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Elevated plus-maze test in handled rabbits at nursing period

Teste do labirinto em cruz elevado em coelhos estimulados tatilmente durante a fase de aleitamento materno

Prueba del laberinto en cruz elevado en conejos estimulados tatilmente durante la fase de lactancia materna

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ABSTRACT

The temperament in animals is a set of behaviors expressed in relation to human handling. The use of positive stimulation can affect the animals cognition, learning, and behavior. This study aimed to compare stimulated and unstimulated rabbits at different ages and its effects on temperament using the elevated plus-maze test. The animals were divided into five treatments (tactile stimulation of the first at 30 days, the first at 10 days, from 10 to 20 days, from 20 to 30 days and unstimulated). The test was performed at 30, 45, 60 and 75 days of age. The stimulated rabbits showed high latency in the open arms, low latency for first entry into the open arm and high behavior frequency while the unstimulated rabbits showed the inverse. By the elevated plus-maze test, customarily used for rats, it was possible to have a better evaluation between stimulated and unstimulated rabbits showing that tactile stimulation during lactation has positive effects and persist throughout the growth of the rabbits.

Key words: elevated plus-maze, tactile stimulation, kits, rabbit production

RESUMO

O temperamento em animais é um conjunto de comportamentos expressos em relação ao manejo humano. O uso de estimulações positivas podem afetar a cognição, o aprendizado e o comportamento dos animais. Este trabalho propôs comparar coelhos estimulados, de não estimulados em diferentes idades e seus efeitos no temperamento utilizando o teste de labirinto em cruz elevado. Os animais foram distribuídos em cinco tratamentos (estimulação tátil do primeiro aos 30 dias, do primeiro aos 10 dias, dos 10 aos 20 dias, dos 20 aos 30 dias e não estimulados). O teste foi realizado aos 30, 45, 60 e 75 dias de idade. Os coelhos estimulados apresentaram alta latência nos braços abertos, baixa latência para primeira entrada no braço aberto e alta frequência de comportamentos enquanto os coelhos não estimulados o inverso. Com o teste do labirinto em cruz elevado, usualmente utilizado para ratos, foi possível ter uma melhor avaliação entre coelhos estimulados e não estimulados mostrando que a estimulação tátil durante a lactação tem efeitos positivos e perduram ao longo do crescimento dos coelhos.

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Palavras–chave: cunicultura, estimulação tátil, labirinto em cruz elevado, láparos,

RESUMEN

El temperamento en animales es un conjunto de comportamientos expresados en relación al manejo humano. El uso de estimulaciones positivas puede afectar la cognición, el aprendizaje y el comportamiento de los animales. Este trabajo propuso comparar conejos estimulados en diferentes edades de no estimulados y sus efectos en el temperamento utilizando la prueba de laberinto en cruz elevada. Los animales fueron distribuidos en cinco tratamientos (estimulación tatil del primero a los 30 días, del primero a los 10 días, de 10 a 20 días, de 20 a 30 días y no estimulados). La prueba se realizó a los 30, 45, 60 y 75 días de edad. Los conejos estimados presentaron alta latencia en los brazos abiertos, baja latencia para primera entrada en el brazo abierto y alta frecuencia de comportamientos mientras que los conejos no estimulados lo contrario. Con la prueba del laberinto en cruz elevado, usualmente utilizado para ratas, fue posible tener una mejor evaluación entre conejos estimulados y no estimulados mostrando que la estimulación táctil durante la lactancia tiene efectos positivos y perdura a lo largo del crecimiento de los conejos.

Palabras clave: cunicultura, estimulación táctil, laberinto en cruz elevado, láparos

Introduction

The way in which an animal deals with a situation, can be categorized in different styles, with a pro-active animal or a reactive animal facing the situation, but showing fear (Verwer et al., 2009). The reactions of an animal can also indicate the welfare through physiological and psychological indicators (Verga et al., 2007). This temperament expressed by animals can change according to the management employed in an animal and can be positive or negative. If the differences in behavior truly reflect differences in reaction characteristics, behavioral responses in a situation must match the behavioral responses in other situations (Verwer et al., 2009). Thus, if the handling leads to different reactions, the behavioral responses may be expressed similarly in various behavioral tests (Verwer et al., 2009).

Tactile stimulation is a positive handling performed during lactation that besides improving the trust of the animal in man may influence cognition, learning ability and social behavior. The stimulation produced increase the brain weight in rats, suggesting that external stimulation can increase the number and size of nerve cells by increasing the amount of DNA and RNA (Lima et al., 1999).

There are several tests to evaluate the effects of stimulation in temperament, through fear responses in relation to human, objects and new environments. Results presented by Zucca et al. (2012) indicated that one minute per day in positive manipulation of kits during the first seven days of life was efficient to reduce the fear of new environments and of the human being.

The elevated plus-maze test is conducted in rats to evaluate diverse effects on reactions of fear and anxiety by interaction of the animal with both open and closed areas of the maze (Pinto et al., 2012). Thus, it was proposed in the study to evaluate the effect of tactile stimulation during the different stages of rabbits lactation using the elevated plus-maze.

Material and Methods

Animals

This study was developed in the Rabbit´s Department at the Faculty of Agrarian and Veterinary Sciences, Unesp Campus Jaboticabal, São Paulo, Brazil. The ration was commercial and pelletized Presence Coelhos (Presence®) for pregnant and lactating females (17% Protein, 2.5% Ethereal Extract, 17% Fiber, 10% Mineral, 1.0% Calcium, 0.5% Phosphorus and 13% Moisture) and Linha do Campo Coelhos (Presence®) for growing rabbits (14% Protein, 1.5% Ethereal Extract, 20% Fiber, 15% Mineral, 1.1% Calcium , 0.5% Phosphorus and 13% Moisture) for growing rabbits, supplied daily in semi-automatic feeders, water ad libitum. The rabbits were housed in suspended galvanized wire cages (80x60x40 cm - 4800cm2) in semi-open shed with East-West orientation.

The reproduction was carried out with 20 matrices of the Botucatu genetic group (Moura et al., 2001), mated with five males of the same lineage and separated into five treatments. Shortly after birth the litters were leveled and randomly distributed among the lactating animals (Fleischhaner et al, 1985; Poigner et al., 2000). Each matrix had free access to the nest throughout the lactation. After birth the kits have been stimulated from 6PM for three minutes. The pelage was marked on the outside of the ear and dorsal region using aniline dye. Weaning occurred at 30 days of age and the rabbits were collectively housed (six animals per cage - 800cm2 / rabbit) according to each treatment, with wooden platforms cages of 56 x 30cm positioned on one side of the cage as environmental enrichment (WRSA, 2009).

Handling and Elevated plus-maze test

The tactile stimulation consisted of containing each kit individually with one hand and with the other stroking its back, with soft, repetitive movements according to each treatment (Riul et al., 1999; Cabral, 2003). Treatment 1: from the the first to 30 days of age (n = 23). Treatment 2: from the first to 10 days of age (n = 26). Treatment 3: from 10 to 20 days of age (n = 21). Treatment 4: from 20 to 30 days of age (n = 25). Treatment 5: unstimulated (n = 23).

The elevated plus-maze test (Handley; Mithami, 1984) was perfomed at 30 (weaning), 45, 60 and 75 days of age. Each rabbit was positioned in the center of the maze, 60 cm above the floor, 60 cm of length, 60 cm of height and 30 cm wide. Input frequency was recorded by visual observation for five minutes. Also was observed the time spent in opened and closed arms, the latency of the first entry and arrival at the end of the opened arms, number of stretched attend postures (SAP) on the opened arm, frequency of dives (head-dips) and frequency of lifting position in the closed arm (rearing) (Almeida et al., 1993).

Data Analysis

Nonparametric one-way ANOVA was used to analyze the latency and number of approaches in SAS (2003), Kruskal-Wallis test to compare treatments and Friedman test for the ages in each treatment with multiple comparisons by Dunn test in GraphPad Prism 4. Level of significance 5%.

Results

The latencies in the open arms differed among treatments at weaning (X2 = 23.94, p <0.001) (Figure 1A), the lower latencies were the stimulated from 10 to 20 days of age. The latencies for stimulated throughout lactation were higher at all ages compared to the other groups. The latency decreased over time within each treatment group but differing in the group stimulated throughout nursing (F = 14.03; p = 0.003) (Figure 1B) and from 20 to 30 days of age (F = 10.3; p = 0.016) (Figure 1C).

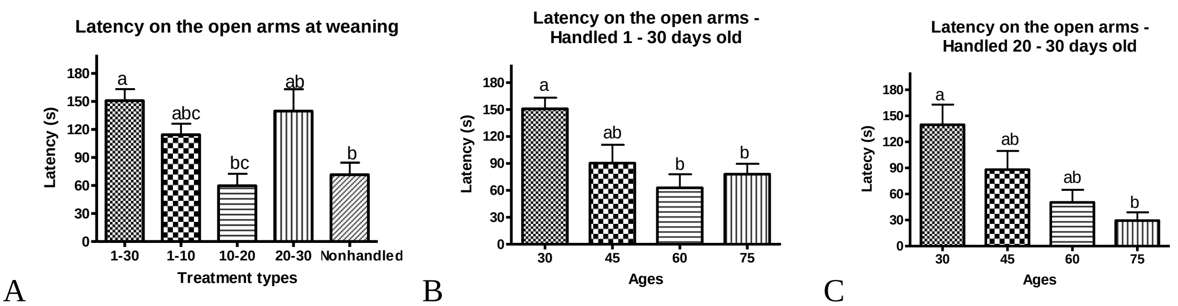


Figure 1. Mean latency time (+ *SE*) on the open arms. Latency handled rabbits at 30 days-old (A). Handling of 1 to 30 days-old (B). Handling of 20 to 30 days-old (C). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The latencies in the closed arms (Figure 2) presented differences between the weaning treatment (Figure 2A) and at 75 days of age (Figure 2B). At weaning (Figure

2A), the kits stimulated from the first to 30 days had lower latency in the closed arms of differing only from the ones stimulated from 10 to 20 days with higher latency (X2 = 12.02; p = 0.02). At 75 days of age (Figure 2B), the kits stimulated during the first 10 days had higher latency differing from all stimulated groups and did not differ from the non-handled group (X2 = 20.81, P <0.001). The latencies increased with time differing for rabbits stimulated from the first to 30 days (F = 13.38; p = 0.004) (Figure 2C) and stimulated ones during the first t 10 days of life (F = 19.73, p <0.001) (Figure 2D).

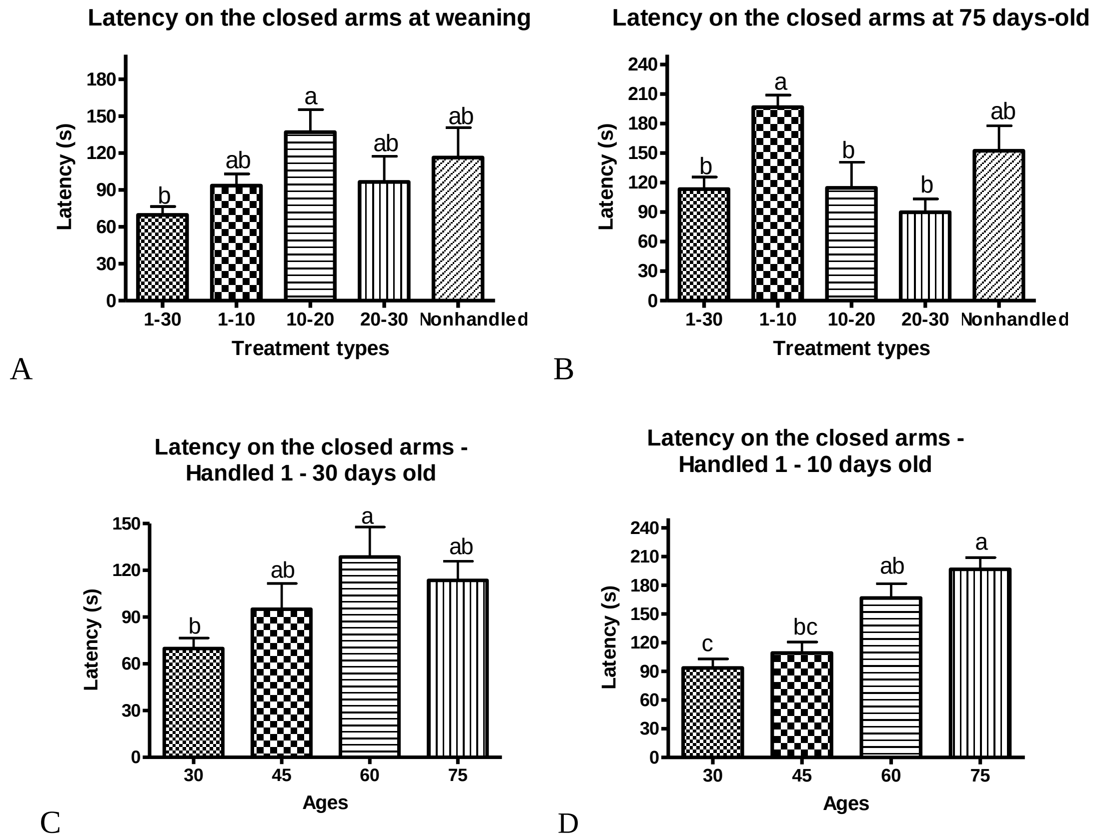


Figure 2. Mean latency time (+ *SE*) on the closed arms. Handled rabbits at 30 days-old (A). Handled rabbits at 75 days-old (B). Handling of 1 to 30 days-old (C). Handling of 1 to 10 days-old (D). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The latencies for the first entry in the open arms did not differ among treatments nor over time within each treatment (p> 0.05), but it was observed that the latencies increase around 60% at 60 days of age and decrease at 75 days.

The arrival at the end of the open arms (Figure 3) showed differences between treatments at 30 and 75 days of age. At weaning the rabbits stimulated since the first day of life showed higher frequencies differing from the ones stimulated from the 10 to 20 days and from the unstimulated rabbits (X2 = 25.83, P <0.001) (Figure 3A). At 75 days unstimulated rabbits showed lower frequencies (Figure 2B) differing from stimulated ones from the first to 30 days of age with higher frequency (X 2 = 14.28; p = 0.006). The frequency decreased with age differing from the stimulated rabbits during the first 10 days (F = 15.18; p = 0.002) (Figure 3C).

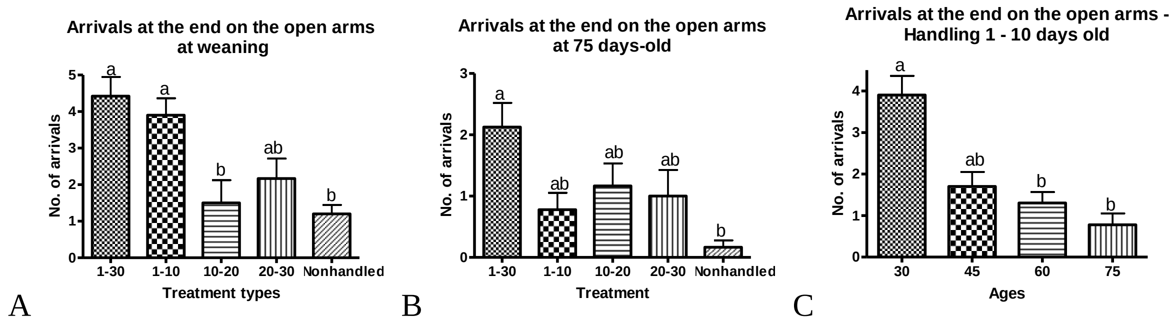


Figure 3. Mean number of arrivals at the end on the open arms (+ *SE*). Handled rabbits at 30 days-old (A). Handled rabbits at 75 days-old (B). Handling of 1 to 10 days-old (C). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The number of attempts to enter the open arms (Figure 4) showed differences between treatments at weaning and 75 days of age and among the ages stimulated for the first 10 days (F = 14.43; p = 0.002) (Figure 4C). At weaning (Figure 4A) the stimulated rabbits in the first 10 days had higher frequencies differing from the other treatments (X2 = 24.19; p <0.001). At 75 days (Figure 4B) stimulated rabbits from 20 to 30 days showed higher frequencies differing from the ones stimulated in the first 10 days with lower frequencies (X2 = 13.78; p = 0.008).

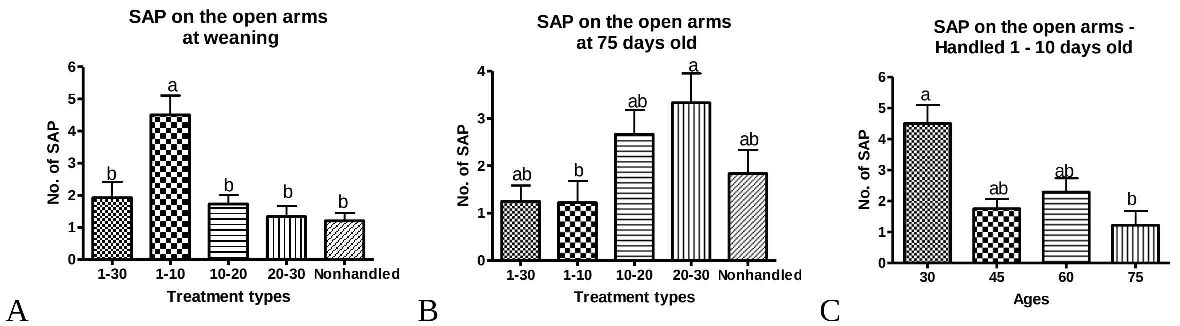


Figure 4. Mean number of stretched attend postures (SAP) on the open arms (+ *SE*). Handled rabbits at 30 days-old (A). Handled rabbits at 75 days-old (B). Handling of 1 to 10 days-old (C). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The number of entries in the open arms (Figure 5) differed at weaning and 75 days of age. At weaning (Figure 5A) the stimulated rabbits from 10 to 20 days had lower frequencies differing from the stimulated ones since the first day that showed higher frequencies (X2 = 21.4, p <0.001). At 75 days (Figure 5B) the stimulated group from 20 to 30 days and unstimulated rabbits had the lowest frequencies differing from the other groups (X2 = 19.93; p <0.001). Over time the frequency of entries into the open arm decreased significantly differing from the stimulated rabbits from the first to 30 days (F = 9.18; p = 0.03) (Figure 5C) within the first 10 days of life (F = 16.32; p = 0.001) (Figure 5D) and non-handled ones (F = 13.30; p = 0.004) (Figure 5E).

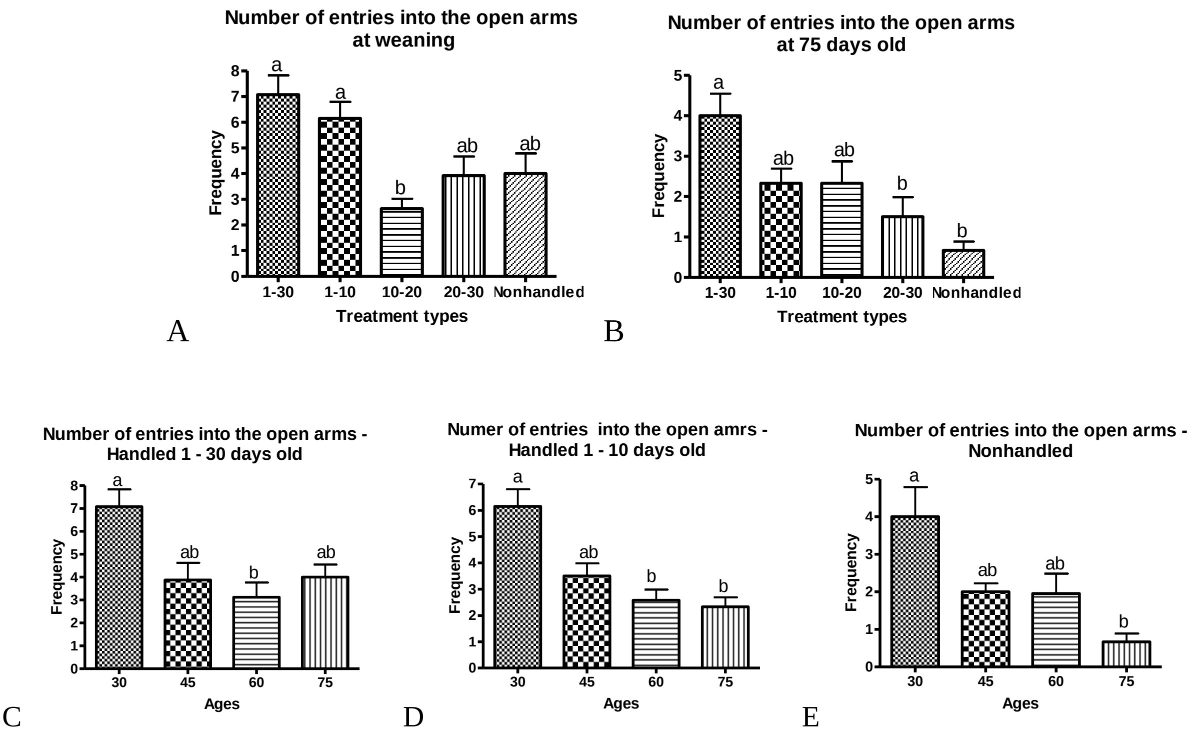


Figure 5. Mean number of entries into the open arms (+ *SE*). Handled rabbits at 30 days-old (A). Handled rabbits at 75 days-old (B). Handling of 1 to 30 days-old (C). Handling of 1 to 10 days-old (D). Non-handled rabbits (E). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

For the frequencies of closed arm entries (Figure 6) there were significant differences at weaning and 60 days old. At these ages the rabbits stimulated at the first 10 days had higher frequencies differing at weaning from the ones stimulated from 20 to 30 days and from the ones non-handed with lower frequencies (X2 = 24.93; p <0.001) (Figure 6A) and at 60 days of age from the non-handled rabbits (X2 = 14.26; p = 0.006) (Figure 6B).

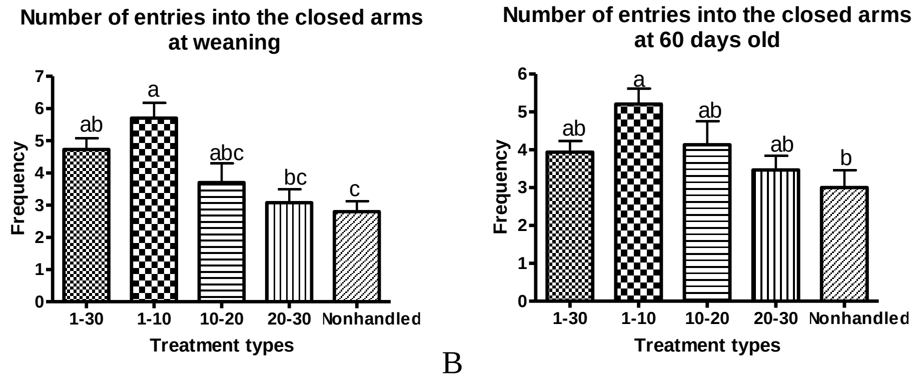


Figure 6. Mean number of entries into the closed arms (+ *SE*). Handled rabbits at 30 days-old (A). Handled rabbits at 60 days-old (B). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The opened arms diving frequencies showed differences at 30, 45 and 75 days old (Figure 7). At weaning the stimulated kits from the first day of life showed higher frequencies differing from stimulated from 10 to 20 days with lower frequencies ($X2 = 17.11$; $p = 0.002$) (Figure 7A). At 45 days of age stimulated from 10 to 20 days of age showed higher frequencies differing from unstimulated ($X2 = 10.87$; $p = 0.03$) (Figure 7B). After 75 days of age the kits stimulated for the whole lactation period showed high frequencies differing from the unstimulated ones ($X 2 = 17.95$; $p = 0.001$) (Figure 7C).

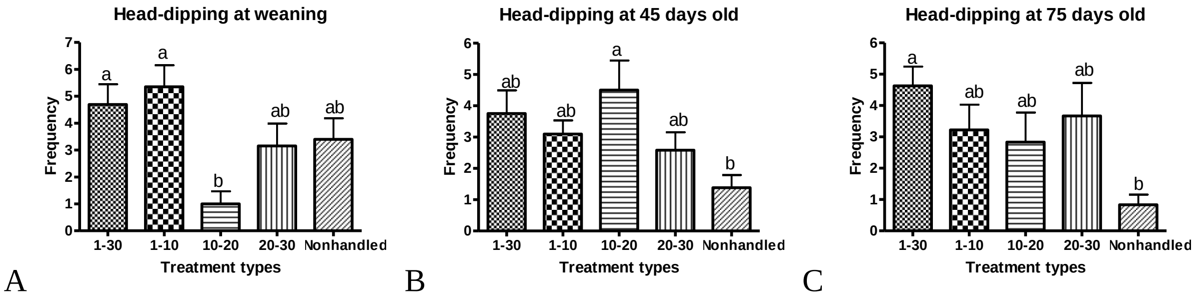
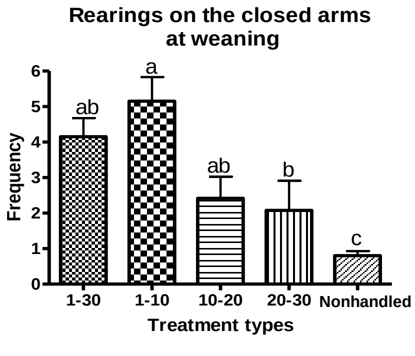


Figure 7. Frequency of head-dipping on the arms (+ *SE*). Handled rabbits at 30 days-old (A). Handled rabbits at 45 days-old (B). Handled rabbits at 75 days-old (C). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

The frequency of rearings on the closed arms differences occurred at weaning among non-stimulated kits that presented low frequencies and the other treatments ($X2 = 28.59$; $p < 0.001$) (Figure 8A). At weaning the kits stimulated during the first 10 days showed high frequencies differing from the frequencies at 45 days of age ($F = 10.41$; $p = 0.015$) (Figure 8B).



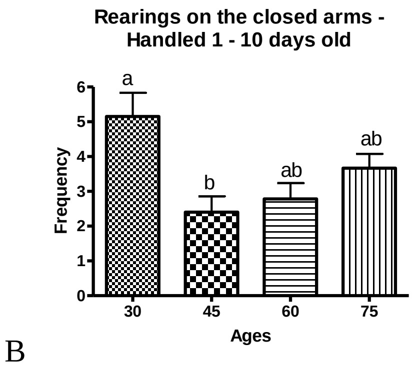


Figure 8. Frequency of rearings on the closed arms (+ *SE*). Handled rabbits at 30 days-old (A). Handling of 1 to 10 days-old (B). Significant differences between groups are indicated with different letters (Dunn's Multiple Comparison Test).

Discussion

It was observed that at 30 days of age the stimulated kits spent more time in the opened arms, differently from the stimulated ones from 10 to 20 days of age. The more intense the exploration of the open arms the less the anxiety (Pellow et al., 1985).

At the 45 days the stimulated kits entered faster in the opened arms in comparison to the non-stimulated ones, which also spent more time in the closed arms representing a high signal of anxiety and fear for the non-stimulated kits.

At the 60 days of age, the latencies for all groups were similar and remained longer in the closed arms however the non-stimulated group took longer for initial entry into the opened arms. At the 75 days of age all groups spent more time in the closed arms. Kits stimulated since the first day of life and from 10 to 20 days of age had lower latencies to first entry in the opened arms and spent more time compared to non- stimulated and stimulated from 20 to 30 days of age which presented higher signal of anxiety and fear.

According to Pellow et al. (1985) and Severiano et al. (2004), the handled animals spent more time in the opened arms than the non-manipulated group, which is an indicative of lower anxiety agreeing with the data found in this study.

The behavior of rearings refers to exploring new environments, the head-dips the risk assessment and stretched attend postures an exploratory attitude (Almeida et al., 1993) which this work the stimulated kits showed higher frequencies compared to non-handled kits even over time.

Regarding the frequencies of behaviors performed at 30 days of age, the kits stimulated since birth had high frequencies in all behaviors and lower frequencies attempts to opened arm entries, signal of low anxiety and fear. At 45 days of age the stimulated animals had higher frequencies than the non-stimulated kits, signal of high anxiety and fear for the non-stimulated ones.

The stimulated kits from the first to the weaning day continued to show signs of minor anxiety and fear at 60 and 75 days of age by having higher arrival frequencies to the end of the opened arms, low frequency of enter attempts on the opened arms, higher number of entries on the opened arms and dives. With time there was no habituation by the non-stimulated rabbits at 75 days old, since they continued to have low frequency of arrival at the end of the opened arms, number of entries into the opened arms and dips, signs of fear and anxiety.

Higher affinity to explore new stimuli was found by Denenberg et al. (1977) in manipulated rabbits that can also be confirmed by the elevated maze test in cross carried out in this work. According to the behavioral tests the stimulated rabbits are less shy animals, less fearful and more curious. Reactive animals have shown long latencies, few approaches and few movements (Verwer et al., 2009).

The results of Zucca et al. (2012) showed that one minute a day handling the kits in the first seven days of life was enough to reduce the fear of new environments and the human being. Verwer et al. (2009) stated that stimulation of kits during lactation is independent of breastfeeding and the rabbit lifetime, it can be very effective in promoting the domestication, less emotional and more cooperative animals. Csatadi et al. (2005) studied the effect of minimal human contact in kits from White New Zealand. They were manipulated half an hour after nursing and they became meek adults independent of the duration of the stimulus.

Bilkó and Altbäcker (2000) found that all animals handled on the first week of nursing showed higher affinity with humans as compared to animals handled on the following three weeks, which were too timid compared to non-handled rabbits. Supposedly the answer to handling is a process of "imprinting" (identifying and learning process in a specific period of the animal life) that begins soon after birth (Pongrácz and Altbäcker, 1999) when the kits learn the smell of humans (Bilkó and Altbäcker, 2000). The process of meekness involves some sort of learning by the animal (Bilkó and Altbäcker, 2000) and fear reducing caused by handling in childhood can be interpreted as simple habituation to humans (Jones and Faure, 1981; Hemmer, 1990; Jones and Waddington, 1992) or a reduction of emotionality (Denelsky and Denenberg, 1967). To Jones (1993), the animals simply learn that the human contact is not necessarily dangerous or unpleasant.

Many authors agree that adaptation to mild stress, which occurred in the postnatal handling of kits, can reduce emotional reactivity or anxiety and may promote a higher ability to deal with stressful situations (Daly, 1973; Meaney et al, 1988; Fernández-Teruel et al., 1990). The lasting effect can be explained by the increased learning ability perhaps associated with interest-induced by stimulation during childhood, which can enhance catecholamine activation (Wilson et al., 1986).

Conclusions

The elevated plus-maze test was efficient to express the temperament of rabbits and differentiate stimulated rabbits from unstimulated rabbits. Unstimulated rabbits showed fear and anxiety indicating that the tactile stimulation improves the reaction of rabbits related to stress in new environments and human-rabbit relationship.

Acknowledgment

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