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Limited Environmental Enrichment Blunts Methamphetamine Sensitization in Young Mice

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Abstract

Two experiments examined the role of limited environmental enrichment on behavioral sensitization to methamphetamine in Swiss-Webster mice. Researchers hypothesized that limited environmental enrichment would slow the rate of sensitization for enriched mice. In Experiment 1, mice (enriched) were exposed to enrichment chambers (6 hours/day x 15 days) or remained in their home cages (non-enriched controls) (Enrichment phase). Mice were then injected with methamphetamine (0.5 mg/kg) once a week for 4 weeks (Sensitization phase). In Experiment 2, mice were exposed either to limited environmental enrichment (6 hours/day x 15 days or 6 hours/day x 5 days) or remained in their home cages (non-enriched controls) during the Enrichment phase followed by methamphetamine sensitization. The results of both experiments indicated that enriched mice, regardless of the amount of environmental enrichment, demonstrated a slower rate of sensitization compared to the non-enriched mice, suggesting that limited environmental enrichment blunted the behavioral effects of methamphetamine.

Keywords: environmental enrichment, sensitization, methamphetamine, mice

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Although there are many theories derived from empirical study, one widely supported psychobiological theory of drug addiction, the Incentive-Sensitization theory, provides insight into abusive drug behavior (Berridge & Robinson, 1993; Ostafin, Marlatt, & Troop-Gordon, 2010). This theory suggests that multiple drug exposures "sensitize" the mesolimbic dopaminergic system (Berridge & Robinson, 1993; Nakagawa, Suzuki, Nagayasu, Kitaichi, Shirakawa, & Kaneko, 2011). In other words, a given stimulus presented multiple times will progressively elicit a greater reaction from a subject. Sensitization is a key indication of the development of drug addiction and drug relapse in non-humans and humans alike, because it increases the level of "wanting" or drug craving (Berridge and Robinson, 1993; Sharpe, Klaus & Beckstead, 2011; Solinas, Thiriet, Rawas, Lardeux, & Jaber, 2009). Researchers in the field of behavioral pharmacology continue to examine the connection between the rewarding nature of drugs and sensitization (Vezina, 2007).

In animal models, *behavioral sensitization*, or the enhanced locomotor activity following continued exposure to a drug, is examined to understand drug abuse. Methamphetamine, a psychomotor stimulant, increases locomotor activity following initial administrations (Hirabayashi & Allam, 1981). Locomotor activity also increases progressively over time with continued presentations of methamphetamine (Rauhut & Bialecki, 2011). Researchers have explored environmental factors that influence the development of behavioral sensitization, as a means of assessing non-biological factors that contribute to drug addiction in humans (Stairs & Bardo, 2009). To this end, an organism's rearing environment has been identified as one of the integral, non-biological factors that

contribute to sensitization. Specifically, "enriched environments" have been shown to blunt the behavioral effects of drug exposure.

Two of the earliest pioneers of environmental enrichment research were Mark Rosenzweig and Edward Bennett (Breedlove, Watson, & Rosenzweig, 2010). The environmental enrichment paradigm they used in their research included larger cages, access to wheels and novel toys of different shape and color, and social environments (Siviy & Panksepp, 2011; Solinas, et al., 2009). Rawas et al. (2009) also made a point of defining an enriched environment as having both social and sensory stimulation, sometimes associated with novelty, which seems to be an important component of enriched environments. As demonstrated, the common characteristics of environmental enrichment include larger spaces, novelty, active movement, and social stimuli.

Initial research in the field of enrichment elucidated a multitude of effects on neural structure and cognitive processes. Rosenzweig and Bennett (1972) determined that several neurophysiological changes occur following exposure to an enriched environment, such as an increase in the number of glial cells and weight of the occipital cortex (Diamond et al., 1966; Rosenzweig & Bennett, 1972). Stairs and Bardo's (2009) review illuminated several neuroanatomical changes for enriched animals, such as an increase in cortical thickness, size of cell bodies, number of dendrites, and number of capillaries in the brain. An enriched environment also creates behavioral changes; it has been shown to facilitate learning, problem solving and memory (Diamond et al., 1966; Rosenzweig & Bennett, 1972; Rosenzweig & Bennett, 1996).

Stemming from Rosenzweig and Bennett's work, other researchers have explored the effects of environmental enrichment on the behavioral responses to drugs of abuse in animals

using behavioral sensitization and conditioned place preference (CPP) tasks (Rawas, et al., 2009; Solinas, et al., 2009). The paradigms used in these experiments determined sensitization by comparing rates of locomotor activity over time following the presentation of a drug. If a subject progressively responded with markedly higher rates of activity, then the sensitizing effect of the drug was considered high. Bardo et al. (1995) found that rats in enriched environments had a more robust response following the initial injection of amphetamine. Over time, however, the same rats did not develop sensitization as strongly as the control subjects. These findings suggest that environmental enrichment affects the rate of sensitization as well as the rewarding nature of drugs (Rawas, Thiriet, Lardeux, Jaber, & Solinas, 2009).

Solinas et al. (2009) conducted their environmental enrichment research using a CPP paradigm, where mice were placed in a box with two chambers connected by an alley. After subjects became acclimated to and were pre-tested in the CPP box, mice were injected with either cocaine or saline and put into one of the two compartments. Researchers determined the acquisition of a CPP by subtracting the amount of activity recorded during pretest from the activity during the test days. The findings demonstrated that environmentally enriched mice show retarded rates of CPP learning compared to non-enriched mice (Solinas, et al., 2009). A number of findings demonstrate that enriched environments decrease the discriminative effects that a drug normally has on CPP and impede behavioral sensitization (Rawas, et al., 2009; Zakharova, Miller, Unterwald, Wade, & Izenwasser, 2009). These studies influenced researchers to suggest that environmental enrichment has a protective neurocognitive effect on subsequent drug-taking (Stairs & Bardo, 2009). In other words,

environmental enrichment may act to fortify the reward circuit and prevent drugs from having more robust effects.

To date, all of the studies that have assessed the effect of environmental enrichment on drug sensitization have employed enrichment paradigms in which rodents are reared, *continuously* in an enriched environment and compared rodents not reared in such an environment. For example, Rawas et al. (2009) exposed rodents to a continuous enriched environment and demonstrated that environmental enrichment hinders the rewarding effects of heroin. This paradigm involved rearing mice for two months, after weaning, in an enriched environment, which included larger cages and running wheels for 24 hours a day. In addition, mice were socially housed (four per cage) and objects of different shape and color were changed each week. It is not known, however, if more limited environmental enrichment will produce similar, drug-blunting effects as continuous, environmental enrichment does. Rosenzweig and Bennett (1972) found that access to as little as two hours of environmental enrichment for 30 days had the same effects as 24 hours of enrichment for 30 days on problem-solving, maze tasks. Thus, more limited environmental enrichment *may* produce outcomes similar to continuous environmental enrichment with respect to altering the behavioral actions of drugs of abuse.

Experiment 1

Experiment 1 examined the effect of limited environmental enrichment on methamphetamine sensitization in mice. To this end, mice that were exposed to an enriched environment with limited access (4/cage; 6 hours/day x 15 days) and group-housed were compared to mice that were solely group-housed (4/cage; non-enriched controls) and remained in their home cages for the duration of the Enrichment phase. Then, all mice

received repeated exposure to methamphetamine once a week for 4 weeks. Researchers hypothesized that limited access to an enriched environment would decrease the initial locomotor activating effects of methamphetamine. Furthermore, limited environmental enrichment was expected to blunt or slow the development of sensitization to methamphetamine compared to non-enriched mice.

Method

Subjects. Thirty male, young (~ 21 days old) Swiss-Webster mice (up to 12 g) were obtained from Charles River Laboratories (Raleigh, NC) and given *ad libitum* access to food and water for the duration of the experiment. Mice were kept on a 12:12 (light/dark) cycle and lights came on at 0900 h. Upon arrival, the mice were acclimated to the animal colony, including human handling, for 7 days. The experiment conformed to the guidelines established by the *NIH Guide for the Care and Use of Laboratory Animals* (1996 Edition) and was approved by the Dickinson College Animal Care and Use Committee.

Drugs. Methamphetamine HCl (Sigma, St. Louis, MO) was prepared in a physiological saline solution. Methamphetamine (0.5 mg/kg) was injected subcutaneously (SC) at a volume of 10 ml/kg. The drug dose had been used previously in behavioral experiments with mice and shown to produce locomotor activation without considerable stereotypic behavior (Hirabayashi & Allam, 1981). Furthermore, this dose of methamphetamine had not been shown to produce neurotoxicity. The methamphetamine dose is expressed as its salt weight.

Apparatus. Enriched environments that were used consisted of four transparent glass aquaria, measuring 50.8 cm x 26 cm (L x W), and were used during the Enrichment phase only. Similar to previous research using enriched environment, each aquaria was equipped

with spinning wheels and toys, such as Legos, and was larger than the home cages. The toys were changed daily. Paper bedding lined the floor of the aquaria.

The locomotor activity chambers consisted of fifteen open-field activity chambers (MED-OFA-510; Med-associated, Vt., USA) and were used during the sensitization phase (Figure 1). The walls of the compartments were constructed of Plexiglas and the inside dimensions were 27.9 cm x 27.9 cm (L x W). Locomotor activity was determined by breakage of 16 infrared photo beams and recorded by an IBM personal computer (MED-PC Activity Software) located in the same room. The chambers were cleaned with a disinfectant solution after mice were removed from the chambers.

Procedure. Following an acclimation period of 7 days, mice were separated into two groups for the duration of the experiment. One group of mice (n = 16) was given access to an enriched environment for 6 hours/day (Monday-Friday) for 15 days (Figure 2). Mice were placed in the enriched environments in cohorts of 4. The control, non-enriched mice (n = 15) remained in their home cages during this phase and did not receive any enrichment beyond that experienced due to social play in their home cages (Figure 3). Mice were videotaped for the first two hours of the six-hour period on Days 1 and 15 of the Enrichment phase.

Following the enrichment phase, mice underwent a sensitization period. Mice were placed in the locomotor activity chambers for a total of 8 hours/week for 4 weeks for Experiment 1. Mice were placed in locomotor activity chambers for 3-hour drug-free periods. This period of time was intended to assess baseline group differences in locomotor activity. Moreover, a long baseline period would ameliorate potential group differences in baseline activity prior to methamphetamine exposure to facilitate group comparisons following methamphetamine exposure. After 3 hours in the chamber, the computer program was

paused and subjects were injected (SC) with methamphetamine (0.5 mg/kg). Following the injection, each mouse was returned to its locomotor activity chamber and the computer program was resumed for a 5-hour period, completing the 8 hours. Upon completion of the sensitization phase, mice were euthanized.

Data Analysis. A two-way repeated-measure analysis of variance (ANOVA) was conducted on the between-subjects factor, Group (Enriched vs. Non-enriched), and the within-subjects factor, Session Hour (1 – 8), for the activity data during Weeks 2 and 3. Group differences during specific hours were examined using independent-samples *t*-tests. A follow-up two-way (Group x Week) ANOVA also was conducted to determine group differences in the rate of methamphetamine sensitization. Unless otherwise indicated, all differences were considered significant when $p < 0.05$.

Results

As a result of experimenter error and equipment malfunction, a considerable amount of data was lost in Experiment 1. Only data for half the mice in each experimental condition (n = 8 Enriched and n = 7 for Non-enriched mice) were available for Weeks 2 and 3. Thus, the analyses below focused on these data.

During the Week 2 of sensitization, there was no significant Group x Session Hour interaction and there was not a significant main effect of Group (Figure 4). These results suggest that group differences were not detected during any portion of the 8-hour activity session. However, there was a significant main effect found for Session Hour, $F(1, 7) = 28.39$, $p < .05$, $\eta^2_{partial} = .06$, suggesting that locomotor activity varied during the 8-hour session. Indeed, an inspection of Figure 4 shows that mice were initially active during Hour 1 (novelty-induced exploration), followed by a decrease and subsequent increase in

locomotor activity after an injection of methamphetamine (i.e., Hour 4). Moreover, as the mice metabolized the methamphetamine, locomotor activity decreased (i.e., Hours 5-8). However, Week 3 of sensitization yielded significant results (Figure 5). There were significant main effects for Group and Session Hour, $Fs > 9.20$, $ps < .05$, $\eta^2 s_{partial} > .40$. There was also a significant Group x Session Hour interaction, $F(1, 7) = 4.082$, $p < .05$. Following the initial two-way ANOVA for Week 3, independent samples *t*-tests were conducted on group activity differences between individual hours. Enriched mice were less active during the Session Hour 4 of Week 3 compared to non-enriched mice, $t(14) = 3.20$, $p < .05$, $d = -1.6$, suggesting that enriched mice showed a blunted locomotor-activating response to methamphetamine. Similarly, Session Hour 5 also was statistically different between enriched and non-enriched mice, $t(14) = 2.39$, $p < .05$, $d = -1.2$.

Using a paired-samples *t*-test, researchers also found a significant difference between Weeks 2 and 3 during Hour 4 for the non-enriched mice, $t(7) = 2.45$, $p < .05$, but not the enriched mice (Figure 6).

Discussion

In Experiment 1, researchers found a significant interaction between Group and Session Hour for Week 3, but not Week 2. Further analysis found that locomotor activity was significantly greater during Hours 4 and 5 of Week 3 for the control, non-enriched mice compared to the enriched-mice. Hours 4 and 5 marked the period that followed the injection of methamphetamine, suggesting that enriched mice were less responsive to the locomotor-activating effect of methamphetamine compared to non-enriched mice. Moreover, locomotor activity increased during Hour 4 between Weeks 2 and 3 for non-enriched mice, providing evidence that non-enriched mice sensitized to the methamphetamine. Locomotor activity,

however, did not change for enriched mice between Weeks 2 and 3, suggesting that enriched mice did not sensitize to the methamphetamine.

Experiment 2

In Experiment 2, researchers examined if even *less* environmental enrichment decreased the locomotor-activating and sensitizing effects of methamphetamine in young mice. To this end, mice received exposure to either environmental enrichment (6 hours/day for 15 days or 6 hours/day for 5 days) or no environmental enrichment (non-enriched control group). Researchers expected to find the sensitization of enriched mice, regardless as to the length of exposure, to become blunted compared to the non-enriched control mice.

Method

Subjects, Drugs and Apparatus. The animals, drugs and apparatus used in Experiment 2 were identical to those used in Experiment 1.

Procedure. The environmental enrichment phase for Experiment 2 was very similar to that of Experiment 1. However, unlike in Experiment 1, mice in Experiment 2 were divided into three groups: non-enriched controls (n = 10), 15-Day enriched (n = 10), and 5-Day enriched (n = 10). Once in groups, mice were acclimated and given access to an enriched environment (15-Day enriched and 5-Day enriched groups) for 6 hours/day or kept in home cages (control) in cohorts of 5. The subjects in the 5-Day Enriched group began their week of 6-hour access to environmental enrichment during the week directly before the sensitization phase. The subjects in the 15-Day Enriched group also underwent the enrichment phase during the 3 weeks directly before sensitization. This way both groups completed their enrichment just prior to sensitization training.

In Experiment 1, group differences were detected after 3 weeks of sensitization training. Thus, in Experiment 2, the sensitization period lasted for only 3 weeks rather than 4, as in Experiment 1. Sensitization training was otherwise identical to that of Experiment 1.

Data Analysis. Similar to the Experiment 1, Experiment 2 also was analyzed using a two-way ANOVA on the between-subjects factor, Group (15-Day enriched vs. 5-Day enriched vs. Non-enriched) and the within-subjects factor, Session Hour (1-8) for Weeks 1-3. Multiple *t*-tests also were used to examine group differences at individual time points (hours). Similar to Experiment 1, a follow-up Group x Week ANOVA also was conducted to determine group differences in the rate of methamphetamine sensitization. All differences were considered significant when $p < 0.05$.

Results

One mouse from the 15-day enriched condition died during the course of the experiment. This mouse's data was excluded from all analyses. Also, the data for 8 mice in the 15-day enrichment condition was lost during Week 1 due to computer malfunction and these data were not included in any analysis involving Week 1. Furthermore, an initial analysis of the data revealed no significant differences between the 5- and 15-Day enriched mice (data not shown). (For the sake of brevity, these analyses are not reported.) Furthermore, in order to increase statistical power, 5- and 15-day enriched groups were collapsed into one group of enriched mice for the subsequent analyses reported below.

During Week 1 of sensitization, there was a significant interaction between Group and Session Hour, $F(1, 20) = 3.75$, $p < .05$, as well as significant main effects of both Group and Session Hour, $Fs > 8.14$, $ps < .05$, $\eta^2 s_{partial} > .03$. The activity between groups during Session Hours 1, 2, 4, 5, 6, and 7, during Week 1, were significantly different between

enriched and non-enriched mice, *ts* (20) > 2.09, *p*s < .05, *ds* > .93. (Figure 7), suggesting that enriched mice were more active during baseline (Hours 1-2), following methamphetamine exposure (Hour 4) and following methamphetamine withdrawal (Hours 5-7) compared to non-enriched mice.

The Week 2 of sensitization yielded significant results. There were significant main effects for both Group and Session Hour, *Fs* >5.40, *ps* < .05, $\eta^2 s_{partial}$ >.17, as well as a significant Group x Session Hour interaction, F (1, 28) = 2.26, p < .05. During Week 2, Session Hours 1 and 4 were significantly different between enriched and non-enriched conditions, *ts* (20) > 2.19, *ps* < .05, *ds* > .90, suggesting that enriched mice were more active at baseline (Hour 1) and following methamphetamine exposure (Hour 4) compared to non-enriched mice (Figure 8).

However, the results of Week 3 revealed only a main effect of Session Hour, F (1, 20) = 42.46, p < .05, $\eta^2_{partial}$ =.61, but no main effect of Group or Group x Session Hour interaction. Following the initial two-way ANOVA for Week 3, independent samples *t*-tests were conducted on group activity differences between individual hours. Only group differences were detected during Session Hour 1 between enriched and non-enriched conditions, t (20) = 2.50, p < .05, d = 1.02, suggesting group differences still existed during the baseline period of Week 3 (Figure 9).

Group locomotor activity was significantly different between groups during Weeks 1 and 2 during Hour 4, *ts* (20) > 2.19, *ps* < .05, suggesting that enriched mice were more active than non-enriched mice during these weeks (Figure 10). Furthermore, the Hour 4 analysis of the sensitization period between the two groups demonstrated that locomotor activity for the enriched and non-enriched animals was significantly greater within groups during Week 3

compared to Week 1, *ts* (11) > 3.17, *ps* < .05, suggesting that both enriched and non-enriched mice sensitized to the repeated injections of methamphetamine.

Despite the fact that both showed sensitization, an inspection of Figure 10 seems to suggest that the rate of sensitization was faster for non-enriched mice compared to enriched mice. Thus, a follow-up analysis, involving independent-samples *t* tests, was conducted on the difference scores (Week 3 –Week 1) for each group. While no reliable group differences were detected, difference scores tended to be greater for non-enriched compared to enriched mice, suggesting that non-enriched mice were more responsive to the sensitizing effects of methamphetamine compared to enriched mice (Figure 11).

Discussion

In Experiment 2, the 15-Day and 5-Day enriched groups had very similar locomotor activity levels and rates of sensitization, suggesting that even low levels of environmental enrichment can produce the same effect as longer and continuous enrichment. However, unlike Experiment 1, enriched mice were 1) more active during the baseline and 2) showed an enhanced locomotor-activating effect following methamphetamine administration compared to non-enriched. Despite the enriched mice being nominally more active than the non-enriched mice during the first two weeks during Hour 4, enriched mice seemed to sensitize more slowly compared to the non-enriched mice following repeated methamphetamine administration, a result similar to that of Experiment 1

Summary and Concluding Discussion

In Experiment 1, enriched mice were less responsive to the locomotor-activating effect of methamphetamine and failed to sensitize to methamphetamine following repeated methamphetamine administration. These results are consistent with initial hypotheses and

corroborate recent environmental enrichment studies. For example, Puhl, Blum, Acosta-Torres, and Grigson (2012) demonstrated that environmental enrichment leads to a significant reduction in the number of high-drug taking rodents compared to control conditions. Researchers have also found that environmental enrichment was able to block the development of sensitization to ethanol (Rueda, Teixeira, Yonamine, & Camarini, 2011). The research that Puhl et al. (2012) and Rueda et al. (2012) conducted substantiates current findings for continuous environmental enrichment literature that demonstrates the changes in the rewarding effects of a drug and the development of sensitization. Additionally, the results of the two experiments support research that utilized limited environmental enrichment, such as a study conducted by Ranaldi, Kest, Zellner, and Hachimine-Semprebom (2011). This group of researchers discovered that allowing rodents to live in an enriched environment following initial drug presentations was able to decrease drug-related responding. Specifically, Ranaldi et al. (2011) exposed rats to 10 days of environmental enrichment following a 10-day break from a cocaine self-administration phase. Their environmental enrichment paradigm was similar to that used in the two present experiments, including running wheels and novel toys. After the 10 days of environmental enrichment, Ranaldi et al. (2011) found that enriched animals responded less during extinction trials. Thus, the results of Experiment 1, suggest that limited, similar to continuous, environmental enrichment, blunts the sensitizing effects of drugs of abuse.

In Experiment 2, researchers found exposure of mice to either 5- or 15-days of environmental enriched produced no differences in their responses to the sensitizing effects of methamphetamine. The absence of a difference between these two groups suggests that 15 days of environmental enrichment conveys no additional benefit beyond the 5 days of

enrichment. Moreover, this result suggests that very little amounts of environmental enrichment may be needed to blunt the sensitizing effects of drugs of abuse. Indeed, Rosenzweig and Bennett (1972) found that as little as two hours/day for 30 days of environmental enrichment enhanced performance on a learning and memory task.

In Experiment 2, researchers found that enriched mice were *more* active during the baseline period (Hours 1-2) and following methamphetamine administration (Hour 4) compared to non-enriched mice. The baseline differences, illustrated by the heightened rates of locomotor activity for enriched mice compared to non-enriched mice, continued into Week 2, but not Week 3. Surprisingly, this is the opposite of what was found in Experiment 1. These results, however, are consistent with research conducted by Stairs et al. (1995), who found that enriched animals had a greater initial locomotor response to amphetamine compared to control animals. The discrepancies between Experiments 1 and 2 could be due to differences in the season during which the two experiments were conducted or to the general activity levels of the groups of mice used (i.e. some mice might just be more physically active). That is, locomotor activity levels were greater in Experiment 1 compared to Experiment 2 for non-enriched mice (compare Figures 6 and 10). Alternatively, outliers in the data of Experiment 2 could represent individual differences that altered the overall findings. For example, one animal responded with 142, 0, 502 centimeters of distance traveled following the presentation of methamphetamine (compared to the more typical rates that are often in the thousands and tens of thousands centimeters traveled). This suggests that the mouse could have been hypersensitive to methamphetamine and reacted with stereotypy. It has been found that animals very sensitive to the stimulant effects of methamphetamine will display stereotypy, which is characterized by subjects who engage in repetitive motions,

or a repetitive series of motions (e.g., bobbing their heads) (Canales & Graybiel, 2000). This behavior competes with (and decreases) general locomotor activity (Hirabayashi and Alam, 1981). Despite nominal differences between Experiments 1 and 2 with respect to the activity of enriched and non-enriched mice, enriched mice tended to show a blunted rate of sensitization in Experiment 2 similar to Experiment 1. This latter result suggests that limited environmental enrichment attenuated the sensitizing effects of methamphetamine.

Implications. To date, the vast majority of environmental enrichment studies have employed a paradigm in which rodents are continuously exposed to an enriched environmental from a young age. These animal studies have led support for the idea that rearing children in an enriching environment may have a "neuroprotective" effect against subsequent drug addiction (Stairs & Bardo, 2009). That is, being raised in an enriching environment could decrease the likelihood that a child will develop a drug problem as an adult. The results of the current set of experiments suggest that even *very* limited exposure to environmental enrichment in young children may also have a "neuroprotective" effect against subsequent drug addiction. For example, perhaps exposing children to a few hours of enriched environments (i.e. playgrounds, creative classrooms, and sports) could blunt the rewarding aspects of drugs, leading to a decreased likelihood of drug abuse in the future. Limited access and exposure to enriching environments, as demonstrated in this experiment, are more effective than no exposure at all. Environmental enrichment could potentially act as a preventative measure that could prepare humans and non-humans to combat the rewarding nature of drugs. These findings are pertinent to public policy, specifically those relating to education. Policy makers should be more aware of the positive effects of environmental enrichment while deciding whether or not to cut funding for sports teams, recess, and arts

programs. These programs could act as one mitigating factor in whether or not students abuse drugs.

Replication of this paradigm is integral to the understanding of limited environmental research. Unfortunately due to technological impediments and experimenter error, the data sets for these experiments were not complete. Replication will help to verify the current findings. With future study, researchers should also work to more accurately predict *how much* exposure to environmental enrichment is necessary to produce the neuroprotective effects found following continuous environmental enrichment. Research could also be used to observe the effects of environmental enrichment on other behavioral tasks, such as CPP and operant responding. Another avenue of research could examine the potentially differential role of environmental enrichment during different critical periods of development. Additionally, research could utilize different lengths of exposure to environmental enrichment to explore the limits of environmental enrichment mentioned earlier and find the lowest amount of environmental enrichment that can produce significant differences.

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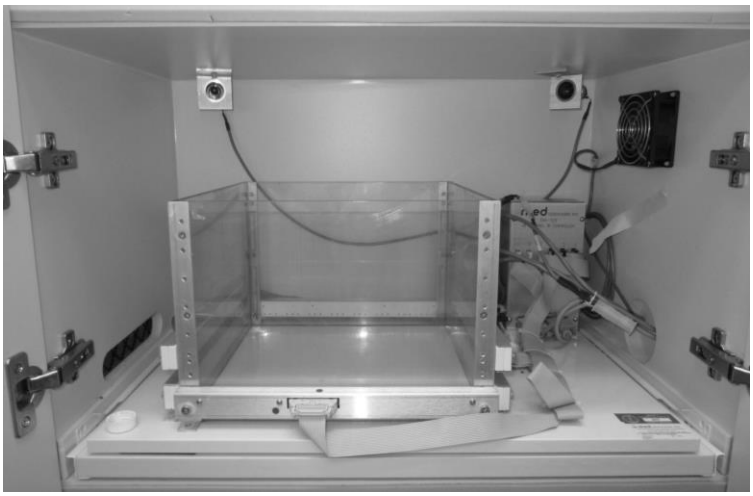

Figure 1: This figure depicts the locomotor activity chambers for Experiments 1 and 2.

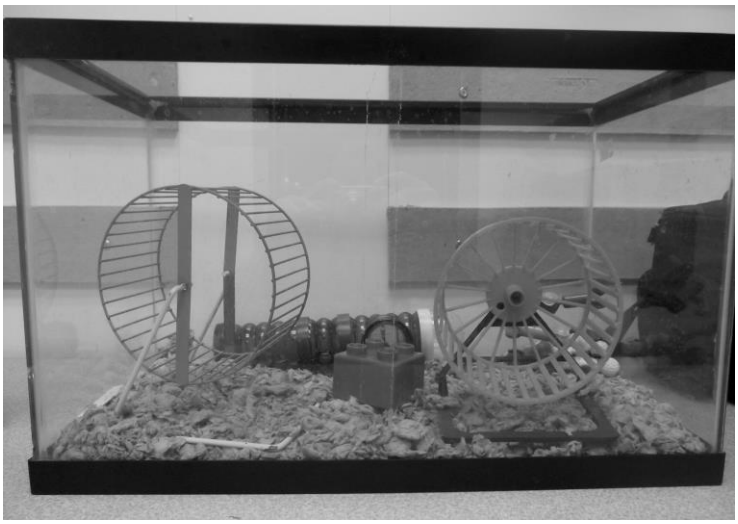

Figure 2: This image demonstrates the enriched condition for Experiment 1 and 2.

Figure 3: This figure illustrates the non-enriched condition and the home cages for each of the subjects for Experiment 1 and 2.

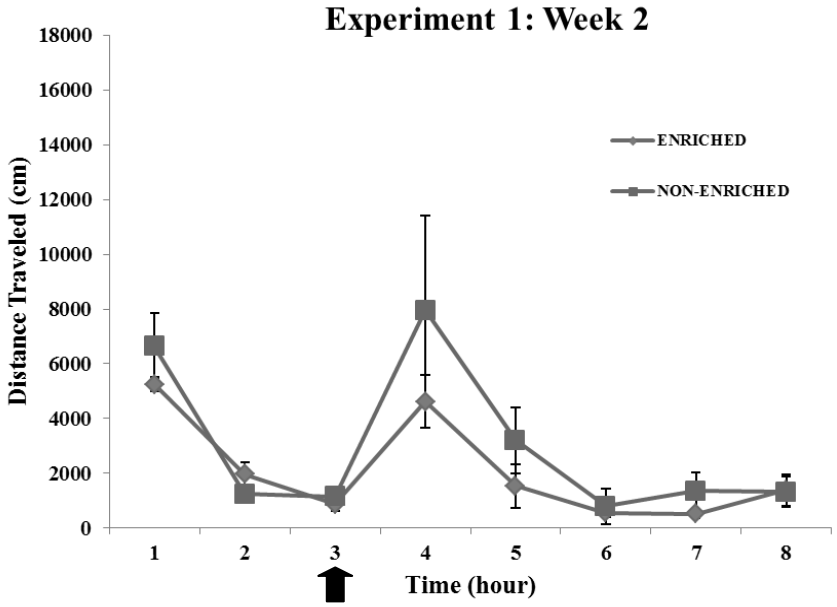

Figure 4. Locomotor activity (distanced traveled in cm) for the enriched and non-enriched control mice during an 8-hour activity session during Week 2 of Experiment 1. Arrow indicates that methamphetamine was injected after Hour 3

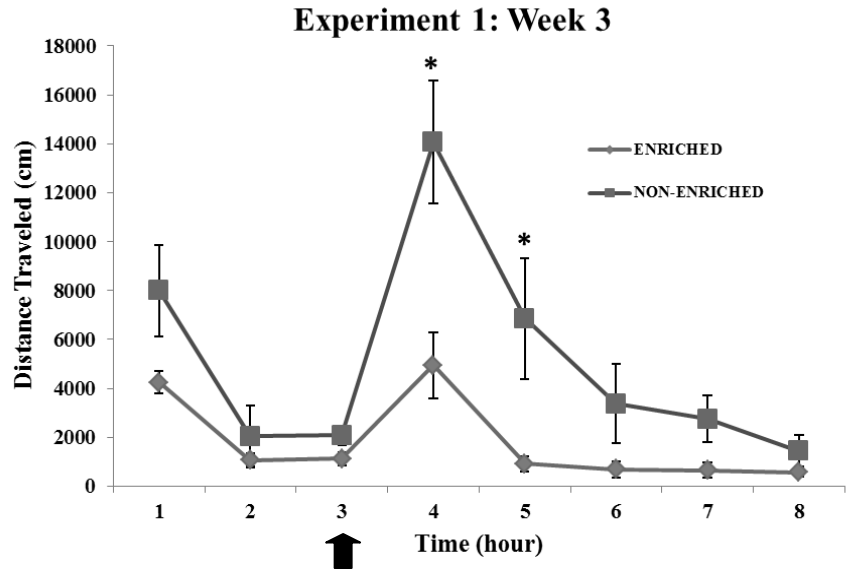

Figure 5. Locomotor activity (distanced traveled in cm) for the enriched and non-enriched control mice during an 8-hour activity session during Week 2 of Experiment 1. Arrow indicates that methamphetamine was injected after Hour 3. Asterisks represent significant differences, $p < .05$.

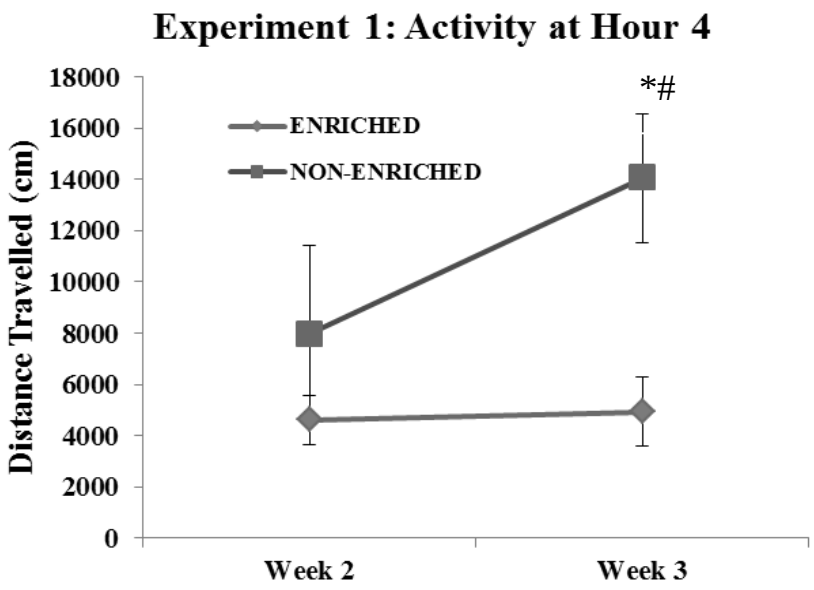

Figure 6. Locomotor activity (defined as distance traveled in cm) for the non-enriched animals was significantly greater during Week 3, compared to Week 2, during Experiment 1, $p < 0.05$. The locomotor activity of the enriched animals did not differ significantly between Weeks 2 and 3. The # symbol denotes a significant difference between Weeks 2 and 3 for non-enriched mice, $p < 0.05$.

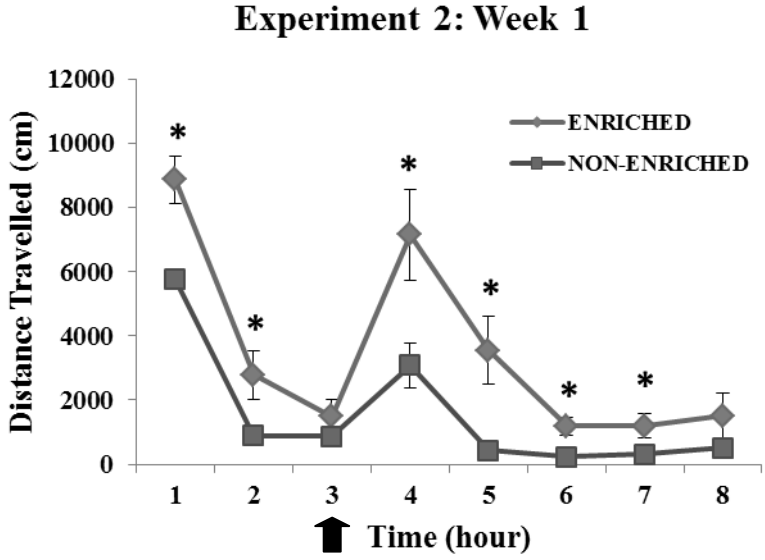

Figure 7. Locomotor activity (distanced traveled in cm) for the enriched and non-enriched control mice during an 8-hour activity session during Week 1 of Experiment 2. Arrow indicates that methamphetamine was injected after Hour 3. Asterisks represent significant differences, $p < -.05$.

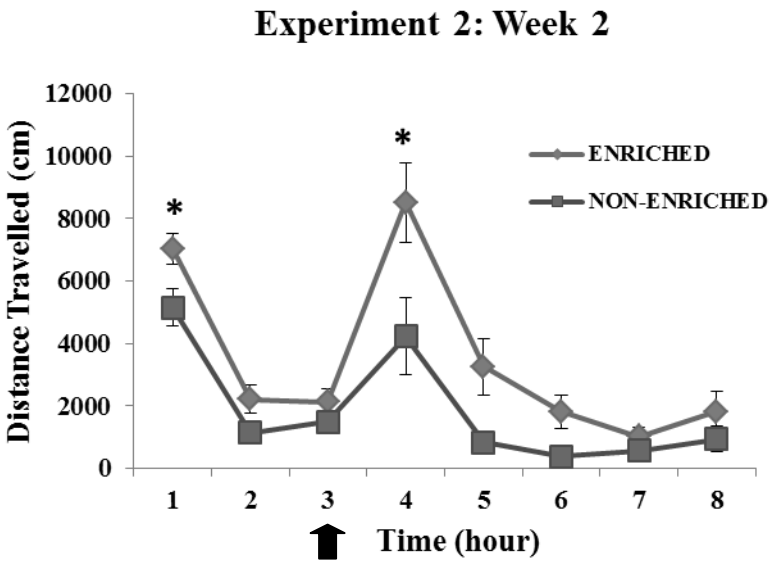

Figure 8. Locomotor activity (distanced traveled in cm) for the enriched and non-enriched control mice during an 8-hour activity session during Week 2 of Experiment 2. Arrow indicates that methamphetamine was injected after Hour 3. Asterisks represent significant differences, $p < -.05$.

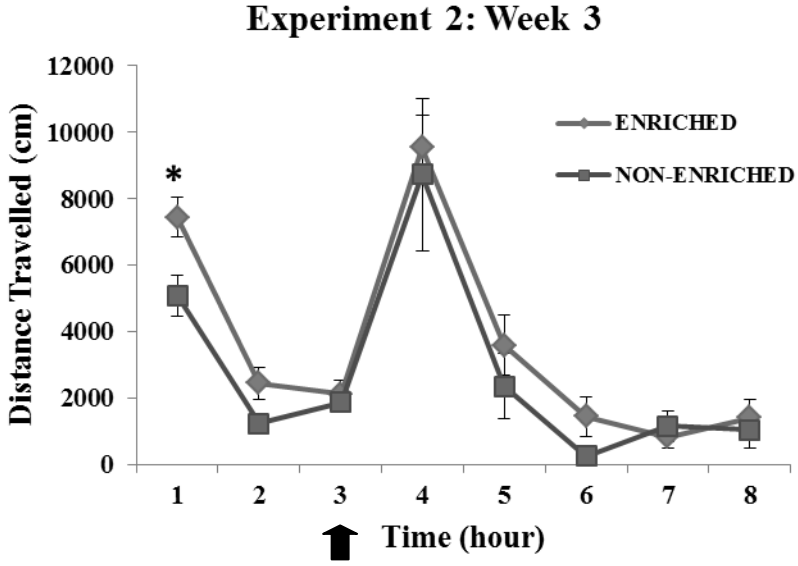

Figure 9. Locomotor activity (distanced traveled in cm) for the enriched and non-enriched control mice during an 8-hour activity session during Week 3 of Experiment 2. Arrow indicates that methamphetamine was injected after Hour 3. Asterisks represent significant differences, $p < -.05$.

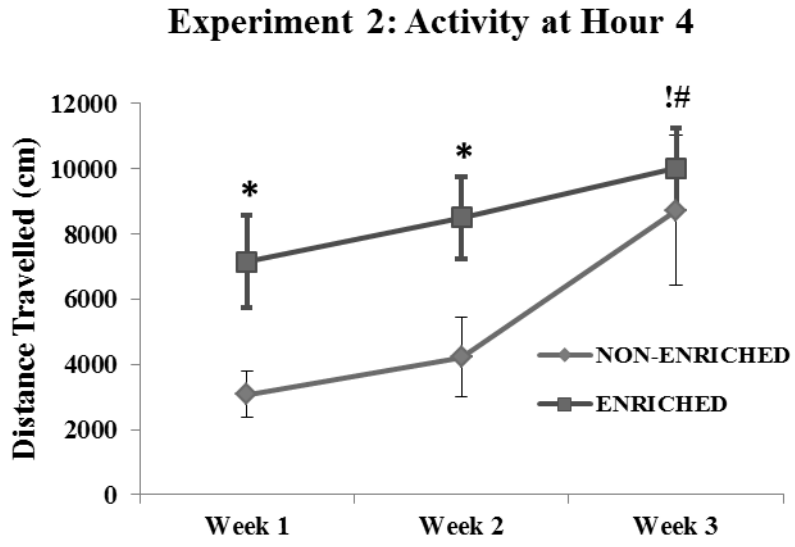

Figure 10. Locomotor activity (distance traveled in cm) for the enriched and non-enriched mice during Hour 4 of Weeks 1, 2 and 3 of Experiment 2. There were significant differences between groups during Week 1 and 2 (asterisks, $p < 0.05$). Locomotor activity, for both enriched (exclamation point) and non-enriched (pound symbol) mice, was significantly different when comparing Week 1 and Week 3 (, *ps* < 0.05).

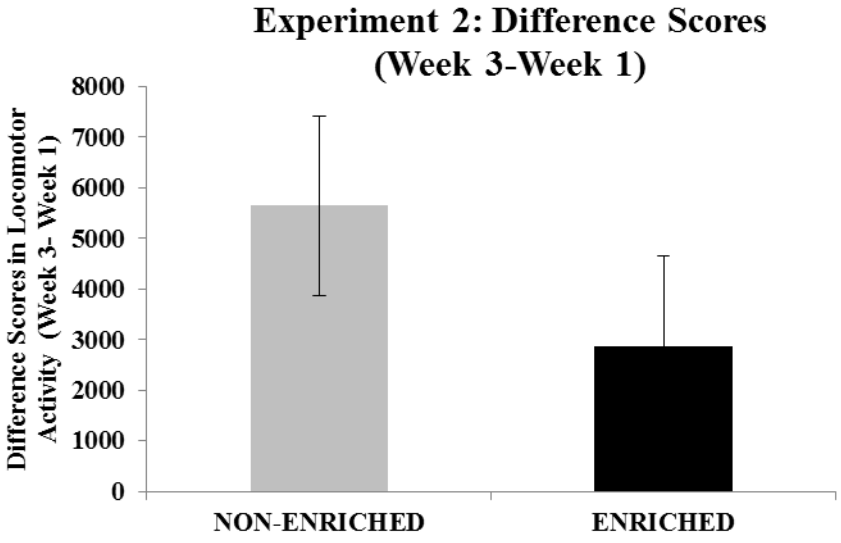

Figure 11. Difference scores (Week 3 – Week 1) for non-enriched and enriched mice during Hour 4 of sensitization training of Experiment 2.