STUDY ON ADAPTIVE CAPACITY AND BEHAVIORAL STRESS OF SEPARATION (MOTHER-JUVENILE) IN RATS WISTAR

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Résumé

Chez l'homme, les facteurs génétiques et environnementaux influent sur la susceptibilité à de nombreuses maladies. En particulier, la qualité des relations entre un enfant et sa mère joue un rôle important pour le développement harmonieux et l'équilibre ultérieur de cet individu. Des perturbations de ces relations peuvent conduire notamment à des désordres psychiatriques graves. De plus en plus, des modèles expérimentaux animaux se développent afin de connaître les perturbations neurobiologiques qui sous-tendent une vulnérabilité à ces troubles. La modélisation chez l'animal reste cependant relative. Le travail présenté dans cet article a été réalisé sur un modèle de séparation mère/nouveau-né chez le rat (Tusrattus) de la souche Wistar. Les rats sont séparés de leur mère et isolés de leurs congénères quotidiennement pendant 5min, 30min et une heure de leur troisième à leurs 14 jours de vie. Des études ont déjà été menées, dans ce modèle, chez des rats devenus adultes. Il s'agissait cette fois d'analyser le comportement de rats à l'âge juvénile. Une première partie présente le contexte général dans lequel s'inscrit le sujet, et regroupe des données bibliographiques concernant les modèles de séparation mère/nouveau-né. La deuxième partie présente les objectifs précis du travail. Elle sera suivie d'une troisième partie décrivant le travail expérimental : les différents tests comportementaux réalisés seront expliqués, puis les résultats obtenus seront discutés, comparés à ceux de la littérature. En fin, les résultats obtenus expliquent que la durée de séparation la plus longue augmente les réponses comportementales, comparés à ceux de la littérature.

***Mots clés** : Capacité d'adaptation, Stress de séparation, facteurs génétiques et environnementaux, rat.*

Abstract

In humans, genetic and environmental factors influence susceptibility to many diseases. In particular, the quality of relationships between a child and his mother plays an important role for the harmonious development and the subsequent equilibrium of this individual. Perturbations of these relationships can lead to such serious psychiatric disorders. More, experimental animal models are developed in order to know the neurobiological disturbances that underlie vulnerability to these disorders. Modeling animal remains relative. The work presented in this paper was carried out on a model of mother / newborn rats (Tusrattus) of the Wistar strain separation. The rats are separated from their mothers and isolated from conspecifics daily for 5 min, 30 min and an hour of their third to their 14th day of life. Studies have already been conducted in this model, in rats became adults. It was this time to analyze the behavior of rats at the age juvénile. The first part presents the general context within forms the subject, and includes bibliographic data models . The second part presents the specific objectives of the work. It will be followed by a third part describes the experimental work. Different behavioral realized tests will be explained, and then the results explain that the longer duration of separation increases behavioral responses, compared to those in the literature.

***Key words**: Adaptive capacity, Stress separation, genetic environmental factors, rat.*

ملخص

إن العوامل الوراثية والبيئية لها تأثير على قابلية الإنسان للإصابه بكثير من الأمراض. خصوصا، فيما يتعلق بنوعية العلاقة بين الطفل وأمه, حيث تلعب هذه العوامل عند الفرد دورا هاما في إتزانه الداخلي ونموه المتناسق . و عند حدوث أختلالات في هذه العلاقات يمكن أن تؤدي إلى ظهور أضطرابات نفسية خطيرة. وبإجراء العديد من التجارب ، حيث يتم تطوير نماذج حيوانية تجريبية من أجل الوصول إلى معرفة الاضطرابات العصبية التي تكمن وراء التعرض لهذه الاضطرابات. العمل المقدم في هذا المقال تم إجراء على نماذج من الانفصال بين الأم / الفئران حديثي الولادة من سلالة Wistar.(Tusrattus) ، حيث يتم فصل الفئران عن أمهاتهم لمدة 5 د، 30 د وساعة، يوميا من اليوم الثالث إلى اليوم الرابع عشر من الولادة. وقد تم إجراء دراسات على هذه النماذج من الانفصال عند مجموعة من الفئران البالغين. والغرض من اختيارنا لهذا الفترات الزمنية الهدف منه التوصل إلى تحليل سلوك الفئران الصغيرة السن. الجزء الأول من هذه الدراسة يعرض السياق العام الذي تم فيه عرض هذا الموضوع، ويتضمن البيانات المرجعية لنماذج الانفصال بين الأم / الفئران حديثي الولادة . الجزء الثاني يعرض الأهداف المحددة للعمل. وسيعقب ذلك جزء ثالث يوضح العمل التجريبي: سيتم شرح الأختبارات السلوكية المختلفة، ولقد أظهرت النتائج أنه كلما زادت مدة الفصل زادت الاستجابات السلوكية، وهذا يتطابق مع المراجع .

***الكلمات المفتاحية:** القدرة على التكيف، إجهاد الانفصال ، العوامل , الوراثية , فئران*

Humans, genetic and environmental factors influence susceptibilité to many diseases, such as cardiovascular, diabetes type II. [1]. In particular, important relational disturbances between the mother and the newborn (uncompensated long separation from the first days of life, lack of care, negatively affect the mother ....) play an important role for the harmonious development and the subsequent balance of the individual. Disturbances of these relationships can undermine the individual and lead to serious illnesses such as psychoses, behavioral disorders, translating into diseases anxious, depressive syndromes, addictive behaviors [2.3, 4.5].

More, experimental animal models are developed in order to know the neurobiological disturbances that underlie vulnerability to these disorders. Modeling animal remains relative. We do not pretend to reproduce the human disease, we simply have access to certain symptoms. Thus, in both animals and humans, perinatal exposure, that is to say, pre-or post-natal in a stressful environment can create a long-term fragility and favor the emergence of behavioral disorders [2,4].

It is fundamentally important in all species, the relationship between the mother and the newborn in the harmonious development of that underlying the relevance of animal models of separating mother / newborn that are being developed .. This separation is an early stress, because it occurs during a period of intense neuronal development, is liable to cause abnormal brain function permanently. These models are studied to try to describe the harmful behavioral and neurobiological consequences of early stress on the development of children. Maternal privation alters at the same time behavior and neurotransmitter systems in many species [6,7].

There are several models of mother / newborns rats separation. Ils diffèrent mainly by the separation time (15 min, 3 hours, 6 hours, 24 hours), and the age of the smaller at time of this separation (from the second to the twenty-first day of life). The modifications induced are different according to the protocol used for separation [6].

A brief maternal separation of 15 minutes per day for 22 days results in a protection of deficits associated with age [8,9], decreased anxiety, and an increase of the negative feedback of the HHS axis. [10, 11, 12, 13]. However, longer periods of separation (greater than or equal to three hours) increases the behavioral responses and the activity of the HHS axis in response to stress [14, 15, 16, 17]. However, it should be noted that some studies show a decrease in anxiety and also the behavior of adult rats after long periods of separation [11, 18, 19].

MATERIALS AND METHODS

Biological Materials

To carry out our work we used the white male adult rats (Tusrattus) of the Wistar strain from the Pasteur Institute in Algiers. The gestation lasts 20 -23 days and ranges from 8 -14 youth. They weigh 5-7g, they are hairless blind and open their eyes between le12th and 16th day. The coat grows completely at 10 day. Youngsters can be separated from the mother from about 20 days.

Pubertal rats are between 50 and 60 days after birth in both sexes, testicular descent occurs before puberty, usually around the age of weaning.

Animals recognize each other by smell. Rats also mark their tracks with their urine. They are first acclimated to the conditions of our Animalerie for the duration of the experiment in polyethylene cages (temperature: 24 ± 1 ° C and illuminance: 12L/12D.) The food is in the form of pellets (UAB , Algeria). Food and water were provided ad libitum, it can meet the needs of the animal.

Methods

Separation protocol

There are several models mother / newborn rats separation. They differ essentially in the separation time (5 min, 30 min one hour). Changes induced differ according to the separation protocol used [6]. Four pregnant females were housed individually. The day of birth of newborn was appointed as J0. The protocol of maternal separation began at the j3- until the j14. A J3, were carried out a lots of 6 to 12 rat, which were attributed to mothers randomly so that all mothers have the same number of children (n = 4), and divided into four groups experimental: Lot T: no treatment, lot 5: separation 5 min, lot 30: separation 30 min, lot1 : separated one hour.

Behavioural analysis (Observation in natural situations and in experimental tests)

Evaluation of exploratory behavior in a stressful context of the open field test (Fig 01).

The open field was used a large box generally square, rectangular or circular shape. The open field is also often used to assess anxiety, including additional measures: defecation, the time spent at the center of the field, in the first five minutes of activity [20].

Each rat was placed initially in one of the four corners of the open field, the head oriented towards the corner. His behavior was observed for 5 min. Five parameters were measured by the experimenter :

1-Time spent in the center exprimed in seconds, **2** - Time spent in the preferred exprimed in seconds, **3**-The total number of adjustments (animal positioned on his two hind feet, straight, in equilibrium in the void or against the wall), **4**-The total number of grooming, **5** - The total number of bowel movements.

Test of cross labyrinth (more mize) (Fig 02)

In the test, each rat was initially positioned in the center of the labyrinth at the intersection of the four arms [21], The head oriented towards one of the open arms , and he had free access to the four arms for a period of 5 min. The labyrinth was placed in a noise isolated room. The experimenter visualized the behavior of rats outside the

room with a video camera, he reported the number of visits and time spent respectively in the BO and the BF, in visit was recorded when the rat had the four lugs in one arm.The results were expressed as:

1- Number of entry in the open arms and closed arms, **2**- Number of entry in the proximal parts of the open arms and closed arms, **3**- Number of entry in the distal parts of open arms and closed arms, **4** - Number of turnaround, **5** - Past time (s) in the center, **6** - Past time (s) in the open arms (BO) and closed arms (BF), **7** - Turnaround time (s), **8** - Number of defecation, **9**- Number of grooming (urine).

Evaluation of the appetitive behavior in the test sugar water consumption

The process of testing the sugar water consumption is as follows: The animals were placed in individual cages, with two standard bottles containing either water for consignments of animals (control, 5min, 30min, 1h) or a water sugar solution (at a concentration of 40mg sugar / L of water) was placed at their disposition for a period of 15 days. The amount of water in the bottle before and after consumption was measured to assess the respective amounts drunk [22].

Fig1: Pregnant used in the openfield test

Fig 2 : Device used in the tests of labyrinthe (test of the ss)

The results are expressed in the form of mean ± standard deviation (m ± SD).

The comparison between the means is carried using the Student T test. The significance of the results is estimated by analysis of variance a classification criteria (ANOVA). The difference between experimental groups was considered significant at $p < 0.05$.

RESULTS

1 - Behavioral parameters of the control and treated lots registered on open field (Table 01)

From these results we notice that in the open field test, rats lot 1 spend much more time in the peripheral part than in the central part intake to controls ($p < 0.05$) rats, lot 1 move much intake to controls ($p < 0.05$) against the rats of lots 5 and 30, they do not exist a significant difference compared to controls.

Table 01: Behavioral parameters of the control and treated lots registered on openfield (n = 4, m ± s)

Rats	Lot T	Lot 5(ns)	Lot 30(ns)	Lot 1
A	41.66 ± 4.31	38.01 ± 14.99	70 .47 ± 4.23	260.78±6.46*
B	2.43 ± 0.56	2.27 ± 0.49	2.11 ± 0.33	0.53± 0.03*
C	256.57 ± 23.35	249.37 ± 23.41	228.43±22.87	38.81 ± 4.87*
D	4.73 ± 0.67	4.33 ± 0.57	3.93 ± 0.56	0.37 ± 0.12*
E	0.25 ± 0.46	0.21 ± 0.31	0.25 ±0.5	0± 0.50.25

A: Time in the periphery (s), **B :** Time in the center (s), **C:** Immobility time (s), **D:** Number of defecations, **E :** Number of grooming (urine)

2 - Parameters of behavioral control and treated lots are registred more mize (Table 02)

From these results we notice that the more test-mize, lot 1 rats spend much more time in the open arms and the center than in the closed arms by intake controls. ($p < 0.05$), against the rats of the lots 5 and 30, they do not exist a significant difference compared to controls.

3 - Quantities of sugared water consumed due to the stress of separation in the test of sugared water consumption (ml) (Table 03)

Our result shows that the consumption of sugared water is very highly significantly higher among in rats separated during an hour than non-separated rats ($p < 0.001$), cons, they do not exist significant difference in lots 5 and 30. But the consumption of normal water is highly significant decreased in in rats separated during an hour than non-separated rats ($p < 0.01$) and very highly significantly reduced in rats for 30 minutes separated intake that rats are not separated ($p < 0.001$).

Table 02 : Behavioral parameters of the control and treated lots registered on more mize (n = 4, m ± s)

Rats♂	Lot T	Lot 5(ns)	Lot 30(ns)	Lot 1
Number of Entering in the open arms	1.17 ± 0.23	2.46 ± 0.25	3.08 ± 0.98	5.37 ± 1.39*
Number of entered in the closed arms	11.07 ± 0.76	12.09 ± 0.99	13.17 ± 1.28	16.46 ± 2.58*
Time spent in the center (s)	64.21 ± 13.11	71.33 ± 13.05	76.37 ± 12.98	141.95 ±16.11*
Time spent in the closed arms (s)	221.37 ± 18.29	211.47 ± 18.17	201.39 ± 18.06	117.15 ± 7.93*
Time spent in open arms (s)	15.47 ± 3.98	17.37 ± 4.09	23.17 ± 6. 15	41.88±8.19*
Turnaround time (s)	0.09±0.02	0.07 ± 0.05	0.14 ± 0.11	0.05 ± 0.04
Number of turnaround	7.25±1.5	0.66 ± 1.11 ns	7.00 ± 5.61	3.5 ± 2.38 ns
Number of defecations	0.5±0.57	0.22 ± 0.44 ns	2.80 ± 3.03	4.00 ± 2.16 ns
Number of grooming (urine)	1.25±0.95	0.25 ± 0.5 ns	0.2 ± 0.44	6.33 ± 3.53*

Table 03 ***:*** Quantities of sweetened water consumed after the stress of separation in the test sweetened water (ml) (n = 4, m ± s)

Rats	Lot T	Lot 5 (ns)	Lots 30	Lot 1
sugared water (ml)	194.54±91.58	216.92±27.19	231.15±38.52	453.43±31.76***
normal water (ml)	274.54±66.72	253.46±38.80	151.92±62.93***	234.37±37.5**

DISCUSSION

The concept of vulnerability to behavioral problems created by early stress, as a long separation mother / newborn animals. The prolonged isolation in a different environment, modified maternal behavior in the cage or a combination of these. [19]. These models engender disturbances such as increased anxiety and dependence to psychostimulants. Neurobiological analyzes performed in adult animals show that many neuronal systems (stress hormones, dopamine, norepinephrine, GABA, peptides) of certain structures of the limbic system are perturbed [13,23, 24, 25].

The groups of rats showed a similar motor activity in 24 hours, with motif inactivity according to the circadian rhythm provided, motor activity decreases at light phase and increasingly gloomy period. On the other hand, in field trials, the rats 5, 30 and 1h showed similar locomotor activity at the periphery of the open field, which suggests that separate rats were more anxious than the control animals. This would be consistent with recent data indicating the occurrence of anxiety-like behavior as a consequence of maternal separation [14, 15, 16, 17].

However, other studies have failed to observe the anxiety-like behavior in the open field test [11] or other tests measuring emotional behavior [19]. This difference can be explained by the different strains used and the age of the tested rats (6week-old Sprague-Dawley rats [11]), lineage and age have been shown to be important factors in stress reactivity [26, 27]. Neurochemical alterations reported in the maternal separation model could be related to changes in hormone and neurotransmitter involved in anxiety and stress-related behavior, such as corticotropin-releasing factor (CRF), serotonin and gamma-aminobutyric acid (GABA) systems [14, 15, 24, 28]. Functional interactions between CCK, CRF, GABA and serotonin have been described in several brain structures related to anxiety and stress [29, 30, 31].

The data of the literature show that the opioid system in the rodent, as in monkeys, very much involved in the development of relations between the mother and the smaller [32, 33]. Opioids lead to positive reinforcing effects (they promote an approach behavior or consumption) at an early age: breastfeeding induced release of opioids in the newborn, which would allow him to consider nursing as having a properties of rewards and would promote the associations between mother, The breastfeeding is the status of recompense [34]. Cholecystokinin (CCK), in turn, coordinates digestion, metabolism and growth of the developing child, and also appears to participate in non-nutritive aspects of relations between the developing child and his mother. [35].

Separated a mother / newborn could lead the animal to a malfunction of the activity neuropeptidergic systems with disturbances in the formation of associations between maternal bonding and the state of rewards [36, 37].On the neurobiological level, researchers have highlighted a decrease in activity of the opioid system. (This system is activated, for example, during sugar ingestion - or very strong manner by taking morphine or heroin - by inducing a production of endogenous opioid witch produces _ peptides bound to pleasure.). However, the decrease in activity of this system leads to gradually subject to higher consumption. Sweetened water is palatable to rodents, measuring consumption possible to evaluate the hedonic state/ hedonic animals.

Consumption of sweetened water separate rats is increased, particularly when rats underwent additional stress of isolation. This sweet water consumption by these rats returns to normal after injection ofL365, 260. Separate newborn rats therefore exhibit in adulthood an hypersensitive to the effects of reward (sweetened water), these data suggest the existence of a partial brain opioid system dysfunction.

The greater sensitivity of the separated rats to the effects of rewards and reinforcing effects of morphine could be explained by hypofunction of the endogenous opioid system (decreased synthesis or liberation of opiates ...) which results in an hypersensitive opioid receptors (increase quantity of receptors and the efficiency of transduction pathways receptor), leading to increased sensitivity of animals to morphine. Several arguments go in this direction. First, the administration of RB101, an inhibitor of the two principal enzymes (aminopeptidase N, neutral endopeptidase) responsible for the inactivation of enkephalins (which belong to the opioid system), is contrary to what happens in non- separated rats incapable to increase the consumption of sweetened water with separated rats.

This absence of effect suggests that it is the tonic or phasic liberation of enkephalins, which is decreased in separated rats because the RB101 prevents the degradation of enkephalins only when they are released into the synaptic cleft [32, 38].

CONCLUSION

The effects of stress on behavioral and adaptive capacity in rats we can move forward a certain number of tracks which remain to be explored.

The stress of separation installs a fairly convincingly anxiety and a depression that interfere with their laps with all of cognitive and immune function.It would therefore be wise to digging these aspects by promoting the tests for storing and exploration of the impact of stress on the reward circuit. A similarity seems to settle between this type of stress and drug addiction.

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