ADOLESCENT SOCIAL ISOLATION ALTERS REWARD-SEEKING BEHAVIOR AND INCREASES ΔFOSB EXPRESSION IN MICE

by
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Abstract

Social isolation (SI) is an environmental stressor that has been shown to disrupt sleep, increase depressive and anxiety-like symptoms, and serve as a risk factor for the development of psychiatric illness. Most studies of social isolation in humans have investigated the effects of isolation in older adults, but there is a trend of increasing isolation and loneliness in adolescents. Animal models of isolation indicate that adolescent social isolation can have life-long effects on neurology and behavior. This project used a mouse model of adolescent social isolation to establish a behavioral and molecular profile of the adolescent SI phenotype. We found that ΔFosB, a transcription factor associated with chronic stress, is increased in the prelimbic and infralimbic cortices of adult male mice that had experienced adolescent social isolation. We also found that male mice that had experienced adolescent social isolation display a behavioral profile of elevated reward-seeking behavior as measured by touchscreen-based versions of a continuous performance test and progressive ratio task.

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List of abbreviations

CPT:	Continuous performance test
FR:	Fixed ratio
ITI:	Intertrial interval
LH:	Limited hold
mPFC:	Medial prefrontal cortex
P[number]:	Postnatal day [number]
PL/IL:	Prelimbic/infralimbic cortex
PR:	Progressive ratio
SD:	Stimulus duration
SI:	Social isolation

Chapter 1 – Introduction

1.1 Social isolation

Social isolation (SI) is a well-established source of environmental stress in social organisms. [1] Social connections are critical to overall health in several species, with levels of social integration being useful as predictors of mortality. [2] Thus, a lack of social connection altogether presents a strong health risk.

In humans, studies of the effects of SI have primarily focused on SI in older individuals. [3] SI in this age group has been considered a public health concern for a long time, as the patterns of degradation of social connections are readily apparent. This body of work has shown that even short-term isolation can result in disruptions to sleep, attention, and cognitive function. [4] However, more recent demographic studies have begun to show a trend of increasing SI among adolescent individuals. [5]

Adolescence is a period characterized by rapid physical and social development [6] that is also associated with increased sensitivity to stress. [7] Due to the developing nature of the brain during adolescence, it is thought that the effects of SI experienced by adolescents may be different from or more severe than the previously established effects. [8]

Social isolation research has historically been complicated by the objective differences between perceived isolation, or loneliness, and physical separation from other people. [9] Loneliness has been historically studied as a psychological issue compared to the external state of isolation, but both have similar effects in the short term. [10] The two

are also difficult to separate in demographic studies, as it is difficult for these studies to reach people who are physically isolated. [11]

Loneliness and social isolation are associated with high rates of morbidity and mortality, even beyond other environmental risk factors. [12] Social isolation has been linked to increased risk of several psychiatric illnesses. Longitudinal studies of loneliness and SI associate them with risk for cognitive impairment, dementia, and several psychiatric illnesses, including schizophrenia. [1]

Depressive symptoms were strongly associated with loneliness in a longitudinal study. [9] This study used self-reported measures of loneliness combined with the CES-D, a commonly used questionnaire to measure depression, and found that loneliness significantly predicted the presence of depressive symptoms, including irritation, impaired attention, and disrupted sleep.

Isolation is known to have extensive impacts on sleep. [13] Studies investigating links between loneliness and sleep found that wake time after onset, a metric of restless sleep, was elevated in people who self-reported as lonely. Self-reported metrics of poor sleep quality, including decreases in sleep duration and increases in daytime dysfunction, also correlated with self-reported loneliness. [13] Conversely, rodent studies show that increased social interaction improves sleep quality. [14]

The disruption of sleep has several downstream effects as well. Chronic sleep disruption has been shown to elevate cortisol levels, lower glucose tolerance, and increase sympathetic tone. [15] All of these features serve as persistent sources of stress, and corrections to sleep quality may not be sufficient to address them.

As sleep is an important restorative behavior, its interruption by isolation has significant impacts on an individual's ability to maintain several aspects of the body. [16] The simultaneous increase in stressors and decrease in an organism's stress reduction mechanism amplify the effects of stress. The directionality of these factors is unclear – whether isolation disrupts sleep by causing stress or causes stress by disrupting sleep – but the negative effects of isolation are readily observable.

Cognitive function is heavily impacted by isolation and loneliness. Studies of this interaction have focused on older individuals already at risk of isolation, with the added risk of Alzheimer's disease and other cognitive disorders. Loneliness and isolation were found to exacerbate the risk of cognitive disorders. [17]

Prior to the development of disorders, however, isolation and loneliness were still shown to affect cognition. [10] Verbal fluency, immediate recall, and delayed recall were all significantly impaired by isolation and loneliness. Isolation was found to have a stronger effect on these domains than loneliness. In this longitudinal study, baseline reports of elevated isolation and loneliness all showed impacts on fluency and recall, but over time, only increases in isolation were associated with decreases in fluency. Increases in both isolation and loneliness correlated with significant decreases in recall ability.

It is important to note that this study specifically investigated the interaction between isolation and aging, and therefore it is possible that adolescent isolation may affect different cognitive domains.

Early-life adversity, including social isolation, has been shown to increase risk of substance use disorders later in life. [18] This has been observed in humans, where

people with problems with substance use report social isolation, [18] and in animals, where adolescent social isolation promotes drug-seeking behavior in adulthood. [19] Some studies suggest that substance use during adolescence can in turn promote social isolation. [20] This bidirectional causation serves to strengthen the negative effects of both social isolation and substance use.

Several aspects of adolescence render it a particularly susceptible period to the impacts of loneliness. In social animals, adolescence is usually associated with an expansion of one's social connectedness. Studies of adolescence in rats have shown increased preference for social interaction compared to adulthood, [6] and social play behavior peaks during this period in rodents. In humans, this manifests as a gradual shift in reliance on an individual's family to their peers. [21] Adolescent people are sensitive to social stress during this time, as negative experiences with other adolescents is associated with risk of depression in adulthood. [7]

Most human studies in this field have used self-reported metrics of loneliness rather than observations of physical isolation. [10,11,13] The reasons for this are primarily the difficulty in reaching people for study who are disconnected from others, and the possibility that someone with low social connectedness may not perceive themselves as lonely (and conversely, that a highly-connected person may still feel lonely). [22] The nuance separating isolation and loneliness adds depth to studies in humans, but the reliance upon self-reporting in assessing loneliness makes it difficult to assess in animal models.

Bearing this in mind as a potential shortcoming of animal models of social isolation, animal studies are still valuable for the insights they can provide into the neurological and cognitive impacts of SI.

1.2 Animal models of social isolation

Studies of social isolation in animals have primarily been conducted using rats and mice. Some of the earliest studies of SI in rats showed a distinct behavioral profile similar to the one seen in humans, including anxiety-like behavior and aversion to novel environments. [23] These studies also showed that the length and timing of isolation impacted its outcome, with certain periods of isolation having long-lasting and irreversible effects and other periods having only temporary effects.

Further research has refined this timing into several critical periods of brain development, during which SI stress can cause long-lasting changes in various parts of the brain. One of the most commonly studied critical periods of brain development in mice consists of postnatal days 21-35 (P21-35). SI during this period has been shown to affect development of the prelimbic and infralimbic cortices as well as the amygdala. [24] The effects of adolescent SI are observable in adulthood, even following resocialization after the isolation period.

Commonly observed effects of SI in mice include anxiety-like behavior, impulsivity, sleep disruption, addiction-like behavior, and increased locomotor activity, with SI during P21-35 also causing observable neurological changes, including decreased myelination of the PL/IL. [25]

Behaviorally, development during P21-35 corresponds with the highest levels of social play with littermates. [8] This behavior is thought to be important for the development of the PL/IL, as SI during this period also negatively affects sociability in isolated mice.

Isolation extending beyond P35 has also been studied. SI of mice from P21-49 is associated with increased locomotor activity and depressive behavior, which was attenuated by administration of tropisetron, a nitric oxide synthase inhibitor. [26]

SI of mice from P21-63 was found to cause changes in the gut microbiome leading to decreased oxytocin receptor in the mPFC. [27] This model also found that administration of an oxytocin receptor antagonist negatively affected social behavior and increased anxiety-like behavior.

Although all of these studies encompass the period of P21-35, it is unclear if isolation extending beyond day 35 was necessary to cause the specific changes observed in those studies. It is possible that extended periods of isolation can affect multiple areas of brain development and combine to produce the observed results.

Studies comparing the timing of isolation have found that isolation periods occurring later in life do not produce behavioral changes as strong as those caused by adolescent isolation, and that animals experiencing later isolation are more responsive to drugs addressing the induced behavioral changes. [28] Therefore, it is accepted that the developmental impacts of isolation necessary and sufficient to cause a persistent SI phenotype occur during P21-35.

Although the neurological and behavioral features of the isolation phenotype are well known, the mechanisms connecting the experience of isolation to these effects remain

unclear. Other factors of an individual's environment may prevent or exacerbate the effects of isolation, and internal factors such as sex or developmental progress may be involved in mediation of the individual's response to isolation stress.

Dopamine metabolism is a potential mechanism interrupted by social isolation stress. [8] SI causes changes in dopamine metabolism in the brains of mice in a region-specific manner, including an increase in the nucleus accumbens. Developmental changes to dopamine metabolism can impact reward learning later in life.

1.3 Environmental stress

Several features of the isolation phenotype point to dysregulated stress response as a possible candidate. Social isolation is one of many examples of environmental stressors. It is important to understand the features that SI shares with other models of environmental stress, both mechanistically and behaviorally. The effects observed following SI are consistent with other models of chronic stress, including limited bedding and repeated drug exposure.

The Fos family of immediate early genes are a key component of an organism's stress response. [29] FosB and related proteins heterodimerize with Jun family proteins to form activator protein-1 complex (AP-1) which then enters the nucleus where it binds to AP-1 promoter sequences. Genes regulated by AP-1 promoters include GluR2, CDK5, and NFκB. However, drug exposure studies indicate that activation of these genes by AP-1 differs depending on the drug administered. Proteins such as c-Fos and FosB are produced and then quickly degraded via ubiquitin tagging, but occasionally a truncation isoform of FosB will be transcribed lacking these degradation domains. This isoform,

ΔFosB, is consequently much longer lived than FosB, and can accumulate in situations of repeated stress response induction. ΔFosB production is associated with several drugs, including cocaine and amphetamine. The long life of ΔFosB makes it useful as a marker of chronic stress exposure, and is possibly functional in the development of the chronic stress behavioral phenotype as well, as it may serve to prolong the activation of stress response mechanisms after the period of acute stress.

This functional role was implicated by studies that found that viral overexpression of ΔFosB in the mPFC of mice increased stress susceptibility. [30] These studies mirrored similar findings that ΔFosB expression was induced via social defeat stress. [31]

ΔFosB is also thought to play a role in risk of addiction, as it has been observed to increase following drug administration. It also plays a role in addiction by augmenting tolerance to a drug and by promoting reward-seeking behavior. As chronic stress during development is associated with risk of addiction, it is possible that ΔFosB may be acting in this role in the context of adolescent SI.

However, level of ΔFosB alone does not give a complete picture of chronic stress. Comparing ΔFosB levels to FosB levels can illustrate an individual's experience with chronic stress as well as their experience of acute stress. In this case, an elevated ratio of ΔFosB/FosB would indicate a history of chronic stress but a low level of acute stress, and a lower ratio may signify that an individual is experiencing acute stress in addition to their experience with chronic stress. This measure is important to contextualize the features of the stress response phenotype.

Although ΔFosB may not be solely responsible for the isolation phenotype, its roles in chronic stress and reward learning imply that it may be involved in the isolation response mechanism.

1.4 Touchscreen-based cognitive testing

Assessment of the cognitive deficits induced by social isolation, including sustained attention, [32] has traditionally taken the form of operant testing. These modular and repeatable tasks are sensitive to changes in attention and cognition, and have been used diagnostically in contexts of Alzheimer's disease, schizophrenia, and other psychiatric illnesses. [33]

Operant tests also have a rich history in animal behavior studies. However, translatability of tests and results between humans and animals remains a significant challenge. [34] The recent development of touchscreen operant testing allows for closer agreement between human and animal cognitive studies, increasing the potential translatability of animal results.

Touchscreen platforms provide a flexible and translatable platform for performing behavioral tests. As an advancement of traditional operant testing, they allow for more in-depth and nuanced analysis of both the specific cognitive domain being tested and various other attributes that may affect test performance. [35] Several traditional operant tests have been translated into touchscreen versions, providing easily customizable and scalable tools for behavioral research. Touchscreen tasks are ideal for measuring attention and recall, two domains known to be affected by isolation.

Touchscreen tasks provide several advantages over other operant test setups. The additional information collected by cameras, IR beams, and other equipment provides a rich synchronized dataset with minimal possibility of bias on the part of the experimenter. The automated nature of the tasks also minimizes the interaction between the experimenter and the animal, which is particularly beneficial to studies of stress effects.

The continuous performance test (CPT) is an established and translatable testing paradigm, with the earliest usage in humans dating back to the 1950s. [36] It consists of a simple set of rules that are learned by the subject over increasingly difficult stages, where the subject learns to specifically respond to a certain on-screen stimulus and withhold response from other stimuli. The CPT is sensitive to alterations in sustained attention and vigilance, and diagnostically has been used in the context of schizophrenia and Alzheimer's disease. [32]

Performance in the CPT is gauged via a metric known as the discrimination index, or d'. This is a measurement derived from signal detection theory, [37] and is a quantification of an individual's ability to distinguish the correct stimulus (S+) from the other incorrect stimuli (S-). In the context of the CPT, a lower d' is indicative of impulsivity, impaired attention, or a lack of understanding of the rules of the test.

The CPT has been used extensively in rodent studies. A rodent-focused version of a touchscreen CPT has recently been developed [38] and has been demonstrated to be sensitive to attentional differences in mice. In addition to the touchscreen, chambers are equipped with cameras and infrared beams allowing for fine tracking of an animal within a chamber during a test session as well as the test itself. The observed negative impact

of SI on attention makes the CPT an ideal assay for interrogating cognitive and behavioral features of the SI phenotype.

Progressive ratio and fixed ratio tasks are used to assay changes in motivation using a simple touchscreen-based task. [35] They are simpler than the go/no-go schematic of the CPT, and only ask the subject to touch the screen a set number of times to complete a trial. As the names suggest, fixed ratio tasks require a fixed number of touches in each trial, where progressive ratio tasks require an increasing number of touches for each trial. The primary metric used to analyze performance in a progressive ratio task is the break point, or the highest number of touches committed to complete a trial within a test session. A higher break point is associated with increased effort and motivation. Progressive ratio tasks are used to investigate changes in reward behavior, another cognitive domain impacted by the SI phenotype.

The depth of the datasets generated by touchscreen tasks, their wide applications, and their established usage in both human and rodent contexts make them an ideal platform for high-throughput cognitive testing in rodents and translating any findings to a human context.

1.5 Social isolation and public health

Loneliness and social isolation have been recognized as serious public health issues by governments around the world. [39] In addition to the known risk of isolation in older individuals, the increased interest in isolation in other age groups has led to findings that illustrate the risk of SI in those groups. This also includes the discovery that isolation or loneliness in parents can impact the health of their children and vice versa. [40]

One of the immediate secondary effects of the quarantine measures implemented against the COVID-19 pandemic was a sharp increase in social isolation. Several studies have already been conducted on the impact of quarantine-related SI on mental health on a population level, and have found increases in depression, anxiety, and sleep disruption consistent with prior SI studies. [12]

Isolation was already being monitored as a high-impact public health issue by many countries even before the pandemic, which provided important perspectives on the possible outcomes of extended SI. [41]

However, it is still too early to assess the impact that quarantine-related SI will have had on adolescents during the past year. As the world moves past the acute phase of the pandemic, it will be important to monitor the social and mental health of adolescent people for the long-term effects of isolation. These effects may not emerge fully until adulthood, so any findings about potential interventions against isolation stress will be valuable.

1.6 Project summary

With the increased interest in the effects of adolescent social isolation and the advances in cognitive testing in mice, we decided to use a mouse model of postweaning social isolation to investigate the neurological, behavioral, and cognitive changes induced by the environmental stress of isolation.

We investigated ΔFosB as a neurological marker of chronic SI stress in chapter 2. Comparing several areas of the brain in male and female mice, we found that SI male

mice showed an increased ratio of ΔFosB/FosB in the prelimbic/infralimbic cortices, suggesting that males are particularly susceptible to chronic SI stress.

In chapter 3, to investigate the behavioral and cognitive effects of adolescent SI, we used a mouse-oriented version of a touchscreen CPT. Following the negative effect that SI has on attention, we hypothesized that the performance of SI mice on the CPT would be impaired. However, we found that SI males actually had significantly improved performance. SI females were not significantly different from group-housed females.

Following the finding of increased performance in the CPT, we assayed male mice in a progressive ratio regimen to screen for changes in reward-seeking behavior that may have interfered with CPT performance. From the CPT results and the connections between SI and addiction risk in humans, we hypothesized that reward-seeking behavior may have been increased in SI male mice. This was confirmed by a significant increase in break point in SI males in progressive ratio testing.

The findings of increased ΔFosB/FosB, increased CPT performance, and increased reward-seeking behavior as measured by progressive ratio task add depth to the current profile of the effects of adolescent SI. The male-specific nature of these findings is also notable, and may guide further investigation into the adolescent SI phenotype.

Chapter 2 – Post-weaning social isolation increases ΔFosB/FosB protein expression in sex-specific patterns in the prefrontal cortex and hippocampus in mice

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Abstract

Social isolation is a growing public health concern across the lifespan. Specifically, isolation early in life, during critical periods of brain development, increases the risk of psychiatric disorders later in life. Previous studies of isolation models in mice have shown distinct neurological abnormalities in various regions of the brain, but the mechanism linking the experience of isolation to these phenotypes is unclear. In this study, we show that ΔFosB, a long-lived transcription factor associated with chronic stress responses and drug-induced neuroplasticity, is upregulated in the medial prefrontal cortex and hippocampus of adult C57BL/6J mice isolated for two weeks post-weaning. Additionally, a related transcription factor, FosB, is also increased in the medial prefrontal cortex in socially isolated females. These results show that short-term isolation during the critical post-weaning period has long-lasting and sex-dependent effects on gene expression in brain, and that FosB/ΔFosB expression provides a potential mechanistic link between adolescent social isolation and the associated neurological abnormalities.

2.1 Introduction

Social isolation (SI) during childhood and adolescence is an adverse event that increases the risk for developing several psychiatric disorders later in life, including anxiety, depression, and schizophrenia [1]. SI has long been recognized as a public health issue among older populations [3], but recent evidence reveals it is a growing problem among adolescents and young adults [42]. The potential disruption of critical developmental processes compounds the negative consequences of SI for this age group. Despite the wealth of epidemiological data available on the detrimental effects of SI, little is known about the underlying neural mechanisms through which SI increases risk for psychiatric disorders [1].

Some of the observed effects of SI include impaired cognition, increased risk for substance use disorders, and increased anxiety and depression. Some of these effects are also observed in situations where stress response proteins are dysregulated. The transcription factor FosB is transiently expressed in response to external stimuli, including environmental stress, but in situations of persistent stress, the truncated and longer-lived isoform ΔFosB tends to accumulate [43]. High levels of ΔFosB are observed in response to chronic drug exposure and are associated with addiction-like behaviors in rodents [29]. Increases in ΔFosB are also observed in chronic social stress models, specifically in repeated social defeat stress [30].

In social defeat animal models, ΔFosB elevation is observed in the medial prefrontal cortex (mPFC), a phenomenon associated with increased susceptibility to social stress, and increased anxiety- and depression-like behaviors. Viral-mediated

genetic overexpression of ΔFosB produces a similar behavioral profile, which suggests that the transcription factor plays a causal role in the phenotype.

ΔFosB/FosB induction has been studied in the context of rodent SI models, but, to our knowledge, not in models of post-weaning/adolescent isolation [31,44]. Here we assessed the expression of ΔFosB/FosB in the mPFC, hippocampus, and striatum, three regions involved in the behavioral response to stress, after exposure to a validated model of post-weaning isolation [25,45,46]. The establishment of a link between adolescent social isolation and a well-studied stress response mechanism presents a valuable addition to the field of neuroscience.

2.2 Materials and methods

2.2.1. Mice

Male and female C57BL/6J mice (6-7 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and set up as breeding pairs. Only one litter from each breeding pair was used in these experiments. The isolation procedure used for these studies was adapted from the procedure described by Makinodan and colleagues [25]. Briefly, pups were weaned at postnatal day 21 (P21) and either housed in same sex groups of three for the duration of the experiment or singly housed from P21-P35 and then rehoused with another isolated littermate for the duration of the experiment (Figure 2.1).

2.2.2. Immunoblotting

At P63, mice were anesthetized with isoflurane and then decapitated. The brain was removed and the mPFC (infralimbic and prelimbic subregions), hippocampus, and

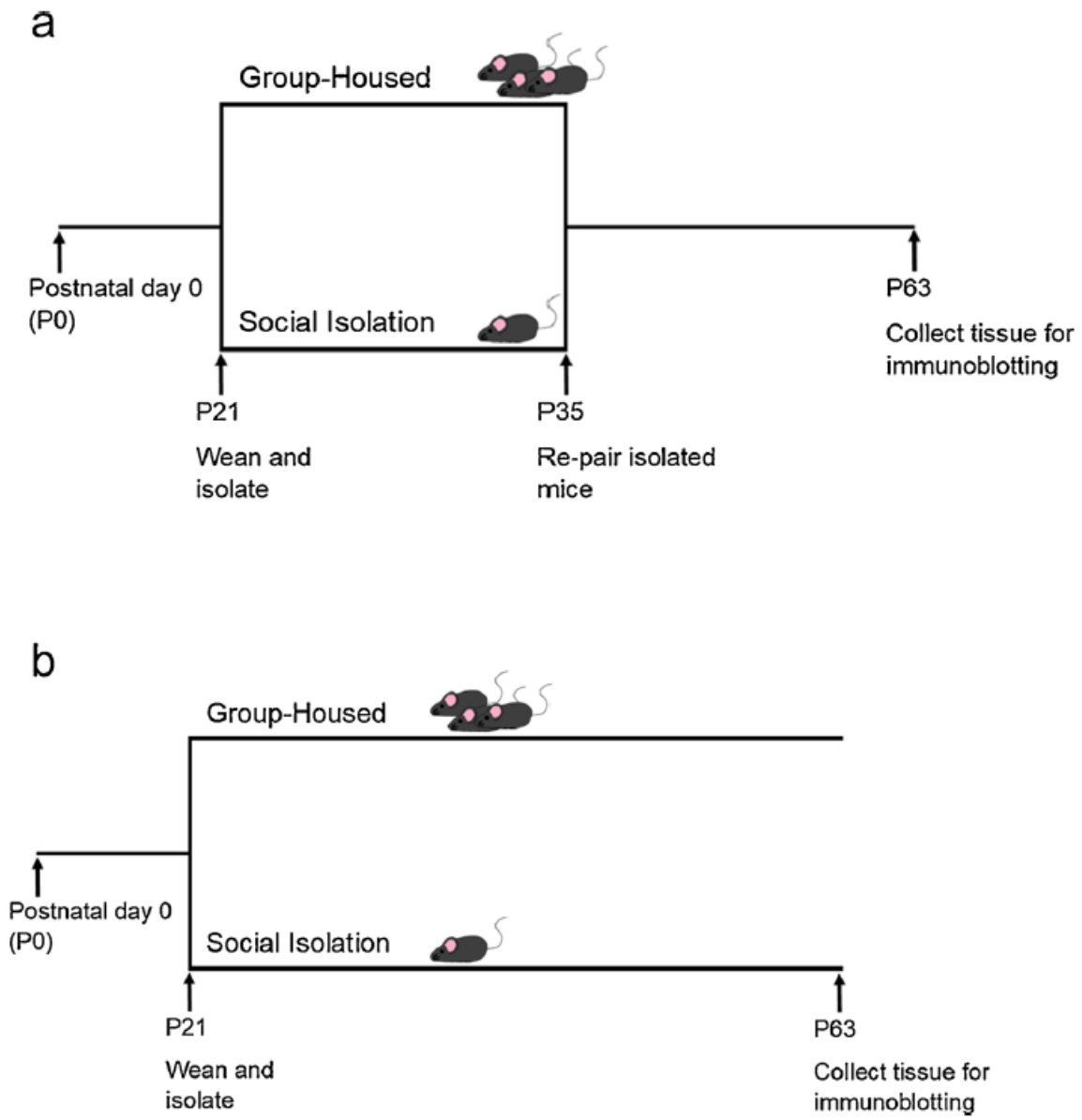


Fig. 2.1. Post-weaning isolation protocols. (a) Mice are weaned at P21 into group-housed cages (3 mice/cage) or isolation cages. On P35, isolated mice were repaired with previously isolated littermates. Brain tissue was collected at P63. (b) A second isolation protocol where mice were weaned at P21 into group-housed cages (3 mice/cage) or singly housed until tissue collection at P63.

striatum were dissected using previously described methods [47] and flash frozen on dry ice and stored at -80°C until processing. The tissue was homogenized and sonicated in T-Per lysis buffer (Thermo Scientific, Rockford, IL, United States). The protein concentration of the samples was determined using a Pierce BCA Protein Assay

Kit (Thermo Scientific, 23225). 40 µg of protein was separated by NuPAGE 4-12% Bis-Tris Protein Gels (Invitrogen, NP0335BOX) and transferred to a nitrocellulose membrane with an iBlot® Transfer Stack (ThermoFisher Scientific, IB301001). After blockade with Odyssey blocking buffer (LI-COR, Lincoln, NE, USA; 927-40000) for 1 h at room temperature, the membrane was incubated with the rabbit monoclonal anti-FosB primary antibody (dilution: 1:1000, product number: 2251S, Cell Signaling Technology, Danver, MA, USA) at 4 °C overnight. ΔFosB/FosB levels were normalized to beta-actin, so membranes were also incubated with a mouse monoclonal anti-beta actin primary antibody (dilution: 1:5000, product number: ab8226, Abcam, Cambridge, MA, USA). After TBST washing three times for 10 minutes each wash, the membrane was incubated with the corresponding secondary antibodies (goat anti-rabbit IRDye® 800CW and goat anti-mouse IRDye® 680LT (dilutions: 1:15,000, LI-COR) for 1 h at room temperature. The western blot protein bands were captured by Odyssey CLX and analyzed by Image Studio software (V3.1, LI-COR).

2.3 Results

2.3.1. ΔFosB *protein is elevated in the mPFC and hippocampus following SI stress*

We found that ΔFosB protein levels were increased in the mPFC ($F_{1,33}$ = 16.54, p = 0.0003) and hippocampus ($F_{1,33}$ = 4.666, p = 0.0381) of adult mice exposed to post-weaning SI compared to group-housed littermates. (Figure 2.2) There were no effects of sex on ΔFosB levels in either region (mPFC: $F_{1,33}$ = 0.0270, p = 0.8704; Hippocampus: $F_{1,33}$ = 0.7638, p = 0.3885) or sex X housing interactions (mPFC: $F_{1,33}$ = 0.0270, p = 0.8704; Hippocampus: $F_{1,33}$ = 0.9484, p = 0.3372). There were no significant effects of

sex ($F_{1,33}$ = 0.0986, p = 0.7555), housing ($F_{1,33}$ = 1.427, p = 0.2408), or sex X housing interactions ($F_{1,33}$ = 0.0986, p = 0.7555) on ΔFosB in the striatum (Figure 2.2).

2.3.2. FosB protein is increased in female mice exposed to SI stress

We also measured the amount of FosB protein in the same three brain regions. There was a significant interaction between sex and housing on FosB in the mPFC ($F_{1,33}$ = 5.708, p = 0.0228). *Post hoc* analyses showed that male mice exposed to SI have less FosB compared to female mice exposed to SI (p = 0.0160). There were no other significant pairwise differences between the groups. There were no significant effects of sex, housing, or sex X housing interactions in the hippocampus or striatum. (Figure 2.2 g-i)

2.3.3. ΔFosB/FosB ratio is higher in the mPFC of male mice exposed to SI stress

Due to the changes in ΔFosB and FosB protein levels across multiple regions, we also analyzed the relative changes in these two proteins within individual mice. We found a significant interaction between sex and housing on ΔFosB/FosB ratio in the mPFC ($F_{1,33}$ = 8.585, p = 0.0061). *Post hoc* analyses showed that the interaction was driven by an increase in the ratio in SI males compared to all of the other groups (p = 0.0017 compared to group-housed males and p < 0.0001 compared to both group-housed females and SI females). (Figure 2.2 j-l)

2.3.4. Six weeks of isolation from weaning until tissue collection increases both ΔFosB and FosB in male mice

In order to test whether the changes in ΔFosB and FosB protein levels were caused by the isolation or the reintroduction to group housing, we measured protein from group

housed and continuously isolated (P21-P63) male mice. We found that isolation increased ΔFosB in the PL/IL (t_8 = 3.481, p = 0.0083) and hippocampus (t_8 = 2.724, p = 0.0261) of adult mice exposed to postweaning SI compared to group-housed littermates while production no change in the striatum (t_8 = 0.1665, p = 0.8719; Figure 2.3 d-f). Social isolation produced a similar increase in FosB protein in the PL/IL (t_8 = 3.258, p = 0.0116) and hippocampus (t_8 = 3.299, p = 0.0109) with no increase in the striatum (t_8 = 0.1665, p = 1.119, Figure 2.3 g-i). The changes in both ΔFosB and FosB led to no change in the ΔFosB/FosB ratios.

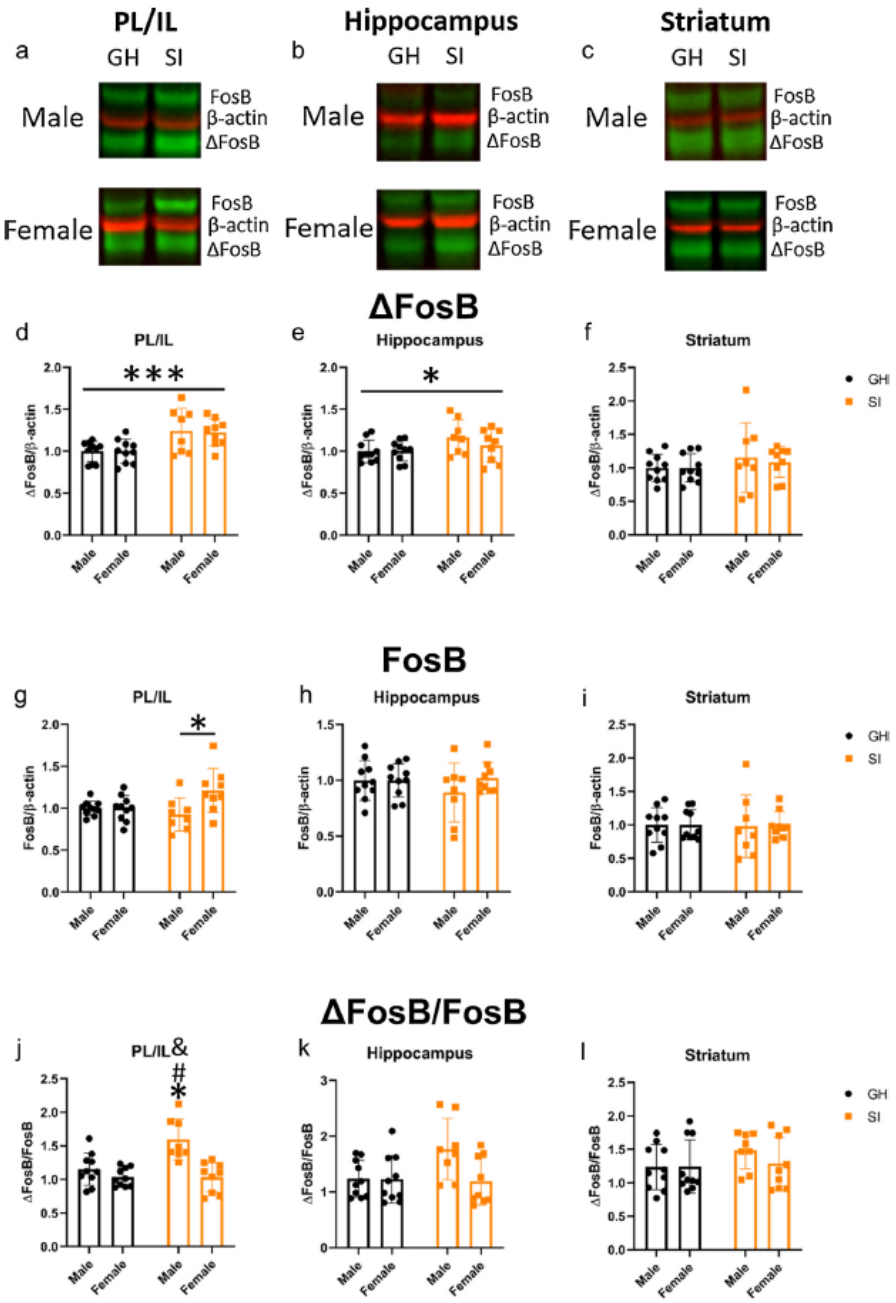


Fig. 2.2. Δ*FosB and FosB protein levels in*P*63 mice following SI (P21-35).* SI increases ΔFosB protein in the PL/IL (representative western blots in panel a and quantification in panel d) and hippocampus (representative western blots in panel b and quantification in panel e) of male and female mice compared to their group-housed (GH) littermates. There were no significant differences in the striatum (representative western blots in panel c and quantification in panel f). FosB protein was increased in SI female mice compared to SI male mice (g). There were no significant changes in FosB protein in the hippocampus or striatum (h-i). SI males had higher ΔFosB/FosB ratios compared to the other three groups in the PL/IL (j). There were no differences in either the hippocampus or striatum (k-l). n = 10/GH males, 10/GH females, 8/SI males, and 9/SI females. Data are normalized to the GH male group and represent the mean ± SEM. **p* < 0.05; ****p* < 0.001; #*p* < 0.05 compared to the GH male group; & < 0.05 compared to the GH female group.

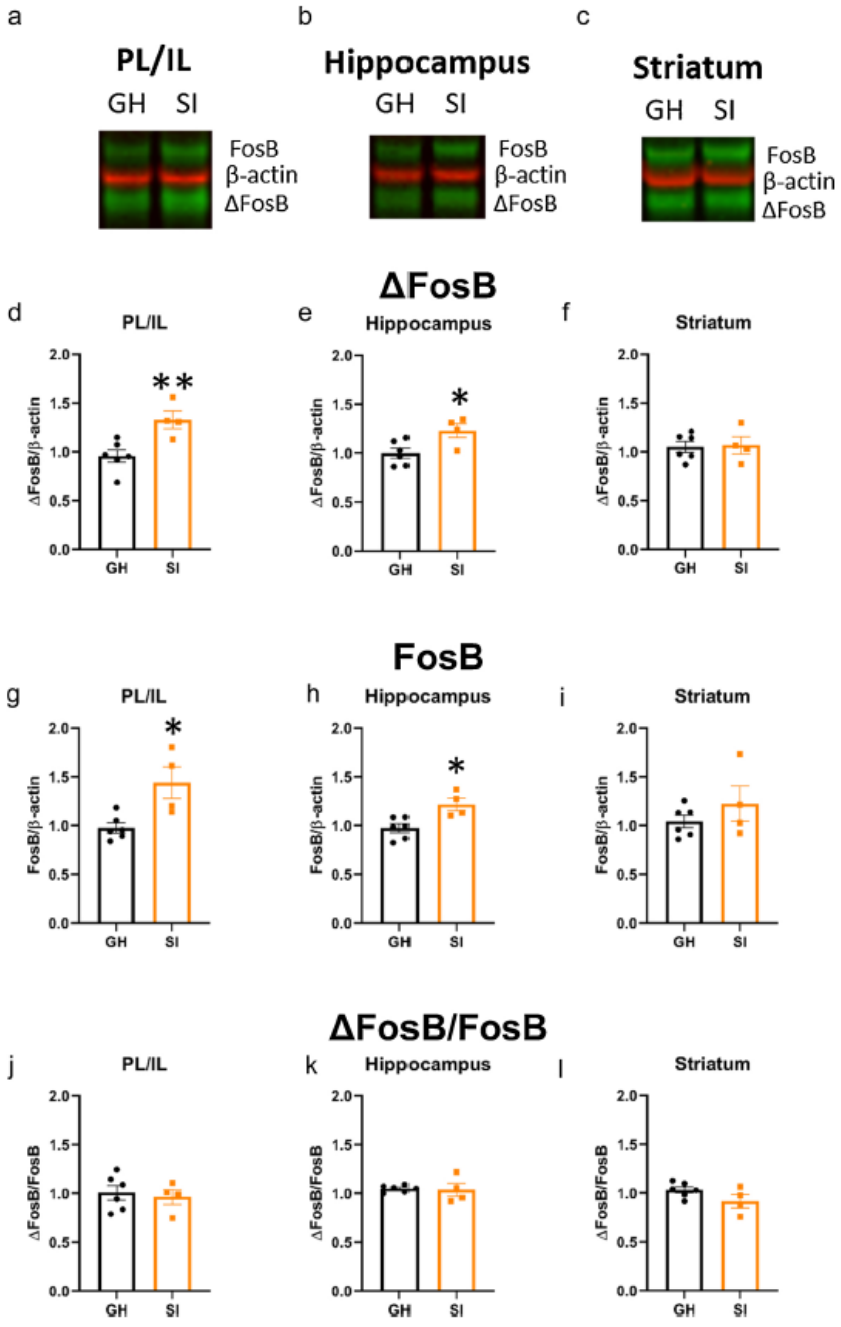


Fig. 2.3. Δ*FosB and FosB protein levels inP63 male mice following SI (P21-63).* SI increases ΔFosB protein in the PL/IL (representative western blots in panel a and quantification in panel d) and hippocampus (representative western blots in panel b and quantification in panel e) compared to their group-housed (GH) littermates. There were no significant differences in the striatum (representative western blots in panel c and quantification in panel f). FosB protein was increased in the PL/IL and hippocampus (g-h). There were no significant changes in FosB protein in the striatum (i). Due to increases in both ΔFosB and FosB there were no changes in the ΔFosB/FosB ratios (j-l) n = 6/GH males and 4/SI males. Data are normalized to the GH male group and represent the mean ± SEM. *$p < 0.05$; **$p < 0.01$.

2.4 Discussion

In this experiment, we measured the levels of ΔFosB and FosB protein in the mPFC, hippocampus, and striatum of mice exposed to transient post-weaning SI. We found that ΔFosB protein expression is a long-term marker of SI in this model. Specifically, ΔFosB protein is increased in the mPFC and hippocampus of adult mice (P63) that were socially isolated from P21-P35. Additionally, there were sex X housing interactions. In the mPFC, SI females had more FosB protein than SI males, but not group-housed females or males. We also measured the ΔFosB/FosB ratio in the three brain regions and found that SI male mice had more ΔFosB relative to FosB in the mPFC compared to the three other groups. Interestingly, these changes in ΔFosB/FosB expression are present weeks after the termination of the SI stress, suggesting there are long-lasting effects of SI that are not mitigated by a return to group-housing.

Increased ΔFosB protein is a hallmark of exposure to multiple types of stress. For example, ΔFosB levels are elevated following exposure to chronic, but not acute administration of multiple drugs of abuse in rats and mice [48]. Chronic restraint and unpredictable stress increase ΔFosB expression in the brain as well [49]. Moreover, chronic exposure to seemingly beneficial perturbations including wheel-running and antidepressants increase ΔFosB expression as well [50,51].

ΔFosB expression, particularly in the nucleus accumbens, is associated with augmented responses to drugs of abuse. Early experiments utilizing overexpression of ΔFosB indicated that high levels of the transcription factor in the nucleus accumbens increases the responsiveness of mice to the rewarding and locomotor-activating effects of cocaine [52]. Additionally, ΔFosB is associated with increased self-administration of

cocaine and inhibition of the aversive effects of kappa opioid receptor activation [53,54]. Taken together, the combined effects of increased reward sensitivity and decreased aversion could support increased drug-seeking and susceptibility to addiction. The increase in drug-seeking associated with ΔFosB expression appears to be a general effect because it is seen with multiple drugs of abuse, including opioids [55].

A previous study [46] established that adolescent social isolation negatively affects neuronal excitability in the PFC. It is possible that ΔFosB is mediating this effect in some way, especially since ΔFosB is known to decrease neuronal activity in the mPFC in other chronic stress models [30]. Similarly, studies of ΔFosB in the hippocampus show that overexpression of ΔFosB produced anxiety-like behavior [56], and this combined with our observation of increased ΔFosB in the hippocampus are consistent with ΔFosB playing a role in chronic stress mediation. A study of FosB knockout mice (but not ΔFosB) showed underdevelopment in the hippocampus at ten weeks as well as depressive behavior [57], highlighting the importance of proper regulation of these two proteins in adolescent brain development.

To our knowledge, our study is the first to investigate changes in ΔFosB/FosB following transient post-weaning isolation, but there have been reports in other models of social isolation. Isolation for eight weeks on adult female prairie voles increased ΔFosB/FosB immunohistochemistry in the basolateral amygdala [44]. Interestingly, prolonged social isolation in adult mice decreases ΔFosB in the nucleus accumbens and increases susceptibility to the detrimental effects of social defeat stress [31]. We found no change in ΔFosB or FosB protein in the striatum. The discrepancy may be due to our sampling of the entire striatum, including both dorsal and ventral (nucleus

accumbens) subregions, and/or the difference in the age of the mice during isolation. There may be fundamental differences in the long-term effects of SI depending on when the isolation occurs.

We found significant changes in ΔFosB/FosB protein in the mPFC. The post-weaning period we examined is critical for the development of the prefrontal cortex in rodents [8,58]. Chronic administration of drugs of abuse have been shown to increase ΔFosB in the PFC [48]. Additionally, chronic treatment with the antipsychotic haloperidol increases ΔFosB in the PFC and the increase in ΔFosB is associated with cognitive disruption [59]. Chronic social defeat stress also increases ΔFosB in the mPFC [60]. These data suggest that the increased ΔFosB produced by social isolation stress may be associated with detrimental behavioral effects in these mice.

Female mice exposed to isolation also had elevated levels of FosB. Most studies investigating ΔFosB/FosB have utilized immunohistochemistry and antibodies that bind both ΔFosB and FosB, making it impossible to determine which variant was responsible for the signal. Here, we used western blotting techniques that allowed us to separate ΔFosB and FosB by size and quantify relative changes between the two variants [61]. It is unclear what the differential effects would be of having either elevated ΔFosB alone or in combination with FosB. If the induction of FosB is related to acute stress and ΔFosB is a marker of a previously terminated stressor, the different response between males and females may represent a critical difference in the downstream effects of social isolation. One study utilizing mutant mice with variable levels of ΔFosB and FosB indicates that relative expression patterns may produce differential behavioral effects. Specifically FosB can antagonize the effects of accumulated ΔFosB [62].

This study represents an initial characterization of the long-term effects of transient post-weaning SI. The role, if any, of ΔFosB/FosB in the behavioral or neurobiological alterations produced by this model are unknown [25,46,63]. While this study did not investigate behavioral aspects related to ΔFosB/FosB changes, future studies will address whether expression of genes regulated by ΔFosB/FosB are also modulated by isolation and whether there is a causal relationship between ΔFosB/FosB activity and the SI behavioral phenotype.

Chapter 3 – Post-weaning social isolation increases reward-seeking behavior in a sex-specific manner in mice (Preprint)

Abstract

Social isolation is a growing concern in public health. Although isolation at any age is harmful, previous studies have shown that isolation during adolescence, correlating with critical periods of brain development, can increase risk for psychiatric illness later in life. In this study, we tested a mouse model of adolescent social isolation using multiple translatable touchscreen-based cognitive assays – a continuous performance test (CPT) which measures sustained attention, and a progressive ratio (PR) battery which measures reward-seeking behavior. We found that adolescent SI works in a sex-specific manner, increasing the reward-seeking behavior of male mice and having little measurable effect on female mice.

3.1. Introduction

Social isolation (SI) is a stressor with significant acute and chronic effects, including increased risk for developing psychiatric disorders. Specifically, there is a clear link between SI and anxiety, depression, substance use disorder, and schizophrenia [1]. Despite the confirmation of SI as a risk factor, the biological link between SI and psychiatric disorders is unknown.

There is an extensive literature on the effects of SI on older people (Reviewed in [3]), but recent studies have identified increases in the prevalence of SI and loneliness among children and adolescents. SI in younger people is particularly important for disorders with significant neurodevelopmental components, including schizophrenia and substance use disorders. Given the plastic state of the brain during childhood and adolescence, SI may have different or greater effects compared to SI in adults.

SI has also been studied in animal models, and both chronic and acute isolation have been associated with anxiety- and depression-like behavior in rodents. SI during postnatal days 21-35, roughly correlating developmentally to ages 11 to 18 in humans, [64] was shown to be necessary and sufficient to show distinct deficits in myelination in the prelimbic and infralimbic cortices. Social play peaks during this period, and is necessary for the expression of appropriate social behavior in adulthood. [25] We previously reported findings of increased levels of ΔFosB, a transcription factor associated with chronic stress, following postweaning SI during postnatal days 21-35. ΔFosB has also been implicated in studies of addiction. [29,31]

Touchscreen-based tasks provide a flexible and sensitive platform to investigate cognitive changes. The continuous performance test (CPT) is a widely-used and translatable behavioral assay sensitive to changes in sustained attention. [38] Fixed ratio and progressive ratio tasks can similarly detect changes in motivation and reward-related behavior. Here, in a mouse model of adolescent social isolation, we used a cognitive testing battery consisting of CPT and PR assays to interrogate the effects of adolescent SI on attention and motivation in adulthood.

We found that adolescent SI significantly affects performance in CPT, PR, and FR assays. Male SI mice demonstrated higher sensitivity in the in the CPT, which is usually indicative of improved attention, However, male SI mice also have higher breakpoints and total responses in the PR and FR tasks, indicating increased reward-seeking behavior. Together, these results suggest that improved performance in the CPT in SI mice may result from increased reward-seeking and motivation for highly palatable food. ΔFosB

3.2. Materials/methods

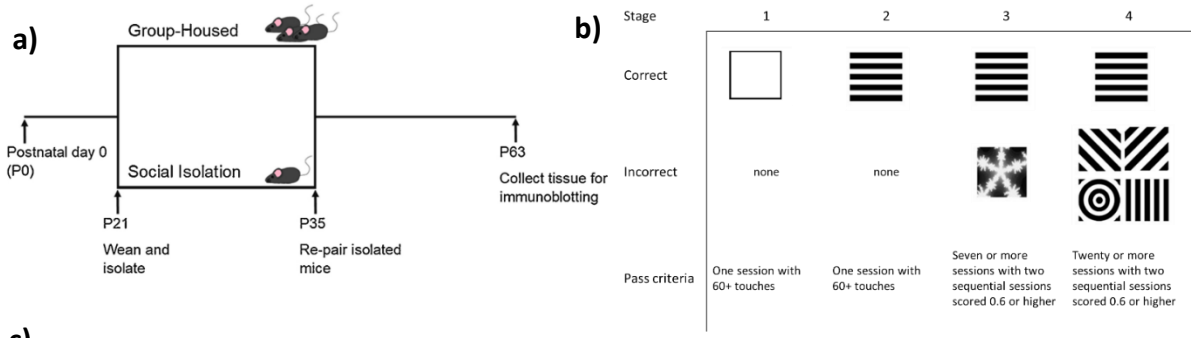
3.2.1 Mice

Breeding pairs of C57BL/6J mice were purchased at 6-7 weeks old from The Jackson Laboratory (Bar Harbor, ME, USA). Only one litter from each breeding pair was used in these experiments. Pups were raised according to an isolation protocol adapted from one used by Makinodan and colleagues [25]. Pups were weaned at postnatal day 21 (P21) and housed either in isolation or in a group of three same sex littermates. Isolated

mice were rehoused with another same sex isolated littermate at P35 for the duration of the experiment (Figure 3.1a).

3.2.2 Handling, food restriction, and habituation

At P63, mice were placed on food restriction and briefly handled for three days. Food amounts were set to keep the animals at no less than 85% of their free feeding weight. During the initial handling days, mice were also introduced to a Bussey-Saksida testing chamber (Lafayette Instrument, Lafayette, IN, USA) for habituation. Mice were placed in the chamber, and 1 mL of reward was placed in the feeding tray. The habituation schedule consisted of a 30-minute session where the screen was responsive to touch, but touches were not rewarded. Mice were considered habituated when they had undergone at least three habituation sessions and had fully consumed the reward during at least one session.



c)

Test	FR1	FR2	FR3	FR5	PR4	FR1	FR5	PR4
Min. sessions	1	1	1	3	7	1	3	7

Figure 3.1. Isolation rearing schematic and behavioral testing battery. a) Isolation rearing. Mice are weaned at postnatal day 21 and assigned to either group housing or isolation. At day 35, isolated mice are rehoused with another isolated littermate. Testing began at day 63. b) Continuous performance test (CPT) progression and scoring. c) Motivation test progression. Session amounts shown are number of passing scores required before moving onto the next stage.

3.2.3 Continuous performance test (CPT)

CPT training consists of four stages (Figure 1b, adapted from Kim).

In stage 1, mice were trained to touch a visual stimulus (white square). The square was displayed (stimulus duration) for 10 seconds. The stimulus duration was followed by a 0.5 second limited hold (LH) period during which the screen was blank, but a touch would still yield a reward. Upon interacting with the stimulus, a one-second 3 kHz tone would sound, a small amount of reward would be dispensed, and the reward tray would be illuminated. Head entry into the reward tray was detected by an infrared (IR) beam, and upon head entry the intertrial interval (ITI) of 2 seconds would begin. If the mouse did not interact with the stimulus, the ITI would begin immediately following the LH period. The next trial would begin immediately following the ITI. The criterion for advancement to stage 2 was obtaining 60 rewards within a single 45-minute session.

In stage 2, the white square pattern was replaced with either horizontal or vertical bars – the mouse's assigned positive stimulus, or S+. The S+ was counterbalanced within groups. The SD was reduced from 10 seconds to 2 seconds, and the LH was increased from 0.5 seconds to 2.5 seconds. The criterion for advancement was the same as stage 1.

In stage 3, a negative stimulus (S-; snowflake pattern) was added. Each trial had a 50% chance of being either an S+ or S- trial. SD and LH were identical to stage 2, but ITI was increased to 5 seconds. Interacting with S- during the SD or LH would not yield a reward and would start the ITI. The criteria for advancement to stage 4 were a minimum of seven sessions, during which at least two consecutive sessions had a discrimination

index (d') score of 0.6 or higher. A discrimination index is a measurement derived from signal detection theory [37] that is used to distinguish meaningful response to stimulus from noise. The discrimination index was calculated as follows:

$$d' = z\left(\frac{correct}{correct + miss}\right) - z\left(\frac{mistake}{mistake + rejection}\right)$$

In stage 4, the snowflake S- pattern from stage 3 was replaced with four new S- patterns. The S+ had a 30% chance of appearing, and the remaining 70% was split among the four S- images. Interacting with S- in this stage triggered correction trials, which consisted only of S- trials until the mouse withheld response. Parameters and scoring criteria were identical to stage 3, but animals were tested for twenty sessions.

3.2.4 Fixed ratio (FR)/progressive ratio (PR) testing

FR/PR testing consisted of several stages of varying difficulty. (Figure 3.1c)

Fixed ratio 1 (FR1) required a mouse to touch a white square, similar to CPT stage 1 without the stimulus duration timer. The session ended after 45 minutes, or when a mouse obtained 30 rewards within one session. After a mouse obtained 30 rewards within a single session, they progressed onto the next stage.

FR2 and FR3 followed the same rules as FR1, but with the respective number of touches per reward.

FR5 consisted of multiple sessions in which the mouse had to touch the square five times to obtain a reward. The same timing and cutoff rules from the previous stages

applied, but mice were required to obtain 30 rewards during each of at least three sessions before advancing to progressive ratio testing.

Progressive ratio 4 (PR4) followed the same parameters as the previous FR stages, but the number of touches required for a reward increased by 4 each time a reward was obtained. The initial PR4 phase consisted of seven sessions, and during each session the break point of each mouse was recorded. The break point was defined as the highest number of touches committed within one session to obtain a reward.

Following the initial PR4 phase, mice were again tested on FR1, but the 30-reward cap was removed. Three similar sessions of FR5 followed. The amount of rewards obtained per session was recorded.

After the free-feeding sessions, mice underwent another seven-session PR4 phase.

3.3. Data analysis

3.3.1 CPT

Discrimination indices from the last ten sessions of stage 4 were used in analysis. ANOVA tests were used to assay for results of sex and rearing condition on CPT performance.

3.3.2 FR/PR

Break points from the fourteen total PR4 sessions were used in analysis. T tests were used to compare group-housed males to isolated males. The numbers of rewards collected during the unlimited FR1 session between PR4 blocks were similarly compared.

3.4. Results

3.4.1 SI did not affect progression through the early stages of the CPT

Stage 1 of the CPT trained mice on the basic mechanism of the test chamber. Neither rearing nor sex affected acquisition of test rules in this stage (Rearing: $F_{1,23} = 0.5490$, $p = 0.4662$; Sex: $F_{1,23} = 0.5957$, $p = 0.4481$). In stage 2, each mouse tested reached criterion in a single session. In stage 3, neither rearing nor sex affected the number of sessions required to reach criterion (Rearing: $F_{1,23} = 0.0820$, $p = 0.7772$; Sex: $F_{1,23} = 0.0820$, $p = 0.7772$), or the d' score upon reaching criterion (Rearing: $F_{1,23} = 0.9956$, $p = 0.3288$; Sex: $F_{1,23} = 0.1988$, $p = 0.6599$).

3.4.2 SI improves performance in CPT stage 4 compared to group-housed controls

We found that d' scores during stage 4 of CPT was increased in SI mice (Figure 3.2, $F_{1,23} = 7.060$, $p = 0.0141$). Sex did not significantly affect performance in CPT ($F_{1,23} = 0.8541$, $p = 0.3650$). However, the difference between SI males and group-housed males was greater than that between SI females and group-housed females (Male: $p = 0.0668$; Female: $p = 0.9138$).

3.4.3 SI did not affect reaction time in males or females

Reaction time (RT) was measured between S+ presentation and screen touch (correct reaction time), S- presentation and screen touch (mistake reaction time), and screen touch and reward collection. Neither sex nor rearing significantly affected correct RT (Figure 3.3a, Rearing: $F_{1,23} = 0.1155$, $p = 0.7371$; Sex: $F_{1,23} = 2.440$, $p = 0.1319$). Mistake RT did not show a rearing effect ($F_{1,23} = 0.2791$, $p = 0.6023$), but did show a

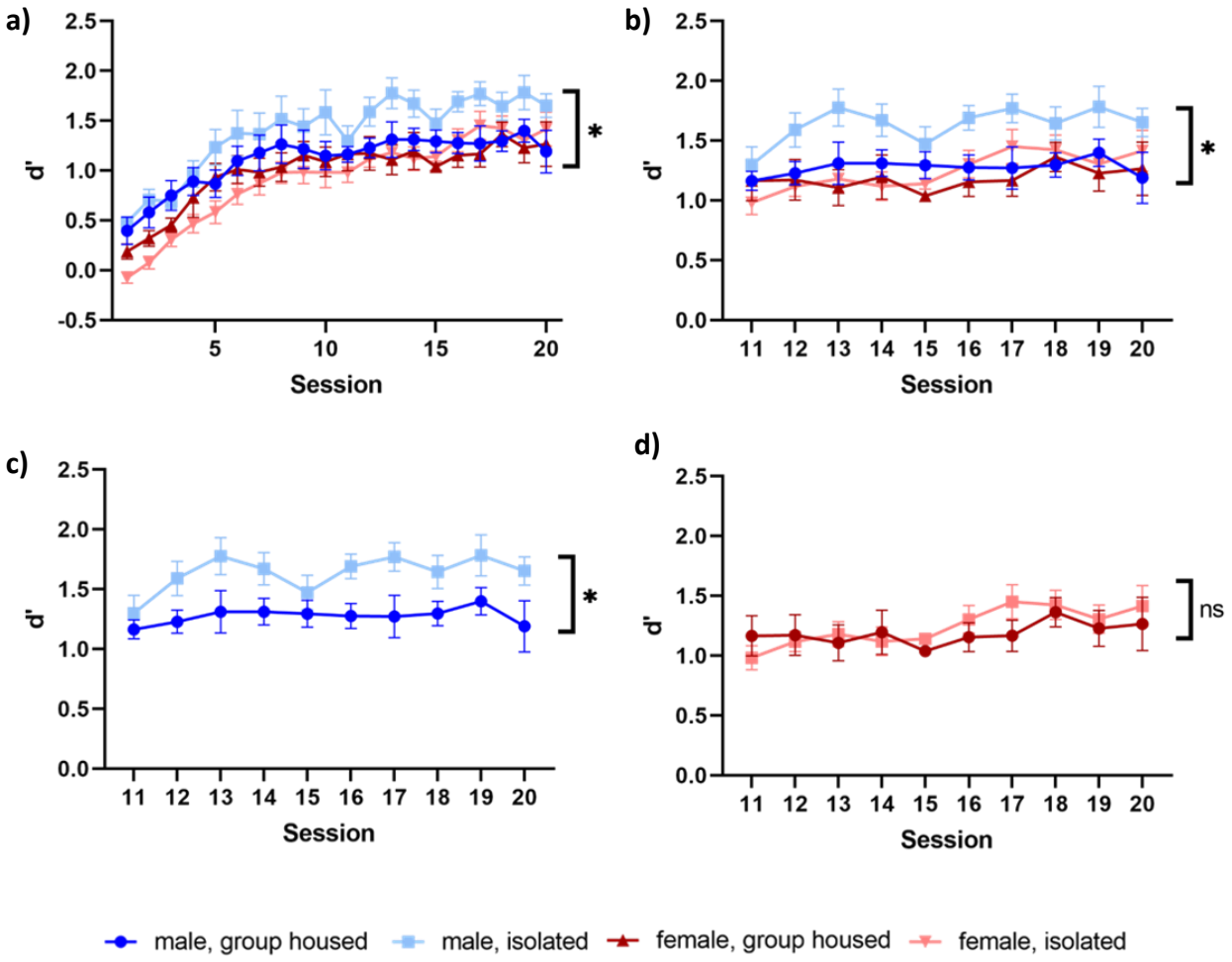


Figure 3.2. Performance in the continuous performance test is affected by SI (P21-35) in males. a) Discrimination index (d') over the twenty sessions of stage 4 of CPT of the four groups. b) Discrimination index over the last seven sessions of stage 4 of CPT of the four groups. c) SI did not affect d' in females. d) SI increased d' in males. n = 6 GH males, 7 SI males, 6 GH females, and 8 SI females. Data are mean ± SEM. *p < 0.05

sex effect, with females being slower to commit a mistake response than males (Figure 3b, $F_{1,23} = 5.915$, $p = 0.0232$). Reward collection time like correct RT was not affected by rearing ($F_{1,23} = 1.801$, $p = 0.1927$) or sex (Figure 3c, $F_{1,23} = 4.277$, $p = 0.0501$). This result was most likely driven by one group-housed male with a much larger mean reward collection time than any other mouse across rearing conditions (63.29 sec vs an average of 3.11 sec for the other mice).

3.4.4 Time bin analysis of CPT

CPT data was analyzed in three 15-minute bins (0-15, 15-30, and 30-45 minutes) to detect any changes in task performance over the duration of a session. (Figure 3.4) We found that performance was consistent among time bins for each rearing condition, with SI males still scoring significantly higher than the other three conditions. This effect reached significance in the first (Figure 3.4a, $F_{3,23} = 4.458$, $p = 0.0131$), second (Figure 3.4b, $F_{3,23} = 3.971$, $p = 0.0204$) and third (Figure 3.4c, $F_{3,23} = 3.527$, $p = 0.0309$) bins.

3.4.5 Male SI mice have higher break points in PR4 compared to group-housed controls

After observing that SI males scored higher in the CPT, a test sensitive to changes in attention, we tested males in a progressive ratio regimen, a battery sensitive to changes in motivation and reward behavior. This test allowed us to determine whether the observed CPT performance was being driven by cognitive changes in the SI phenotype or changes in reward-seeking behavior. We found that male mice that had undergone post-weaning SI expended more effort to obtain rewards during PR4 trials compared to group-housed littermates. (Figure 3.5a, $F_{13,11} = 3.430$, $p = 0.0113$)

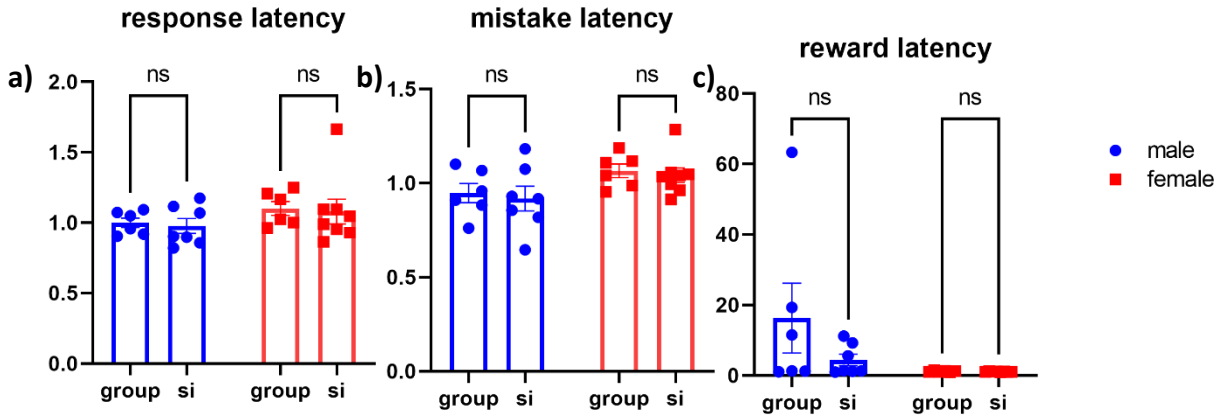


Figure 3.3. Response latency in the continuous performance test is unaffected by sex or rearing. a) Latency to respond to S+ (hit) in stage 4 of CPT. b) Latency to respond to S- (mistake) in stage 4 of CPT. c) Latency to reward collection after responding to S+ in stage 4 of CPT. n = 6 GH males, 7 SI males, 6 GH females, and 8 SI females. Data are mean ± SEM. *p < 0.05

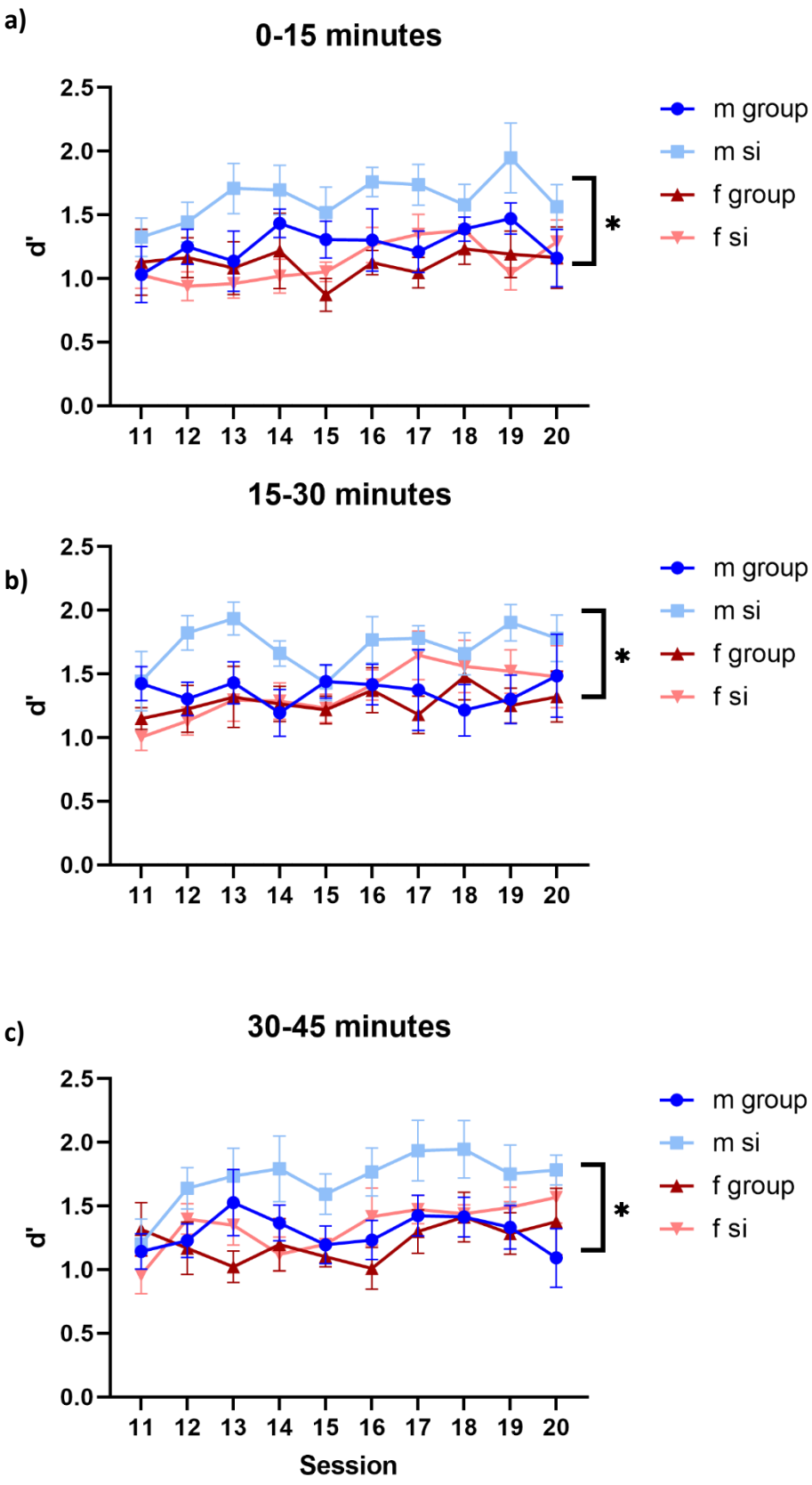


Figure 3.4. Time bin analysis of stage 4 of continuous performance test. a) 0-15 minutes. SI males scored significantly higher during the first 15 minutes of the CPT sessions. ($p = 0.0084$) b) 15-30 minutes. Neither rearing nor sex had a significant effect on performance during the second 15-minute bin of CPT sessions. ($p = 0.0722$) c) SI males scored significantly higher during the last 15 minutes of the CPT sessions. ($p = 0.0196$) n = 6 GH males, 7 SI males, 6 GH females, and 8 SI females. Data are mean ± SEM. *$p < 0.05$

3.4.6 Male SI mice consume more reward in an uncapped FR1 session compared to group-housed controls

The motivation testing battery included an unlimited FR1 session between blocks of PR4 sessions to eliminate potential reward satiety as a confounding factor. We found that while both GH and SI mice consumed more rewards during the FR1 session than during the PR4 sessions, thus confirming that they were not reaching reward satiety, SI mice consumed significantly more rewards than their group-housed littermates. (Figure 3.5b, $F_{13,11} = 1.349$, $p = 0.0037$) The number of rewards received during this session also exceeded the amount of rewards received during any one session of the CPT, indicating that satiety was not a confounding factor in that test either.

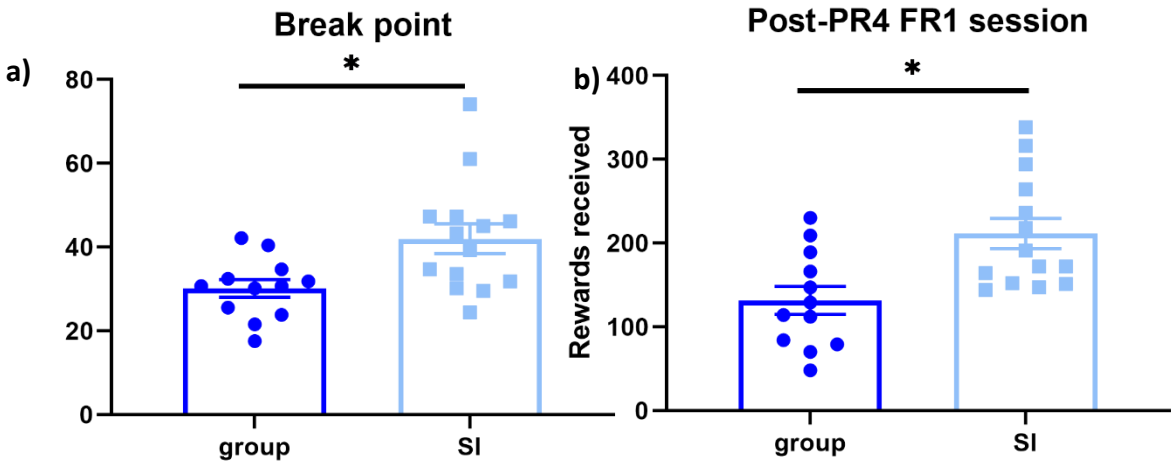


Figure 3.5. SI increases reward-seeking behavior in males as measured by a fixed/progressive ratio behavioral regimen. a) Average break point over seven PR4 sessions is increased in SI males. b) Reward intake during an unlimited FR1 session is increased in SI males. n = 12 GH males and 14 SI males. Data represent mean ± SEM. *p < 0.05

3.5. Discussion

Adolescent social isolation causes measurable behavioral changes that persist through adulthood. Using a continuous performance test, we found that SI seemed to cause an improvement in attention and vigilance. SI also causes increases in reward-seeking behavior in males as measured by a progressive ratio regimen. The effects of SI appeared to be sex-specific, as the performance of female mice in the CPT was unaffected by SI. These results together form a robust profile of the effects of adolescent SI on behavior.

3.5.1 Adolescent SI improves attention in males

This study investigated the effects of adolescent SI on sustained attention via CPT, which has established usage in rodent studies and human diagnostics. Adolescent SI male mice scored significantly higher on the late stages of the CPT than group-housed males and both group-housed and SI females. Group-housed and SI females did not score significantly differently on the CPT.

To further characterize how mice engaged with the task, we analyzed the CPT session data using 15-minute time bins. Performance throughout each CPT session was consistent among the rearing conditions, with male SI mice scoring significantly higher than the other rearing conditions in all three bins. Female SI and group-housed mice again were not significantly different from each other.

Higher scores in CPT are usually associated with improvements in sustained attention, but most studies of SI indicate impaired attention as a key feature of the phenotype. In response to this finding, we considered performance in the CPT as an interaction

between the mouse's willingness to engage in the task and their interest in the food reward. If SI impaired attention but also increased reward-seeking behavior, this may read as an increase in CPT score depending on the weight of each change.

3.5.2 Adolescent SI increases reward-seeking behavior in males

To further investigate this interaction between attention and motivation, we utilized a fixed-ratio/progressive-ratio regimen. Only males were tested due to the fact that females did not show a significant effect of isolation in the CPT. Progressive ratio tasks measure effort committed to obtain a reward without the cognitively demanding rules of the CPT, so the PR results would possibly allow us to further refine the potential implications of the CPT results.

SI males showed increased effort in the PR, which is interpreted as increased reward-seeking behavior. This validates our hypothesis following the initial CPT results, that any changes to attention caused by SI interact with significant changes to motivation. This finding also supports the known links between SI in humans and substance use disorders. [8]

3.5.3 Adolescent SI differentially affects males and females

The apparent resilience to SI stress in females was another finding of interest. Several other studies have found sex-specific responses to early life stress [65,66], including other findings where males were seemingly more affected or uniquely affected by the imposed environmental stress. It is possible however that the cognitive tests chosen did not interrogate domains affected by SI in females.

3.5.4 Behavior effects in the greater context of adolescent SI effects

A previous study from our group [67] focused on the molecular effects of adolescent SI and found that the stress response protein ΔFosB was upregulated in SI males. One of the largest remaining questions about the SI phenotype is how an organism translates the experience of SI stress to the observed behavioral effects, and our findings about ΔFosB combined with the behavioral effects found in this study are new pieces of the potential biological pathway between SI stress and the observed changes.

Increased reward-seeking behavior has also been observed following overexpression of ΔFosB. [29,31] However, to concretely establish ΔFosB as the mechanism activated by SI to increase reward-seeking behavior, it would be necessary to perform a study similar to this one utilizing a knockdown of ΔFosB. If SI does not cause changes in reward-seeking behavior in the absence of ΔFosB, then it is most likely that this is the molecular mechanism mediating the development of the adolescent SI phenotype.

3.6. Conclusions

This study demonstrates the effects of postweaning SI on the reward-seeking behavior of male mice. However, the mechanism leading to this behavioral profile is still unclear. Future studies will attempt to further characterize the interaction between the molecular and behavioral effects of postweaning SI.

Chapter 4 – Discussion

This project has established distinct behavioral and neurological features of the postweaning social isolation model in mice. We have shown chronic activation of stress response mechanisms readable as increased ΔFosB/FosB in males, and increased reward-seeking behavior in males measurable via CPT and progressive ratio touchscreen testing. These add to the growing understanding of the effects of postweaning social isolation in mice, which includes several other neurological and behavioral deficits.

This project also provides a next step in understanding the biological mechanisms mediating the response to social isolation. Particularly, the findings that isolation leads to increased ΔFosB as well as increased reward-seeking behavior can be combined with the reports that ΔFosB alone increases reward-seeking behavior [30] to conclude that isolation affects reward-seeking behavior via mechanisms involving ΔFosB. The full pathway is likely much more complicated, but the implication of chronic stress mechanisms in the development of the isolation phenotype can be used as a basis for studies seeking to further clarify the mechanism. Further experiments can confirm whether or not ΔFosB plays a causative role in the phenotype by RNAi knockdown or any other method of interfering with the production or function of ΔFosB.

Although the findings of increased reward-seeking behavior are in line with literature surrounding social isolation, it will be important to confirm that we truly observed reward-seeking behavior and not simply food-oriented behavior. Social reward value may also be altered in SI mice. [68] Some studies show increased sociability following

certain periods of adolescent social isolation, but it is unclear if this is a function of altered reward behavior.

The establishment of molecular handles and testable behavioral features provide ideal conditions for assessing the effects of therapeutic drugs in treating or preventing the impacts of isolation. Design of therapeutic interventions can vary along several parameters, including targeted mechanism, timing, whether the intervention is a drug or a change in an animal's social context, and many others. For example, a future study could investigate whether a potentially therapeutic drug interfering with chronic stress mechanisms is more effective when administered during the isolation period to prevent the development of the isolation phenotype, or during adulthood to alter the extant isolation phenotype.

The implication of chronic stress mechanisms as being critical to the development of the isolation phenotype allows for the treatment of chronic stress as a possible avenue to prevent or ameliorate the isolation profile. Reward-seeking behavior, with its connection to addiction research, is a well-known behavioral domain that can provide a platform for further investigation into the isolation phenotype.

The sex-based differences of the model provide a basis for further investigation. The experiments in this project showed blunted measures of chronic stress in females compared to males, and no changes in attention or reward-seeking behavior in females as measured by CPT. The incomplete understanding of the mechanisms affected by isolation stress make these measures difficult to definitively interpret, as females may be responding to isolation stress in other ways. It is possible that females are naturally

resilient to the stress of social isolation, but it is important to probe other aspects of the phenotype to confirm this.

Clarifying the sex-based differences of the model is particularly important in translating these findings to any interventions in humans. Many pediatric psychiatric illnesses, including attention deficit disorders, can present differently based on sex, which has led to imbalances in diagnoses in boys and girls. [69] It is unclear if this is a result of natural differences in occurrence rates or an artifact of biases in diagnostic profiles. Researchers and clinicians have been aware of this bias for some time and have moved to correct it, [70] but the relatively young field of research in adolescent social isolation has the opportunity to build this bias correction into its foundation.

Translatability of these results is also immediately relevant in the wake of the COVID-19 pandemic, in which people across the world experienced social isolation as a result of quarantine measures. Several studies have already confirmed that quarantine produced effects in adults consistent with social isolation, [12] but the relatively undeveloped understanding of the effects of isolation in adolescents makes this difficult to confirm in all age groups. However, the long-term and irreversible impacts of adolescent isolation compared to the more treatable effects of isolation in adulthood make this age group critical to monitor moving forward.

The delay between adolescent isolation and the emergence of observable changes can guide the development of therapeutic interventions. Following the isolation of quarantine, measures such as periodic sampling of self-reported loneliness and screening for associated behavioral effects outside of the context of isolation can provide a picture of the long-term effects of isolation. These effects may take decades

to emerge, but consistent monitoring, continued research of the adolescent SI model, and integration with preexisting public health measures against loneliness may help mitigate the negative effects.

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Publications and Abstracts

Peer-reviewed publications
Noback M, Zhang G, White N, Barrow JC, Carr GV. Post-weaning social isolation increases ΔFosB/FosB protein expression in the prefrontal cortex and hippocampus in mice. Neuroscience Letters, 2021 https://doi.org/10.1016/j.neulet.2020.135423
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Presentations

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Abstracts

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