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This paper received a QuaesitUM outstanding paper award.

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Age-Dependent Effects of Social Isolation on Behaviors Related to Anxiety and Addiction in Mice

**Faculty Sponsor**

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## Abstract

Reduced social interaction is associated with an increased occurrence of anxiety and substance use disorder. Using open field testing in mice, this study addressed two questions: 1) Can the behavioral effects of adolescent isolation be reversed through social interaction in adulthood? 2) Does social isolation during adulthood have a similar detrimental effect as adolescent isolation? The study included 4 groups: mice that remained in enriched housing from adolescence to adulthood (environmental enrichment: EE), mice that were group housed during adolescence then isolated as adults (EE-ISO), mice that remained isolated from adolescence to adulthood (ISO), and mice that were isolated during adolescence then group housed as adults (ISO-EE). ISO-EE and EE-ISO mice responded more similarly to EE than ISO mice, indicating that the behavioral effects of adolescent isolation were reversed through social interaction in adulthood and that isolation during adulthood does not have the same impacts as adolescent isolation.

## Introduction

Almost 1 in 5 adults in the United States live with a mental illness (Substance Abuse and Mental Health Services Administration Report, 2019). Of these mental illnesses, anxiety and substance use disorder are 2 of the most common. In the US, 18% of the adult population has reported annual anxiety symptoms, with 22% of those suffering from severe symptoms (Shirneshan et al., 2013). Anxiety is often defined as "a psychological, physiological, and behavioral state induced in animals and humans by a threat to well-being or survival, either actual or potential" (Steimer, 2011, p. 496). Anxiety is an excessive, medically unexplained, worry that occurs more days than not, over at least a period of 6 months and causes impairments in other aspects of functioning (American Psychiatric Association, 2017). Symptoms of anxiety can be nervousness, tenseness, restlessness, or having feelings of impending danger, panic, or doom. In more severe cases anxiety can lead to panic attacks, concentration issues, and even gastrointestinal problems. 7.2% of adults in the United States have been diagnosed with substance use disorder, and nearly 75% of those fell under alcohol use disorder. Substance abuse disorder is a pattern of maladaptive, repeated drug or alcohol use that often interferes with health, work, or social relationships with an inability to control their use of the drug (American Psychiatric Association, 2017). Research identifying risk factors associated with these disorders can be valuable for both prevention and treatment.

Animal studies are particularly useful for identifying risk factors given that researchers can control and manipulate sensitive variables (Stevens & Vaccarino, 2015). Animal studies allow us to understand neural substrates and disorders associated with adolescent experiences via the manipulation of many variables (Jaworska & MacQueen, 2015). Mice in particular are widely used in scientific research due to them being compact, cost-effective, easily available and conserve ~99% of human genes and physiologically resemble humans (Dutta & Sengupta, 2016). The lifespans of mice and humans differ significantly with mice average lifespan of 24 months while humans average 80 years (~24 months and ~80 years, respectively); however, similar molecular mechanisms of aging and patterns of disease pathogenesis are observed in mice and humans. Faster aging patterns in mice enables research on various life stages in a shorter time period. Mice are weaned at 3 weeks and reach the onset of puberty at 4 weeks and sexual maturity at 8-12 week (10 weeks average); therefore, adolescence in mice is often defined as the age of 3-10 weeks (Dutta & Sengupta, 2016). Adolescence in particular is an important developmental period for both physiological and neurobiological changes. In humans, stress during childhood and adolescence is 1 environmental factor

that has been associated with both anxiety and substance use disorder in adulthood (Watt et al., 2017). In rodents, social isolation during adolescence has been shown to increase release of the stress hormone corticosterone and induce behaviors related to anxiety (Lopez & Laber, 2015; Lukkes, et al., 2009) and increased drug-seeking (Alexander et al., 1978; Walker et al., 2019).

Open field testing is a common way of assessing behaviors associated with anxiety and substance use disorder. During open field chamber testing, time spent in the center of the chamber and exploratory behaviors are recorded. In mice, decreased time spent in the center is associated with increased levels of anxiety, as mice prefer to stick to the edges of the chambers (Sáenz, Villagra & Trías, 2006). Exploratory behavior is measured with locomotor activity (total distance traveled within the chamber) and rearing (number of times mice stand on their back legs). Increased exploratory behaviors has been correlated with an increased likelihood of drug self-administration, a well-established measure related to substance abuse disorders (Mitchell, Gao, Hallett, & Voon, 2016). Preliminary studies in our lab have shown that mice that have been isolated during adolescence, which ranges from 3 weeks old to 12 weeks old, spent less time in the center which is an indication of increased anxiety and displayed increased locomotor activity and rearing which both indicate an increased vulnerability to drug abuse. These findings are similar to results from other labs (Pietropaolo, Feldon & Yee 2008; Seibenhener & Wooten, 2015). This leads us to ask two questions related to impact of social isolation on these behaviors:

1) Can the behavioral effects of adolescent social isolation be reversed through social interaction in adulthood?

2) Does social isolation during adulthood have the same detrimental effect as social isolation during adolescence?

Both of these questions aim to understand the importance of developmental stage on the effects of an environmental stressor. Neural plasticity has been shown to be more dynamic during adolescence compared to adulthood (Watt et al., 2017). Therefore, regarding the first question, the neural changes induced by adolescent isolation may not be reversible with adulthood interventions. For example, Pietropaolo, Feldon & Yee (2008) found that social isolation during early post weaning phase (3-7 weeks) induces behavioral changes, such as startle reactivity, isolation-induced hyperactivity, and enhanced locomotor response that cannot be reversed via subsequent resocialization. Also, early environmental stress has been shown to cause a biological response of altering DNA methylation, which in turn affects transcriptional activity of DNA and can have long-term impacts (Walters & Kosten, 2019). However, Solinas et al. (2008) found that after 30

days of environmental enrichment behavioral sensitization preference for cocaine was eliminated in previously isolated mice, supporting the idea that environmental stimulation and social interaction can eliminate an already-formed drug propensity due to isolation.

The second research question addresses whether social isolation during adulthood has the same detrimental effect as isolation during adolescence. Adolescence is a key developmental window during which stressful experiences may result in long-term alterations of brain structure and functioning (Burke et al., 2017). However, moving mice from environmentally enriched to non-enriched conditions increased the rewarding effect of cocaine and increased neural functioning associated with anxiety and emotional distress (Nader et al., 2012). Smith et al. (2017) found that enrichment removal in rats, who during adolescence had an enriched environment, presented features of depression that otherwise are only rarely observed in animal models. This same study also found that these symptoms of stress, hyperphagia and weight gain, were unique to enrichment removal as the removal of other rewards did not produce the same symptoms (Smith et al., 2017). These findings indicate that positive life conditions during early life stages, if not maintained during adulthood, may have negative emotional consequences and associations with anxiety and substance use disorder (Nader et al., 2012).

The current study aims to expand on previous results and address the above-mentioned research questions. We hypothesized that the mice raised in isolation and transferred to an enriched environment would have reduced behavioral phenotypes related to anxiety and substance use disorder compared to mice raised in isolation. In other words, we expect that enrichment during adulthood could reverse the measured behavioral effects of adolescent isolation. We also hypothesize that the mice raised in an enriched environment and transferred to isolation would have increased behavioral phenotypes related to anxiety and substance use disorder compared to mice left in enrichment. Thus, we expect that adult isolation will induce similar behavioral changes as seen with adolescent isolation. Currently, gaps exist between our understanding of adolescent brain development and the clinical research involving adolescents with emerging, or later established, psychiatric disorders (Jaworska & MacQueen, 2015). The findings from this study will elucidate the importance of developmental stage in mediating anxiety, and addiction related behavioral effects of social isolation. This information can be beneficial to programs aiming to prevent or treat anxiety and/or substance use disorder.

## Methods

This experiment was approved by the University of Memphis Institutional Animal Care and Use Committee (IACUC) and was conducted in accordance with the guidelines set by the Public Health Service (PHS).

### Subjects and Housing Conditions

Sixty-four female C57 mice were obtained from Jackson Laboratory (Bar Harbor, ME) at 3 weeks old and were randomly divided into 4 housing condition groups. Female mice were exclusively used in the present study due to male mice co-habitation following isolation often resulting in fighting, injury, and/or death of the subjects (Kappel, Hawkins & Mendl 2017). Given that adolescence in mice is defined as between the ages of 3-10 weeks (Dutta & Sengupta, 2016), the housing conditions were designed to persist from the beginning of adolescence to adulthood. Socially enriched environments (EE) included 4-5 mice per cage. Twenty-five mice remained in EE, from 3 to 15 weeks old, throughout the study. Isolated environments (ISO) consisted of 1 mouse per cage. Twenty-one mice remained in ISO throughout the study, again from 3 to 15 weeks old. Nine mice were isolated during adolescence, 3 to 12 weeks, then moved to EE for 6 weeks. The mice in this group will be referred to as ISO-EE (isolated to enriched housing). Nine mice were housed in EE during adolescence, 3 to 12 weeks, then moved to ISO for 6 weeks. The mice in this group will be referred to as EE-ISO (enriched to isolated housing). See Table 1 for a breakdown of the experimental groups. All housing conditions were in a temperature-controlled room (21±1° C) with food and water available ad libitum, on a 12 hr light/dark cycle (lights on at 0600). Cages were cleaned and bedding refreshed consistently twice per week.

*Experimental Groups*

| Adolescent Housing Condition | Adult Housing Condition | N |
|---|---|---|
| EE | EE | 25 |
| EE | ISO | 9 |
| ISO | ISO | 21 |
| ISO | EE | 9 |
| | **Total** | **64** |

**Table 1.** Housing conditions. EE: environmentally enriched; ISO: isolated.

### Procedure

On the day following the end of the allotted housing condition period, open field testing was conducted once on each mouse. On the day of testing, mice were individually caged in the testing room in a dark, soundproof cabinet

45 minutes before being placed in the open field chamber. This habituation period was to prevent exposure to aversive stimuli and to get the mice used to the testing environment. Open field testing occurred consistently in the morning, between the hours of 0800 and 1100. Four open field chambers were available for simultaneous testing of individual mice. The mice were placed in the front center of the open field, and the infrared recording button started at the same moment of placement. The mice were allowed to freely roam in the chamber for 20 minutes while locomotor behavior was quantified by infrared beam breaks and distance traveled within the chamber. HamiltonKinder SmartFrame™ (Hamilton Kinder, Poway, CA), with a 4 x 8 photo beam strip and a 4 x 8 photo beam rearing attachment, was used for open field testing in junction with MotorMonitor version 4.14 (HamiltonKinder, Poway, CA) recording the locomotor behavior. The walls of the open field chamber were clear Plexiglass, and lighting included a 15-W bulb. The center area of the open field chamber was set in the software to measure 12.05 cm x 22.86 cm. This central area was located 6.03 cm from the left and right walls and 11.43 cm from the front and back walls. After each 20 min. recording session, the mice were returned to their home cages. The open field chambers were cleaned with 10% isopropyl alcohol and allowed to dry before a new mouse as introduced to the chamber.

#### Data analysis

Three dependent variables were measured using open field testing: time in center of chamber, overall locomotor activity, and number of rears. For each dependent variable, a one-way between-subjects ANOVA with Tukey HSD post hoc test was used to determine differences between the 4 housing conditions (EE, EE-ISO, ISO, and ISO-EE).

## Results

#### Time in Center

A one-way between-subjects ANOVA was conducted to compare the total time (in sec) spent in the center of the chamber between housing condition groups. Housing conditions significantly affected the time spent in the center [$F(3,60) = 7.850$, $p < .001$] (Figure 1). A post-hoc Tukey HSD test indicated a near significant difference between ISO and EE mice ($p = .069$), with ISO mice spending less time in the center compared to EE mice, indicating increased anxiety in ISO mice, as expected. No statistical difference was observed between EE and EE-ISO mice ($p = .803$), indicating that moving mice to isolation during adulthood did not alter anxiety-related behavior. Interestingly, the ISO-EE mice spent a significantly increased amount of

time in the center compared to both the EE mice (p = .031) and ISO mice (p = .000), indicating decreased occurrence of anxiety-related behavior in the ISO-EE mice.

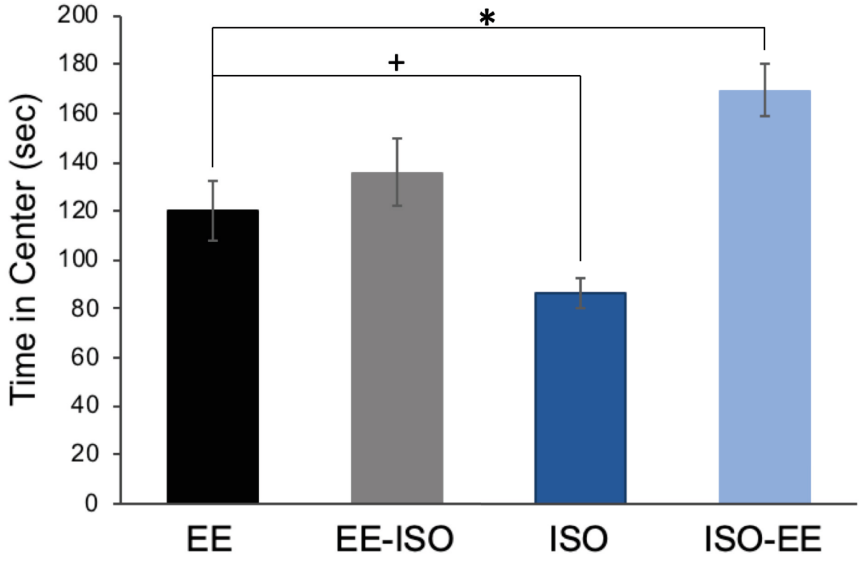


**Figure 1.** Time in Center

Increased time in the center is an indication of reduced anxiety. No differences were observed between EE and EE-ISO mice. A trend in the data suggested that ISO mice were more anxious than EE mice (+ p = .069 compared to EE). ISO-SH mice were less anxious than EE mice (* p < .05 compared to EE). EE: environmentally enriched housing during both adolescence and adulthood; EE-ISO: environmentally enriched housing during adolescence and isolation in adulthood; ISO: isolation during both adolescence and adulthood; ISO-EE: isolation during adolescence and environmentally enriched in adulthood.

#### Locomotor Activity

A one-way between-subjects ANOVA was conducted to compare the effect of housing conditions on the locomotor activity (distance travelled in cm). Housing conditions significantly affected locomotor activity [F (3,60) = 5.898, p = .001] (Figure 2). Specifically, ISO mice displayed significantly increased locomotor activity relative to EE mice (p = .001), as expected. However, neither EE-ISO nor ISO-EE significantly differed from EE mice in regards to locomotor activity (p = .692 and .622, respectively).

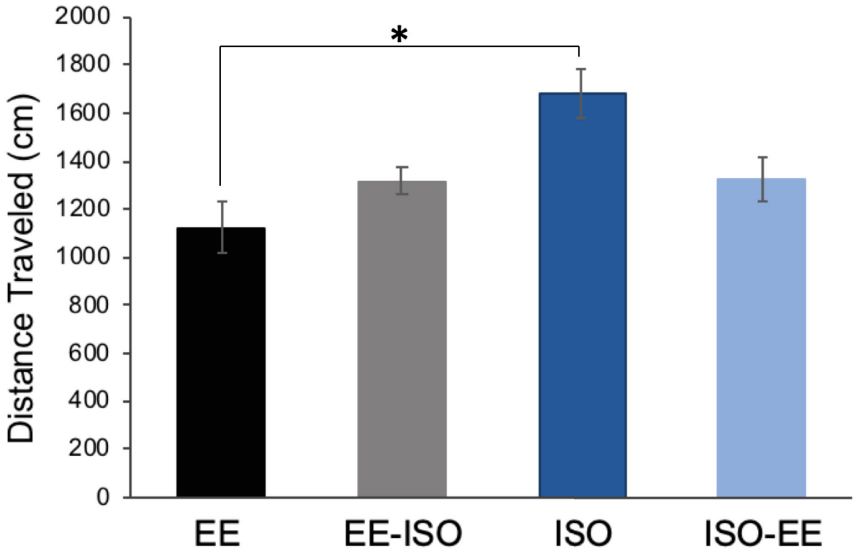


**Figure 2.** Locomotor Activity

Increased locomotor activity indicates increased exploratory behavior. No differences were observed between EE, EE-ISO, and ISO-EE mice. ISO mice exhibited increased locomotor activity compared to EE mice (* $p < .05$ compared to EE). EE: environmentally enriched housing during both adolescence and adulthood; EE-ISO: environmentally enriched housing during adolescence and isolation in adulthood; ISO: isolation during both adolescence and adulthood; ISO-EE: isolation during adolescence and environmentally enriched in adulthood.

#### Number of Rears

A one-way between-subjects ANOVA was conducted to compare the effect of housing conditions on the number of rears. Housing conditions significantly affected the number of rears [$F(3,60) = 8.951$, $p < .001$] (Figure 3). As expected, ISO mice had a significantly increased number of rears compared to EE mice ($p = .003$). EE-ISO also showed an increase in their number of rears relative to EE mice ($p < .001$). However, no rearing differences were observed between ISO-EE and EE mice ($p = .693$).

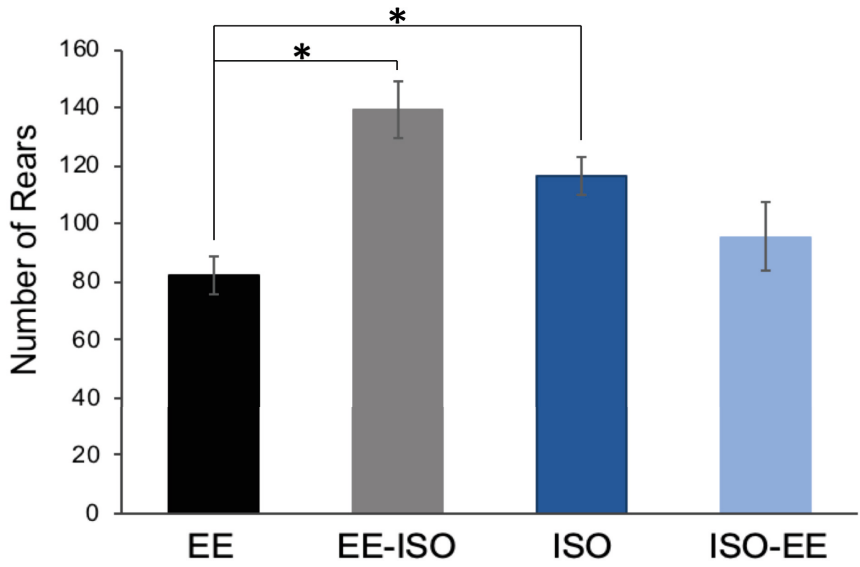


**Figure 3.** Number of Rears

Increased rearing indicates increased exploratory behavior. No differences were observed between EE and ISO-EE mice. EE-ISO and ISO mice exhibited increased rearing activity compared to EE mice (* $p < .05$). EE: environmentally enriched housing during both adolescence and adulthood; EE-ISO: environmentally enriched housing during adolescence and isolation in adulthood; ISO: isolation during both adolescence and adulthood; ISO-EE: isolation during adolescence and environmentally enriched in adulthood.t

## Discussion

For mice, isolation during adolescence is known to induce behaviors associated with anxiety and substance use disorder. The purpose of this project was to determine if the behavioral effects of adolescent social isolation can be reversed through social interaction in adulthood; and if social isolation during adulthood has the same detrimental effect as social isolation during adolescence. It was hypothesized the mice raised in isolation and transferred to an enriched environment (ISO-EE) would have reduced behavioral phenotypes related to anxiety and substance use disorder compared to mice that remain in isolation (ISO). In regards to the second question, we expected that the mice raised in an enriched environment and transferred to isolation (EE-ISO) would have increased behavioral phenotypes related to anxiety and substance use disorder compared to mice left in enrichment (EE). Regarding behavioral phenotypes, the anxiety-related behavior measured

in the current study was time spent in the center of the open field chamber. Decreased time spent in the center is associated with increased occurrence of anxiety-related behavior (Sáenz, Villagra, & Trías, 2006). Exploratory behavior (as measured with locomotor activity and rearing in the open field chamber) was used as an indication of propensity for drug abuse. Increased exploratory behaviors correlates with hyperdopamineric functioning and increased drug-seeking behaviors (Beninger, 1983).

As expected, ISO mice spent less time in the center and displayed increased exploratory behaviors compared to EE mice. These findings support many previous studies in which adolescent isolation in rodents induced increased occurrence of behaviors related to anxiety and increased drug-seeking and drug responses (Pietropaolo, Feldon & Yee, 2008; Rodríguez-Ortega, & Cubero, 2018; Seibenhener & Wooten, 2015). Regarding the first research question of whether EE during adulthood can reverse the behavioral effects of adolescent isolation, we found that ISO-EE mice behaved more similarly to the EE mice than the ISO mice. ISO-EE mice actually spent more time in the center compared EE mice (indicating reduced anxiety), and ISO-EE mice displayed similar exploratory behaviors as EE mice. These findings indicate that moving isolated mice to EE housing did reverse the observed isolation-induced behavioral deficits; and support the findings of Rodríguez-Ortega and Cubero (2018) in which adulthood access to EE blunted isolation-induced operant responses in the cue-related reinstatement of heroin seeking in rats. Studies such as these are important for clinicians treating disorders centering around stressful events and/or negative social interactions during adolescence.

Regarding the second research question of whether social isolation during adulthood has the same detrimental effect as social isolation during adolescence, our results showed that EE-ISO mice did not significantly differ in their time spent in the center of the chamber nor in their locomotor activity compared to EE mice. As mentioned previously, locomotor activity in the novel open field often signals mesolimbic dopamine functioning, similar to that of drugs of abuse, and can be used as a correlation link between addiction related behaviors in mice (Beninger, 1983).

Thus, our findings indicate that adulthood isolation does not have the same effect as adolescent isolation, given that the time in center and locomotor activity of the EE-ISO group more closely resembled that of the EE mice rather than the ISO mice. Reduced neural plasticity in adulthood may prevent environmental stressors from impacting behavior to the same extent as is possible during adolescence, a time in which neural changes are more rampant (Watt et al., 2017). However, we did find that the EE-ISO mice exhibited an increased number of rears compared to EE mice. Previous

studies have shown that moving mice from EE to non-enriched conditions increased the rewarding effects of cocaine, increased neural functioning associated with emotional distress, and increased occurrence of behaviors associated with depression (Nader et al., 2012; Smith et al., 2017). It is possible that the isolation paradigm of the current study was not stress-inducing enough to alter the behaviors of the adult mice. The mice were isolated, in a cage by themselves, but still able to see, hear, and smell mice from other cages. Future studies could use total isolation and/or measure corticosterone levels to determine whether the isolation induced a stress response.

## Conclusion

In conclusion, the findings of the current study indicate that adolescent isolation was associated with increased behavioral phenotypes of anxiety and substance use disorder in female mice, but these behavioral changes seem to be reversible and age dependent.

The data supported the hypothesis that the mice raised in isolation and transferred to an enriched environment (ISO-EE) would have reduced behavioral phenotypes related to anxiety and substance use disorder compared to mice that remained in isolation, but the data did not support the hypothesis that the mice raised in an enriched environment and transferred to isolation (EE-ISO) would have increased behavioral phenotypes related to anxiety and substance use disorder compared to mice left in enrichment. Instead, adult isolation did not seem to have a major impact on the measured behaviors.

It should be noted again that the current study was only conducted in females. Male and female mice have been shown to react differently to altered social interactions during both adolescence and adulthood (see Walker et al., 2019), but repeating these experiments with a focus on the physical stimuli rather than the social stimuli may be useful in males. Nonetheless, the present findings highlight the importance of EE during adolescence and the potential use of EE in adulthood to rescue isolation-induced behavioral phenotypes. The present findings also support the use of social and environmental stimuli in therapeutic programs for disorders related to anxiety and substance abuse.

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