rats in forced swim test, while the effect of fluoxetine was only observed after its subchronic and chronic administration. With the combination of nicotine and fluoxetine, no synergetic actions were found.

LOCOMOTOR ACTIVITY

One of the behavioral changes initially observed in CLI - treated rats was the increase in activity during adulthood measured in the open field test (Hartley et al., 1990; Hilakivi, L., Sinclair, & Hilakivi, I., 1984; Mirmiran et al., 1983). Gaztelu, Montes, Barrenechea, Romero, and Saiz-Ruiz (1996) found that the increase in activity depend on the dark/light phase in which the test starts. When the animals were tested in the light phase they found increase in activity, but no changes were observed when the animals were tested in the dark phase. As this increase in activity was not found in the central area of the apparatus, is possible that the animals were not exploring the place, but perhaps were trying to escape of the experimental area. The increase in activity was age dependent, which could be interpreted as an inverted U-shaped curve; due to the finding that activity has a peak at four months.

The same rats treated with CLI or vehicle were tested in two apparatus, the open field

and one which monitored total spontaneous motor activity called Digiscan, at different ages (Hartley et al., 1990). In the open field, the increase in activity in the peripheral area was replicated at 4 and 6 months of age. However, in the other apparatus, the increase in activity occurred only at three months of age (Maciag et al., 2006).

Together, these results suggest that the animals are not only hyperactive in an acute stressful situation like the open field test, but also in a relatively normal situation where the test is longer, and that the changes depend of the phase of the day that the test began.

Two effective treatments for depression were tested regarding locomotor activity in an open field test with CLI rats: the administration of imipramine or four days of REM sleep deprivation (Vogel et al., 1990a). The first treatment significantly reduced the activity of CLI - treated animals. In contrast, REM sleep deprivation increased the activity of CLI - treated animals. These preliminary results indicate that treatment with imipramine normalizes the hyperactivity of CLI-treated animals found in this test, but with other treatments the alteration is accentuated.

PLEASURE SEEKING BEHAVIOR

One of the most relevant symptoms of depression is the inability to experience pleasure (DSM IV, 1994). To evaluate this, different indicators of non sexual pleasure seeking have been used: intracranial self-stimulation, with one or several intensities of stimulus; the sucrose or saccharin consumption during 24 hours; and the exploration of a novel object in an open field. Compared to controls, CLI-treated rats presented less intracranial self-stimulation at 7 months of age, but not at 4 and 5 months. No differences were found between treatments in consumption of different concentrations of sucrose when the animals were evaluated monthly from 3 to 11 months of age. However, in another experiment, at seven months, it was found that CLI - treated animals consumed less saccharin solution than controls (Vogel et al., 1990a, 1990b).

Neonataly CLI treated rats consume less of a 1% sucrose solution than controls in a preference test that was performed during two hours with animals deprived of food and water for 18 hours, and that had received previous training of 48 hours of exposure to water and a 1% sucrose solution (Bhagya et al., 2008).

On the other hand, a procedure of consummatory Successive Negative Contrast (cSNC) in animals that had been neonataly treated with CLI was performed (Ruetti, Justel, & Mustaca, 2008). In this experiment, the experimental animals had access during ten daily trials of 5 minutes each to a reward of high magnitude (32% sucrose solution, pre-shift phase) and then suddenly downshifted to a lower magnitude one (4% sucrose solution, post-shift phase), while the control group had always access to the less preferred solution. With the unexpected incentive downshift, the animals suppressed the consummatory behavior compared to the control group. During downshift, the CLI group, like the vehicle group, reacted with a decrease in the consummatory behavior with respect to those animals that always received the 4% solution, but the CLI - animals experienced a recovery of the negative contrast in the second day of downshift. In contrast, the animals neonataly injected with vehicle recovered after three days of contrast.

Finally, CLI - treated animals showed less exploration of a novel object in the open field test at the age of 3 and 4 months, but these differences disappeared when they reached 5 and 6 months of age (Vogel et al., 1990a).

SEXUAL BEHAVIOR

One of the negative effects of serotonin reuptake inhibitors, like CLI, is the abnormalities in sexual behavior as a decreased libido and failures to reach orgasm or to ejaculate (Hendrick, Gitlin, Altshuler, & Korenman, 2000).

Male Wistar rats neonataly treated with CLI also exhibited sexual deficiencies in adulthood, expressed as a lower number of

mounts and ejaculations (de Boer, Mirmiran, van Haaren, Louwerse, & van de Poll, 1989; Feng et al., 2003; Maciag et al., 2006; Mirmiran et al., 1980, 1981, 1983). A decrease in the ejaculations of male Syrian hamsters treated with the oral administration of CLI in adulthood were also founded (Boscarino & Parfitt, 2002). In the same study, it was shown that the daily oral administration of CLI for two weeks to pregnant female Syrian hamsters provoked in adult male offspring, with respect to non-treated controls, a greater number of penetrations before reaching the first ejaculation. Sexual alterations were replicated in the Long-Evans strain (Neill et al., 1990). In addition, it was shown that deficits were reversed with imipramine administration and REM sleep deprivation, both treatments used as antidepressants (Vogel, Neill, Kors, & Hagler, 1990).

No alterations were found in the activity of the Hypothalamic-Pituitary-Gonadal (HPG) axis and the level of testosterone was normal. For this reason, it was excluded that the sexual dysfunction of these animals was due to physiological alterations relative to testosterone, it would be interesting to further investigate whether other hormones involved in the HPG axis are implicated or if this deficit is more related to alterations in motivation or reinforcement systems (Bonilla-Jaime et al., 2003).

Three different drugs were administered to evaluate the recovery of sexual performance in CLI - treated rats: yohimbine (a selective alpha-2 blocker), 8-OH-DPAT (a selective agonist of 5HT_{1A}) and oxotremorine (a selective agonist of muscarinic receptors; Bonilla-Jaime, Retana-Márquez, & Velázquez-Moctezuma, 1998). The administration of oxotremorine and yohimbine induced a slight improvement, while 8-OH-DPAT restored sexual performance to normal levels in CLI - treated subjects.

Lower intracranial self-stimulation, lower consumption of saccharin or sucrose, and alterations in the sexual behavior support the idea that CLI-treated animals have a diminished capacity for pleasure and could have damage the basic system of reinforcement or pleasure. Furthermore, some antidepressive

treatments reverse alterations in sexual performance.

Nevertheless, we must be cautious with this conclusion. The decrease in intracranial self-stimulation was shown in a specific period of the development of the subjects; the differences in the consummatory behavior of saccharin and sucrose were demonstrated in two publications and also during a limited age period. Regarding the exploration of a novel object, the differences also appear to be space and temporal specific. In addition, the test was performed in an open field, where the animals had already shown differences in their stress related behavior (Hartley et al., 1990). On the other hand, novelty seeking is considered to be associated with the control of impulses, and does not only relate to an aspect of reinforcement seeking (Ballaz, Akil, & Watson, 2007; Zheng, Tan, Luo, Xu, Yang, & Sui, 2004). Besides, the disruptions in sexual behavior were due to the consummatory aspect of this behavior, the motivation phase was not evaluated yet. Nonetheless, the positive findings encourage the further evaluation of this animal model.

VOLUNTARY CONSUMPTION OF ALCOHOL AND NICOTINE

Clinical studies suggest that depression facilitates the alcohol abuse and that nicotine improves depressive states. This allows treatment with nicotine to reduce the alcohol consumption in depressed subjects. It is a documented fact that nicotine provokes antidepressive effects (Salin-Pascual, de la Fuente, García, & Drucker-Colin, 1995; Salin-Pascual & Drucker-Colin, 1998; Salin-Pascual, Rosas, Jiménez-Genchi, Rivera-Meza, & Delgado-Parra, 1996). With these ideas in mind Martínez-González, Próspero-García, Mihailescu, and Drucker-Colin (2002) treated male rats with CLI during neonatal days and tested several behaviors with or without nicotine treatment when the animals reached the adulthood. Effectively, CLI-treated rats consumed more alcohol than controls. Nicotine treatment induced a decrease in alcohol consumption in CLI-treated rats, which was

similar to the consumption of control rats. In control animals, the application of nicotine had no effect on this parameter.

Hilakivi, L. and Hilakivi, I. (1987) found that rats neonatally treated with DMI show an increase in immobility in the forced swim test, and this was shortened with alcohol-treatment, suggesting that alcohol had an antidepressant-like effect on these animals. These studies are promising to test the relationship between alcoholism, depression, and its possible treatments.

AGONISTIC RESPONSE

CLI - treated rats showed abnormalities in the agonistic response that could be reverted with treatment for depression. One of the first studies evaluated the antidepressant effect on the fighting response induced by electric shocks; in this case two animals were placed together in a chamber and received electric shocks (Vogel, Hartley, Neill, Hagler, & Kors, 1988). The rats were evaluated during two weeks and immediately before the beginning of treatment (baseline). It was found that CLI-treated animals were significantly less aggressive than control groups. Then, the animals received two antidepressive treatments: 4 days REM sleep deprivation, or the administration of imipramine. Both treatments caused an increase in the fighting response of CLI treated animals in comparison to controls without treatment.

The diminished aggressive response of CLI - treated rats was replicated (Martínez González et al., 2002). In this case, antidepressive treatment with nicotine was applied. CLI - treated animals that received nicotine presented higher levels of aggression similar to control animals.

LEARNING

Depression in humans is associated with alterations in cognitive processes. Several clinical studies demonstrated that depressed subjects have alterations in the hippocampus,

which causes a deficit in memory and learning (McEwen & Sapolsky, 1995; Sapolsky, 2000).

To our knowledge, two studies tested the cognitive capacity of CLI - treated rats. The first one studied the effects of REM sleep deprivation on the learning of passive avoidance task in rats neonatally treated with CLI (Prathiba, Kumar, & Karanth, 2000). There were three groups: CLI-treated rats received a treatment of REM sleep deprivation in adulthood during four consecutive days, a CLI treated control group (without REM sleep deprivation) and a group injected with vehicle during neonatal days remained without treatment. The rats were later tested in a passive avoidance task. The results showed that neonatally - CLI treated animals without REM sleep deprivation had an improvement in the task, while the CLI rats with REM sleep deprivation behaved as animals that received vehicle neonatal treatment. The authors showed that rats neonatally treated with CLI improved the retention in the passive avoidance task; and the deprivation of REM sleep reverse this improvement, which according to the authors indicates that these animals had an increase in the sensitivity of cholinergic system.

The second study evaluated the performance of CLI - treated rats, saline controls and intact controls in an eight arms radial maze, which is a more complicated learning and memory task (Bhagya et al., 2008). CLI - treated rats exhibited a profound deterioration in learning compared to controls.

PHYSIOLOGICAL ALTERATIONS

NEUROENDOCRINAL ALTERATIONS

In human depression, neuroendocrinal alterations are presented as the deregulation of the Hypothalamic-Pituitary-Adrenal axis (HPA - McEwen, 2000; Sapolsky, 2000). The experimental results were ambiguous with respect to the function of this axis in CLI-treated animals.

One way of evaluating HPA axis function is using a dexametasone suppression test (DST). There is evidence indicating that people with endogenous depression present abnormalities in REM sleep associated with a non suppressive response to DST, and higher basal levels of cortisol (Asnis et al., 1983; Willner, 1985). Therefore, a direct relation would exist between abnormalities in REM sleep, DST and depression. In this respect, it was studied if animals treated with CLI demonstrated a normal response to DST, and whether this response was affected by REM sleep deprivation (Prathiba, Kumar, & Karanth, 1998). When the animals reached 3 months of age, the authors evaluated corticosterone levels before and after the administration of DST, and also later, REM sleeps deprivation during four consecutive days. It was found that, before treatment, CLI - treated animals had significantly higher basal levels of corticosterone in comparison to control animals. After the administration of DST in CLI - treated animals, there was a significant increase in corticosterone levels, and a non suppressive response. On the other hand, REM sleep deprivation reduced corticosterone levels in CLI animals in comparison to control animals that were not exposed to this. The animals that received REM sleep deprivation did not differ from controls in corticosterone levels. In this way, neonatal treatment with CLI affected the ability to suppress corticosterone levels after the administration of DST, and increased basal levels in experimental subjects. This finding constitutes the first evidence that neonatal treatment with CLI induces deregulation of the HPA axis.

At present, there is also data indicating the existence of a hypoactivation of the HPA axis that is not consistent with the validity of the model that is being shown. The neonatal administration of CLI induced, in adult animals, a lower response of the HPA axis under a stressful situation (Ogawa, Mikuni, Kuroda, Muneoka, Mori, & Takahashi, 1994). Specifically, CLI-treated rats were exposed for seven days to physical restriction during two hours and blood samples were

taken in different periods of time: before the restraint in housing chambers, during, and after exposure to the stressor. It was found that, compared to appropriate controls, that CLI - treated animals did not differ in corticosterone basal levels, but presented a lower increase in plasma levels of this hormone during exposure to the stressor, and returned faster to basal levels after the stress ended. In accordance with these results, it was found that the HPA axis was affected by neonatal treatment with CLI (Bonilla-Jaime et al., 2003; Feenstra, van Galen, Te Riele, Botterblom, & Mirmiran, 1996). The corticosterone levels before and after copulation were measured. The corticosterone basal level was the same in CLI - treated animals and controls, but after copulation, there was a lower increase in experimental subjects with respect to controls. The conclusion of the study was that the adrenal response normally provoked by sexual performance was significantly diminished in CLI - treated animals.

To summarize, while it is true that some experimental results found that CLI - treated rats have a higher basal level of corticosterone than controls, others found that not only do they differ in basal level, also during the stress situation; circulating corticosterone increases less and returns more rapidly to basal levels than control groups. For this reason, we can conclude that if alterations in the HPA axis indeed exist in CLI - treated animals, it is still unclear in which way the deregulation is manifested.

BODYWEIGHT

Another symptom that is found in the DSM IV for depressive disorder is the increase or decrease in subjects' weight. There is evidence of this type of alteration in neonatally CLI - treated animals. Different studies found that experimental animals in adulthood have lower weight in comparison to control groups (de Boer et al., 1989; Hansen & Mikkelsen, 1988; Maciag et al., 2006; Mirmiran et al., 1983; Yoo, Bunnell, Crabbe, Kalish, & Dishman, 2000). This weight difference could be

because CLI is an anorexigenic compound (Hansen & Mikkelsen, 1988). However, no studies have tested this hypothesis.

NEUROPHYSIOLOGICAL ALTERATIONS

Several lines of evidence suggest that a reduction in central serotonergic system activity is involved in the physiology of depression. The compounds that block serotonin reuptake are effective antidepressants (Hansen & Mikkelsen, 1988). Furthermore, the different treatments commonly used as antidepressants improve serotonergic transmission (Vázquez-Palacios et al., 2005). In connection to this, the hypothalamic concentration of serotonin was 20% diminished when experimental animals were compared to their controls at one year of age (Feenstra et al., 1996). A significant increase in the expression of "5HT transporter mRNA" in the dorsal raphe nucleus (DRN) after the administration of selective and non-selective reuptake inhibitors of serotonin was founded (Hansen & Mikkelsen, 1988). Regarding these results, the indicators of neuronal serotonergic firing in the DRN was diminished in experimental animals compared to their controls, which is consistent with the hypothesis that serotonergic neurotransmission is diminished in depression (Kinney, Vogel, & Feng, 1997; Yavari, Vogel, & Neill, 1993). Furthermore, neonatal administration of CLI or citalopram induced changes in the serotonergic system, which includes a reduction in the immunoreactivity in the DRN of the limited enzyme of serotonin production (Maciag et al., 2006). The authors argue that because the citalopram is a selective serotonin reuptake that shows no affinity for other sites they can say that the changes in serotonergic transmission during a critical period of development of the organism is the responsible for the changes, such as depression found in adult subjects.

On the other hand, it has been shown that nicotine is related to the serotonergic system (Mihailescu, Palomero-Rivero, Meade-Huerta, Maz-Flores, & Drucker-Colin, 1998; Seth,

Cheeta, Tucci, & File, 2002). As demonstrated in another study, nicotine has antidepressive effects, which suggests that these effects could involve serotonergic transmission (Vázquez-Palacios et al., 2005).

Several evidences indicate that in depression, there is an increase in cholinergic activity. There are antecedents in which neonatally CLI - treated animals present altered sensitivity of cholinergic receptors in adulthood (Prathiba et al., 2000). Based on these results, an experiment was performed to study the activity of a soluble form of acetylcholinesterase (ACHE) in the hippocampus of CLI - treated animals in comparison to controls (Mavanji & Datta, 2002). In the frontal lobe, the opposite was found: CLI - treated animals showed significantly lower levels of AChE. There were no differences between treatments when AChE levels were measured in the brain stem, hypothalamus, and in the septum.

One previously mentioned study concerning sexual alterations and their reversal, suggested that CLI - treated animals have cholinergic and adrenergic systems alterations, specifically in the muscarinic receptors, while the serotonergic system seems to be preserved at least when dealing with alterations found in sexual performance (Bonilla-Jaime et al., 2003).

DISCUSSION

The reviewed data, although including some contradictory results, allows us to make the following conclusions. Adult animals exposed to CLI in neonatal days showed alterations in REM sleep, lower weight, disruptions in locomotor activity, less intracranial self-stimulation, lower saccharin and sucrose consumption, less suppression of the consummatory behavior, sexual alterations in males, higher alcohol consumption, disruptions in the agonistic response and in learning compared to untreated animals. Several of these abnormalities could be reversed with those treatments that are effective for treating depression in humans (antidepressive drugs, nicotine and REM sleep deprivation treat-

ment). These results were obtained in male rats of different strains and in hamsters, and at different months, the majority of them at 3-4 months, and some of them after the sixth, this could be because some changes were caused with the decrement in the age of the animals, further research is needed to elucidate this issue (Bonilla-Jaime et al., 2003; Frank & Heller, 1997; Hartley et al., 1990; Neill et al., 1990; Vogel, G. & Vogel, F., 1982; Vogel et al., 1990a, 1990b).

In the methodological level two experiments showed that the oral administration of CLI provoked alterations in the sexual performance of adult hamsters. Potentially it could be a valuable method that needs to be evaluated in other behaviors and other rodents. If positive results were obtained then there would be a decrease in the common problems associated with handling and injections, reducing possible sources of stress caused by the procedure per se and improving the animal quality of life (Boscarino & Parfitt, 2002).

On the other hand, only one experiment showed that prenatal administration caused an alteration in sexual performance of the adult hamster (Boscarino & Parfitt, 2002). This result, although minor, deserves to be further considered and because of its clinical implications.

Neuroendocrinal alterations analogous to those found in human depression were also discovered in CLI - treated rats, although the data is contradictory. These include HPA axis alterations; while some authors found hypoactivity others found hyperactivity (Asnis et al., 1983; Bonilla-Jaime et al., 2003; Feenstra et al., 1996; Ogawa et al., 1994; Willner, 1985).

Other results support the hypothesis that alterations found in CLI - treated animals are due to alterations in serotonergic transmission during a critical period of development, such as the neonatal stage; more specifically, a reduction in the hypothalamic concentration of serotonin, like a decrease in the neuronal firing in the dorsal raphe nucleus (Feenstra et al., 1996; Frank & Heller, 1997; Kinney et al., 1997; Yavari et al., 1993). An increase in cholinergic activity was also found (Bonilla-

Jaime et al., 2003; Prathiba et al., 2000; Velázquez-Moctezuma et al., 1993), although the data in this field is not as vast as that found in relation to the neurotransmission of serotonin.

All of these results suggest that rats treated with CLI during neonatal days present alterations in adulthood analogous to human depression, but also the data indicates that is not yet a valid model. Further research is needed, and we have to be cautious with the conclusions because there is some evidence suggesting that this is a promising model but other does not support its validity.

As commonly occur with all animal models in psychopathology, certain human characteristics cannot be evaluated; in this case, recurrent thoughts of death or suicidal thoughts, emotionally depressed state, feelings of uselessness. Furthermore, in this case, some deficiencies appear in limited periods of time, others cannot always be replicated, and others require crucial experiments to verify the mechanism of alteration.

Clinical investigations with humans are revealing, but had difficulty in identifying the etiology of the disorders, since the majorities are correlational studies with very little control of variables (Papini, Wood, Daniel, & Norris, 2006). Animal models allow for greater empirical control, a greater possibility of manipulation of behavioral, neurophysiological, genetic or psychological variables (Mustaca & Kamenetzky, 2006). Additionally, these models lets us to consider psychopathologies as determined behavioral processes which mechanisms can be scientifically understood (Hunziker & Pérez-Acosta, 2001) and to study the brain mechanisms implicated in the pathogenesis and treatment of the disorder that, for ethical reasons, are not possible to study on human subjects. Research with mice and rats constitute a valuable instrument if is considered that humans as well as rodents evolved from common mammalian ancestors (Papini, 2003). On the other hand, animal models also present disadvantages like those discussed previously.

Therefore, if a model like the neonatal administration of CLI achieves the reproduction of some symptoms, neurophysiolog-

ical and behavioral alterations of depression, the advantages are invaluable. In this sense, neonatal treatment with CLI is very promising.

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