Video Article

Biomarkers in an Animal Model for Revealing Neural, Hematologic, and Behavioral Correlates of PTSD

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Abstract

Identification of biomarkers representing the evolution of the pathophysiology of Post Traumatic Stress Disorder (PTSD) is vitally important, not only for objective diagnosis but also for the evaluation of therapeutic efficacy and resilience to trauma. Ongoing research is directed at identifying molecular biomarkers for PTSD, including traumatic stress induced proteins, transcriptomes, genomic variances and genetic modulators, using biologic samples from subjects' blood, saliva, urine, and postmortem brain tissues. However, the correlation of these biomarker molecules in peripheral or postmortem samples to altered brain functions associated with psychiatric symptoms in PTSD remains unresolved. Here, we present an animal model of PTSD in which both peripheral blood and central brain biomarkers, as well as behavioral phenotype, can be collected and measured, thus providing the needed correlation of the central biomarkers of PTSD, which are mechanistic and pathognomonic but cannot be collected from people, with the peripheral biomarkers and behavioral phenotypes, which can.

Our animal model of PTSD employs restraint and tail shocks repeated for three continuous days - the inescapable tail-shock model (ITS) in rats. This ITS model mimics the pathophysiology of PTSD [17, 7, 4, 10]. We and others have verified that the ITS model induces behavioral and neurobiological alterations similar to those found in PTSD subjects [17, 7, 10, 9]. Specifically, these stressed rats exhibit (1) a delayed and exaggerated startle response appearing several days after stressor cessation, which given the compressed time scale of the rat's life compared to a humans, corresponds to the one to three months delay of symptoms in PTSD patients (DSM-IV-TR PTSD Criterian D/E [13]), (2) enhanced plasma corticosterone (CORT) for several days, indicating compromise of the hypothalamopituitary axis (HPA), and (3) retarded body weight gain after stressor cessation, indicating dysfunction of metabolic regulation.

The experimental paradigms employed for this model are: (1) a learned helplessness paradigm in the rat assayed by measurement of acoustic startle response (ASR) and a charting of body mass; (2) microdissection of the rat brain into regions and nuclei; (3) enzyme-linked immunosorbent assay (ELISA) for blood levels of CORT; (4) a gene expression microarray plus related bioinformatics tools [18]. This microarray, dubbed rMNChip, focuses on mitochondrial and mitochondria-related nuclear genes in the rat so as to specifically address the neuronal bioenergetics hypothesized to be involved in PTSD.

Video Link

The video component of this article can be found at https://www.jove.com/video/3361/

Protocol

1. Animal Behavioral Model of PTSD

1. Subjects: Male albino Sprague Dawley rats (Taconic Farms, Derwood, MD) are used, weighting 150 to 200 g at the time of administration of the stress protocol.
2. Measurement of food, water intake and body weight gain: On arrival in the laboratory rats weighing 100 ± 25 g are housed two to a cage (cage size: 45x24x20 cm). Cage padding substrate (woodchips) are changed twice weekly. Animals are maintained on a reversed 12-hr light cycle (lights on: 1800/0600 and off: 0600-1800) in a temperature (22 ± 4 °C)- and relative humidity (30-70%)-controlled environment one week before experiments. Water and standard pelleted chow (Harlan (2018) 18% protein Rodent diet, Global diets, Harlan Teklad) are freely available in the home cages, and bodyweights are recorded daily 3 days before, 3 days during and 3 days after the cessation of stress.
3. Acclimation: Rats are acclimated for three days to both the animal facility and an acoustic startle chamber. To acclimate them to the acoustic startle chamber, animals are handled in it for 5 min each day for three consecutive days prior to the initial measurements.
4. Stressed protocol: The animals are equally assigned to each group based on their body weight and baseline startle response. Testing is done on two groups of animals; each group consisting of 16 animals. Group 1 receives the stress protocol, and Group 2 is the control.

The stress exposure protocol is a continuous 2-hr procedure, where each session consists of restraint ("inescapable") and tail-shocks, repeated once a day over 3 consecutive days. Stressing is done in the morning (within the window of 0800 and 1200). Animals are restrained by being immobilized in a ventilated plexiglass tube. Forty electric shocks (2 mA, 3 sec duration; Animal Test Cage Grid Floor Shocker, Coulbourn Instruments, USA) are delivered to their tails at semi-random intervals of 150 to 210 sec (Graphic State Notation software, Habitest Universal Link, Coulbourn Instruments, USA). Stimulation at 2 mA was chosen because it is aversive, but not painful, when the stimulating output is placed across the experimenter's finger. Electrode gel is applied using Q-tip to form a thin layer of conducting gel between electrode and the skin of the rat's tail. The electrode clips are adjusted and connected to the tail to ensure a good connection without affecting the blood circulation of the tail.

5. Acoustic Startle Response (ASR): Acoustic startle response measurement was conducted with a Startle Response Acoustic Test System (Coulbourn Instruments, Columbus, Ohio, USA) [3]. This system consists of weight-sensitive platforms in a sound-attenuated chamber. The transducer, which is a strain gauge, in each of the startle platforms requires calibration before use. First, the coupler must be in the DC coupled mode for calibration. In this mode, the coupler's output directly follows the input from the platform, the mode required for calibration with static weights. The transducer is switched to the AC coupled mode during experiments so that only rapid change in force, which indicates startle response, is output. The animals' movements in response to sound stimuli are thus measured as a voltage change by a strain gauge inside each platform and recorded as the maximum response occurring within 200 ms of the onset of the startle-eliciting stimulus.

There are six types of stimulus trials: 100 dB alone, 100 dB with pre-pulse, 110 dB alone, 110 dB with pre-pulse, pre-pulse alone and no stimulus control. Each trial type was presented eight times. Trial types are presented in random order to avoid order effects and habituation. Inter-trial intervals range randomly from 15 to 25 sec. Among the eight trials only the maximum values are collected in the results and finally adjusted with the animal body weight of the same day. Animals are tested one day before stress or other treatment as a baseline reading and 1, 7, 14 and 21 days following the final day of the consecutive 3 days of the stress procedure or other treatment.

6. Data analysis:
   1. ASR: The amplitude of acoustic startle response tested each time is represented as "% of baseline", which is calculated using the equation: % of baseline = (absolute amplitude /baseline absolute amplitude) ×100%. For each test day, ANOVAs for repeated measures are performed on startle amplitudes with factors of stress status and drug dosage using SPSS (Version 16) software. Tukey, Bonferroni, or Dunnett tests are used to assess significant post-hoc differences between individual groups. The data are represented as mean ± S.E.M.
   2. Growth: Body weight and food and water consumption are measured on Day -1 preceding stress and on Days 14, 21 and 30 following completion of the stress protocol. Statistical analysis: Food and water intake data are subjected to one-way repeated-measures analysis of variance (ANOVA). To analyze the changes in food consumption rate over time, the amount of food intake is further divided by the body weight of the subject to minimize the variance in body weight induced by the stress protocol and initial difference in the body weight between the groups. All procedures are conducted under the approval in accordance with the Institutional Animal Care and Use Committee (IACUC).

2. Brain Dissection

1. Instruments & Materials:
   1. Anesthesia jar: This is a glass jar with lid, measuring about 6 inches high and 5 inches in diameter, such as an "apothecary jar", "cotton ball jar", or "condiment jar."
   2. Guillotine for rats, such as supplied by Kent Scientific.
   3. Vibratome 1000:
      1. Cut razor blade in half longitudinally, wash oil off with alcohol, insert in clamp.
      2. Fill bath with ordinary crushed ice.
      3. Place the tray longitudinally.
      4. Wipe the tray of moisture before placing a drop of cyanoacrylate.

   4. Rongeurs (Examples are a Micro Friedman rongeur from Miltex, cat. no. 17-4801 or a Pearson rongeur from Fine Science Tools cat. no 16015-17).
   5. #3 or #5 jewelers forceps (sharp to pull off dura).
   6. Small scissors with sharp points (Vantage V95-304 is an example).
   7. (2) Flat stainless steel mini spatulas, such as used to transfer powdered chemicals to a balance scale. The broad end is bent forward so as to fit between telencephalon and calvarium.
   8. Spoon to transfer brain block. (A plastic soup spoon from the cafeteria works fine.)
   9. Scalpel #10.
   10. Double edge razor blade (carbon steel):
      1. One half for Vibratome.
      2. One half for dissection.

   11. Two glass Petri dishes, ~ 3.5 inches and 4 inches in diameter, such that one fits inside the other. Crushed ice in the bottom dish. Two filter papers in the top dish. Move slice to required positions by moving the filter paper.
   12. 100 ml beaker.
   13. Eye dropper to irrigate brain with ice chilled a CSF. (Glass Pasteur pipette with narrow end inserted in 1/2 inch wide latex rubber bulb works well.)
   14. Crushed ice tray: About 5 inches high, 20 inches long, 10 inches wide. Contains:
      1. Stick all the instruments in the crushed ice.
      2. Low calcium / high magnesium aCSF in plastic bottle that can be banged to crush the ice.
      3. Cyanoacrylate.

   15. Low calcium / high magnesium artificial cerebrospinal fluid (aCSF):

1. In mM: 125 NaCl, 2.5 KCl, 0.5 CaCl$_2$*2H$_2$O , 2.0 MgCl$_2$*6H$_2$0, 1.2 NaH$_2$PO$_4$*H$_2$O, 25 NaHCO, 11 Glucose.
2. In plastic bottle that can be banged to crush the ice.
3. Chill for 20 min in -80 °C freezer to make it into a slush.

16. Cotton swabs.
17. Filter paper: Whatman 42.5 mm (Cat no. 1001 042).
18. Mini centrifuge tubes to collect smaller sections of tissue.
19. Wells plate to collect larger structures and sections of tissue.
20. Paper towel.
21. Red biohazard bag.

2. Anesthesia:
    1. Anesthesia jar, gauze pads, isoflurane, metal forceps, are positioned in a fume hood.
    2. Rat is placed in anesthesia jar.
    3. Dampen gauze pad with isoflurane and place in the anesthesia jar.
    4. Wait until pedal reflex - the withdrawal of the paw when the web is pinched with forceps - or corneal reflex disappears.
    5. Decapitate: Grasping rat with one hand around thorax from behind, decapitate rat with guillotine as near to the occiput as possible. The atlas will still usually remain attached to the skull.
    6. Blood Collection: Squeeze rat at thorax to prevent blood from spurting from carotid arteries. Gradually relax grip so as to control the flow of blood into the collection container.

3. Removal of the Brain:
    1. Quickly cut muscle and any remaining vertebrae away from the basiocciput with the small scissor.
    2. Using rongeur and beginning at the foramen magnum, cut away the occipital condyles and the basiocciput.
    3. Midline scalp incision with scalpel.
    4. Using the scissors, cut a mid sagittal incision in the occipital and parietal bones. Grasping the cut margin of the skull with the rongeurs, break it back like an egg shell away from the midline, being careful to minimize any contact with the brain. The midline cut and break back may be done in stages.
    5. As the brain is further exposed, it is critical to douse it with ice chilled aCSF to preserve the integrity and vitality of the tissue.
    6. Similarly break away temporal and frontal bones.
    7. With fine forceps, tear away the dura, making sure to get the tentorum cerebelli.
    8. With mini spatula inserted under the frontal lobe raise the brain from the calvarium just enough to put tension on the cranial nerves.
    9. Transect the cranial nerves with the scissor.
    10. Lift the brain entirely from the skull and let drop into the beaker of iced low calcium / high magnesium aCSF. Leave a minute or more to cool so that the tissue will be solid, the structures clearly visible, and the health of the tissue will be preserved.
    11. With the plastic spoon, transfer the brain onto the filter paper in the ice chilled Petri dish, ventral side up.

4. Dissection of the Brain:
    1. Make a coronal transection with razor blade at the middle cerebral artery (**Figure 2A**).
    2. Save frontal lobe temporarily in ice chilled low calcium / high magnesium aCSF in 100 ml beaker.
    3. Flip brain so that the dorsal surface is up. Transect the brainstem at the juncture of the midbrain and diencaphalon. The resulting brain block is seen in **Figure 2B**.
    4. Drop the blocked cerebrum back into iced low calcium / high magnesium aCSF.
    5. Using mini spatulas, dissect cerebellum from medulla/pons. Save in media appropriate for intended analysis.
    6. Return the blocked cerebrum to the Petri dish. Using two filter papers, lift the brain by placing one filter paper on the ventrum. With it, place the posterior coronal plane of the blocked cerebrum on the edge of the second filter paper. With this filter paper place the anterior cut plane of the blocked cerebrum on a drop of cyanoacrylate on the Vibratome cutting plate, cortex facing the blade.
    7. With the Vibratome, cut off just enough of the caudal brain so that the hippcampus shows (**Figure 2C**).
    8. Take a section, 2,400 μ thick in a 125 g rat, 2,700 μ thick in 200 g rat. Transfer the section using a cotton swab onto the filter papers on the Petri dish (**Figure 2D**).
    9. Cut and save cingulate cortex with the razor blade.
    10. With a dental spatula, push into the distal edge of the corpus callosum to cut it, then peel off the isocortex.
    11. From its ventolateral margin, peel off the hippocampus (**Figure 2E**).
    12. Save the midbrain.
    13. Take a second section, 2,400 μm thick in 120 g rat, 2,500 μm thick in 200 g rat. Place the section on the filter papers on the Petri dish (**Figure 2F**). Alternatively, five 500 μm sections could be taken at this point for electrophysiology.
    14. Cut and save cingulate cortex with the razor blade.
    15. Make lateral cuts with the razor blade at the internal capsule to remove the isocortex (**Figure 2F**).
    16. Make oblique cuts with the razor blade to remove the amygdali (**Figure 2F,G**).
    17. Make a box cut with the razor blade to remove the hypothalamus. The lateral cuts are lateral to the ventromedial nucleus of the hypothalamus, which is visible. We make the dorsal cut just above the third ventricle or ventral tegmental area (**Figure 2H**).
    18. Push spatula into the corpus callosum and pull away the small piece of isocortex attached to the hippocampus. Place the narrow end of the spatula ventral to the hippocampal commissure and raise dorsally to pull off the hippocampus.

5. Preservation of RNA and Protein in Samples of Brain Tissue and Blood:
    1. Media for RNA assay:
        1. Brain: RNAlater RNA stabilization reagent, #76106 in Eppendorf tubes.
        2. Blood: PAXgene Blood RNA Tube (PreAnalytix, Quagen).

    2. Media for protein (CORT) assay from blood: Solution d-HBSS, w/o Ca2+ and Mg 2+, (Quality Biological, Inc).
    3. Storage:

1. Collected brain tissue is first stored in a basket of dry ice for no more than 12 hr and then in a -70 °C freezer for use within 6 months, the duration of the study.
2. Collected blood is kept at room temperature overnight to allow reagents to penetrate, after which it is stored in a -70 °C freezer for use within 6 months, the duration of the study.

3. Gene Microarray of Mitochondrial & Mitochondria-related Nuclear Genes

To study rat mitochondrial functions in brain tissues, we have recently developed the rat mitochondrion-neuron focused microarray (rMNChip) and bioinformatics tools for rapid identification of differential pathways in brain tissues [18]. rMNChip contains 1,500 genes involved in mitochondrial functions, stress response, circadian rhythms and signal transduction. The bioinformatics tool includes an algorithm for computing of differentially expressed genes, and a database for straightforward and intuitive interpretation for microarray results.

1. Purification of total RNA from tissues (Cat# GPM-Kit 2011-1, GenProMarkers):
    1. Rat brain tissues of specific nuclei in RNA*later* RNA stabilization reagent (Qiagen).
    2. Tissue (30 mg in 600 µl GPM-L/B buffer) homogenization using Ultra-Turrax T8 on Dispergierstation (IKA Labortechnik).
    3. Centrifuge in 1.5-ml tubes at Sorvall 21,000 rpm for 15 min.
    4. Decant the supernatant into a spin column with a 2-ml collection tube.
    5. Centrifuge the column-tubes at 15,000 rpm for 3 min, discard the flow-through.
    6. Add 700 µl GPM-B/W buffer to each of the spin columns, centrifuge the column- tubes at 16,000 rpm for 15 sec, discard the flow-through.
    7. Add 500 µl GPM-W buffer to each of the spin columns, centrifuge the column- tubes at 16,000 rpm for 15 sec, repeat once.
    8. Place the spin column in a new 1.5 ml collection tube.
    9. Add 30 µl DEPC-treated water to each of the spin columns, centrifuge at 16,000 rpm for 1 min, repeat once.
    10. Measure RNA concentration, adjust the concentration to 1 µg/µl with DNase- & RNase-free water, the samples are ready for microarray labeling.

2. Microarray labeling and hybridization (Cat#Array 900, Genisphere):
    1. 1.0 µg rat total RNA is used for cDNA synthesis and microarray labeling following the manufacturer's instructions.
    2. Microarray hybridization is conducted on an rMNChip at 65 °C for 12-16 hr as previously described. [18]

3. Washing Hybridized Microarray Slides (Cat#GPM0101-6, GenProMarkers):

Washing Solution	Volume	20 X SSC	10%SDS	ddH$_2$O
0.5 X SSC/0.01 SDS	500 ml	12.5 ml	0.5 ml	up to 500 ml
0.5 X SSC	500 ml	12.5 ml		up to 500 ml
0.1 X SSC	500 ml	2.5 ml		up to 500 ml
0.01 X SSC	500 ml	0.25 ml		up to 500 ml

During the washing the slides should be protected from light. Do not let slides dry out at any stage.
    1. Wash slides in 250-ml Solution 1 in a glass coplin jar (Cat #: 70312-20, Electron Microscopy Sciences, Hatfield, PA) at room temperature by agitating the jar on a BioShaker (Molecular Technologies Inc., St. Louis, MO) until all of the cover slips fall off the glass slides (It takes <3 minutes).
    2. Wash the slides with 250-ml Solution 2 in another jar by agitating the jar for 3 min.
    3. Wash slides with 250-ml Solution 3 in another jar by rotating the jar for 3 min, repeating this step twice.
    4. Wash slides with 250-ml Solution 4 in another jar for 10 sec. Immediately dry the slides by centrifuging the jars placed on the PN11779 plates and ST-H750 rotor in a Sorvall Super T21 centrifuge at 1,000 rpm for 5 min.
    5. Place the washed slides in a box for scanning as soon as possible.

4. Microarray image scan and analysis using ScanArray Express Microarray Scanner (PerkinElmer) following the instruction manual and focus on:
    1. Microarray image scanning: easy scan vs. scanning protocol.
    2. Effects of LASER power, PMT and normalization methods on data outcomes.
    3. Alignment of GAL file onto image.
    4. Alignment of spots to grids precisely.

5. Data analysis: The customized computational procedures for microarray data analysis include microarray image evaluation, data filtering, spot size correction, inclusion, normalization and comparison as described previously [18]. In addition, the methods for ontology, pathway and network analyses are the same as described previously in order to assure the generation of reproducible and verifiable microarray results [2, 22, 25, 19, 20, 8, 23, 18].

4. Blood Sample Collection and Plasma CORT Concentration Measurement

1. Pre- or post-stress tail blood samples from anesthetized animals are collected on Day-1, 14, 21 and 30 into heparinized tubes for determination of circulating CORT levels.

2. Trunk blood samples are also collected into heparinized tubes after animals are euthanized. Plasma is extracted and stored at -70 °C until analysis.
3. Media for plasma extraction:
    1. Blood samples for RNA extraction are collected using PAXgene Blood RNA tubes (PreAnalytix, Quagen)
    2. Blood samples for DNA extraction are collected using PAXgene Blood DNA tubes (PreAnalytix, Quagen).
    3. Blood samples for protein analysis are collected using Lithium Heparin Capillary Collection tube (200 µl) from MaketLab #ML5601.

4. Plasma CORT concentration is tested and analyzed with Active Rat Cort EIA (Diagnostic Systems Labs Inc. http://www.beckmancoulter.com/) as follows:
    1. Mark the microtitration strips to be used.
    2. Prepare Rat CORT Enzyme Conjugate Solution by diluting Rat CORT Enzyme Conjugate Concentrate in the Conjugate Diluent.
    3. Pipette 25 µl Standards, Controls and Unknowns into wells.
    4. Add 100 µl Enzyme Conjugate solution to each well using a semi-automatic dispenser. Gently tap the well holder 5-10 sec.
    5. Add 100 µl Rat CORT Antiserum to each well using a semi-automatic dispenser.
    6. Incubate the wells at room temperature, 25 °C, on a shaker set at 500-700 rpm for 60 min.
    7. Aspirate and wash each well 5 times with Wash Solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
    8. Add 100 µl of the TMB Chromogen Solution to each well using a semi-automatic dispenser.
    9. Incubate at room temperature 15-20 min on an orbital microplate shaker set at 500-700 rpm. Watch the color change.
    10. Add 100 µl Stopping solution to each well using a semi-automatic dispenser.
    11. Shake plate by hand 5-10 sec.
    12. Read the absorbance of the solution in the well within 30 min.
    13. Filter is set at 450 nm. *EIA kits can also be purchased at Immunobiological Laboratories (www.ibl-america.com).
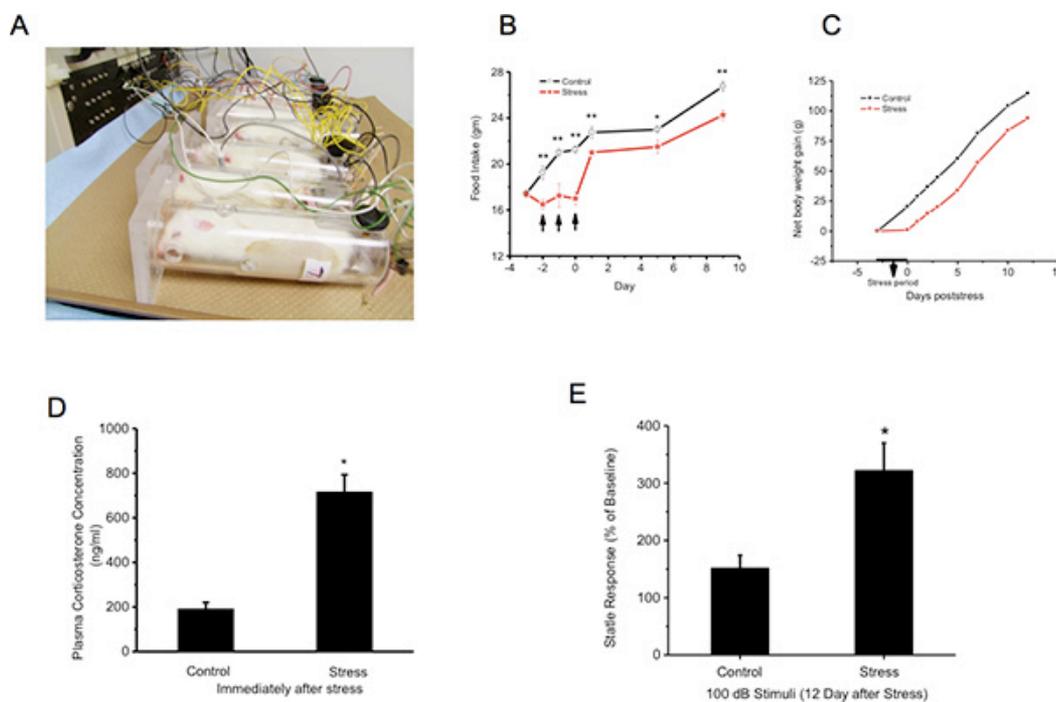
5. Representative Results

Figure 1. Rats are restrained and exposed to tail shock. Subsequent body weight, plasma corticosterone concentration, and acoustic startle response are measured.**A**: Stress Exposure: Animals are restrained by being immobilized in a ventilated plexiglass tube. Forty electric shocks (2 mA, 3 sec duration;) are delivered to their tails at semi-random intervals of 150 to 210 sec. **B** and **C**: Stress retards gain in body weight during growth: Body weight and food and water consumption are measured immediately prior to stress (Day -3), on the day of the three days of stress and then every other day there after up to Day 14. The lack of gain of body weight during stress is never compensated. **D**: Stress increases plasma corticosterone concentration. **E**: Acoustic Startle: Animals are tested one day before stress (day-1) as a baseline reading and 12 days following the final day of the consecutive 3 days of the stress. Data for each group - Stress and Control - are expressed as percent of acoustic startle on day 12 relative to day -1. Stress markedly increases the acoustic startle reflex. Click here to view larger figure.
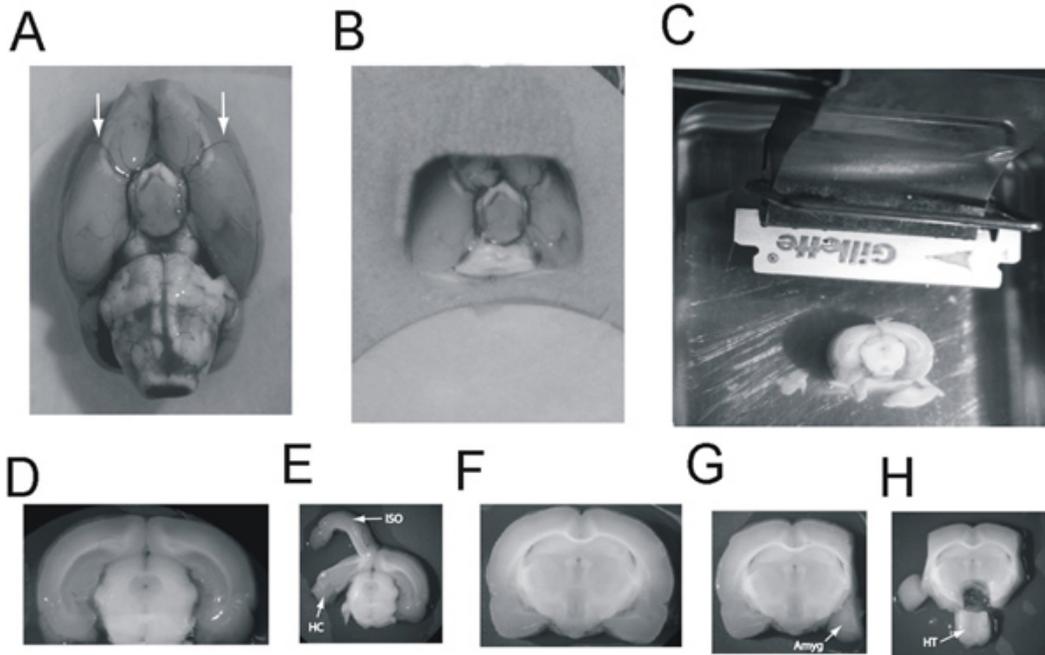
Figure 2. Dissection of the amygdala, hippocampus, and hypothalamus from the brain slice. **A**: Rat brain, ventral view: Arrows point to the middle cerebral arteries. **B**: The brain block, ventral view, ready to be transported to the Vibratome. **C**: The brain block glued to the vibratome tray, caudal side up, cortex facing the blade. The caudal brain has already been cut away exposing the caudal hippocampus. The block is now ready for the 2,500 μm slice containing the major part of the hippocampus to be taken. **D**: The 2,500 μm thick slice containing the caudal hippocampus. **E**: The isocortex (ISO) is peeled from the hippocampus (HC) and the hippocampus peeled from the midbrain. **F**: The 2,500 μm thick slice containing the amygdala and the rostral hippocampus. **G**: The isocortex has been excised and the amygdala (Amyg) resected. **H**: The hypothalamus (HT) is excised and displaced. Click here to view larger figure.
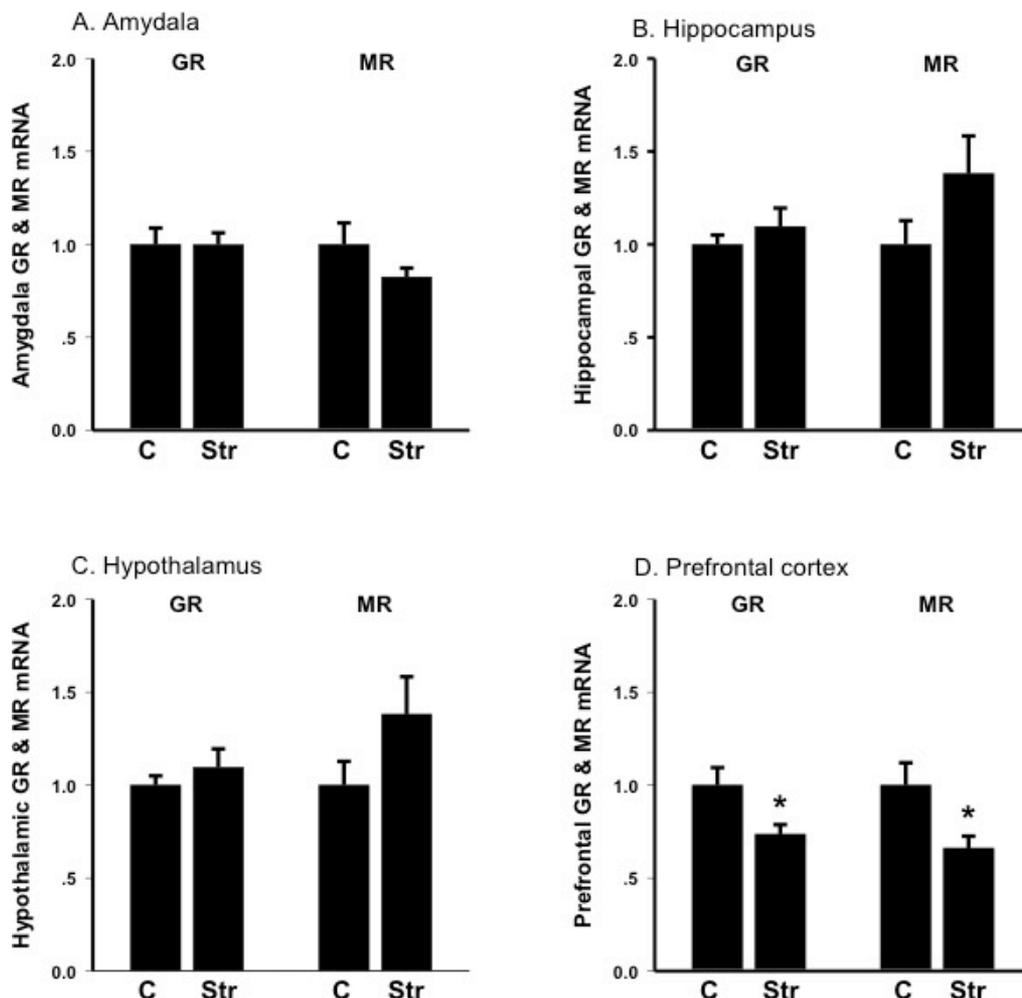
Figure 3. Expression levels for mRNA of the glucocorticoid receptor (GR) and minerocorticoid receptor (MR) in amygdala, hippocampus, hypothalamus, and frontal cortex before (C, control) and after (Str) tail-shock stress. Units are proportion of control; bars are S.E.M. **A**: Amygdala: Stress decreases minerocorticoid receptor mRNA expression. **B**: Hippocampus: Stress increases minerocorticoid receptor mRNA expression. **C**: Hypothalamus: Stress increases minerocorticoid receptor mRNA expression. **D**: Frontal Cortex: Stress decreases glucocorticoid receptor mRNA expression as well as minerocorticoid receptor mRNA expression.

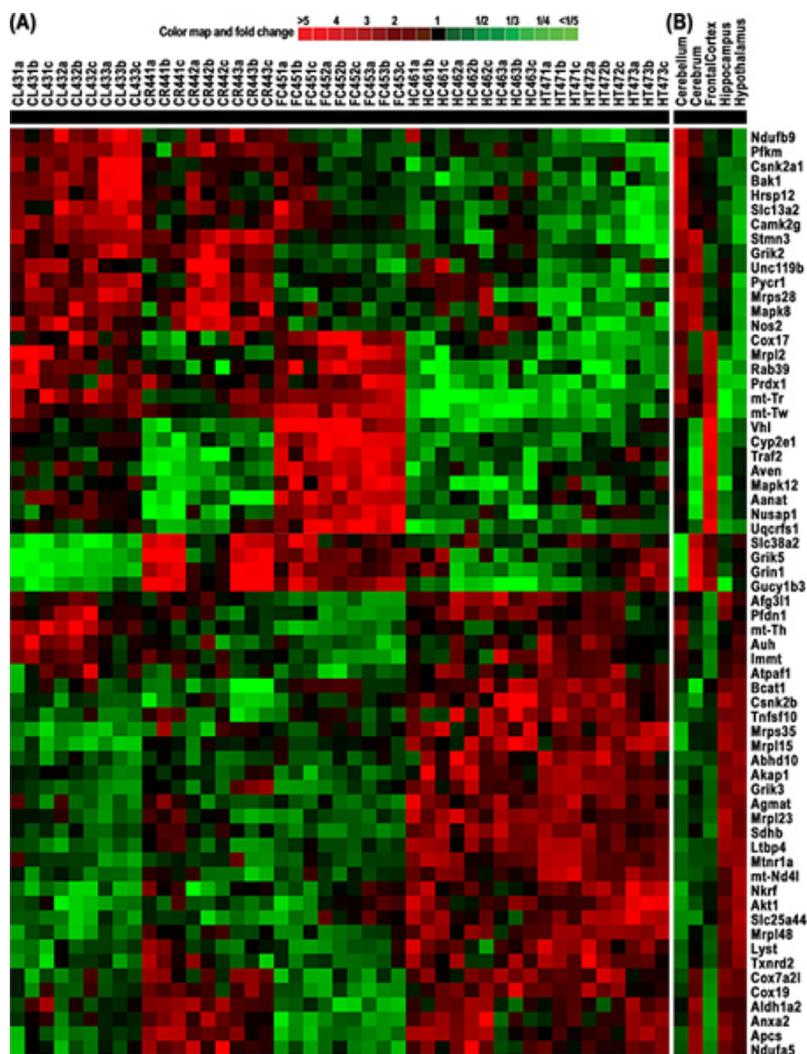| The (121bp) PCR primers for rat GR are:	1f.CCACTGCAGGAGTCTCACAA 1rAACACCTCGGGTTCAATCAC
The (99 bp) PCR primers for rat MR are:	1f.GCCTTCAGCTATGCCACTTC 1rAACGTCGTGAGCACCTTTCT

Figure 4. Cluster and heatmap of RNA differentially expressed from 64 genes derived from 5 rat brain tissues, including cerebellum (CL), cerebrum (CR), frontal cortex (FC), hypothalamus (HT), and hippocampus (HC). Color map indicates fold changes in down- (green) and up- (red) expressed genes. (A) Cluster and heatmap of the normalized signal intensities of 9 measurements for each of 64 genes derived from 15 microarray experiments for these five brain tissues. The expression of each gene was measured by technical triplicates and experimental triplicates. (B) Cluster and heatmap of the mean RNA levels of these 9 measurements of each of the 64 genes. These results show clear differences in mitochondrial gene expression and therefore related functions, which support our hypothesis that different brain regions have different energy demands. Click here to view larger figure.

Our application of the rMNChip and bioinformatic tools led to identification of a cluster and heatmap of 64 genes with differentially expressed RNA derived from 5 rat brain tissues including cerebellum (CL), cerebrum (CR), frontal cortex (FC), hypothalamus (HT), and hippocampus (HC) (**Figure 4**). These data demonstrate the clear differences in mitochondrial gene expression and therefore related functions. The results demonstrate that the different brain regions demand different amount of energy in order to carrying out corresponding brain functions.

Discussion

The diagnosis of PTSD is based on self reported psychiatric symptoms (DSM IV) by potential subjects. No well defined biomarker is currently available to access the pathophysiological status of potential PTSD patients. PTSD is a disorder provoked by life threatening traumatic events and the main psychiatric symptoms remain present in the survivor's life for months and even years after the initial events. The most prominent and persistent symptoms revealed in patients with PTSD are hypervigilance, delayed exaggerated startle response [14, 15, 16] and an apparent compromise of the HPA axis. In humans these symptoms remain, or appear with a delay of three months, after cessation of the traumatic stressor [24]. Our current model for biomarker studies of PTSD employs restraint and tail shocks repeated for three continuous days (2-hr sessions of 40, 2 mA tailshocks) - the inescapable tail-shock model (ITS) in rats weighing 150 gram. This ITS model has been shown to mimic to a substantial extent the pathophysiology of PTSD [17, 7, 4, 10]. Our lab and other labs have verified that the ITS model of stress in rats induces behavioral and neurobiological alterations that are similar to those found in PTSD subjects [17, 7, 10, 9]. Specifically, these stressed rats exhibit (1) a delayed and exaggerated startle response appearing several days after stressor cessation, which given the compressed time scale of the rat's life compared to a humans, corresponds to the one to three months delay of symptoms in PTSD patients(DSM-IV-TR PTSD Criterian D/E [13]), (2) enhanced plasma corticosterone (CORT) for several (10) days, indicating compromise of the hypothalamopituitary axis (HPA), and (3) retarded body weight gain after stressor cessation, corresponding to the dysfunction of metabolic regulation of PTSD. There is no evidence in

the literature that fox odor, predator exposure, or fear potentiated startle response, exhibit these persistent behavioral and neuroendocrinologic phenotypes associated with PTSD.

Rats exposed to a single stress session (1DS) have exhibited transient, but not the persistent abnormalities displayed by 3DS rats [17] The present experiment compared the startle response of 3DS and 1DS rats 4, 7, and 10 days after stressor cessation. Consistent with previous work, stressed rats exhibited elevated basal plasma CORT levels the first day post stress [17]. These CORT levels were sensitive to the number of stressor exposures with higher CORT levels in 3DS rats than in 1DS rats. As for startle response, the 1DS rats exhibit an exaggerated startle response 7 days post stressor, whereas startle sensitization only becomes apparent 10 days post stressor in 3DS rats. Thus, the appearance of an exaggerated startle response after stressor cessation appears to be related to the number of stress session exposures. The 3DS stressed model appears to be useful to gain insight into the altered expression of biomarkers associated with the symptoms of PTSD and the key measurable behavioral phenotypes associated with the timing following the cessation of stress. Genomic results presented provide proof of principle for applying rMNChip and bioinformatics tools to identify differential pathways, and gene and protein biomarkers, which will greatly facilitate systems-biological study and understanding of molecular mechanisms underlying complex and multifactorial neurologic disorders, including PTSD.

While our paradigm does not delve into the cognitive and more complex behavioral aspects of PTSD, we note that altered sleep patterns in the ITS model [1] correspond to the difficulty falling and staying asleep and the nightmares of PTSD patients [11] (DSM-IV-TR PTSD Criteria D [13]), and the deficiencies in escape/avoidance learning and learning of an appetitive task in the ITS model [12] corresponds to the poor concentrations and memory deficits of PTSD [5] (DSM-IV-TR PTSD Criteria C [13]). The current model correlates well with the key symptoms characteristic of PTSD and provides a good model for correlating peripheral biomarkers of PTSD, which can be collected from patients, with central, mechanistic biomarkers, which cannot [6].

Disclosures

No conflicts of interest declared.

Acknowledgements

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