Effects of Early Handling on Rat Performance in a Radial Arm Maze Task

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Abstract

Early life experiences can produce notable differences in the behavioral development of rats. The two heavily researched experience conditions are early handling (EH), which serves as a minor stressor by removing the rat pup from the mother for a brief period of time, or maternal separation (MS), which is an isolation condition that serves as a major stressor. The present study used 27 Wistar rat pups split into a non-handled condition and 15-minute EH condition. After the handling and weaning, the rats were tested in a treat-baited radial arm maze task to assess the differences in performance. It was hypothesized that the male rats in the EH condition would exhibit the best performance, while the female rats of the EH condition would exhibit the worst performance. The data analysis revealed that the hypothesis was not supported: there were no significant differences in the performances of either the male or female rats, or the rats in the handled or non-handled conditions. It is suggested the radial arm maze task does not measure memory development in the same way that other assessments do.

Literature Review

Early life experiences, such as exposure to stressors, have significant influences on the development of rat brains and, in turn, on adult behavioral phenotypes of rats as well; too much stress can cause damage (Frankola, Flora, Torres, Grissom, Overstreet, and Dohanich, 2010). In most research on early life experiences, maternal separation (MS) serves as a stressor: the dam (mother) is taken from the pups for an extended period of time, making the pup lose out on maternal nourishment and comfort. Findings show that maternal separation can be quite damaging to the development of pups. Of course, when damage is possible to be avoided or mitigated, it is advantageous to do so. One of the best methods of risk avoidance is prevention, which only works after the identification of risk factors (Kosten, Kim, & Lee, 2012). Early handling (EH) of rats is one method used in many studies to increase stress tolerance. These short separations from the dam serve as minor stressors that the rats become accustomed to and lessen the animal's stress response. The pup is taken from the dam and placed individually into a small container for a brief period of time. The maternal behavior increases during the period immediately after the pup is returned to the dam (Frankola et al., 2010). The aim of the present research is to better understand the extent of the consequences of these experiences.

Kosten et al. (2012) collected the findings of research that investigated ideas that early life stress can inhibit proper development in rats. Of particular interest here are hormonal and neuronal effects of early life manipulations, behavioral effects and motor coordination, spatial/relational memory tasks, hippocampal development, and sex differences.

Frankola, Flora, Torres, Grissom, Overstreet, and Dohanich (2010) found that varying degrees of maternal separation can influence development, especially focusing on cognitive development. They also acknowledge that female rats tend to be less cognitively impaired by chronic stress, so they expected males to perform worse on experimental tasks. In this study, rat pups were placed into one of three groups: control, 15-minute handling, or 180-minute maternal separation. The handling condition served as a positive-environment group, while the 180-minute maternal separation (MS) condition serves as a negative-environment group. Frankola et al. (2010) hypothesized that the rats in the MS group would exhibit the lowest performance on object recognition tasks, Y-maze tasks, and reference and working memory versions of the Morris Water Maze. The object recognition task consisted of introducing a rat to an environment with two identical stimuli. The rats were then removed, environments cleaned, and one of the objects was replaced with a new object. Interest in novel stimuli was measured by how long the animal spent investigating the new object. The Y-maze was similar: instead of having objects in the environment, the rat was in a Y-shaped chamber with one arm blocked. After the initial period in the chamber, the rat was removed and later reintroduced to the environment with all three arms of the Y open (spatial memory was recorded by measuring how much time the rat spent in the previously closed arm).

In the majority of the tests, the male handling group performed better than the male MS group. In contrast, the female MS group typically performed better than the female handling group. Overall, male MS performed significantly worse than all other conditions. Frankola et al.'s hypothesis was supported: separation of pups and dams caused developmental impairment in pups, which produced significantly worse performance than both the control and handling conditions, except in the female pups. The researchers involved with Frankola et al. believe that this effect may be attributed to the dam's androgen production, which causes them to attend more to their male offspring. Because the female pups naturally receive less attention from the dams, it is possible that the maternal separation simply does not influence the females as strongly (Frankola et al., 2010).

Other researchers have focused attention on behavioral aspects of the influence of handling and maternal separation. Spivey, Barrett, Padilla, and Gonzalez-Lima (2008) used Holtzman rats because of their tendency to show depressive-like behavioral responses to stress. In this study, the groups consisted of control, 15-minute early handling (EH), and a group with an extended MS period of 6 hours. The experiments conducted were open-field activity and defensive withdrawal in a light-dark apparatus. Open-field activity is a measure of the rat's exploratory behavior and involves monitoring the amount of time spent roaming the environment in comparison to staying in one position. The defensive withdrawal trials consisted of a lit, open-air chamber connected to a covered and dark auxiliary chamber. Anxiety-like behavior was measured by time spent in the covered auxiliary chamber.

Results showed that in every test, the Holtzman rats of the MS group showed significantly lower levels of open-field activity than the other groups: they were more inhibited, less active, and more fearful. The EH group showed increased risk-taking behavior, which suggests that handling and maternal interaction aid in the coping of stress (Spivey et al., 2008).

Further research shows that postnatal stress may influence outward behavior, such as activity, anxiety, and exploration (Skripuletz, Kruschinski, Pabst, von Hörsten, & Stephan, 2010). In the majority of the tests, the MS group performed, yet again, in the direction of increased anxiety-like behavior, less exploratory responses, and inhibited behavior (results determined through usage of open-field activity testing, hole-board exploration, and activity in the home cage). Skripuletz et al. acknowledges that other research that suggests there is no difference in behavior (2010). However, Farkas, Reglodi, Gaszner, Szogyi, Horvath, Lubics, Tamas, Frank, Besirevic, and Kiss (2008) determined that while motor coordination and reflex development may be considered behavioral characteristics, no significant difference in performance was found between the control and the MS groups.

The previous studies (Frankola et al., 2010; Spivey et al., 2008; Skripuletz et al., 2010; and Farkas et al., 2008) all highlight the behavioral influences of handling and MS. Generally, the mild stressor of handling increases performance, while the severe stressor of MS impairs it. Now that a generic trend has been clearly detailed, it is logical to explore the neurological basis for these effects.

The hypothalamus-pituitary-adrenal axis (HPA axis) plays a great role in the down-regulation of the stress response. In the HPA axis, the hippocampus receives the cortisol (a neurotransmitter that greatly influences stress response) and begins to reestablish equilibrium. Meaney, Aitken, Bodnoff, Iny, and Tatarewicz (1985) showed that pre-weaning handling had a significant effect on hippocampal development of rats. In this study, the rats were split into handled and non-handled groups, with each group being divided again into social and isolated housing (handling and housing served as the only variables). After 135 ±15 days, rats were euthanized in order to study the targeted brain regions: frontal cortex, hippocampus, hypothalamus, amygdala, septum, and pituitary. The only developmental differences were found in the hippocampi of the handled rats. In these rats, 34-37% more glucocorticoid receptors were present in the hippocampus. The presence of more receptors aids the rat because it promotes a faster return to baseline levels after the termination of a stressor. The findings suggest that early postnatal handling influences the development of the glucocorticoid receptor system in the hippocampus and frontal cortex (Meaney et al., 1985). A review of the literature by Kosten et al. (2012) shows great support for this trend with most other studies finding similar conclusions.

Plescia, Marino, Navarra, Gambino, Brancato, Sardo, and Cannizzaro (2013) put female rats through object recognition tests as well as the Morris Water Maze after a period of handling (postnatal days 2-21). The results were in concordance with those of Meaney et al. (1985): that early handling aids in hippocampal development. Plescia et al. (2013) showed that early handling condition rats showed a recognition index over 50% in object recognition tests (preference to novelty), which was greater than the control group. During the Morris Water Maze portion of the research, EH females displayed a significant decrease in escape latency and path length. The researchers concluded that brief separation (EH) from the dam increases the maternal care, which helps in the development of hippocampal function and HPA axis response. Overall, Plescia et al. (2013) shows that EH is able to improve declarative, reference, and working memory.

Winkelmann-Duarte, Padilha-Hoffman, Martins, Schuh, Fernandes, Santin, Merlo, Sanvitto, and Lucion (2011) studied the differences in neuronal structures (neurons and glial cells) between rats in control and handled conditions. The rats in the control condition were completely left alone, while the rats in the handled condition were only briefly held by experimenters each day. Winkelmann-Duarte et al. (2011) compared development by having two sets of euthanized specimens. Some of the rats were euthanized on day 11, while others were euthanized later on day 90. The density of neurons in the handled group were higher than in the non-handled group as well as the density of glial cells, but neither of these results had a significant main effect of age. Cellular proliferation also increased in the hippocampi in the handled rat pups (Winkelmann-Duarte et al., 2011).

Sandstrom and Hart (2004) and Sandstrom (2004) used a radial arm maze to determine the effect of early experience on hippocampal development. The rats control condition stayed with the mother, and rats in the isolation condition were placed in a new environment without the dam or any other pups for six hours. The radial arm maze used in these studies consists of an apparatus with a central room that has twelve arms leading away from the center. Some of the arms have food at the ends of them, and some of them don't. The radial arm maze measures the number of reference memory errors and working memory errors. Once the trial periods have begun, a rat's choice to traverse an arm without food was qualified as a reference memory error, and a choice to traverse a previously visited arm qualified a working memory error. Sandstrom and Hart determined that the number of working and reference memory errors were greater in the isolation condition (six hours of daily isolation) rats than the control condition for the first three trials, but not for the last three trials.

At the end of the trials, the rats in both conditions exhibited the same level of performance, which indicated a significant main effect of block (number of trials completed), also known as a learning effect. Sandstrom and Hart also measured the corticosterone levels of the rats with blood samples. Sandstrom and Hart found significant main effects for treatment condition and age: the rats in the isolation condition had higher levels of corticosterone during early life as well as in adulthood (Sandstrom & Hart, 2004).

Sandstrom (2004) later replicated this study, but focused on the influences of biological sex. There was no difference between the sexes in the elevation of corticosterone: both male and female rats in the isolated condition had higher levels than the rats in the control condition. As for performance on the radial arm maze, the findings replicated those of Sandstrom and Hart (2004). The males exhibited a lasting impairment in working memory and the females did not show as many long-term consequences as a result of isolation stress. The performance of the females was generally unaffected by the isolation condition. They showed no impairment of memory retention during the radial arm maze trials. The Sandstrom (2004) study clearly indicates that sex is a factor in the consequences of early-life stressors.

The research in this field has heavily supported the idea that early life experiences (exposure to stress through maternal separation) can have an influence on the neural and behavioral development in rats. Meaney et al. (1985) and Plescia et al. (2013) identified a connection between early handling and hippocampal development, both neurologically and behaviorally. In their rats, they had increased glucocorticoid receptor sites and performed better in spatial relation tasks. Winkelmann-Duarte et al. (2011) found a clear relationship between development of neuronal structures and early-life environment. Sandstrom and Hart (2004) determined that isolated rats have higher levels of corticosterone, a chemical interacting with the hippocampal role in the HPA axis response to stress. Sandstrom's (2004) findings supported that of Frankola et al. (2010): females were less affected by early neonatal stressors. This research clearly indicates that early life stressors in the form of isolation impair both performance on memory tasks as well as neurological development, although the effect is not as strong in female rats as it is in male rats.

The research reviewed here has shown several trends: separation between pups and dams typically produces impaired task performance, depressive and anxiety-like behavioral phenotypes, and impaired brain development, especially in the hippocampus; early handling helps to build stress tolerance, improves working and reference memory, produces more risk-taking behavior, and aids in the development of the brain (and, again, primarily in the hippocampus); female rats of the MS condition tend to perform better than male rats overall.

The aim of the present study is to determine the influences that early life experiences can have on radial arm maze performance. The purpose of this study is to replicate the work done by Frankola et al. (2010), which found that early handling improved performance in object recognition tasks, Y-maze tasks, and reference and working memory versions of the Morris Water Maze. In this study, a radial arm maze task will be used to examine these effects. Consistent with previous results, it is predicted that EH will improve performance on the radial arm maze task (total number of arm entries, total number of errors, latency of maze completion, and number of unique arm visits in first eight decisions) and that the males will outperform the females.

Methods

Subjects

Twenty-seven Wistar rat pups (with dams) from three different litters were obtained from Charles River Laboratories. They were delivered on postnatal day (PND) 10. All animals had unrestricted access to food and water during resting periods and were housed in a room with a maintained 12h on/off light cycle at a constant temperature. Housing consisted of a clear plastic container roughly the size of a shoebox: 30cm in long, 18cm wide, and 10cm tall. On PND 15, the rats' tails were marked with a permanent marker in order to differentiate groups more easily. Pups were weaned by PND 21. From PND 21 and on, the animals were on a diet of standard lab chow (LabDiet 5001) and maintain free access to water. On PND 21, rats were separated from the dam and lived in cross-condition pairs.

Apparatus

The radial arm maze for this experiment was custom-built. The central platform had a diameter of 27.5cm, with eight 47cm long arms. Arms were 10cm wide with walls 15cm tall; the ends of the arms had a recessed cup for food that is .5cm deep and 35mm in diameter. Arms were covered with plexiglass sheets to prevent rats from climbing out. The maze was constructed from lightweight wood and finished with a clear coat to prevent splinters and to make for easy cleaning.

After approximately four days of dieting, the rats were introduced to the radial arm maze. The apparatus had eight arms extending away from a central platform at equal angles. The maze was placed in a well-lighted room with extramaze stimuli present.

Procedure

The experimental design is based on a study conducted by Meck, Church, and Olton (2013).

Handling condition

On PND 15, pups were divided and assigned to a control or a handling condition. Animals in the control condition were left alone with the dams. From PND 15 to PND 21, the animals designated to the handling condition were removed from the dam and placed into a sawdust-lined Styrofoam cup for a 15-minute interval once a day. During this same 15-minute period, the rats of the control condition were left alone. The animals were weighed on PND 21.

Training

On PND 22, the rats had restricted access to food until they reach 80% of their weight from the beginning of the restriction period. After reaching the target weight, the weight was maintained throughout the experiment by giving food for approximately 1 hour after testing (Olton, Meck, & Church, 2013).

For the first two days of training, food pellets were scattered along all arms of the maze. Each pair of rats was given 15 minutes to explore the maze and eat the pellets. For these two days, the rats were placed in the maze in pairs, as they are more focused on the task than when they are placed in the maze individually. During the next two days, the pellets were only placed at the ends of the arms, and then in the food cups. This stage persisted until each rat ran to the ends of the arms and ate the pellets. Testing began after this stage, on PND 27.

Testing

Each rat had one session in the maze for each of two days. Food pellets were placed only in the food wells at the end of the arm; the rat was placed in the center platform. The rat traversed the arms to find food pellets. An arm traversed was determined to occur when the rat had all four paws inside the arm. If the arm had food, and the animal ate the food, the decision was considered correct. If the rat revisited an arm, the decision was marked as an error. If the rat visited an arm with food and didn't eat the food, the decision was also be marked as an error. The trial continued until the rat had located and consumed each food pellet, or until the ten-minute time limit had been reached. Performance time was measured with a stopwatch – the timer began when the rat makes its first decision and was stopped when the rat consumed the final pellet.

Data Analysis

The test consists of two independent variables: a controlled variable of handling condition and a subject variable of the rat's sex. As for dependent variables, results were assessed as total number of arm entries, total number of errors, latency of maze completion, and number of unique arm visits in first eight decisions.

The data were analyzed with a series of 2x2x2 factorial analyses of variance, with handling (handled or not handled) and sex (M, F) treated as between subjects variables, with a separate analysis for each of the three dependent variables. If there were significant interactions, individual pairwise comparisons would be made.

Results

The independent variables for this study were days, treated as a within subject variables, and handling treatment (handling or no handling) and sex, treated as between subjects variables. The dependent variables were analyzed using a 2x2x2 mixed factorial ANOVA.

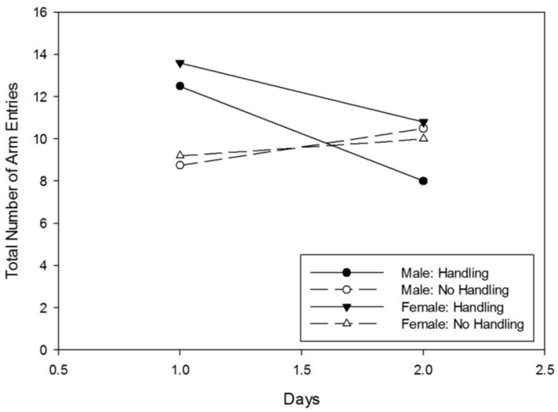

Figure 1. Effects of Within Subjects Variables (days) and Between Subjects Variables (sex and treatment condition) on the Total Number of Arm Entries

The results for number of arm entries are shown in Figure 1. From the first day of testing to the second day, the rats in the handled condition showed a decrease in total number of arms entered, while the rats in the not handled condition showed an increase in total number of arms entered. While the numbers of arm entries were different per treatment on the first day of trials, the difference in the numbers of arm entries on the second day were negligible. The analysis of the data show that there was a significant Days x Treatment Condition interaction for the total number of arm entries, $F(1, 23) = 7.27$, $p<.05$. There were no significant main effects or significant interactions with the other variables. It should be noted that there were a few extreme scores for a couple of the subjects on day 1, but the performance was much more "normal" on day 2. This may have contributed to the Day x Treatment Condition interaction.

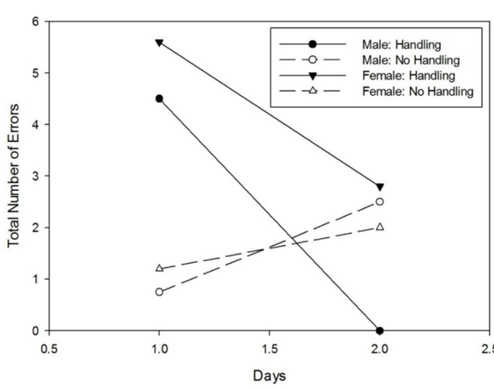

Figure 2. Effects of Within Subjects Variables (days) and Between Subjects Variables (sex and treatment condition) on the Total Number of Errors

The results for the number of errors are shown in Figure 2. On the first day of testing, the rats in the handled condition made several more errors than those in the not handled condition. On the second day of testing, the number of errors of each group was more similar. An analysis of variance revealed a significant Day x Treatment Condition interaction for the total number of errors made, $F(1, 23) = 7.27$, $p<.05$. There were no significant main effects or significant interaction with the other between subject variables.

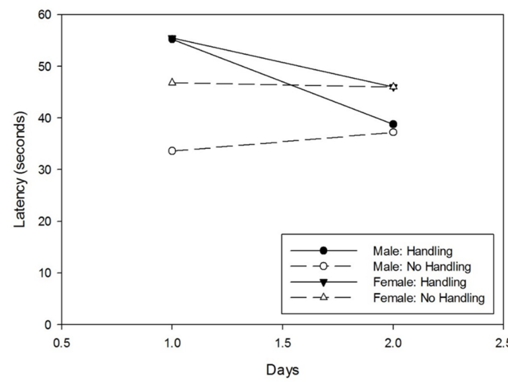

Figure 3. Effects of Within Subjects Variables (days) and Between Subjects Variables (sex and treatment condition) on the Latency of Maze Completion

The results for the latency of maze completion are shown in Figure 3. Though somewhat higher on day 1 for the handled rats, the latencies of the individual groups were similar on the second day. There was a significant Day x Treatment Condition interaction for the latency of maze completion, $F(1, 23) = 5.354$, $p<.05$. There was also a significant main effect for days, $F(1, 23) = 5.015$, $p<.05$. There were no significant interactions with the other between subject variables.

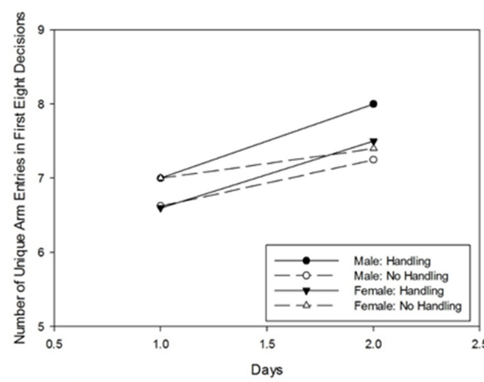

Figure 4. Effects of Within Subjects Variables (days) and Between Subjects Variables (sex and treatment condition) on the Number of Unique Arm Visits in First Eight Decisions

The results for the number of unique arm visits in the first eight decisions are shown in Figure 4. Every subject group performed similarly for each day of testing. The analysis of the data revealed that there were no significant differences between any of the groups. There were no significant main effects or significant interactions with any of the subject variables.

Discussion

The hypotheses for this study were that the rats in the early handling condition would outperform the rats in the control condition, and that males would outperform the females. The hypotheses were not supported. There was no evidence that either condition or sex outperformed the other. On the second day of testing, all of the rats produced similar numbers for each dependent variable.

One of the limitations of the study was the amount of time available for completion of the study. If more time were available, the opposite early life experience (maternal separation) could have been analyzed as well, possibly producing some numbers that could support the hypotheses. A more specific time-related limitation was how old the rats were for each stage of research. For example, Plescia et al. (2013) had the rat pups in the early handling stage from PND 2 to 21, and then didn't start any training until PND 55. Winkelmann-Duarte et al. (2011) performed the handling stage between PND 1 and PND 10. It is necessary to consider that the days for the handling condition were not early enough to produce lasting effects that would be present in testing, but Sandstrom and Hart (2004) found evidence that early life stressors specifically in the third postnatal week affected the rats enough to produce observable differences in testing. Sandstrom (2004) also didn't begin the dieting stage until the rats were three months of age. The article does not describe what the rats were doing between PND 21 and roughly PND 90, but perhaps this period of growth is also critical. Meck et al. (2013) also didn't start training period until PND 90 for the radial arm maze procedure. It is entirely possible that the age of the rats during testing has an influence on the presented differences between experimental conditions; the training and testing in this research may have been so early that the effects could not have been produced.

A potential contributory factor for the results inconsistent with the hypotheses was how the rats were housed and trained. Sandstrom and Hart (2004) housed their rats in same-sex pairs and had a pre-training period for the maze in which 4-5 rats were placed in the maze at a time with pellets scattered all over the arms before beginning individual training. The present study housed the rats in pairs based on their tail markings: each rat was housed with a rat from the opposite experimental condition regardless of sex, and they were placed in the maze in pairs before individual training. There is no certain evidence that the housing pairs or size of pre-training groups would affect later performance, but it should be considered.

Potential future research should be extensive enough to include a maternal separation group as well as an early handling group. Although this experiment was not intended to be invasive, a follow-up study should be. Sandstrom and Hart (2004) euthanized some of the rats from each condition during the isolation period in order to run blood tests and determine if the isolation condition is producing a greater stress response than the control group by analyzing the levels of corticosterone. It is possible that the early handling group was not consistently stressed at the proper amount to produce increased stress tolerance.

The results of the present research contradict the trends observed by Frankola et al. (2010) in a design that reflects that of Sandstrom et al. (2004) and Sandstrom and Hart (2004). As the hypotheses were not supported, one can conclude that either some of the previously described factors contributed to an unsupported hypothesis, or it is possible that the radial arm maze task does not measure memory development in the same way that other assessments do.

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