Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles

Supplemental Information

Supplementary Methods

Experimental Subjects

Male 8 week-old C57BL/6J mice, and 6-month-old CD1 retired breeders, were maintained on a 12h light-dark cycle (lights on 7 am) at 22-25°C with food and water *ad libitum*. C57BL/6J mice were housed 5 per cage except following defeat experiments when mice were singly housed. All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Mount Sinai. All behavioral testing occurred during the animals' light cycle. Experimenters were blinded to experimental group and testing order was counterbalanced.

CSDS, Behavioral Testing and Drug Treatment

An established CSDS protocol was used to induce depressive-like behaviors in mice (1,2). C57BL/6J mice were subjected to 10 daily, 5-min defeats by a novel CD1 aggressor, then housed across a plexiglass divider to allow continued sensory contact. Control mice were housed separated from other control mice by a plexiglass divider and rotated to a different cage daily. Social-avoidance behavior was assessed with a novel CD1 mouse in a two-stage social-interaction (SI) test. In the first 2.5-min test (no target), the experimental mouse was allowed to freely explore an arena (44×44cm) containing a plexiglass and wire mesh enclosure (10×6cm) centered against one wall. In the second 2.5-min test (target), the experimental mouse was returned to the arena with a novel CD1 mouse enclosed in the plexiglass wire mesh cage. Time spent in the 'interaction zone' (14×26cm) surrounding the plexiglass wire mesh cage, 'corner

zones' (10×10cm) and 'distance travelled' were measured by video tracking software (Ethovision 3.0, Noldus).

To determine inclusion in antidepressant or saline treatment, resilient and susceptible mice were identified by their respective preference for, or avoidance of, interaction with a novel mouse 24h after day 10 of defeat. Resilient mice were defined as those that spent more time in the interaction zone with target present than absent *and* total time spent interacting with target >60s. Susceptible mice were defined as those that spent less time in the interaction zone with target present than absent *and* with a total time spent interacting with target <60s.

Susceptible mice were split into 3 groups and treated with either saline, ketamine or imipramine. Based on previously established effective treatment regimens, ketamine was administered acutely and imipramine chronically (1,3). Saline-treated susceptible mice and resilient and control mice were i.p. injected once daily with saline for 14 days; imipramine-treated mice were i.p. injected once daily with 20 mg/kg imipramine for 14d; ketamine-treated mice were i.p. injected once daily with saline for 13d, and on day 14 were injected once with 10 mg/kg ketamine.

24h following the final injection, mice were subjected to a second SI test (SI2). Mice were defined as "responders" to imipramine or ketamine treatment if they spent more time interacting with target following antidepressant treatment *and* had an increase of >20s in interaction time from SI1 to SI2. Mice were defined as "non-responders" if they spent less time interacting with target following treatment *or* had an increase of <10s in interaction time from SI1 to SI2. Saline-treated resilient and susceptible animals were included in transcriptome-wide analyses if they continued to meet the SI1 criteria in SI2. All control animals were included in downstream analysis.

RNA Isolation, Library Preparation and RNA-Sequencing

In order to profile resting-state gene expression changes following ketamine or imipramine treatment and limit the contribution of acute effects of behavioral testing on gene expression, mice were killed 2 days following SI2. Previous work has established that antidepressant treatment induces lasting effects on brain function and behavior (3-6). Brains were removed, coronally sliced and NAC, PFC, HIP and AMY tissues were rapidly dissected and frozen on dry ice. Tissue from 2 mice were pooled for each sample for n=3-5 biological replicates for each brain region and phenotype. RNA isolation, qPCR and data analyses were performed as described (7). Briefly, total RNA was isolated with TriZol reagent (Invitrogen) and purified with RNAeasy micro kits from Qiagen. All RNA samples were determined to have 260/280 and 260/230 values ≥1.8.

RNA integrity was assessed using either an Agilent 2100 Bioanalyzer with the RNA 6000 Nano assay or an Agilent 2200 TapeStation with the R6K ScreenTape (Agilent, Santa Clara, CA). Average RIN values were above 8. Libraries were prepared using the TruSeq RNA Sample Prep Kit v2 protocol (Illumina, San Diego, CA). Briefly, the cDNA was synthesized from poly-A-selected and then fragmented total RNA using random hexamers, followed by end-repair and ligation with sequencing adaptors. The libraries were size selected and purified using AMPure XP beads (Beckman Coulter, Brea, CA). Barcode bases (6 bp) were introduced at one end of the adaptors during PCR amplification steps. Library size and concentration were measured by Bioanalyzer or Tape Station (Life Technologies, Grand Island, NY) before sequencing. Libraries were sequenced on the Illumina HiSeq 2500 System utilizing V3 chemistry with 50 base pair paired-end reads at the Mount Sinai Genomics Core Facility.

Statistical and Bioinformatic Data Analysis

Differential expression analyses

Sequencing short reads were aligned to the mouse mm9 reference transcriptome using Tophat2 (8). Read count normalization and gene expression estimation were done by HtSeq. We filtered for protein coding and long non-coding RNA and then summed raw counts across all samples and removed the bottom 25% to remove very low expressed genes and performed pair-wise differential expression comparisons using Voom Limma (9). A nominal significance threshold of fold change > 1.3 and $p < 0.05$ was used.

Enrichment analyses

Overrepresentation of gene ontologies (GOs) was assessed through Fisher's exact test corrected for multiple testing using MSigDB (Broad Institute; http://www.broadinstitute.org/gsea/msigdb/index.jsp).

Enrichment between gene lists was analyzed using the GeneOverlap R package, which compares gene lists to one another using the list of all genes detected in the experiment (i.e., brain-expressed genes) as the background of the multiple pairwise Fisher's exact tests (www.bioconductor.org/packages/release/bioc/html/GeneOverlap.html). Similar results were obtained by use of the Jaccard index (not shown).

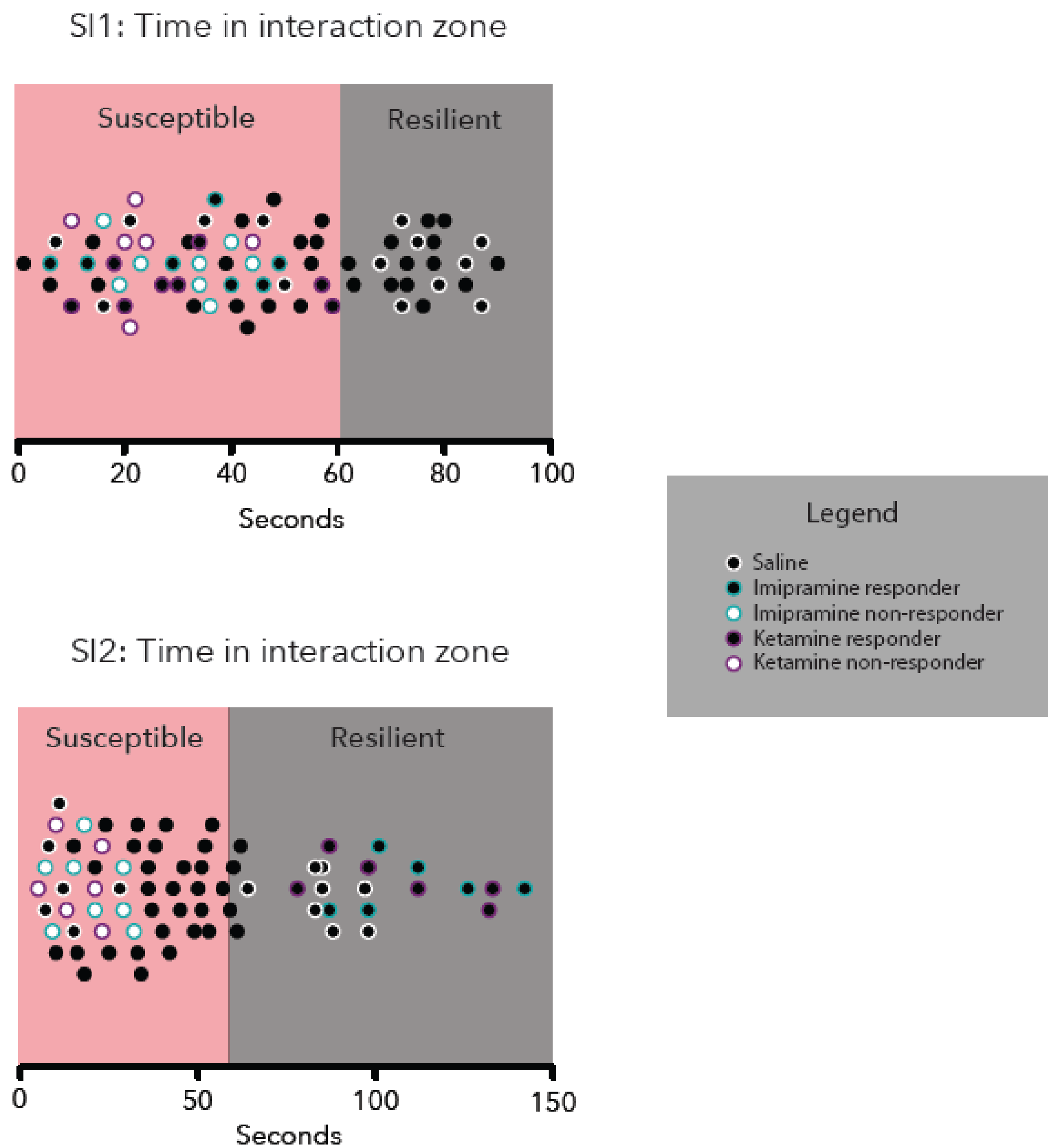

Figure S1. *Social Interaction Cohort Data*. (a) Time spent in interaction zone in Target test (sec) illustrated for experimental cohort demonstrating the split between resilient mice (n=22; gray background >60 sec) and susceptible mice (n=55; red background <60s). A similar split was observed in the first social interaction test (SI1) 24h after CSDS and in the second social interaction test (SI2) on day 27.

Legends for Supplemental Tables (see Excel files)

Table S1. Lists of differentially expressed genes represented in Figure 1G in prefrontal cortex.

Table S2. Lists of differentially expressed genes represented in Figure 1G in nucleus accumbens.

Table S3. Lists of differentially expressed genes represented in Figure 1G in amygdala.

Table S4. Lists of differentially expressed genes represented in Figure 1G in hippocampus.

Table S5. Lists of differentially expressed genes in prefrontal cortex, nucleus accumbens, amygdala and hippocampus that are (a) consistently regulated by ketamine and imipramine treatment response or non-response, as represented in Figure 2 (b) similarly regulated by ketamine or imipramine response as compared to resilience, as represented in Figure 3, and (c) oppositely regulated by ketamine or imipramine response as compared to susceptibility, as represented in Figure 5.

Supplemental References

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