Video Article

A New Method for Inducing a Depression-Like Behavior in Rats

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Abstract

Contagious depression is a phenomenon that is yet to be fully recognized and this stems from insufficient material on the subject. At the moment, there is no existing format for studying the mechanism of action, prevention, containment, and treatment of contagious depression. The purpose of this study, therefore, was to establish the first animal model of contagious depression.

Healthy rats can contract depressive behaviors if exposed to depressed rats. Depression is induced in rats by subjecting them to several manipulations of chronic unpredictable stress (CUS) over 5 weeks, as described in the protocol. A successful sucrose preference test confirmed the development of depression in the rats. The CUS-exposed rats were then caged with naïve rats from the contagion group (1 naïve rat/2 depressed rats in a cage) for an additional 5 weeks. 30 social groups were created from the combination of CUS-exposed rats and naïve rats.

This proposed depression-contagion protocol in animals consists mainly of cohabiting CUS-exposed and healthy rats for 5 weeks. To ensure that this method works, a series of tests are carried out - first, the sucrose preference test upon inducing depression to rats, then, the sucrose preference test, alongside the open field and forced-swim tests at the end of the cohabitation period. Throughout the experiment, rats are given tags and are always returned to their cages after each test.

A few limitations to this method are the weak differences recorded between the experimental and control groups in the sucrose preference test and the irreversible traumatic outcome of the forced swim test. These may be worth considering for suitability before any future application of the protocol. Nonetheless, following the experiment, naïve rats developed contagion depression after 5 weeks of sharing the same cage with the CUS-exposed rats.

Video Link

The video component of this article can be found at <https://www.jove.com/video/57137/>

Introduction

Tests carried out in recent times have suggested that psychiatric illnesses could be easily spread to healthy individuals via contagion¹. In this case, it is termed social contagion and is spread through affect, attitude, or behavior. This only requires one depressed individual to interact with one or more healthy individuals, thereby facilitating the sharing of emotion. Social relationships are hence a very important component of mood, as they define the transfer of emotions from one individual to the other, by way of mimicry and "emotional contagion". Time frames for contagion to take effect vary², inevitably depending on the severity of the emotion and the strength of resistance of the recipient.

The significant consequences of emotional contagion have ensured that studies made in the past mainly focused on the negative aspects. The outcome of negative effects ensured contagion depression received enormous attention, with studies showing that contagion depression increases the likelihood of families and friends of a depressed individual exhibiting depressive behaviors^{3,4,5,6}.

There are both personal and economic reasons for tackling depression. It commonly causes morbidity; and its lifetime incidence is between 13.3 and 17.1% in the United States⁷. The World Health Organization files show that depression is fourth on the list of global diseases with huge burdens, occurring in people of all genders, ages, social backgrounds, and is equally capable of inflicting poor health, impacting the ability to interact with others^{8,9,10,11}, and causing excess disability^{12,13}. 850,000 lives are estimated to be lost to depressive suicide each year¹⁴. Patients are typically prescribed antidepressant medications or advised to undergo cognitive behavioral therapy. These treatments help about 60-80% of patients. However, dealing with the disease still poses a big problem; treatment is not available to all depressed patients. For those who get treatment, some suffer side effects, while others poorly comply with guidelines¹⁵. The number of patients resistant to treatment is up to

about 40%¹⁴. With depression, the economy has frequently suffered in the form of expensive treatment, a decrease in the workforce, and early retirements¹⁶. An estimated annual loss of \$44 billion in the United States is caused by depression, accounting for almost half of the country's lost productivity¹⁷. Expensive treatment requires careful medical attention, which incurs a variety of increased medical costs and necessitates anticipation of undesirable outcomes, as well as poor response to treatment¹⁸.

Having not come across an already proven animal model to study the depression-contagion mechanism, its prevention, and treatment, this hypothetical animal protocol was used for the first time. It suggests that through cohabitation with CUS-exposed rats, healthy rats tend to express depressive behaviors. The main objective of this experiment was to establish a laboratory procedure that highlighted the transfer of depression, via contagion, from CUS-exposed rats to healthy ones. Next, results were evaluated to determine if depression-contagion was limited only to depressive symptoms, or if it was related to other mood disorders, such as anxiety. The ultimate goal of the experiment is to draw closer to better understanding the mechanism of depression-contagion in the race to develop new therapeutic approaches¹⁹.

Protocol

The following procedure was conducted according to the recommendations of the Declaration of Helsinki and Tokyo and the Guidelines for the Use of Experimental Animals of the European Community. The experiments were also approved by the Animal Care Committee at the Ben-Gurion University of the Negev.

1. Preparing Rats for the Experimental Procedure

Note: For the experimental procedure, select male Sprague-Dawley rats with no overt pathology, each weighing between 300 and 350 g.

1. House three rats per cage, with chow and water available *ad libitum*, and allow for at least two weeks of adaptation, alternating between 12 h of daylight and 12 h of darkness.
2. Before initiation of the experiment, test all rats for the presence of depressive-like behaviors using a 3-day **sucrose preference test (refer to step 4)**.
3. Exclude rats that are perceived to display depressive-like behaviors and those that are housed in the same cages as such rats, so as to avoid possible spread of depression to unaffected rats before the experiment has begun first, eliminate rats that test positive for depression; then, eliminate rats that are housed in the same cages as the depressed rats.
4. If necessary, exclude additional rats at random to create final groups of 60 rats for the depression experiment, 30 rats for the contagion group, and 30 rats for the control group.
5. Tag rats (using numbered stickers), throughout the experimental procedures. Temporarily transfer rats to single cages during the experiments (in the same room and dark cycle for the CUS, Sucrose and open field tests, and in a different room and dark cycle for the forced-swim test and for one of the stressors (refer to 2.1.7)) and return them to their original social groups at the end.

2. The Procedure for the Induction of Depression in Rats

Note: In order to induce depression, subject the 60 rats in the CUS-exposed group to several manipulations of **CUS** for five weeks²⁰.

1. Expose rats daily to any 2 of the following 7 stressors, in a random order; to one stressor during the day and to the other at night, for 5 consecutive weeks.
 1. House the rats in groups (six rats, instead of the usual three, per cage for 18 h);
 2. Place the rats in a tilted cage (45° along the vertical axis for 3 h);
 3. Deprive the rats of food (18 h);
 4. Deprive the rats of water (18 h) and expose them to empty water bottles immediately following a period of acute water deprivation (1 h);
 5. Place the rats in a wet bedding cage (with 300 mL of water spilled in the bedding) for 8 h (night cycle);
 6. Expose the rats to continuous lighting (24 h) and reversed light/dark cycle (12 h) twice per week;
 7. Place the rats in a hot environment (40°C, 5 min - night cycle), with another apparatus used to increase the heat.
2. Confirm the development of depression by performing a **sucrose preference test (refer to step 4)**.
Note: The protocol can be paused after each of the above steps. Additionally, if any aberrant behavior is displayed by any rat, then, eliminate the rat and any other rat that might have been affected by the erratic rat.

3. The Procedure for Establishing Depression-Contagion in Naïve Rats

1. Establish 30 social groups with rats from the CUS-exposed (60) and depression-contagion (30) groups:
 1. House two CUS-exposed rats per cage in 30 different cages.
 2. Add one rat from the depression-contagion group to each cage containing two CUS-exposed rats.
2. Allow **cohabitation for 5 weeks**, under standard conditions, with chow and water available *ad libitum*.
3. After 5 weeks of cohabitation, subject all groups to the sucrose preference, open field, and forced-swim tests in the order shown in **Figure 1**.

4. The Sucrose preference test²¹

1. During the dark cycle, place the rats in individual cages in the same room as the housing.
2. Place a bottle of 100 mL 1% (w/v) sucrose solution in each cage for 24 h, and allow for adaptation.

3. Remove the bottles and deprive the rats of food and water for 12 h.
4. Place two bottles, one containing 100 mL of sucrose solution (1 %, w/v) and the other, 100 mL of tap water, in each cage for 4 h.
5. Record the volume (in mL) of both the consumed sucrose solution and water, and calculate the affinity to sucrose preference as follows:
Sucrose preference (%)

$$= \frac{\text{Sucrose consumption (mL)}}{\text{Sucrose consumption (mL)} + \text{water consumption (mL)}} \times 100\%$$

5. The Open Field Test

Note: The standard open field test is commonly used to assess locomotor, exploratory, and anxiety-like behaviors, and behavioral responses to novelty in laboratory animals^{22, 23}. This test has also been shown to analyze depressive behaviors²⁴. The open field test examines two opposing parameters; the rodents' fear for the bright light shining at the center of the field against their expressive desire towards novelty. When anxious, rodents tend to avoid discovery and stay put by the walls (thigmotaxis). This anxiety is determined by the degree of averting the brightly lit central field. The open field, consisting of a black lusterless acrylic box (120 cm × 60 cm × 60 cm), is divided into the central part (25%) and the rest of the box (75%). This test takes place in the same room as the housing during the dark cycle. A video camera, suspended about 200 cm above the field, is used for recording the open field test^{22, 23}.

1. Clean the apparatus with 5% alcohol prior to the introduction of each animal. Place the rat at the corner of the open field facing the wall.
2. Record the rat's behavior with a video camera for 5 min.
3. Analyze post-recordings of the following parameters: total travel distance, velocity in the central part of the field, and time spent in the central part of the field.

6. The Forced-Swim Test

Note: The principle of the forced swim test is based on the fact that when rats are forced to swim in a confined space, they eventually give up and remain immobile, occasionally moving their bodies in order to avoid drowning^{21, 23}. Due to the enormity of the already assembled apparatus, this test was carried out in the different room during the dark cycle.

1. For habituation, place the rat in a glass cylinder (100 cm tall, 40 cm in diameter, and 40 cm deep) containing water at room temperature, for a 15 min swimming session.
2. Remove the rat from the cylinder, dry with paper towels, and place in a warm cage for 15 min.
3. Return the rat to its home (original) cage;
 Note: The protocol can be paused here. Also, if any rat in the course of this test is drowning, pull it out immediately.
4. On the following day, videotape a 5-min swim session and analyze post-recordings of the following parameters: Immobility, Climbing, and Defecation.

Representative Results

The sucrose preference test: After exposing rats to 5 weeks of CUS for induction of depression, and then subsequently exposing healthy rats to the CUS-exposed rats for a further 5 weeks, both sets of rats displayed depressive-like behaviors at the end of the experiment (**Figure 2**). The evidence of this behavior was seen in the reduced preference for sucrose by the depressed rats, after CUS ($65 \pm 2.8\%$, $p < 0.001$, **Figure 2**), when compared to the 30 rats in the control group ($101 \pm 7\%$, (**Figure 2**). After 10 weeks, the 60 CUS-exposed rats demonstrated a statistically significant change ($72 \pm 3.3\%$, $p < 0.001$, **Figure 2**) when compared to the 30 rats in the control group ($95 \pm 3.4\%$, **Figure 2**), as did the 30 depression-contagion rats ($76 \pm 4.7\%$ $p < 0.001$, **Figure 2**), after 5 weeks of cohabitation with the CUS-exposed rats.

The open field test: Rats in both the CUS-exposed and depression-contagion groups had decreased total travel distances (depression-contagion - $p < 0.05$ and CUS-exposed - $p < 0.01$, **Figure 3A**), and decreased mean velocities (CUS-exposed - $p < 0.05$, **Figure 3C**), compared to the control group. The time spent at the center of the open field by rats from the control group and the two experimental groups was non-significantly different (**Figure 3B**).

The forced-swim test: Results from the evaluation of immobility were as expected. Both CUS-exposed and depression-contagion rats showed extended immobility following the forced swim test. However, a significantly extended time of inactivity was registered only in the CUS-exposed rats ($p < 0.01$) when compared with the control group (**Figure 4A**). In another investigated forced-swim test parameter, climbing time, both groups of CUS-exposed and contagion-depression rats exhibited highly limited climbing properties ($p < 0.01$) when compared to the control group (**Figure 4B**). The assessment of the rate of defecation resulted in the registration of high quantities of feces for both experimental groups ($p < 0.01$), compared with control (**Figure 4C**).

Figure 1: The experimental protocol design. Shows the order and timing of the respective experimental protocols. [Please click here to view a larger version of this figure.](#)

Figure 2: The sucrose preference test. The percentage change in the sucrose preference test significantly decreased in the 60 CUS-exposed rats ($65 \pm 2.8\%$ vs. $101 \pm 7\%$, $p < 0.001$) compared to the 30 rats in the control group, after 5 weeks of CUS. There was a significant percentage change in the sucrose preference test in both the 60 CUS-exposed ($72 \pm 3.3\%$, $p < 0.001$) and 30 depression-contagion ($76 \pm 4.7\%$, $p < 0.001$) rats after 10 weeks, compared to the 30 rats in the control group ($95 \pm 3.4\%$). Statistical analysis was done with one-way ANOVA with Bonferroni's *post hoc* test. The data are presented as percentages of the baseline and expressed as mean $\pm$ SEM. [Please click here to view a larger version of this figure.](#)

Figure 3: The open field test. Both the depression-contagion ($68.1 \pm 6.5\%$, $p < 0.05$, **Figure 3A**) and CUS-exposed rats ($59.6 \pm 5.7\%$, $p < 0.01$, **Figure 3A**) had decreased total travel distances, compared with control rats ($100 \pm 13\%$, **Figure 3A**). No significant differences were found for time spent by all 3 groups of rats in the central part of the open field (**Figure 3B**). The CUS-exposed ($75.4 \pm 6\%$, $p < 0.05$, **Figure 3C**) and depressed-contagion ($88 \pm 5.6\%$, $p < 0.005$, **Figure 3C**) rats both had decreased mean velocities, but a significant change was recorded only in the CUS-exposed group, compared to rats in the control group. In all 3 tests, the Kruskal-Wallis, followed by Mann-Whitney test were used. The data are presented as percentages of the control group and expressed as mean $\pm$ SEM. [Please click here to view a larger version of this figure.](#)

Figure 4: The Forced-swim test. The CUS-exposed ($151 \pm 3.3\%$, $p < 0.01$, **Figure 4A**) and depression-contagion ($107 \pm 6.7\%$, **Figure 4A**) rats were both found to be highly immobile, but a significant change was recorded only in the CUS-exposed group, compared to the rats in the control group. The CUS-exposed ($46 \pm 5.5\%$, $p < 0.01$, **Figure 4B**) and depression-contagion ($64 \pm 5.4\%$, $p < 0.01$, **Figure 4B**) rats both expressed reduced climbing times. The defecation rates of the 60 CUS-exposed rats ($278 \pm 32\%$, $p < 0.01$, **Figure 4C**) and 30 depression-contagion rats ($131 \pm 37\%$, **Figure 4C**) were significantly greater compared to the defecation rate of the 30 control rats ($100 \pm 22.5\%$, $p < 0.01$). *Post hoc* analysis found no significant difference between the depression-contagion rats and the controls, but showed a significant difference between CUS-exposed compared with control rats ($p < 0.01$, **Figure 4C**). Statistical analysis was done with one-way ANOVA. The data are presented as percentages of the control group and expressed as mean $\pm$ SEM. [Please click here to view a larger version of this figure.](#)

Discussion

According to the results obtained with the application of this protocol, healthy rats, like humans, were negatively affected by depressed rats when housed together over an extended period. The contagiously depressed rats were affected by their already depressed counterparts after five weeks of cohabitation, establishing a distinct animal depression-contagion model for the first time. An earlier study with pigs had also suggested shared emotional statutes between depressed and healthy pigs²⁵.

The induction of depression in rats was achieved with the application of CUS. This method expresses features common to every day's socio-environmental stressors. Archives show that CUS is the most used method of inducing depression in animals. The results obtained with this method share strong similarities with clinical symptoms and depressive-like behaviors. If it is properly used on rats, the rats can express all known depressive signs²⁶. The ability to bring out all depressive behaviors when applied on rats validates CUS as a reliable depression-inducing method with high predictability and construct validity^{27,28}. The CUS method is equally used as an animal depression model to analyze cellular and molecular mechanisms of depression's pathophysiology, as well as to study the mechanism of action of antidepressants^{29,30,31,32,33}.

The sucrose preference test applied after cohabitation of depressed and naïve rats enabled the evaluation of the spread of depression, resulting in the expression of depressive behaviors in both groups of rats after 5 weeks (**Figure 2**, **Figure 3**, **Figure 4**). The forced-swim test²⁰ and the open field test^{34,35,36,37,38,39,40} were also used to investigate depression in rats⁴¹. These methods mirror an extensive range of behavioral irregularities. The fact that there is no specific mechanism of contagion means that conscious and unconscious components could be advanced as potential hypothetical outcomes. Unconsciousness could manifest through mimicry⁴², by way of healthy rats copying body movements of depressed rats. Movements most likely to be reproduced are such as facial expressions and the neuron system⁴³. Consciousness, on the other hand, could arise by means of communication methods. One such method is co-rumination⁴⁴.

Although the healthy rats became depressed when sharing housing with the depressed rats, depressed rats also became less depressed, compared to their state after CUS, as a result of cohabiting with their healthy counterparts. This follows a pattern that was observed in earlier findings when depressed college students were able to contagiously become undepressed after spending time with their healthy roommates⁴. There is, therefore, not only a negative impact with contagious depression, but also a reciprocal effect. Depressed individuals have a negative bearing on healthy ones, while they themselves slightly recover from depression by virtue of being exposed to non-depressed colleagues. Such a finding might be a boost to psychiatry in tackling both depressed individuals and populations.

Prior to this test, animal models of depression-contagion were nonexistent, and even though a breakthrough was made here, this test has its limitations. It can be considered somewhat weak for a few reasons. The difference between the sucrose uptake and water uptake in the supposedly depressed rats, when compared to controlled rats, is not highly significant and is not demonstrative of a highly depressed state in the experimental rats. Also, the use of the forced-swim test would not be advisable if better alternative options can be found. The damages caused by this test are traumatic, permanent, and irreversible. Consequently, animals subjected to the forced-swim test cannot be recycled or used for further experiments. Regardless of the drawbacks of the method, it remains a pioneering method that produced decent results.

Much has been said and done to come to terms with depression, but it has remained a major cause for concern, with the increasing number of life-threatening situations highlighting its severity in society today. The complex nature defining the interactions between humans makes it problematic to systematically evaluate depression contagion. Thus, an advanced understanding of an animal model could be key to unlocking the mechanism of depression in the human population, subsequently establishing therapeutic answers to this problematic disease.

Disclosures

The authors have nothing to disclose.

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