**Methods**

***Behavior***

*Sucrose Preference Test.* Experimenters were blind to the rat’s group assignment. At PND 90 the rats were tested for anhedonia-like behaviors using the sucrose preference test (SPT) as originally proposed by Willner P, Towell A, Sampson D, Sophokleous S, Muscat R [1]. First, animals were given free access to two 500 mL bottles of 1% sucrose solution for 24-hour acclimation. One of the bottles was then replaced with 500 mL of water for another 24-hour acclimation. On day 3, food and water were removed from the cage for 24 hours. Finally, the animals were housed individually and were given 500 mL of both sucrose solution and water, as well as free access to food. After 8 hours, the remaining volume of water and sucrose solution was recorded. Percent sucrose preference was calculated as volume of sucrose solution consumed per total volume of liquid consumed, as described by Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z [2]. In order to study changes relevant to anhedonia, MS animals with reduced sucrose preference behavior were selected for further behavior testing and molecular biology experiments. 10 male and 10 female animals (5 control and 5 MS of each sex) were used for molecular biology and sequencing analysis. A subset of these animals (n=3 per group) was tested for other depression-related behaviors in order to assess the extent by which the MS procedure induced a depressive-like state. Following SPT, the elevated plus maze (EPM) and forced swim test (FST) were administered. All behaviors were compared across groups using 2x2 ANOVA.

***Sequencing***

Total RNA prepared from brain tissue was used to prepare miRNA sequencing libraries for each sample. miRNA sequencing libraries were quality tested with Agilent 2100 Bioanalyzer. The quality score, Q, was greater than 89% for all samples indicating a high probability of base calling accuracy during sequencing. The samples were then denatured with 0.1M NaOH, captured on Illumina flow cells, amplified, and sequenced for 51 cycles on the Illumina NextSeq 500. The raw sequencing data was checked for quality (FastQC), trimmed (cutadapt), and aligned (miRDeep2) to the reference genome. counts per million (CPM) was calculated from expression reads using the R package, edgeR.

***miRNA Sequencing Analysis***

Using *R* software (v 3.6.0), we applied a 2x2 ANOVA to test for the effect of MS and sex on miRNA expression (i.e. CPM) in each brain region. We further considered miRNAs which exhibited both high significance (p<0.05) and fold change values (fc > 1.3). Then, in order to determine the covariation of miRNA expression across the three brain regions, we conducted miRNA-miRNA correlations between the significantly altered miRNAs. We used the *cluster* (https://CRAN.R-project.org/package=cluster) package in R to apply divisive hierarchical clustering to miRNA-miRNA correlation values using the DIANA algorithm [3]. By this algorithm, at each clustering division, the value with the highest dissimilarity from other values is used to create a new cluster. All values more similar to the new cluster than the original, were moved to the new cluster and the process continued iteratively until each value belonged to a unique node. There is evidence that this algorithm produces more biologically relevant clusters from gene expression data when compared to other unsupervised clustering methods [4]. We used the silhouette method (*factoextra* R package; https://CRAN.R-project.org/package=factoextra) to determine the optimal number of clusters for each analysis. We additionally created a clustering with *k* set to 3 in order to determine whether 3 clusters would parse the miRNA coexpression by brain region or by some other property such as function. *Dendextend* in R (https://cran.r-project.org/package=dendextend) was used to calculate entanglement scores in order to compare hierarchical clustering patterns between groups. Entanglement represents the quality of the alignment between two clustering trees. Scores can vary from 0 to 1 with lower scores representing better overall alignment. While entanglement is independent of cluster number, visualization using tanglegram requires that each hierarchy contain the same number of clusters. Thus, *k* was arbitrarily set to the 3 clusters.

***In Silico Gene Target Prediction and Gene Ontology***

MiRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) software was used to assess validated gene targets of all significantly altered miRNAs and to *predict* gene targets of significantly altered and strongly correlated miRNAs across brain regions. Of the significantly altered miRNAs in the main effect of MS and the interaction, we selected miRNAs which were highly correlated with at least 4 miRNAs in different brain regions or were correlated with miRNAs with further correlations to other miRNAs creating an interconnected network. miRNAs which were only correlated with less than 3 miRNAs in a single brain region were not included. Genes which were predicted in 6 or more prediction software (miRWalk, MircoT4, miRanda, miRBridge, miRDB, miRMap, miRNAMap, PICTAR2, PITA, RNA22, RNAhybrid or Targetscan) on miRWalk, were included in the Ingenuity Path Analysis (IPA) path analysis. We used predicted gene targets for subsequent analysis due to the limited number of validated targets available (13 in MS and 31 for the interaction of MS and Sex). IPA was used to further filter relevant genes associated with psychological disorders and to create miRNA-gene target interaction maps.

Predicted gene targets were used for GO analysis. We determined highly represented ontology terms using Metascape [5]. Metascape hierarchically clusters terms into parent groups and represents them as a clustered GO map. ShinyGO (v.0.61) was used to assess the top 30 cellular component GO terms (FDR correction at 0.05) and bubble plots were created in R based on the ShinyGO output[6].

**References**

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