Video Article

Applying Stereotactic Injection Technique to Study Genetic Effects on Animal Behaviors

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Abstract

Stereotactic injection is a useful technique to deliver high titer lentiviruses to targeted brain areas in mice. Lentiviruses can either overexpress or knockdown gene expression in a relatively focused region without significant damage to the brain tissue. After recovery, the injected mouse can be tested on various behavioral tasks such as the Open Field Test (OFT) and the Forced Swim Test (FST). The OFT is designed to assess locomotion and the anxious phenotype in mice by measuring the amount of time that a mouse spends in the center of a novel open field. A more anxious mouse will spend significantly less time in the center of the novel field compared to controls. The FST assesses the anti-depressive phenotype by quantifying the amount of time that mice spend immobile when placed into a bucket of water. A mouse with an anti-depressive phenotype will spend significantly less time immobile compared to control animals. The goal of this protocol is to use the stereotactic injection of a lentivirus in conjunction with behavioral tests to assess how genetic factors modulate animal behaviors.

Video Link

The video component of this article can be found at http://www.jove.com/video/52653/

Introduction

The mouse has been widely used in neurobiology because it is easy to manipulate genetically. Gene knockout techniques allow researchers to investigate how each genetic factor shapes mouse behaviors. Moreover, the cre-loxp system provides a valuable tool for tissue- and cell type-specific gene knockout in mice, which enables researchers to study gene function in different tissues.[1] However, in practice, expression patterns of cre promoters are difficult to control, and so far many established cre drivers have not achieved region-specific expression.[2,3]

Alternatively, stereotactic injection is a method that targets specific brain regions of the adult mouse. By injecting genetically engineered viruses expressing cDNA or shRNA, region-specific modulation of gene expression can be achieved. Although the brain size of each mouse varies, the location of specific brain regions can be determined using stereotactic coordinates set from landmarks on the skull of the mouse brain. The most commonly used landmarks are bregma, lambda, and the interaural line. Using coordinates obtained from a brain atlas,[4] the exact location of each brain area can be identified by the antero-posterior (A/P), medial-lateral (M/L), and dorsal-ventral (D/V) axes from bregma/interaural line intersection. Typically viruses injected into the brains of mice are tagged with either red, or green fluorescent protein (RFP or GFP), so that injections can be confirmed by fluorescent microscopy.

Behavioral assessments of mice are especially necessary for basic research of psychiatric disorders. Symptoms of psychiatric disorders in patients typically involve abnormal behaviors. Some of these human behaviors are evolutionarily conserved and can be directly mimicked and observed in the mouse. For example, depression can be modeled in the mouse by measuring behavioral despair. People with depression often feel as though nothing they do will ever help, a symptom that can eventually lead to suicide. In rodents, this can be modeled using the Forced Swim Test (FST), which measures the amount of time a mouse swimming versus floating in a pool of water (viewed as giving up). This paradigm is validated by rescuing the phenotype with anti-depressants.[7,8,9] Mice which have received anti-depressants will spend significantly less time immobile compared to untreated controls. Another behavioral test, the Open Field Test (OFT) is designed to assess locomotion in mice, and additionally can be used to analyze the anxious phenotype in mice.[5,6] This test is based on the premise that mice feel safer when they are close to the wall in a novel open field. Wild type mice will eventually explore the novel environment, as they are curious animals. However, spending less time in the center of the field indicates anxiety in the mouse, as the mouse will not be able to overcome the initial fear brought on by a novel environment. The anxiety of the mouse, as quantified by the amount of time spent in the center of an open field, can be compared to clinical anxiety in humans, which is present in many psychiatric disorders.

The combination of stereotactic injections with behavioral paradigms is a novel way to alter the expression of a specific gene in a targeted brain area. The effect of modulated gene expression on mouse behaviors can then be determined. In contrast to whole brain knockout, this method is particularly useful as it only targets specific brain areas. In addition, stereotactic injections are typically performed in the adult wild type mouse, therefore, endogenous gene expression has been maintained throughout developmental stages. This method will avoid the confound effect if the gene is required for survival during the embryonic or postnatal stage of development. One major limitation is that the experimental mice need

to go through an invasive surgery, in which the skulls of mice have to be opened. Moreover, the degree of gene modulation is determined by the titer and efficiency of the virus. The virus needs to be injected into the correct region using stereotactic coordinates, which requires special instruments. Verification of the correct injection site can only be completed post mortem.

This method has been previously used to test the involvement of a specific gene in various neurological diseases. For example, viral mediated RNAi targeting the *Th* gene (which allows dopamine to be synthesized) was injected into the substantia nigra compacta, and locomotor behavior analysis was conducted.[10] Another study used stereotactic injection of a lentivirus silencing DISC1 to assess mouse behavior in relation to schizophrenia. Knockdown of DISC1 led to increased locomotion in response to novelty (parallels positive symptoms in schizophrenia), and greater immobility in the FST.[11] Similarly, an additional study found that 5-HT_{1B} overexpression led to increased exploratory behavior in the OFT, consistent with an anti-anxiety phenotype using this method.[12] Stereotactic injections can deliver cre virus to induce recombination in cre-loxp mice. This method was used to selectively delete the Y2 receptor in the amygdala and the bed nucleus of the stria terminalis. Upon behavioral analysis, these mice were found to an anti-depressive phenotype when the gene was deleted in the central amygdala, but no phenotype when the gene was deleted in the basolateral amygdala or the bed nucleus of the stria terminalis.[13] Thus, this technique provides a unique tool to study the genetic effect on animal behaviors.

Protocol

NOTE: All protocols involving animals were followed in accordance with the animal care guidelines of The Pennsylvania State University, IACUC #44057

1. Lentivirus Production

NOTE: The day before transfection, LentiX-293 cells should be at 80% confluency.

1. Rinse cells with DMEM just before transfection and incubate in 10 ml of DMEM/10% FBS with Penicillin (100 IU/ml) and Streptomycin (100 µg/ml) for 5 min at RT.
2. Dilute DNA and Polyethylenimine (PEI) (1 mg/ml) in 1:3 ratio (1 µg of DNA: 3 µl of PEI) in 1 ml of DMEM and vortex for 1 min. The volume of DNA added is dependent on the concentration of the DNA. Incubate at RT for 10 min.
    1. For example, use 20 µg of DNA plasmid, comprised of 10 µg of vector plasmid, 5 µg of psPax2[14] plasmid, 5 µg of vesicular stomatitis virus(VSV) with 60 µl of PEI per dish.
3. Mix the DNA and PEI solution again by vortexing for 1 min. Incubate at RT for 10 min.
4. Add 1/10th volume of total culture medium of DMEM (*i.e.* 1 ml for 10 ml culture medium in a 100 mm dish). Add 1 ml of DNA-PEI mixture drop by drop into the dish and swirl the dish around until to the culture medium is well-mixed with the DNA.
5. Incubate for 6 hr at RT and remove the supernatant. Add 5 ml of culture medium.
6. 48 hr after transfection, collect viral supernatant and spin at 627 x g for 5 min at 4 °C in a 50 ml tube. Filter 30 ml of viral supernatant through a 0.45 µm syringe filter into an ultracentrifuge tube.
7. Add sterile water to balance the tubes, and cover tubes with small piece of parafilm. Spin tubes at 11,249 x g for 120 min at 4 °C. Remove liquid using a vacuum tip.
8. Add 100 µl of cold PBS the tube. Gently rock the tube at 4 °C O/N.
9. To re-suspend the virus, pipette the PBS added in step 1.8 over the pellet 10 times, being careful not to touch the pellet with the tip. The pellet will not be re-suspended until this is complete.
10. Aliquot the virus at 10-20 µl per tube, flash-freeze in liquid nitrogen and store at -80 °C.

2. Stereotactic Injection

2.1) Preparation of Instruments

1. Place a pair of scissors, blunt-end forceps, a needle holder, scalpel, cotton swabs, and a cloth in a sealed pack with a sterilization indicator, and autoclave prior to surgery. Additionally, obtain 10% povidone iodine, 70% ethyl alcohol, absorbable sutures, a heating pad, artificial tears, a glass bead sterilizer, drill, a drill bit, 2 disposable syringes, an injection syringe, electric razor, analgesic (ketoprofen), anesthetic (avertin), gloves, and a stereotactic apparatus with injection pump.
2. Make 1.25% avertin solution fresh that day, filter in a sterile hood using a 0.2 µm sterile syringe filter, and place into a sterile serum vial. Mix 2.5 g of 2,2,2-tribromoethyl alcohol into 5 ml of tert-amyl alcohol, then dissolve in 200 ml water. Keep the pH below 5.[15]

2.2) Preparation of the Mouse

1. Administer avertin based on the weight of the mouse (375 mg/kg).[15] Inject the mouse with the avertin via an intraperitoneal (IP) injection, and then place back into its cage until fully anesthetized.
2. Give an analgesic via IP injection (ketaprofen, 5 mg/kg), and place artificial tears on the eyes of the mouse to prevent drying.
3. Ensure that the mouse is fully asleep by pinching their foot. If the mouse responds to the foot pinch, then give more anesthetic (in doses of 50 µl). Shave the head of the mouse using an electric razor. Shave the area to be operated on (typically from just behind the ears, to the top of the snout), and the surrounding area.
4. Place the mouse into the stereotactic apparatus. Latch the front teeth onto the anterior clamp, and lower the clamp and tighten it so that the jaw is fully secure.
5. Insert the ear bars into the ear canal to fully stabilize the head. Be careful not to insert the ear bars in too far, which could damage the inner ear. Once finished, the body of the mouse should be able to be moved slightly without disrupting the position of the head.
6. Place a heating pad under the mouse in order to regulate the mouse's body temperature throughout the procedure.

7. Clean the surgical area thoroughly. Using a cotton swab, rub 10% povidone iodine in a circular motion, starting from the middle of the surgical site and moving outwards. Then, use a cotton swab to rub 70% ethyl alcohol on the surgical site in the same fashion. Repeat two times.

2.3) The First Incision

1. Once the mouse is prepped, open the sterile equipment bag. Change gloves before touching the instruments. Take out the sterile cloth and place the instruments on the cloth. If any instrument touches an un-sterile surface, use the glass bead sterilizer for 15 sec to sterilize it.
2. Take the scalpel in the dominant hand and blunt-end forceps in the non-dominant hand. Use the blunt-end forceps to gently grip the skin of the mouse, and make an incision using the scalpel. Begin the incision about 1.5 cm above the ears (toward the nose) and extend to about 0.5 cm below the ears. Extend the incision vertically down the middle of the mouse's head to expose bregma (**Figure 1**).
3. Once bregma is visualized, take a sterile cotton tip to gently remove any blood covering the surface of the skull. Use two cotton tips to push the skin toward the side of the head.

2.4) Equipment Setup

1. After any blood is removed from the surface of the skull, and bregma is clearly visualized, prepare the syringe (concentration of 10^8 TU/ml, volume of 1 µl). In the fume hood, fill the syringe with the virus to be injected. Ensure that no bubbles are inside. Place the syringe in the stereotactic apparatus, making sure that it is fully secured.
2. Slowly lower the syringe until it is right above the surface of the skull, so that the tip of the sterile syringe needle is set to the intersection of bregma and the interaural line. Set this point as zero, and determine coordinates from this point.
3. Depending on the brain area of interest, vary the stereotactic coordinates. Determine these coordinates by utilizing a brain atlas[4]. Once the coordinates are determined, move the needle of the syringe to match those coordinates. Target the dentate gyrus using the coordinates: M/L= +/- 1 mm, A/P= -1.82 mm. Lower the needle to right above the skull to visualize where the hole needs to be drilled.
4. Raise the syringe slightly, take the drill and place the drill bit right above the target drill site at about a 45° angle to the skull. Begin drilling, and keep drilling until the skull gives way. When the skull gives way, a drop in resistance can be detected. Be careful not to drill into the brain, so as to prevent cortical damage.
5. Take a sterile cotton tip and wipe away any blood from the hole.
6. Lower the syringe so that the tip sits right on the surface of the brain (not the skull). Set the D/V coordinate (depth) to 0. Lower the syringe to the desired depth, based on the brain atlas coordinates. To reach the dentate gyrus, set D/V to -1.79 mm.
7. Start the injection at a rate of 0.2 µl/min. Watch to ensure that the syringe does not slip lower than the desired depth. If this occurs, gently raise the syringe to the desired depth again.
8. After the injection is finished, wait about 2 min to ensure that any residual virus has been absorbed. Slowly raise the syringe and use a cotton tip to remove any liquid from the injection site.
9. Repeat for the other hemisphere.

2.5) Suturing

1. Remove the sterile suture and needle from its packaging and grip the needle using the needle holders. Face the pointed edge of the needle away from the needle holder. Hold the blunt-end forceps in the non-dominant hand, and use it to grip the skin on the mouse. Start with the dominant hand.
2. Gently push the suture through the skin and use the blunt-end forceps to grab the skin on the other side of the incision. Pull the suture material through until about 0.75 inch remains.
3. Let go of the needle, and use the blunt-end forceps to wrap the suture around the needle holder one time, then, grab the 0.75 inch remaining suture with the needle holder, and pull the suture through, so that the needle and needle holder end up on the side of the head opposite to that which they were on originally. This should form a knot.
4. Repeat this step three more times, alternating the side of the head the needle holder ends up on each time.
5. Cut the suture and repeat steps 2.5.1-2.5.3 until the incision is closed.

2.6) Post-operative Care

1. Gently remove the ear bars from the mouse, and remove its jaw from the anterior clamp.
2. Keep the mouse on the heating pad until it wakes up and moves around on its own. Monitor the mouse daily, and keep and eye out for signs of pain, such as not grooming, eating or drinking. Give additional analgesic when deemed necessary.

3. Open Field Test

3.1) Set Up

1. Obtain an empty square, plastic arena with dimensions 50 cm x 50 cm, with 50 cm high walls (but can be slightly larger). Clean the arena with 70% ethyl alcohol prior to the beginning of the experiment. Make sure the ethyl alcohol has fully dried before placing a mouse into the arena.
2. Place the arena on the floor, and try to ensure the lighting is set to minimize shadows and glare.
3. If using tracking software, position the camera directly overhead of the open field, approximately 1.5 ft above the open field (far enough away to full encompass the whole arena, but close enough to have a clear view of the mouse).
4. Set up a zone in the center of the box (approximately an area of 30 cm x 30 cm).[16] To set a zone, click on the zone 1 tab in the side panel.
    1. Select a shape outline in the upper panel, and outline the perimeter of the center zone. Select add zone, and click on the inside of the center zone. Using the program prompts, name the zone (this will be referred to as 'center' for this procedure). If the behavioral test will be scored by hand, make sure the bottom of the field is divided into 10 cm x 10 cm squares, marked using tape.
5. Set up the software so that the settings are correct, and the mouse is easily visible on the screen. Accomplish this by adjusting the detection settings on the machine to be compatible with the testing arena (lighting) and mouse color.

NOTE: When the settings are correct, the system should track only the mouse, and not any shadows or urine/fecal matter. The specific software settings depend on the lighting of the arena, and the color of the mouse.

3.2) Acclimation and Test

1. Move experimental mice into the behavioral room 1 hr before the test in order to acclimate them to their surroundings. Leave the mice in their cage for the acclimation.
2. Turn on the camera to tape the behavioral task and place the mouse toward the middle front of the arena so that the mouse is facing the wall.
3. Set the timer for 5 min, and step away from the arena. If possible, leave the room so as to not accidentally influence the mouse.
4. Once 5 min are up, remove the mouse and place them in their cage.
5. Use 70% ethyl alcohol to thoroughly clean out the field before proceeding to the next mouse. Make sure the ethyl alcohol has fully dried.

3.3) Scoring

1. Consider the mouse as being in the center of the field when all four paws are inside the middle 30 cm x 30 cm.
2. Quantify the amount of time the mouse spends in the center. Alternatively, determine this time using tracking software (such as Noldus ethovison).
    1. To do this, go to the analysis tab, and click on “movement” under analysis profiles. Delete the categories currently set, and then select “in zone” under the location tab. Select the center zone. Next, select “analysis output” (under the results tab) to view results. A table listing the time spent immobile for each trial should appear.

3. Otherwise, score the OFT by hand. The open field should have 25 10 cm x 10 cm squares outlined on the bottom of the open field (as outlined in step 3.1.3). Quantify the amount of time the mouse spends in the center, in addition to the number of entries into the center of the arena.[16]
   NOTE: The center 30 cm x 30 cm area is considered the center of the open field. A mouse is regarded as inhabiting the center region if all four paws are inside the 30 x 30 cm area.

4. Forced Swim Test

NOTE: Allow a minimum of five days between behavioral tasks.

4.1) Set Up

1. Obtain a 2 L clear bucket, and fill with 22 °C water. Place the bucket on a table, and try to ensure the lighting is set to minimize shadows and glare.
2. If using tracking software, position the camera directly overhead of the bucket.
3. Set up software so that the mouse is easily visible on the screen.

4.2) Acclimation and Test

1. Move experimental mice into the behavioral room 1 hr before the test to acclimate the surroundings. Leave the mice in their cage for the acclimation.
2. Turn on the camera to tape the behavioral task. Gently, place the mouse in the middle of the bucket of water. Make sure not to drop the mouse in. Slowly lower the mouse, so its front feet touch the water first. Take care to prevent their head from submerging.
3. Set the timer for 6 min, and step away from the behavioral area.
4. Once 6 min are up, remove the mouse from the bucket, and wipe off excess water before placing the mouse back in their cage.

4.3) Scoring

1. If using tracking software, set percent immobility to 11%, as suggested by Noldus (this should be the default setting), to quantify time floating vs. swimming.
2. To quantify using the tracking system, go to the analysis tab, and click on movement under analysis profiles. Delete the categories currently set, and then select mobility state, on the left side panel (under individual behavior). Set immobility to 11%.
3. Select track visualization, under the results tab. Select analysis output (under the results tab) to view results. A table listing the time spent immobile for each trial should appear.
4. If hand scoring, measure the time the mouse spends swimming or climbing, and amount of time the mouse spends immobile. In addition, measure the latency to the first time immobile. See **Figure 2** in Results Section**.**

Representative Results

Accurate stereotactic injection relies heavily on setting the correct coordinates. The tip of the needle used to inject the virus should be set directly on the intersection of bregma and the interaural line (**Figure 1**). It is helpful to use a stereomicroscope to ascertain whether or not the needle is placed correctly. When looking through the microscope, the needle should be positioned so that if the virus were injected, it would land directly on the intersection of bregma and the interaural line. That is, the opening in the syringe needle should be placed directly above the intersection. In this study, a lentivirus expressing shRNA against RBM8a, a core factor in the exon junction complex, was injected into the dentate gyrus. The lentivirus also expresses RFP to label infected neurons. **Figure 2** shows that the virus was injected into the correct region as indicated by the red signal (**Figure 3**).

To achieve significance in behavioral tasks, the sample size should range from 12-15 mice per group. Behavioral tasks can be conducted two weeks after stereotactic injections. In the OFT, an anxious phenotype is present if mice spend significantly less time in the center of the open

field compared to the control group. The results indicate that knockdown of RBM8a in the dentate gyrus of adult mice leads to anxious behaviors (p <0.05, **Figure 4**). In the FST, there was no observable difference in immobility between control and experimental mice, suggesting that RBM8a knockdown in the dentate gyrus does not affect anti-depressive behavior (**Figure 5**). Statistical analysis consisted of performing a two-tailed, two sample-unequal variance t-test.

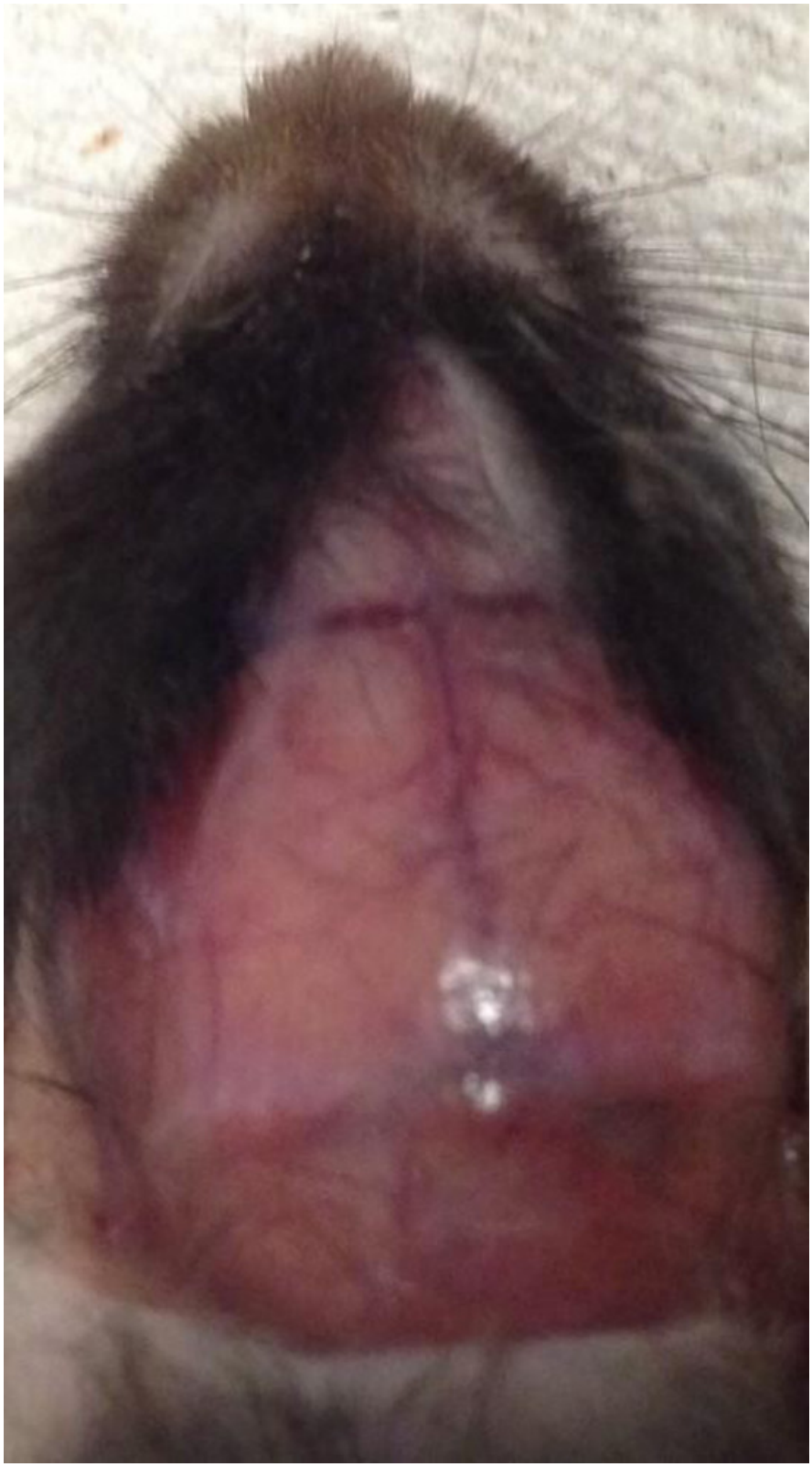

Figure 1: Intersection of Bregma and the Interaural Line. For stereotactic injections, the syringe needle should be lined up with the intersection of bregma and the interaural line. This point is set at zero.

	Transfection	Make Virus	Stereotactic Injections	Open Field Test	Forced Swimming Test
Day	1	3	4	18	25

Figure 2: Schematic Illustrating the Timeline for the Entire Experiment.

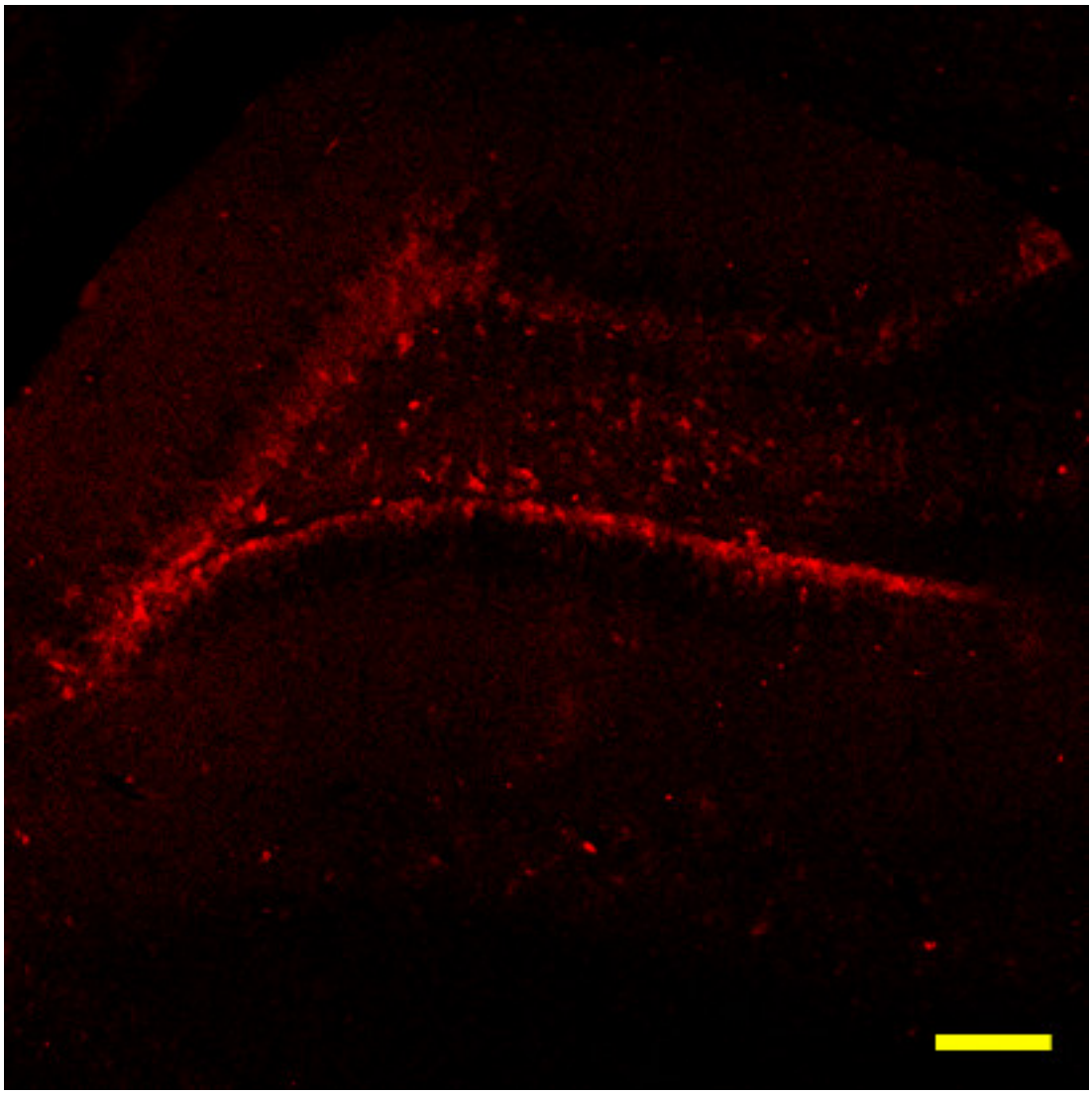

Figure 3: Representative brain slice illustrating successful injection of virus into the dentate gyrus. The scale bar = 100 µm.

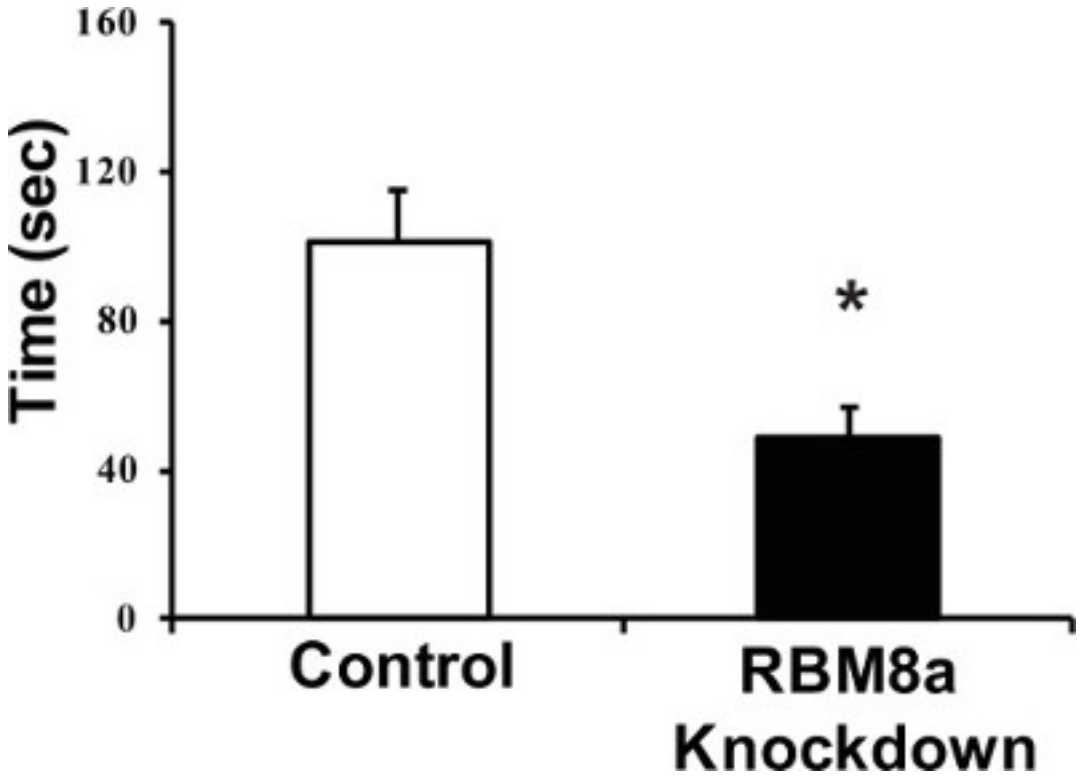

Figure 4: The Amount of Time that Control and Experimental Mice Spent in the Center of a Novel Open Field. Experimental mice spend significantly less time in the center of the arena, indicating an anxious phenotype (N = 9-11, *, T_{18} = -2.72, p <0.05, mean SEM)

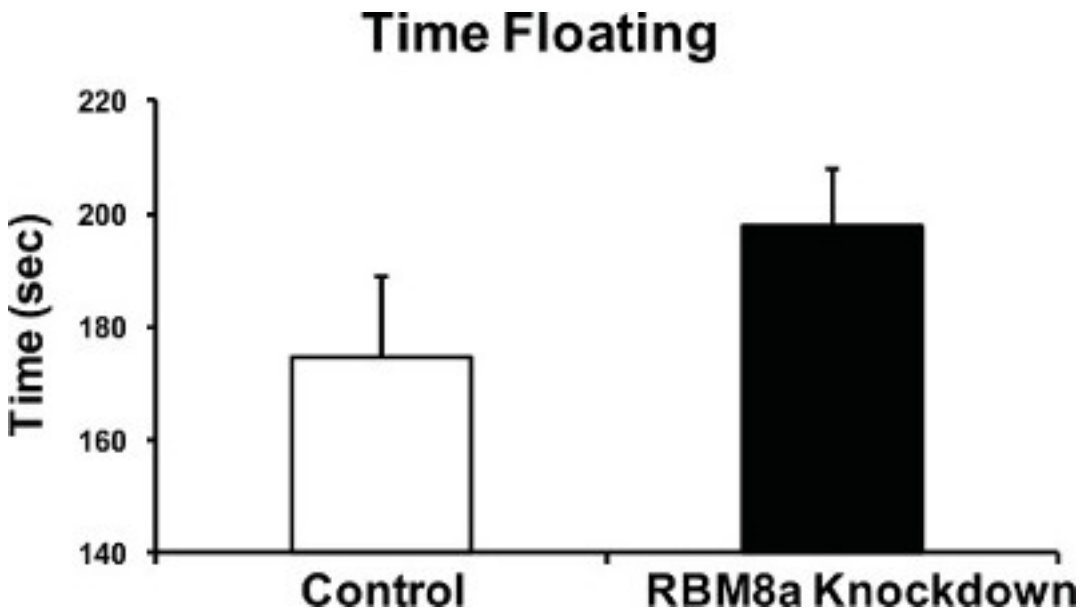

Figure 5: The Amount of Time that Control and Experimental Mice are Immobile in the FST. Experimental mice do not differ from controls in time spent immobile (N = 9-11, T_{18} = 1.25, p >0.05, mean SEM)

Discussion

Successful stereotactic injections rely on three factors: keeping the mouse alive, setting the correct zero point for coordinates (tip of the needle on the intersection of bregma and the interaural line), and setting the right depth to the zero point (tip of the needle just touching the exterior of the brain tissue). The viability of mice is important. Surgery survival can be aided by making sure that the mouse is properly anesthetized and receives adequate analgesic. Pain is known to be a major cause of poor recovery after surgery. Ensuring that the mouse is fully anesthetized throughout the surgery (it should not respond to foot pinches), and giving the correct dose of analgesic (based on the weight of the mouse), should help survival.[17] Additionally, the mouse should be kept on a heating pad throughout the surgery process until it wakes up from the anesthesia. Mice cannot regulate their body temperature when they are anesthetized. Their body temperature will drop significantly during

the surgery process. Even though the length of stereotactic surgery is relatively short, hypothermia could seriously impair mouse survival. Proper suturing technique is also instrumental in conducting a successful surgery. Mice will try to pick at their sutures, so it is crucial to make sure that the sutures are tight enough to prevent removal, but not too tight as to put too much tension on the wound. Four knots in each stitch should ensure that the mouse is unable to remove the suture. Proper suturing is important as an open wound will increase the susceptibility to infections, which would negatively affect any behavioral experiments.

The OFT is a behavioral paradigm designed to assess locomotion and the anxious phenotype in mice. When testing mouse behavior, it is important to be very careful when handling the mice and setting up the arena. In the OFT, the paradigm is designed to pair the mouse's apprehension when placed in a new environment, with its natural curiosity and desire to explore novelty. A wild type mouse will initially be hesitant to enter the center of the open field, where it is more vulnerable, but will eventually do so, due to its innate curiosity. In an anxious mouse, the apprehension of crossing a more vulnerable space (the center of the field) will be greater than their natural curiosity and desire to explore, which will result in significantly less time spent in the center. To make sure the paradigm works correctly, it is important to minimize stress associated with the factors other than the open field. Stress can be minimized by allowing the mouse to acclimate to the behavioral room (so the exterior environment is not a possible confounding variable), and cleaning out the field between mice, to guarantee that all smells associated with the previous mouse have been removed.[18] These steps should help eliminate any differences that are caused by improperly handling mice. In this study, RBM8a knockdown mice spent significantly less time in the center of a novel open field, compared to controls. This is indicative of an anxious phenotype, since the mice are more reluctant to enter the middle of the novel open field, where they are more vulnerable, compared to controls.

The FST seeks to investigate the anti-depressive phenotype in mice. When placed in a bucket of water, mice that spend significantly less time floating versus swimming are considered to have an anti-depressive phenotype. Immobility in the forced swim test is interpreted as behavioral despair (*e.g.* giving up). This paradigm is validated by anti-depressant treatment.[7,8,9] Mice that receive anti-depressant drugs will spend significantly less time floating compared to controls, which is indicative of an anti-depressive phenotype. In this study, RBM8a knockdown mice did not significantly differ from control mice in time spent immobile. This indicates that RBM8a knockdown in the dentate gyrus of adult mice does not elicit a depressive, or anti-depressive phenotype. In our previous study, RBM8a overexpression in the dentate gyrus of adult mice significantly decreases time immobile compared to controls, which indicates an anti-depressive effect.[16]

The stereotactic injections used for representation in this paper were targeted to the dentate gyrus. The dentate gyrus was chosen as the target site, since the gene of interest is highly expressed there in the adult mouse. In addition, the dentate gyrus has been associated with depression and anxiety. For example, one study used *POMC-Chr2* mice to selectively activate the granule cells in either the dorsal or ventral dentate gyrus. Activation of both the dorsal and ventral dentate gyrus led to increased exploration of a novel environment, indicating a potential role of the dentate gyrus in anxiety.[19] A different study found that mice with impaired neurogenesis had increased immobility in the FST, indicating a depressive phenotype.[20] Moreover, activation of Ap oa_1 in the forebrain leads to an anti-depressive effect in the FST, novelty suppressed feeding, and sucrose consumption test. When VEGF was knocked-down in the dentate gyrus of Ap oa_1, this anti-depressive phenotype was lost.[21] These data suggest that the dentate gyrus has a role in the pathophysiology of anxiety and depression, and indicated that the dentate gyrus may be a good area to target for our genetic modulation and behavioral experiments. A lentivirus was used specifically, so that all cells in the dentate gyrus would be infected, as opposed to only dividing cells (retrovirus).

A combination of these three experimental designs (in addition to other behavioral tasks) is a novel way to assess behavioral phenotypes in mice, since a virus can be injected into a specific brain area at specific developmental times. Using stereotactic injections of a lentivirus paired with behavioral tasks only takes a few months to complete and allows researchers to gather preliminary data on the behavioral effects of gene expression in a specific brain area. In contrast, conditional knockout mice often take years to generate. Moreover, cell type preference of viruses can be used to further target cells. For example, retroviruses can only infect dividing cells (such as neural stem cells in the dentate gyrus) whereas a lentivirus infects all cell types.[22,23] Thus, together with co-injection, these widely used and individually validated experimental protocols provide a quick but comprehensive way to test the role of specific gene in a targeted brain area, on mouse behaviors.

Disclosures

The authors have nothing to disclose.

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