LAKE FOREST COLLEGE

Senior Thesis

Contagious Depression: A Social Transmission Hypothesis

by

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The report of the investigation undertaken as a Senior Thesis, to carry two courses of credit in the Department of Neuroscience

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Abstract

Developing a new hypothesis for the pathophysiological mechanism of depression is necessary to further understanding of the disease. The purpose of this study was to test the hypothesis that depression is contagious. I predicted that naïve rats cross-housed with rats subjected to social defeat would develop depressive-like behaviors similar to their socially defeated cage mates. In addition, ΔFosB expression between the two groups should be parallel in brain areas implicated in depression, specifically the infralimbic prefrontal cortex. Trends were observed that supported the hypothesis presented. It is imperative to explore new avenues of research and strive to develop more effective and scientifically informed treatment options for those who suffer from depression.
To those who suffer

Persistent pain and a muddied mind,
Searching the emptiness for a path back to the surface
Pushes me to reach a hand,
And pull you up safely.

An irreplaceable and precious core,
Fights with courage
Against an illness that grips the mind
And paralyzes pleasure.

Know in life,
That you are not alone.

-Hannah Samberg, 2016
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Introduction

Depression is a pervasive psychiatric illness affecting approximately 1 in 6 individuals in the United States during their lifetime. Core symptoms of depression include depressed mood, anhedonia (reduced ability and desire to experience pleasure), irritability, alterations in appetite and sleep, as well as suicidal thoughts and behaviors. Depression is a self perpetuating process characterized by prolonged negative mood, increased sensitivity and reactivity to stress, and interference with adaptability and emotional processing. Deficits in memory and attention have also been shown to occur in depressed patients. Depression is a chronic and widespread problem that affects not only the individual, but also the family and society as a whole. It poses a global threat as a leading cause of burden, making the search for effective treatments a public health priority. This thesis used rats in an experimental study to test the hypothesis that there is a contagion effect associated with depression; that is, whether or not depression can be transmitted through extended contact.

Review of Symptomology and Etiology of Depression

While much of previous research on depression has focused on the symptomology of the disease by measuring behavioral outputs of lab animals after being subjected to various forms of chronic stress, pharmacological alterations, or genetic manipulations, there is less agreement in the literature about the causes. The disease has been shown to arise due to a variety of environmental and genetic causes. It has been shown that 40-50% of the risk for depression in humans is genetic, although the specific genes underlying the disease have yet to be identified, and the remaining 50-60% of non-genetic risk is attributed to early childhood trauma, emotional stress, physical illness, and even viral infections. Research has generated a few leading hypotheses for the neurophysiological mechanisms of depression.
such as the ‘monoamine hypothesis’, which states that depression is caused by decreased monoamine (e.g. serotonin) function in the brain.\cite{8-10} Another hypothesis points to the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and reveals how chronic episodes of stress lead to depression.\cite{11-13} A third hypothesis, the ‘BDNF hypothesis’, points to the depletion of neurotrophic factors that support plasticity in the brain.\cite{14,15} Depressed patients exhibit region specific brain-derived neurotrophic factor (BDNF) decreases while patients receiving antidepressant medications show region specific increases.\cite{14,16,17} In particular, BDNF is up-regulated in the amygdala and nucleus accumbens (NAc) and down-regulated in the hippocampus and medial prefrontal cortex (mPFC) of depressed patients.\cite{17}

Depression has a complex set of behavioral markers and a largely unknown pathophysiology.\cite{18} The monoamine hypothesis by itself is a far too simple explanation for the cause of depression. For example, antidepressants such as monoamine oxidase inhibitors and SSRI\textsc{s} produce immediate increases in monoamine transmission but take weeks to develop their mood-enhancing properties.\cite{19} In addition, while experimental depletion of monoamines in unmedicated depressed patients produces a slight increase in depressed mood, there is no corresponding effect observed in healthy controls, although we would expect that depletion of monoamines in healthy controls would induce depression.\cite{19} Further, studies that deal with rodent stress models have shown that enhancement of neurotransmitters such as dopamine, which we would expect to have a therapeutic effect, can instead have adverse effects by strengthening memories of aversive life events.\cite{20} Despite these contradictions, treating depression with antidepressants has been accepted in the field of psychiatry as a default treatment.\cite{6,21,22}

The three hypotheses I presented, the monoamine imbalance, BDNF depletion, and HPA hyper-activation, may seem discrete, but in reality they are interconnected. It is important to look beyond the apparent monoamine imbalance, at upstream triggers, in an
attempt to get at the root of the pathophysiology. BDNF has been shown to regulate the survival and differentiation of 5-HT (serotonin) expressing neurons, meaning that a decrease in BDNF expression will subsequently have a negative impact on the survival of 5-HT neurons.\textsuperscript{23,24} Additionally, infusion of BDNF \textit{in vitro} increases mRNA levels of the serotonin transporter, and the autoreceptors 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B}.\textsuperscript{25} The intracellular signaling pathway reveals that an increase in BDNF leads to the phosphorylation of tuberin, an upstream suppressor of the mammalian target of rapamycin (mTOR).\textsuperscript{26,27} Once tuberin is phosphorylated, its suppression of mTOR is alleviated, allowing mTOR-mediated translation to occur.\textsuperscript{26,28} So, once BDNF signaling decreases, mTOR-mediated protein synthesis will decrease too. Upstream regulators of BDNF include the HPA axis. Research on the HPA axis indicates that both acute and chronic stress attenuates HPA negative feedback, thus increasing glucocorticoid levels to an unhealthy level.\textsuperscript{16,29,30} Chronically increased HPA activity and glucocorticoid levels will subsequently down-regulate BDNF expression (Figure

\begin{figure}
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\caption{Neurophysiological Mechanisms in Depression. (A) Shows a normal neuron and its innervation by multiple inputs including monoamines (5-HT) and BDNF as well as other types of neurons. (B) Shows how severe stress, or excessive glucocorticoid stimulation caused by hyper-activation of the HPA axis inhibits BDNF signaling, causing a decrease in dendritic arborizations and connectivity with other neurons.}
\end{figure}
This mechanism of glucocorticoid-mediated BDNF expression actually indicates that glucocorticoids inhibit the interaction between the TrkB receptor and Shp2, a tyrosine-protein phosphatase. Since BDNF binds to TrkB, an inhibition of it will lead to an interruption of BDNF signaling. In addition, research has demonstrated a negative correlation between administration of dexamethasone, a glucocorticoid stimulator, and serotonin synthesis. Putting all of this together, HPA hyper-activation inhibits BDNF signaling, subsequently decreasing serotonin availability (Figure 2).

Figure 2. Interaction between the HPA-axis, BDNF, and 5-HT.
Episodes of chronic stress hyper-activate the HPA-axis leading to an inhibition of BDNF signaling, which then downstream leads to a decrease in the survival and efficacy of serotonin expressing neurons. SSRIs, or selective serotonin reuptake inhibitors, are prescribed in the treatment of depression. They act to increase the amount of serotonin in the brain.

Research indicates that BDNF is the middle step between HPA hyper-activation and monoamine deficiency, which is why I initially chose to evaluate BDNF levels as a marker of depression in this study. But, despite numerous efforts to obtain a reliable BDNF primary antibody, I was unsuccessful. Various antibody vendors were experiencing quality issues with the BDNF antibody. I therefore had to change my focus to another indicator. One possibility was to study the expression of the immediate early gene c-fos since many acute
stress studies focus on its role as a marker of acute stress.\cite{34} However, as exposure to stress is sustained, the ability of neurons to express the protein declines.\cite{34} Due to this temporal restriction, c-fos was not a viable option to use as a biomarker for depression for this study. On the other hand, ΔFosB, an indicator of neuronal activation in response to chronic stress, proved to be a more suitable option to measure as a biomarker of a depressive-like state.\cite{35,36} Upregulation of ΔFosB has been identified following exposure to chronic stress in many regions of the rat brain; in particular the infralimbic prefrontal cortex.\cite{36,37} Evidence has shown that the infralimbic mPFC plays a role in regulating the stress response via the HPA-axis, for example, lesion of the infralimbic mPFC has been shown to attenuate HPA responses to acute stress.\cite{38,39}

*Animal Models for Studying Depression*

Scientific study of diseases requires the use of models because the model allows the mimicking of symptoms, making it possible for researchers to probe the essence of a disease in ways that are not possible or practical with human subjects. In the best case, models allow us to answer questions about the cause, progression, and effective treatment options for a disease. Psychiatric illnesses, however, are different from somatic diseases, since emotional distress cannot be verified and measured in animals in the same way bodily dysfunction and abnormality can.\cite{40} Nevertheless, some of the most well validated animal models of clinical depression include both environmental and social stressors, as well as pharmacological manipulation. Most research on psychological phenomenon are carried out using rats. Their ability to learn through conditioning and their socially inclined tendencies create nice parallels to humans.\cite{41} The chronic unpredictable stress model (CUS) entails exposure to a series of well-defined environmental stressors over a period of several weeks.\cite{42} These different stressors, which include cage tilt, isolation, water deprivation, and overcrowding,
are applied 1-2 times per day for several hours. This exposure to chronic mild stress is intended to stimulate a state of anxiety or chronic mild depression in humans that develops gradually over time.\textsuperscript{42,43} Alternatively, the social defeat model involves exposure to social stress. The stress is induced through interaction with an aggressor, where one rat must submit to another that is dominant. The aggressive interaction occurs once a day over varying periods of time, ranging between 5-10 days. This socially induced stress leads to social avoidance, anhedonia, decreased locomotor activity, and increased anxiety.\textsuperscript{44,45} Social defeat stress models may be more relevant to and representative of a human depressed state than an environmentally induced stress, as severe social stress and abuse are significant etiological precursors to the development of clinical depression and other psychopathologies in humans.\textsuperscript{46} In addition to inducing quantifiable behavioral changes, social defeat stress leads to a mimicking of neuronal characteristics of depression.\textsuperscript{20} Finally, Pharmacological alterations, such as corticosterone injections, can induce the molecular markers of depression in the brain, although the construct validity of these models, is fairly low, as they do not parallel the true development of the disease in humans.\textsuperscript{47}

Animal models of mental illnesses such as depression are described as representing ‘depressive-like’ behaviors that correspond to behaviors seen in depressed human patients. These depressive-like behaviors include decreased locomotor activity, decreased exploration, decreased curiosity or willingness to interact with strangers, decreased risk taking, and decreased pleasure seeking.\textsuperscript{48} Together, these measures can shed light on the overall mental state of the rat in terms of how anxious and ‘depressed’ it is. It is important to use multiple measures of behavior to assess depressive-like behaviors, because simply measuring exploratory behavior, for example, may only indicate how anxious the rat is.\textsuperscript{6,46} In contrast, reduced exploration paired with anhedonia and reduced social interaction can indicate that a more complex depressive-like phenotype is present. These behavioral characteristics can be
measured using a variety of methods. In this study, locomotor activity and anxiety were measured using the open field test (OF) and elevated plus maze (EPM); interaction with a stranger rat and anxiety were measured using the social interaction test (SI), and anhedonia was measured by sucrose preference (SP).

*Social Contagion Hypothesis as a Novel Model of Depression*

The challenge in studying psychiatric illnesses is to produce an animal model that accurately represents the induction, development, and progression of the disease. When construct validity is achieved, the pathophysiology and neuronal mechanisms can be studied, leading to the development of realistic and effective treatments. Most current models, while valid, cannot match the complexity of depression. This lack of a comprehensive model can be attributed to the fact that, as stated above, the pathophysiological basis for depression is generally unknown.

In this study, I explore the contagion hypothesis as a novel mechanism of inducing depression and look closely at whether behavioral and molecular characteristics of depression can spread merely through co-habitation. To examine the plausibility of a social contagion hypothesis, in brief, I first induced depression through chronic social defeat, and then housed naïve rats with those that underwent social defeat. Through both behavioral assessment and ΔFosB expression quantification, I was able to evaluate the extent to which a depressive-like state is transferrable during extended social interaction.

The literature, although limited, that implicates the existence of emotional contagion in humans demonstrates social contagion between college roommates, spouses, and couples that live together. College roommates of depressed individuals were shown to become more depressed over the course of a three-week study, in particular, the roommates who had a higher tendency towards “reassurance seeking”. Reassurance seeking could indicate a degree of vulnerability to stress that makes the induction of depression occur. A parallel was
also found between a spouse’s depression and the development of depression in the other spouse. These human studies provide preliminary evidence of emotional contagion.

In order to address my hypothesis that depression is contagious, I predicted that test animals cross-housed with socially defeated animals would be more similar in behavior and ΔFosB expression to social defeat controls than naïve controls. Therefore, cross housed animals should spend less time in the center zone than naïve controls in an open field, spend less time interacting with a stranger during social interaction testing than naïve controls, have a lower sucrose preference than naïve controls, and spend less time in the open arms of an EPM than naïve controls (Figure 5). In addition, cross-housed rats should have increased ΔFosB expression in the infralimbic mPFC and decreased ΔFosB expression in the nucleus accumbens shell compared to naïve controls.

Materials and Methods

1. Experimental Design

All procedures were performed in accordance with the Institutional Animal Care and Use Committee of Rosalind Franklin University of Medicine and Science (protocol #13-10), and followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Male Sprague-Dawley rats (Harlan; age 54-64 days at arrival, total n=48) were used in this experiment. All animals were kept under constant environmental conditions: temperature was maintained between 64-69 degrees and humidity was maintained between 30-70%. Housing rooms were set to a 12:12 reverse light-dark cycle. Upon arrival, animals remained in their cages for a 1-week acclimatization period. After this week of acclimatization, the first part of the experiment began (Figure 3, Manipulation A). Rats were
divided randomly into the depression group or control group. Rats in the depression group were subjected to 10 days of social defeat stress (SD), as described below, to induce depression. Control rats were handled and weighed during the 10 days but were not subjected to any behavioral or stress protocols.

In the second part of the experiment (Figure 3, Manipulation B), rats were divided into three experimental housing groups for a total of 14 days: (a) social defeat control comprised of 3 socially defeated rats, (b) naïve control comprised of 3 naïve rats, and (c) cross-housed comprised of 2 socially defeated and 1 naïve (Figure 4). The third rat in each cage (randomly chosen in the control groups, and the naïve rat in the cross-housed groups) was referred to as the “test” rat. During this period, there were two testing days, day 7 and day 14 of co-

![Figure 3. Timeline of Experimental Design.](image)

habitation, when behavioral tests were performed to measure the development of depressive-like behaviors in the cross-housed test rats. On day 7 of the co-habitation period, all test rats were subjected to open field (OF) and social interaction (SI) testing. On day 14
of the co-habitation period, OF and SI tests were repeated along with sucrose preference (SP) testing and elevated plus maze (EPM) (all behavioral tests described below).

Following the 2-week cohabitation period, test rats were anesthetized and euthanized on day 14 of co-habitation and immunohistochemistry was performed to analyze the tissue (described below).

2. **Inducing depression in rats by social defeat**

   During Manipulation A, rats were exposed to a different Long Evans (Harlan Sprague Dawley Inc.) aggressor rat on 10 out of the next 14 days, in the home cage of the aggressor. The two rat’s contact with each other was limited to a maximum of 15 min. During interaction time, intruder rats showed signs of stress and subordination through vocalizations, flight response, and submissive posture. They were then separated by a wire
mesh cage when one of the following conditions was met: exhibition of a submissive posture, 10 attacks with no submission, 5 minutes with no attacks, or an attack that wounded the rat. Once separated, placement in the wire cage allowed auditory, olfactory, and visual engagement between the resident and intruder to continue. The intruder remained in the wire cage for another 15 minutes, or until the production of 3 vocalizations within 30 seconds. After removal, the intruder rat was placed back into its own home cage. The handling of the control rats involved their placement into a transport cage for 20 min. At the end of each session, rats were returned to their home cage.

3. **Open field test**

Test rats were individually placed in an open field (61 cm × 89 cm) in a room with dim white light (20–25 lx; 5 min) and dim red light. Video was captured with an IR-sensitive camera (Fire-I, Unibrain). The field was divided into 16 boxes (15.2 cm × 14.8 cm) during analysis. The central area zone was defined as the middle four boxes. Exploration in the open field was quantified as the amount of time the rat was in the central area zone of the field during a 5 min period (AnyMaze software, Stoelting Co., Wood Dale, IL). After administration of the test, the data was checked for anomalies to ensure that the software was operating correctly.

4. **Social interaction test**

For the social interaction test, a novel rat was placed in the open field immediately following the open field test (5 min, same conditions as open field). The novel rats had a body weight within 50 g of the test rats. As above, video was captured with an IR-sensitive camera (Fire-I, Unibrain). The video was used to measure the number of rat interactions and the total amount of time in contact. During video replay the experimenter manually recorded
the number of times the test rat approached and interacted with the other rat (defined as exploration of novel rat with nose). Then, during a second video replay, a digital stopwatch was used to quantify the total time of interaction, initiated by the test rat.

5. Sucrose preference test

Sucrose preference testing measured anhedonia in the test rats. The animals were exposed to the sucrose solution overnight prior to testing to acclimatize them to the sucrose. Then on the morning of testing, all rats, except the test rats, were removed from their home cages and placed in an identical cage with access to water and food. Test rats were then water deprived for 2 hours in their home cages on the morning of testing beginning at 7:00am (the beginning of the dark cycle) to maximize the effects of deprivation. Home cages were brought into a separate room for testing. After the period of water deprivation, the test rats were offered a choice of 2% sucrose or drinking water in 20 mL tubes with stoppers and ballpoint sipper tubes and fluid levels of sucrose and water were noted. The test rats freely drank from either bottle for 30 min. Sucrose preference was calculated as a percentage (100 x [volume of sucrose consumed (in bottle A)/total volume consumed (bottles A and B)]).

6. Elevated plus maze

The behavioral impact of stress was assessed in the elevated plus maze (EPM) on day 14 of cohabitation (Figure 5). The EPM (Scientific Designs, Pittsburgh, PA) consisted of four horizontal arms: two open arms (width × length, 4.25"×19.75") and two closed arms (width × length × wall height, 4.25"×19.75"×18"). Arms were elevated 32" off the ground. The animals were placed one at a time in the junction of the four arms, facing the open arm opposite the experimenter. Animal behavior was recorded for 5 min and analyzed by a
personal computer (Dell E6500, Dell, Round Rock, TX) running video-tracking software (Any-Maze, Stoelting, Wood Dale, IL). The time spent on the open arms was measured and used as an indication of anxiety-like behaviors. More anxious rats will tend to spend less time in the open arms versus healthy rats. In addition, the total number of arm entries was measured and used as an indicator of locomotor activity. After administration of the test, the data was checked for anomalies to ensure that the software was operating correctly.

7. Immunohistochemistry staining for ΔFosB

For the immunohistochemistry ΔFosB staining, animals were first euthanized by injection of 400 mg/kg of 8% chlora hydrate (Sigma Aldrich), then injected with 100 units of heparin into the heart (1 unit/µL), and then perfused with 50-100 ml 0.9% saline, followed with 150 ml 2% paraformaldehyde. Brains were removed and fixed for 24 hours in 2% paraformaldehyde, then cryoprotected in 30% sucrose. Sucrose was replaced every 24
14 hours until the brain sunk. Coronal sections (40 µm) were cut on a sliding microtome and placed in 0.1 M Tris-buffered saline (TBS). See Figure 6 for schematic of staining procedure. In brief, sections ranging from Bregma 3.24 mm to -5.88 mm were washed in 0.1 M TBS for 40 minutes (pH 7.4), washed with 1% H202 in 0.1 M TBS then incubated in 3% donkey serum and 1.5% triton in 0.1 M TBS for 1 hour. Staining was performed by incubating sections in primary antibodies (1:1000 dilution anti-ΔFosB; Cell Signaling Technology) in 0.3% triton and 3% donkey serum in 0.1 M TBS for 48 hours at 4°C, and with secondary antibodies (1:500 dilution biotinylated donkey anti-rabbit; Jackson Immuno Research Lab) in 0.3% triton and 3% donkey serum in 0.1 M TBS for 3 hours at room temperature. Then, sections were incubated in avidin-biotin-peroxidase reagent (1:200 dilution, ABC Elite; Vector Labs) for 2 hours at room temperature. Sections were then reacted with a DAB kit (Vector Labs), floated onto gelatinized slides, treated with ethanol and histoclear (Life Technologies), and cover-slipped with histomount (Life Technologies).

Figure 6. Schematic of Immunohistochemistry Procedure.
8. **Cell counting**

Brain areas were determined using the George Paxinos and Charles Watson Rat Brain Atlas (6th edition). Photographs of tissue were taken using Motic Moticam 10 CMOS 10.0MP Color Digital Camera on a VWR microscope at 10x magnification and captured using Motic Images Plus 2.0 software. Cells were counted manually and tracked with ImageJ64 software.

9. **Statistical Analysis**

Raw data for sucrose preference, elevated plus maze, and ΔFosB expression were analyzed for significant differences between groups using a one-way analysis of variance (ANOVA). Raw data for open field were analyzed across days with a two-way ANOVA. A p value of less than 0.05 was considered statistically significant. Table 1 shows the number of animals in each group (n) by measure and the corresponding statistical test that was preformed.

<table>
<thead>
<tr>
<th>Measure</th>
<th>n Values</th>
<th>Statistical Test Performed</th>
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<tbody>
<tr>
<td></td>
<td>Social Defeat Control</td>
<td>Naïve Control</td>
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<tr>
<td>Sucrose Preference</td>
<td>2</td>
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<tr>
<td>EPM</td>
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<td>3</td>
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<tr>
<td>Social Interaction</td>
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<tr>
<td>Open Field (Time spent)</td>
<td>4</td>
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<tr>
<td>Open Field (Number of entries)</td>
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<td>4</td>
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<tr>
<td>ΔfosB Expression</td>
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<td>3</td>
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</tbody>
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Table 1. Number of animals in each group by measure and statistical test performed.

**Results**

Differences in anhedonia, anxiety-like behavior, social interaction, exploration, and ΔFosB expression were analyzed between social defeat control, naïve control, and cross-
housed experimental groups. It is worth noting that there were observable behavioral fluctuations of the aggressor rats from day to day, meaning that the application of social defeat stress over the course of 10 days may not have been as uniform or severe as possible.

1. *Sucrose Preference*

Sucrose preference testing measured anhedonia in the animals. There were no statistically significant differences observed in sucrose preference between groups (Figure 7A; social defeat control n = 2, naïve control n = 2, cross housed n = 6; one-way ANOVA, F(2,7) = 0.2403, p = 0.7926).

2. *Elevated Plus Maze*

Behavioral responses on the elevated plus maze indicated the relative level of anxiety in the animals. There were no statistically significant differences observed in time spent in the open arms between groups (Figure 7B; social defeat control n = 4, naïve control n = 3, cross housed n = 8; one-way ANOVA, F(2,12) = 0.7074, p = 0.5124).

3. *Social Interaction*

There were no observable differences observed in social interaction between groups (Figure 7E). Statistical analysis could not be performed on these data, as there was only one animal in the naïve control group (social defeat control n = 2, naïve control n = 1, cross housed n = 4). One of the trials had to be omitted due to complications during testing.
Open Field

Behavioral responses in the open field test indicated exploration and anxiety-like behavior in the animals. There were no statistically significant differences observed in time in
the center zone between groups (Figure 7C; social defeat control n = 4, naïve control n = 4, cross housed n = 7; two-way ANOVA, Stress treatment x Day, no significant main effect of stress F(2,12) = 1.164, p = 0.3451, no significant main effect of day F(1, 12) = 0.2037, p = 0.6598) or number of entries into the center area zone between groups (Figure 7D; social defeat control n = 4, naïve control n = 4, cross housed n = 6; two-way ANOVA, Stress treatment x Day, no significant main effect of stress F(2,11) = 1.215, p = 0.333, no significant main effect of day F(1, 11) = 0.5597, p = 0.4701).

5. ΔFosB Expression

There were no statistically significant differences observed in ΔFosB expression between groups in the infralimbic mPFC (Figure 8C; social defeat control n = 2, naïve control n = 3, cross housed n = 5; one-way ANOVA, F(2,7) = 0.1635, p = 0.8523).

Figure 8. ΔFosB Staining in Infralimbic Prefrontal Cortex.
(A) Diagram showing the infralimbic prefrontal cortex (II) at Bregma 3.24. Cells were counted from this region. (B) Qualitative photos of staining in II cortex in naïve control, social defeat control, and cross housed animals. (C) Quantified cell counting data.
Discussion

In this study I attempted to measure whether social interaction can influence the transmission of a depressive-like phenotype. Primarily, there were no statistically significant differences in sucrose preference, open field behavior, elevated plus maze behavior, or social interaction behavior between groups. Nevertheless, differences among groups were consistent with my predictions. My first manipulation of social defeat stress presented the following trend: socially defeated rats tended to have a lower sucrose preference than naïve controls and spent less time in the open arms of an EPM than naïve controls. Both trends are consistent with my predictions. The small sample size likely contributed to the lack of statistical significance. There is a large body of literature that indicates the induction of depressive-like behaviors such as anhedonia, decreased locomotion, and decreased exploration following exposure to chronic defeat stress, so I would predict that increasing the number of trials would likely bring my data to a level that might prove statistically significant.44,53

My second manipulation of cross housing seems to have produced an intermediate behavior level between the naïve and social defeat controls. Although the differences among groups were not statistically significant, an intermediate trend was evident, most clearly in the sucrose preference data, but also to an extent in the time spent in the center area zone of an open field, which was again consistent with my original predictions. These results may be strengthened by either extending the period of or intensifying manipulation A, or by continuing manipulation A co-temporally with manipulation B. Perhaps social contagion can only occur if the first manipulation imparts a more severe form of stress. In many studies, 10 days of social defeat is sufficient to induce behavioral changes, but in order to observe a contagion effect perhaps the defeat must persist for a longer period of time. Extended defeat
of up to 5 weeks has demonstrated successful and sustained development of depressive-like behaviors. Increasing the number of days of social defeat from 10 to 15, or even 20 could have an impact on the behavior of those animals, thereby increasing the likelihood of a contagion effect. Due to temporal restrictions I could not carry out a longer period of social defeat. A second way to strengthen the data obtained could be to continue social defeat during the time of cohabitation. This could prove to be more effective rather than having the two manipulations occur during discrete time periods. By relying on a minimum exposure to social defeat in manipulation A, my hope was to observe a baseline of what the contagion effect could look like, while adhering to conservative ethical guidelines of animal stress studies.

In future studies, it could be promising not only to increase the number of days rats are subjected to social defeat in an attempt to achieve a maximum effect, but also to pair the defeat stress with a supplemental stressor such as chronic unpredictable stress (CUS). The only other study exploring the contagion hypothesis in rats (published after my original research proposal), which subjected male rats to 5 weeks of CUS followed by a 5 week period of co-habitation, found that healthy rats developed depressive-like behaviors after cross housing. Specifically, they demonstrated that rats in both the depression group and depression contagion group exhibited decreased sucrose preference and decreased total distance traveled and mean velocity in an open field compared to naïve controls. This study, conducted by Boyko et al., is the first published attempt at developing an animal model to not only test but also successfully demonstrate the hypothesis of contagious depression. Although they were able to illustrate the phenomenon of contagion through the use of CUS, their study has two limitations. First, CUS parallels a more mild state of anxiety similar to a mild chronic depression, or dysthymia, that develops in humans over an extended period of time while defeat stress produces a model that is more relevant to human
major depressive disorder. In addition, social defeat has been used to study the molecular responses to chronic stress as it reliably reproduces known neurophysiological biomarkers of depression such as HPA hyper-activation and selective BDNF and ΔFosB changes. While CUS has also been shown to induce selective ΔFosB and serum BDNF changes, the paradigm mainly produces anhedonic responses as well as some grooming deficits and changes in aggressive and sexual behavior.

Utilizing animal models to study depression is important but also presents various methodological and construct validity issues. Firstly, chronic stress models demand precision in timing and degree of application. Consistent expression of aggression during social defeat is uncontrollable and often generates uneven exposure to stress. Factors that affect the level of aggression include changes in bedding and time of day, although random behavioral fluctuations of the aggressor from day to day also influence the reliability of social defeat. Hyper-aggression can cause physical harm and is deemed unethical but hypo-aggression poses the risk of not developing enough of a depressive phenotype behaviorally or molecularly. The reproduction of depressive-like behaviors in research is difficult due to the high degree of overlap they have with anxiety-like behaviors. Depression and anxiety have high comorbidity clinically, and so logically, the differentiation of their behavioral parallels in animal models is often unclear.

It is necessary to remember that each species operates uniquely and has varying emotional and reactive capacities. From an evolutionary standpoint, depression in rats may simply represent the idea of an involuntary defeat strategy, which is a social phenomenon occurring after an animal experiences defeat in a competition for resources, but may not indicate full blown depression. The depressive-like behaviors exhibited in rats subjected to chronic stress may not allow us to draw conclusions about whether this is actually a depressive state although the brain changes may help to disambiguate this. Decreased
pleasure seeking, psychomotor retardation, and social avoidance are characteristic of an individual with an adaptive disadvantage. Instead of depression being associated with despair as it is in humans, animal models of depression could merely represent a reaction to subordination, either to the environment or other animals. One possible explanation for why it is hard to produce and accurately identify a depressive pathology, versus anxiety, is because animals may be too sensitive to the stress paradigms that are used. Manipulating the environment or social relationships, through CUS or social defeat respectively, may be too harsh, initiating instinctual physiological responses that dictate the animal’s behavior, while depression in humans is more akin to a symphony of behavioral and emotional reactions. The behavioral changes observed in rats are largely due to chronic sympathetic and HPA hyper-activation. In order to precipitate a more complex phenotype paralleling human depression, something less acute, such as the social contagion model, could be useful.

Exploration of the social contagion hypothesis of depression is in its infancy. The complexity of human emotion generates unique and dynamic relationships, making it hard to study the effect of emotional contagion. The possibility of developing a more sensitive way to induce depression in rats without directly manipulating their surroundings through stress is exciting. It could aid in investigating the mechanisms behind the depressed mind and give insight into potential therapeutic targets. It is important to note that Boyko et al. found evidence that the contagion effect could be bi-directional; rats subjected to CUS after 5 weeks of cohabitation with naïve rats exhibited slightly less severe depressive-like behaviors. This indicates that treating depression with a bi-faceted approach of pharmacological drugs and psychological therapy may not be as robust an approach as possible: targeting the social environment of the patient may be vital as well. The contagion hypothesis of depression is a promising option for research and should be considered in the search to better understand the pathophysiology of depression.
References


