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Comparing Components of Enriched Environments Including Objects,
Handling, and Social Experiences to Standard Housed Rats
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Abstract

A number of studies have found that enriched environments facilitate learning. However, there is confusion in the way different researchers have defined enriched environments. Research on enriched environments has included the addition of objects, tunnels, running wheels, as well as other animals or human interaction. The present study sought to clarify the role of different types of enrichment by having rats exposed to one of the common types used in other studies. The types of enrichment studied included giving objects for the rats to interact with, handling the rats, and allowing the rats time in a social environment. These enriched conditions were compared to a control condition in which the rats lived in isolated standard housing. These rats were then placed in an operant conditioning chamber without shaping to determine the speed of acquisition of the bar press response. The dependent measures were time to the first bar press, time from the first to the 20th bar press, and total bar presses over a twelve hour period. It was expected that the animals in the object, handling, and social conditions would acquire the bar pressing response faster than the standard housed animals, although the results did not show any difference. There was some weak evidence from a post hoc analysis that indicated that the standard housed rats may have acquired the bar press response faster than the social condition rats and pressed the bar more total times than the handling condition.

Comparing Components of Enriched Environments including Objects, Handling, and Social Experiences to Standard Housed Rats

The belief that enriched environments enhance learning is an idea that has sparked much interest. While it may seem like common sense that stimulation in an environment helps with the ability to learn, this idea has led to many questions in the laboratory. When compared to standard environments, enriched environments lead to greater learning in rats (Chapillon, Patin, Roy, Vincent & Caston, 2002). In order to understand what determines an enriched environment, it is important to understand standard housing. The rats housed in standard environments are exposed to minimal stimuli and are only cared for as needed. Enrichment has been defined as anything added to the standard living environment which provides stimulation for the animal (Chapillon, 2002). Research on enriched environments has included the addition of objects, tunnels, running wheels, as well as other animals or human interaction. In other words, the animals were stimulated by their surroundings (Chapillon et al., 2002). Therefore it is the addition of this stimulation that has been researched and associated with greater learning. Chapillon et al. even goes as far as to report the animals in the enriched environment not only lead to greater learning but are stimulated enough to result in behavioral and neurological differences from the standard housed animals.

These behavioral differences are displayed as an improvement in performance on various learning and memory tasks and can be demonstrated after limited exposure to an enriched environment. For example, Forgays and Read

(1962) showed that enriched environments enhanced performance of animals (Paylor, Morrison, Rudy & Waltrip, 1992). Also, Paylor et al. (1992) found that only a brief exposure to an enriched environment around the time of weaning appears to be enough to produce functional changes in the process needed to solve some learning and memory tasks. Rats reared in an enriched environment also perform better than isolated or social-reared control animals on learning and memory-dependent tasks (Paylor et al., 1992). The rats in the experiment increased performance time on the Morris water task after only 12 days of the enriched environment. The difference between the standard housed rats' performance and the rats in the enriched environments was significantly different (Paylor et al., 1992). Overall, behavior and performance seemed to increase in each study.

Research has found neurological differences including differences in brain weight, dendritic branching and microanatomy between enriched and standard housed rats. For example, Bourgeon, Xerri & Coq (2004) used a maze and problem solving tasks to test their rats. They then looked closer at the rats' neurological make-up and found that rats in the enriched environments had an increase in the branching of dendrites, and increased synaptic density as well as synapse counts. Through the mazes and problem solving tasks they also concluded that learning and memory are improved because of enrichment.

Furthermore, Greenough, Madden & Fleischmann (1972) found that rats reared in enriched environments differ biologically from rats reared isolated conditions in cortical weight and thickness, distribution of brain enzyme activity, and brain

microanatomy. Greenough, Madden & Fleischmann (1972) also reported that as a whole the total brain weight increases when animals are exposed to an enriched environment. In summary, the relationship between internal changes (i.e. brain weight, dendritic branching, microanatomy) and enriched environments has been shown to have a significant correlation. Because behavior is the outward expression of an inward change, it is important to investigate the measurable outward occurrences. The neurological differences found suggest that there is more to be learned about enriched environments and its effects on learning.

Although studies have shown significant behavioral and neurological differences between enriched and standard housed rats, the term enrichment has been defined in several different ways making it difficult to separate which variables of enrichment are affecting these differences. Some descriptions of enriched environment suggest objects, while others suggest handling, or social housing. Only through disconnecting these variables (such as social interaction, objects, and handling of the animals) and comparing them can a solution or improvement be made. Through close observation and separation of specific objectives the distinction between the effects of enriched environment and coincidences could be deciphered. Greenough (1972) suggested that future research should examine distinct aspects of the studies differently and separately, in other words do not look at the variables collectively but look at them independently.

One different variable used in enriched environment research has been the handling of rats (Levine, 2000). Handling in the majority of the experiments is

described as holding, petting, and stroking of the animal. These actions are clumped together into one term, handling (Chapillon et al., 2002). Positive effects of postnatal handling were described in many behavioral studies using anxiety tests. Compared to controls, handled animals were found to be less anxious in test, such as bar pressing and the like. It seemed that the handled rats were more at ease in what is normally a stressful situation (Chapillon et al., 2002). Daily handling has previously been found to have little or no effect on the gross brain measures affected by enriched rearing (Greenough et al., 1972). Greenough (1972) also found that enriched and handled animals were significantly faster than the impoverished animals in strait alley running. In the presence of free food, handled rats pressed the bar for a greater percentage of their reinforcement supply than did the control or the enriched condition (Nau, Elias & Bell, 1972). In other words, in a cage there were two dishes, one that was full of food and one that only gets food in it when the rat presses the lever. Rats in Nau, Elias, and Bell's (1972) enriched condition ate a larger percentage of their food from the dish that required bar pressing in order to receive food. Therefore, they pressed the bar more and ate more food out of that particular dish.

Social housing is a third variable that has also been included under the category of enriched environments. It has been found that rats housed in complex social environments perform better on various problem solving tasks compared to those reared in isolated environments (Hebb, 1949). Most of the research is inconsistent due to the fact that some studies studying enriched environments house the rats in social housing, while others housed rats individually and yet they

still attempt to compare the two with these confounds. It is difficult to differentiate the importance of the social factor in overall task performance. The Greenough et al. (1972) study housed the handling condition and the deprived condition rats individually, while the enriched or object condition rats were housed 12 rats per cage. In another study, the rats were housed socially in the handling, deprived and non-handled condition (Pryce, Bettschen, Nanz-Bahr & Feldon, 2003). Pham, Soderstrom, Winblad & Mohommed (1999) housed 8 rats per cage in the enriched or object condition, but the deprived condition was housed individually. Although it is clear that social experience does play some role in the enrichment experience, there is no clear comparison of the effects of handling and social experience on the animal's task performance.

Consequently, it has been difficult to differentiate the importance of the handling and social factors in overall task performance, since the variables are not clearly separated. The present study is going to manipulate enrichment from handling, social, and object conditions to gain clarity and tease out the relative contributions of the three variables. Because the research does not clearly define enriched environments, this study is not going to use that term. Enrichment will be divided into three conditions: object, handling and social. These three conditions will be compared to a standard housed condition. Operant conditioning chambers were used during trial periods in order to test a learned behavior, bar pressing. Food deprived rats when placed into an operant conditioning chamber, will press the bar in order to get food. Bar pressing was measured across time. It was expected that the animals in the object, handling, and social conditions would

acquire the bar pressing response faster than the standard housed animals, but there was no basis for predicting any difference in acquisition between the 3 manipulations.

Method

Participants

Twenty-five naïve, 3-6 week-old, male Sprague-Dawley rats from Harlan Labs in Bloomington, IN were used. At the time of arrival, the rats weighed approximately 100 grams. The reason for the specific size of rat is because the rats have passed weaning stage but still are naïve and able to perform. Twenty-three rats were used for data collection because 2 rats died due to dehydration caused by a heating problem in the facility. One rat died in the object condition and was replaced by an extra rat that was sent. The 2nd rat that died was from the social condition and was not replaced due to time constraints.

Once the rats arrived, they were individually caged in a temperature-controlled environment with lights on from 9:00 a.m. to 9:00 p.m. The room had no windows and was lit by florescent lights. The cages were yellow tinted transparent plastic and 8x11x6 inches.

Equipment

Model #84012 operant conditioning chambers, model #80200 pellet dispensers, and model #81335 operant conditioning consuls was used. The consul controlled schedules of reinforcement, recorded responses and reinforcement. The food pellets that were used were NOYES Precision Pellets PJA1-0045.

Cumulative Recorder 654 from Campden Instruments Ltd. was used in order to

record all of the data. The recorder had a pen that marks a straight continuous line on a role of paper when there were no responses, which can be seen in Figure 1. For each bar press, the pen marked a hash mark and moved upward on the paper to show the accumulation of bar presses. All the equipment was purchased from Lafayette Industries. Latex gloves were used to move social rats and all rats into the operant conditioning chambers. Figure 2 shows the objects which were used in the object condition. These objects included 12 1x1x1 inch square blocks, 6 6-inch paint roller tubing, 6 multicolored odd shaped rubber balls, 6 8-inch pieces of rope tied in 1 knot.

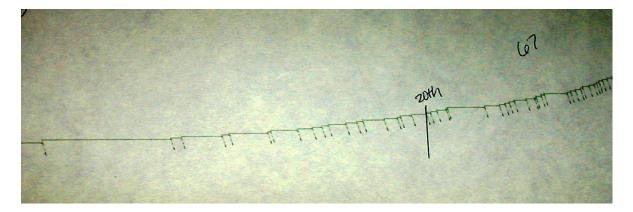


Figure 1. A sample of the paper from the cumulative recorder with the marks of the bar press response across time.



Figure 2. Objects used in the object condition.

Procedure

The animals were housed in the same conditions. First, the bottoms of the 8x11x6 inch cages were lined with cedar shavings. The cages had waterspout in the back of each cage where the animals were able to get water. Some of the rats struggled to drink out of the waterspouts when there was a technical problem with the buildings heat, so water dishes were placed in their cages and changed daily. The food was hung from the top of the cage and each food bin was filled with 6 pellets of food and checked daily to ensure that the rats were eating. The rats were randomly assigned to one of 4 conditions: Object Condition, Handling Condition, Social Condition, and Standard Housing Condition.

Object Condition. The 6 rats in this condition will be housed in separate cages. Each rat had the same 5 objects (2 blocks, 1 ball, 1 tube, and 1 rope) placed in their cages for 20 minutes daily. After the 20 minutes in the condition, the objects were quickly be removed by the researcher carefully so as not to make contact with the rat. The object condition will be carried out for 14 consecutive days. One of the rats in this condition died of dehydration due to a technical difficulty which increased the heat in the animal facility. This rat was replaced with an extra rat that was sent at the same time as all the others.

Handling Condition. The 6 rats in this condition were housed in separate cages. Each rat was handled individually for 20 minutes each day. Each of the two researchers handled the same 3 rats daily. The handling included holding the rats on the researchers' laps while petting and stroking the rats. The rats were not allowed to run freely on the researcher but were not restrained uncomfortably

during the handling. The rats were handled in a caring manner in order to maintain the rats' safety and comfort. The handling condition was carried out for 14 consecutive days.

Social Condition. The condition had 5 rats that were housed in separate, individual, cages. Each rat was placed into a cage with one or two other rats for 20 minutes each day. The rats that were placed together were labeled as groups. Three of the rats were marked with two colors of hi-lighters each day so that the rats could be returned to their original cages. The groups of 2 or 3 were lifted by their tail out of their cage with latex gloves and were placed into a neutral, clean cage. The reason for the neutral cage was to prevent conflict over territory. The bottoms of the social cages were lined with cedar shavings. The rats were left together and the researcher left the room, in order to allow the groups to socialize. After 20 minutes, the rats were separated, picked up by the tale, and placed back into their individual cages. The groups were placed together each day for 14 consecutive days.

Scheduling. Due to equipment limitations with the operant conditioning chambers and the cumulative recorders, a schedule was created in order to collect data. The data was collected in groups of 3 rats. Until the rats were placed in their conditions, they were housed individual in standard conditions. An example of the schedule is seen in Table 1.

Table 1	. Schedule	for	Running	Conditions.

Handling Rats 1-3	Handling Rats 4-6	Object Rats 1-3	Object Rats 4-6	Social Rats 1-3	Social Rats 4-5	Standard Housed	Standard Housed
						Rats 1-3	Rats 4-6
Day 1:	Day 2:	Day 3:	Day 4:	Day 5:	Day 6:		
Began 14	Began 14	Began 14	Began 14	Began 14	Began 14		
days in	days in	days in	days in	days in	days in		
condition.	condition.	condition.	condition.	condition.	condition.		
Day 15:	Day 16:	Day 17:	Day 18:	Day 19:	Day 20:	Day 21:	Day 22:
Run	Run	Run	Run	Run	Run	Run	Run

Standard Housing Condition. The 6 rats in this condition were housed in separate cages and were not touched. The only disturbance they experienced was to add food and place water dishes in the cages of rats that needed water. The process was carried out for 14 consecutive days.

Operant Conditioning Chambers. On day 14 after being in their prospective conditions the rats were deprived of food for 24 hours. Deprivation began at 9:00 p.m. The following day, day 15, the rats were placed into individual operant conditioning chambers at 9:00 p.m. The chamber had one pellet of food in the food dish. The researchers wore latex gloves to pick up each rat by the tail and place it into an operant conditioning chamber. The gloves were worn in order to avoid any previous connection the rats made with the researchers. The operant conditioning chambers were set on a continuous reinforcement schedule. Every time the rat pressed the lever in the chamber the rat was rewarded with a small pellet of food. The rats were left alone in the operant conditioning chamber for 12 hours (9:00 p.m.-9:00 a.m.). This amount of time was determined sufficient after running a pilot study on 2 naïve rats, which were raised in standard housing. During the 12 hours, the cumulative recorder and the

operant chamber recorded each bar press. The cumulative recorder also tracked the accumulation of bar pressing. It recorded from the first to the last of the bar presses. After 12 hours, the rats were removed from the operant conditioning chambers and placed back into their own individual cages. The time from the first bar press to the 20th bar press was measured from the line recorded on the cumulative recorder. The number of bar presses during the whole 12 hours was also recorded and the data will be compared. There was a power outage during one of the nights of data collection. Three rats were affected in the object condition. With all three, data was unable to be collected for total number of bar presses. Time to the 20th bar press was collected for 2 of the 3 rats because they reached the 20th bar press before the power outage. Data was compared through measurement of the increase in height of the pen marks on the cumulative recorder.

Results

Averages were taken across the 4 conditions for each of the 3 measures: time to the 1st bar press, time from the 1st to the 20th bar press, and total number of bar presses. Figure 3 shows the average time it took the rats in each condition to reach the 1st bar press with error bars marking the variance in the data. Figure 4 shows the average time it took the rats in each condition from the 1st to the 20th bar press with error bars marking the variance in the data. Figure 5 shows the average total number of bar presses for each condition over the entire 12 hour period with the error bars marking the variance in the data.

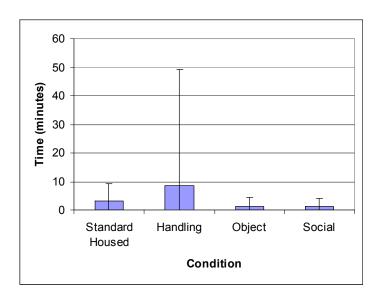


Figure 3. Mean Time to the 1st Bar Press

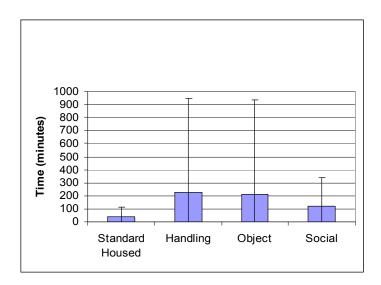


Figure 4. Mean Time from the 1st to the 20th Bar Press

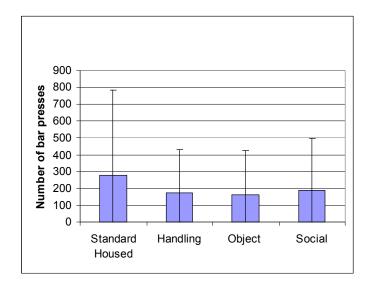


Figure 5. Mean Number of Total Bar Presses

As seen in the error bars for each of the figures, there is large variance in the data. An ANOVA was not used in the analysis of this data because we did not have equal variance and we had a small sample size with N=5 or 6 in each condition. A median test was used. This test calculated the median for the variable, for example, the median for the time to the 1^{st} bar press. The number of cases above and below the median was counted. These numbers were shown in Table 2 for each of the dependent variables. From that data a 2-way Chi-square of those frequencies was calculated.

Table 2. Number of Rats Above and Below the Median in each Condition	Table 2.	Number of	^f Rats Above	and Below the	: Median in each	Condition
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		Standard Housed Condition	Handling Condition	Object Condition	Social Condition
Time to 1 st Bar	# Above	4	4	2	1
Press	Median				
	# Below	2	2	4	4
	Median				
Time from 1 st to	# Above	1	3	3	4
20 th Bar Press	Median				
	# Below	5	3	2	1
	Median				
Total Bar	# Above	5	1	2	2
Presses	Median				
	# Below	1	5	1	3
	Median				

The Chi-square values were not significant. When looking at the time to the 1st bar press, $\chi^2(3, N=23)=3.76$, NS. From the 1st bar press to the 20th bar press, $\chi^2(3, N=22)=4.67$, NS. The total number of bar presses $\chi^2(3, N=20)=5.87$, NS. Even when the means are presented as seen in Figures 1-3, a large difference can be seen between the conditions but the variance is so large that it overwhelms those differences.

When looking at the data in the median table, it became clear that there were a few situations where some effect may be present. These situations are marked by the bold numbers. Because of these potential differences, median tests were used to compare each of the enriched conditions individually to the standard housing condition. When the handling condition was compared to the standard housing condition, there was a significant Chi-square value when examining the total number of bar presses, $\chi^2(1, N=12) = 5.33$, p = .02. This indicates that

compared to the handling condition the standard housed rats pressed the bar significantly more times during the 12 hour period in the operant conditioning chambers. There was no significant Chi-square value when comparing the object condition to the standard housing condition. There was a significant Chi-square value when comparing the social condition to the standard housing condition when examining the time from the 1st bar press to the 20th bar press, $\chi^2(1, N=11)$ = 4.41, p = .04. This indicates that the standard housed rats reached the 20th bar press significantly faster than the social condition rats. These post hoc analyses show some evidence to support that the conclusion that the standard housed animals acquired the bar press response faster than the other conditions, although this evidence is weak. It cannot be concluded that there is any difference between the 3 enriched conditions, but it seems that any enrichment shows some difference from standard housed rats.

Discussion

The present study investigated the effects of enriched experiences (handling, social, and objects) compared to rats residing in standard housing on the acquisition of the bar pressing response in an operant conditioning chamber. This study attempted to determine if enriched environments would lead to a decrease in time to acquire the bar pressing response and in fact hypothesized exactly that. The original hypothesis was not supported by the data. If anything, there was weak evidence to support the opposite of the original prediction. The post hoc analysis of the data indicated that when compared to the social condition

the standard housed rats reached the 20th bar press significantly faster. Also, the standard housed rats pressed the bar significantly more total times than the handled rats. These post hoc results seem to indicate that the standard housed rats may have had some advantage in acquiring the bar pressing response. These results could be the result of chance which means there may have truly been no difference between the 4 conditions. The past research may also be correct in their findings that enrichment benefits learning. The results of this study can be examined from any of these 3 possibilities.

This study had no evidence to support that enrichment helped improve or enhance learning and it may even indicate the opposite. The results show no strong differences between the enriched conditions and the standard housed rats. Also, there seems to be little to no difference among the enriched conditions themselves. If there had been no difference among any of the variables it would appear that enrichment has no effect on learning.

Taking a closer look these results might indicate that the enriched environment, when broken down into simple conditions such as handling, social contact, and object interaction have little effect on a simple task like the bar pressing response used in this study. Also, the results suggest that animals residing in standard housing may positively effect the acquisition of a simple task. For example, the rats in the standard housed condition pressed the bar significantly more times than the rats in the handling conditions. Also, the standard housed rats slightly significant faster time in the bar pressing acquisition when compared to the social condition. In both cases, these results suggest that

the behavioral differences reported between enriched and standard housed rats following these conditions are not to any great extent due to their reaction to these experiences. However, the results also suggest that a standard housed rat compared to the enriched environment animals might be considered preferable in acquiring simple learned behaviors.

As noted in the introduction, the majority of previous research in operant conditioning supports the view that enrichment increases behavior acquisition and performance of tasks. There is no clear explanation of why the standard housed rats may have had an advantage in performing a simple task like acquiring the bar pressing response. However, looking deeper into research one study seemed to contradict the majority. Van der Harst et al. found that standard housed rats are more sensitive to rewards than enriched housed rats as reflected by their anticipatory response. Van der Harst study used classical conditioning to accomplish the experiment. The present study used operant conditioning so Van der Harst's study was not seemingly relevant to refer to. Van der Harst's study entailed pairing a tone with a sucrose reward and measured the amount of behavior during the pairing of the two. For this reason and for the majority of other research suggesting that enrichment improves acquisition and performance of tasks the present study conformed to the majority.

The differences between the standard and enriched conditions are very minimal and one suggestion is that the task complexity played a role the similarity of the rat data. The operant conditioning chamber was not used in any of the research reviewed for this study. The past research had used more complex tasks

such as a water maze or discrimination tasks. Possibly in more complex tasks the standard housed rats were rewarded by the stimulating situation alone and thus distracting from the actual tested task at hand. Conceivably the difference between past research and these findings is the task itself. Further research should be done in order to identify the difference not only in the conditions but also in the acquisition of behavior and/or task performance.

Perhaps past research was correct in stating that enrichment and enriched conditions result in improvements in task performance. The present study had setbacks that might have caused some problems. For example, this study had, at most, six participants in a condition. With such a small number of participants the results were subject to unequal variance.

Not only did the small number of participants weaken the study but so did the measures. First, most of the measures were taken from previous research. For example, the amount of days and time each rat should be in their condition. However, there did not seem to be any previous research which stated how many bar presses could be assumed that the rat had acquired the bar pressing response. The twentieth bar press was assumed to be considered acquisition of the bar pressing response. The assumption came from a pilot study that suggested that at the twentieth bar press the rat had acquired the response. A Pearson correlation was used to determine which measures were correlated with each other. A significant correlation was found between the measure of time from the 1^{st} bar press to the 20^{th} bar press and total number of bar presses, r(20) = -.66, p = .001. This indicates that this measure was an accurate measure of the acquisition of the

bar press response. There was not a significant correlation between total bar press and the time to the 1st bar press, r(20) = -.08, NS. Also, there was not a significant correlation between the time to the 1st bar press and the time from the 1st to the 20^{th} bar press, r(22) = .39, NS. The measures are presumably a central factor in this study.

From this study, future research has many directions to investigate. First, there should be a continuous effort to examine the various aspects and definitions of these enriched environments. It is important to understand which parts of the enrichment lead to improvements. Not only should the definitions be considered but so should the different tasks available. A comparison of tasks may help clarify the issue of task complexity and its effect on learning vs. performance. Lastly, time in each condition should be studied to determine how long is enough to truly enrich the animals in order to improve learning or performance.

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