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Research Article,

Effect of Human Handling during Postnatal Period on Learning and Memory in Adult Rats Tested In the Morris Water Maze

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Abstract:

Human-animal interactions occur in many sectors of livestock production. This study aims to determine whether human manipulation during the critical period could disrupt their long-term spatial memory and learning functions. Forty-four pups rats (22 males and 22 females), were divided into two groups (handling, non-handling: corresponding to the control group). Subsequently, each group was subdivided into two subgroups: male subgroup (Handling Males, H ♂) and a female subgroup (Handling Female, H ♀), which were handled daily for 5 min from birth to weaning. On postnatal day 81, they were subjected to Morris Water Maze test (MWM). The results showed that in both sexes, the manipulated group had spent more time in the quadrants where was the platform. Therefore, human handling during the neonatal period of rats could induce in long term better learning, and greater spatial memory without any negative effects on behavior.

Keywords: Postnatal handling, Learning, Spatial memory, Rat, MWM

1. INTRODUCTION

Several studies have focused on the therapeutic and beneficial effects of animals on humans, but not on the effects of humans on animal welfare, although animal welfare is now a major concern in modern society. In recent years, interest in studying the human-animal relationship in domestic species has increased, particularly for economic reasons. Poor quality of this relationship can have negative consequences on animal behavior.

In recent years , several studies have investigated the effects of neonatal stress on behavior and stress resistance in animal and human models (**Fernandez-Teruel et al., 1997 ; Gallois et al., 2012)**, and have found that prenatal stress can affect the emotional and cognitive ability of rat pups **(Cabrera et al., 1999; Nishio et al., 2001)**, in addition to causing adult-specific alterations in response to aversive situations **(Cabib et al., 1993)**, such as psychosis, behavioral disorders, depressive syndromes, addictive disorders and memory disorders with disruption of the functional maturation of hippocampal networks **(Parker, 1981; Holmes & Robbins, 1987; Canetti et al., 1997; Benoit et al., 2015; Reincke & Hanganu-Opatz, 2017)**. Furthermore, rhesus monkeys raised in an enriched neonatal environment, have superior performance in orientation and motor activity, with fewer temperamental responses (such as fear) compared to rhesus raised in a less rich environment **(Schneider et al., 1991; Bard et al., 2001)**. **Tremblay (2002)** suggested that some types of maternal care improve spatial learning and memory of the offspring by increasing hippocampal development.

The brain of the offspring after birth is very sensitive to environmental factors, epigenetic mechanisms that play a central role in the long-term, even transgenerational **(Gressens & Mezger, 2014)**. Thus, studies on neonatal environmental effects and their interactions on behavior and stress adaptation are of paramount importance to better understand the impact of the early environment effect on the behavioral and cognitive development of adult animals.

Human-animal interactions are sometimes very close and are mainly done through non-verbal language. Indeed, the main element of communication that allows the creation of a relationship between human and animal is physical contact or touch **(Servais, 2007**). Thus, this study aims to determine the effects of long-term human neonatal physical manipulation on learning, and spatial memory of adult rats.

MATERIALS AND METHODS

Animals:

Forty-four albino weaned rats (22 males and 22 females) of Wistar strain obtained from mating six males and six females rats of the same strain were used during experimentation phase. They were provided by the Pasteur Institute in Algiers, Algeria. The rats were housed in polycarbonates cages in a colony room of the Department of Biology, University of Annaba, Algeria, and reared under standard laboratory conditions (temperature of 25 ± 2 °C, humidity 50 ± 5 %, artificial lighting, 12 h light/ 12 h night from 07h00). They were fed on standard rat chow produced by ONAB (national office of animal feed manufacturing), Annaba, Algeria, and supplied with water ad libitum.

Experimental Design:

All experimental procedures described in this work were carried out in accordance with the guide for the care and use of laboratory animals of the University of Badji Mokhtar Annaba, Algeria. Figure 1 presents the experimental design. For the synchronization of estrous cycles in female rats, six males and six females of the same strain were placed in a compartmentalized cage without physical contact for 7 days, which allowed exposure to male pheromones. Males and females were then placed in cages for reproduction. Vaginal smears were examined daily for sperm presence. The males were then removed and the pregnant females were reared in individual cages until parturition. Birth day was noted PND 0, and the offspring remained with their mothers were separated into two experimental groups: handling (H), and non-handling group (NH). The offspring of the (H) group were physically manipulated by the experimenter for 5 min per day till PND 21 (weaning), while, the NH group had no physical manipulation. After weaning, the offspring were separated from their mothers, 11 males and 11 females were randomly selected from each group (NH= 22, H= 22; 11♂/11♀) and rose in pairs in each cage to adulthood under the standard conditions mentioned above. In PND 81, they were subjected to behavioral test to assess spatial memory level, and learning behavior.

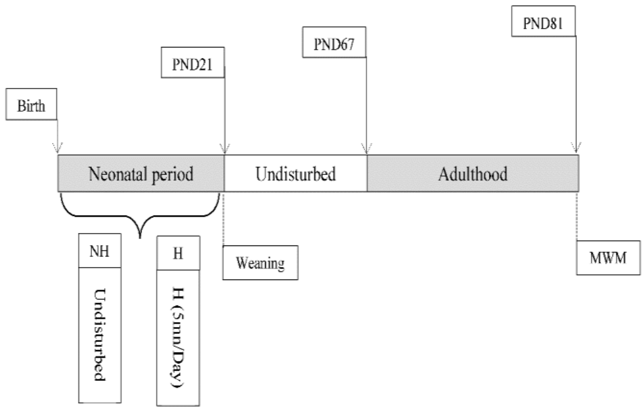

Figure 1. Timeline of the protocol. NH (Non-Handling); rats of this group were left undisturbed. H (Handling); females and males rats were exposed to daily handling session for 5 min.

All rats were kept undisturbed until postnatal day (PND) 21, when they began performing the Morris water maze (MWM) on PND81.

Handling (Physical Manipulation):

The physical manipulation of the offspring was carried out according to touching method described by **Weininger (1954)**, Therefore, the animal is held in the experimenter's left hand and placed against the experimenter's chest, so that the animal is nestled in the palm of the left hand, and with the thumb or fingers, the experimenter caresses the animal's back from head to the base of the tail. The offspring were handled individually, 5 min per day from PND1 to PND21 at 9.00 am by the same experimenter. A thin layer of sawdust was collected from the nest and deposited in the palm of hand to avoid any emotional stress of a new environment.

The Morris Water Maze experiment:

The water maze is one of the most commonly used tests to measure learning and memory, and was developed by **Morris (1984)**. This test is based on the natural tendency of animals which, placed in a stressful and confined environment, attempt to escape **(Morris et al., 1982)**. It enables learning, memory, and spatial working to be studied with great accuracy, and can also be used to assess damage to particular cortical regions of the brain.

The basic procedure for the Morris water navigation task is that the rat is placed in a large circular pool (approximately 1.50 m in diameter and 60 cm high, half-filled with water of 22 ° C ± 1) ,and is required to find an invisible platform that allows it to escape the water by using various cues (Figure 2). The pool is virtually divided into 4 quadrants: North-East (NE), South-East (SE), South-West (SO) and North-West (NO). The test is carried out in 2 stages over 5 consecutive days. The first step is an acquisition or learning phase, it lasts 4 days with 4 tests per day. During this step the platform is always placed in the same place (NO). The starting points vary in the same session, and the starting order varies from day to day (NE, SE, SW, NO). Before the start of the acquisition phase, the rat is placed on the platform for 30 seconds, in this way, he finds his way through cues and learns that there is an escape **(Kahloula, 2010)**. In the acquisition phase, the rat is then placed at the starting point, with its head facing the wall at one of the four cardinal points. The time spent reaching the escape platform is recorded **(Lodiot, 2009)**. Each test lasts 60s. If the rat does not find the platform at the end of the test, he is gently pushed towards it, then left on the platform for 20s, and then got out of the pool and dried. Twenty to thirty minutes between each test are necessary for minimal rest of the animal.

24 hours after the last learning session, spatial memory performance is evaluated during the retention phase: 60s free trial (called retention time), without platform, where we measure the time spent in the quadrant where the platform was previously located.

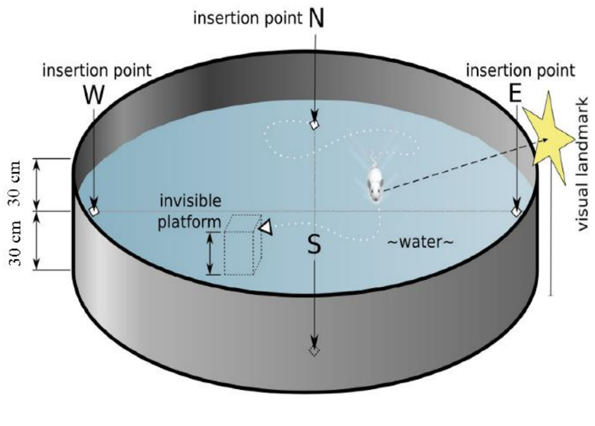

Figure 2. A Morris Water Maze. The animals should remember the position of the hidden (below surface) platform. The size and the visual landmark may be different.

Statistical Analysis:

Student's T-test was used to determine the difference between groups during behavioral tests using Addinsoft XLSTAT-Premium, version 2016. The results were presented as a mean±SEM.

The one-way analysis of variance (ANOVA) test was used to analyze the intragroup sex-effect on rat memory behavior by IBM SPSS Statistics 23.0. At $p<0.05$, the difference was considered significant.

RESULTS

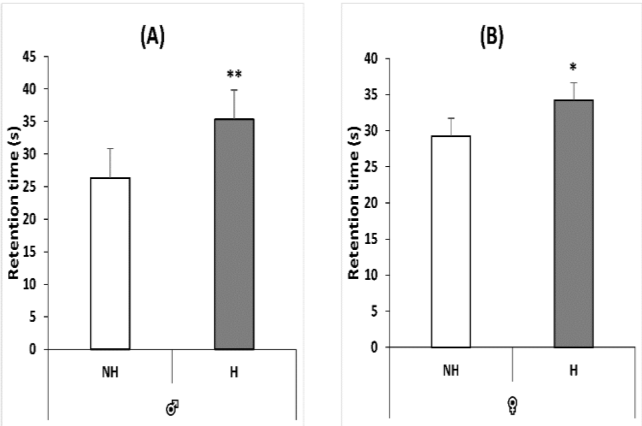

Figure 3. Effect of neonatal handling on the Morris water maze of rats of both sexes. A: retention time (in seconds) of male rats. B: retention time (in seconds) of female rats. of Values are expressed as Mean ± SEM and compared by Student test (Number of rats per group = 22; 11♂/♀). NH: Non-Handling, H: Handling, RS. *Indicates p value is <0.05. **Indicates p value is <0.01. Figure 3 illustrates the spatial memory and learning performance of rats during the Morris water maze test. There was a significant difference between groups in retention time for both sexes. The H♂ group had a higher retention time (35.36s±3.44) than the NH♂ group (26.36±9.44), and the same observation for the female group, indeed, the H♀ group also a higher retention time (34.18±4.03) than NH♀ group (29.27±6.41). The panels A and B revealed a strongly reduced memory behavior in the non-handling rats, compared to the handling group (♂:t= -2.97, p= 0.008; ♀: t= -2.10; 0.048), who showed an increase in the time spent in the quadrant where the escape platform was. **Sex effect on retention time of handled and no handled rats (One-way ANOVA):**

Table 1: Sex-dependent effect on Morris Water Maze in NH rats (One-way ANOVA). NH: Non-Handling group (Number of rats: 22 with 11 rats of each sex). F: Fisher-statistic. DF: degrees of freedom. Sig: significance (p-value).

	NH			
Effect	Mean Square	F	ddl	Sig
Sex	46.55	0.716	1	0.408

Table 1 shows that the sexual factor does not significantly affect the retention time of No handled rats in MWM (p= 0.408, F=0.716).

Table 2: Sex-dependent effect on Morris Water Maze in H rats (One-way ANOVA). H: Handling group (Number of rats: 22 with 11 rats of each sexes). F: Fisher-statistic. df: degrees of freedom. Sig: significance (p-value).

	H			
Effect	Mean Square	F	ddl	Sig
Sex	7.68	0.505	1	0.485

Table 2 shows that the sexual factor does not significantly affect the retention time of Handled rats in MWM (p= 0.485, F=0.505).

DISCUSSION

The purpose of this study was to determine the extent to which human handling, applied from birth to weaning, modifies learning and memory behavior in adult male and female rats, and thus verify whether human interaction during the neonatal period of rats has any impact in adulthood.

Memory is defined as, the process of acquiring, retaining, and then rendering information **(Delacour, 1984)**. From the experimental point of view, in animals, memory obviously cannot be evaluated in verbalized form, however, spatial memory disorders do not necessarily require verbalization in their assessments. This form of memory, which integrates the spatial orientation and learning capacities, is therefore more accessible to evaluate in animals.

It is well known that in early development, central nervous system has a high plasticity and can be very sensitive to even moderate environmental interventions **(Gschanes et al., 1998 ; Inazusta et al., 1999 ; Sternberg & Ridgway, 2003 ; Zhang & Cai, 2008)**, therefore, early life experiences have long-term effects on behavior and stress responsiveness **(Padoin et al., 2001).** Several studies have shown that distinct stimuli during the early life affect the behavioral, physiological and endocrine processes in adult rats **(Casolini et al., 1997; Pham et al., 1999; Wigger & Neumann, 1999; Kalinichev et al., 2002; Knuth & Etgen, 2007; Kosten et al., 2000; Kosten et al., 2004).**

Spatial memory is an important cognitive function, which depends mainly on the integrity of the hippocampus and also of the prefrontal cortex **(Becker et al., 1980; Brito et al., 1982; Meck et al., 1984; Brito & Brito, 1990; Lenck-Santini et al., 2001; Lenck-Santini et al., 2002)**.

Our results report changes in learning behavior and spatial memory in adulthood of rats of both sexes, induced by treatment applied during the neonatal period. We found that neonatal handling improved long-term memory behaviors in male and female rats. The time spent in the quadrant where the platform was located was higher in the handling group, a sign of improved long-term memory capacity **(Morris et al., 1982).** The increased time traveled in this quadrant within the Morris water maze also suggests that learning was more developed in the manipulated rats. This results are consistent with those of **Wilson et al., (1986); Tang & Zou, (2002) ; Akers et al., (2006); Tang et al., (2006)** who report that new external stimuli, such as early tactile manipulation, improve long-term hippocampal potentiation, learning and spatial memory.

Neonatal handling is similar to maternal affection and licking **(Schanberg & Field, 1987)**, and therefore, has a strong influence on normal brain development **(Chiba et al., 2012)**. Tactile stimulation has been shown to affect the function of the hypothalamic-pituitary adrenal axis (HPA) **(Katsouli et al., 2014)**, enhance the adult's ability to adapt to stressful stimuli **(Stamatakis et al., 2009)**, and assist in the recovery of neonatal brain injury **(Rodrigues et al., 2004; Richards et al., 2012)**. Moreover, it has been shown that also postnatal handling in an early life experience could induce neuro-chemical, physical and psychological changes in the offspring. Thus, it allows greater psychological and physiological adaptation to stressed adults **(Cirulli et al., 2003; Pryce & Feldon, 2003; Weaver et al., 2004; Imanaka et al., 2008)**, and to have a long-term effect on adult behavior **(Giachino et al., 2007)**.

No specific sex effect results were observed in the handled group, which is in agreement with the various studies published on memory **(Clark et al., 2000; Chopin et al., 2002; Izaute & Bacon, 2005).** The memory abilities and learning would be more related to age **(Clark et al., 2000).**

In summary, the results presented here provide further evidence on the beneficial effects of neonatal handling on learning and spatial memory. The main conclusion of this work is the beneficial effect of physical manipulation during postnatal brain development on the neurodevelopment of the hippocampus and the prefrontal cortex, regions responsible for learning and spatial memory.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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