Identifying and calibrating the effects of cellular dissociation for transcriptomics in neuroscience

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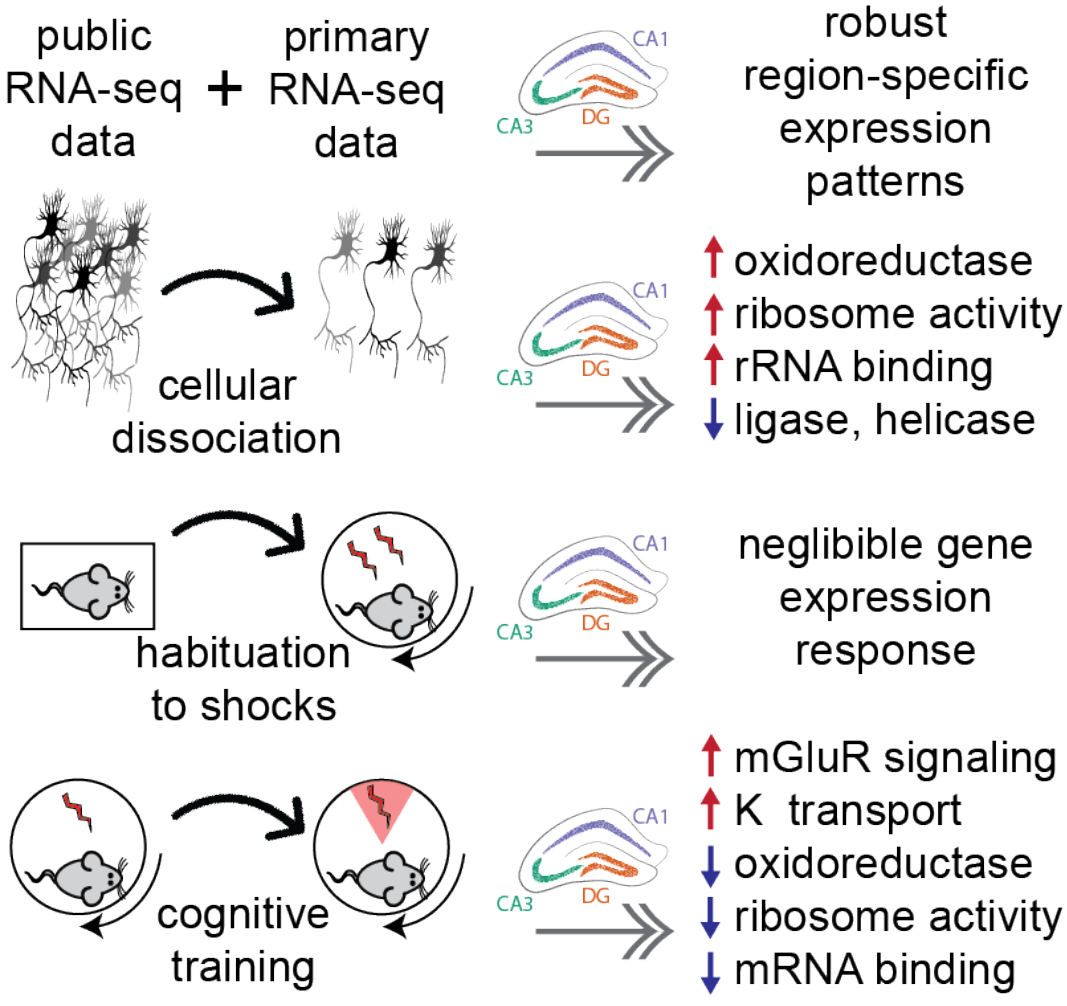
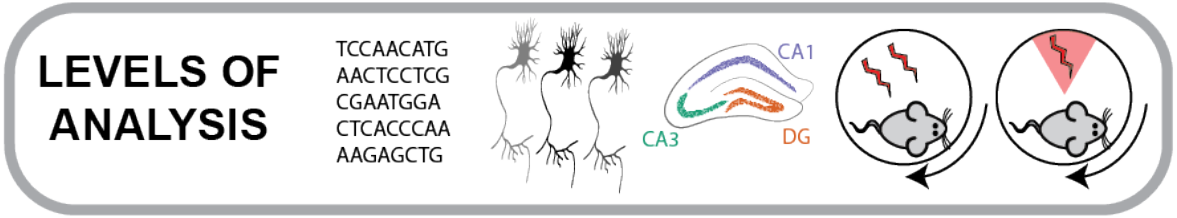
Graphical Abstract



Materials & Methods



HPC FOR RNA-seq

TACC
FASTQC
Cutadapt
Kallisto

REPRODUCIBLE RESEARCH in R

reshape2
dplry
plyr
knitr

STATISTICAL ANALYSES

DEseq2
GO_MWU

DATA VIZUALIZATION

VennDiagram
ggplot2
cowplot
pheatmap
RColorBrewer
Adobe Illustrator

RESEARCH SHARING

GitHub
Zenodo
GEO
FigShare
BioRxiv
Peer Review
Medium
YouTube
SlideShare
Cirricula

Figure 1

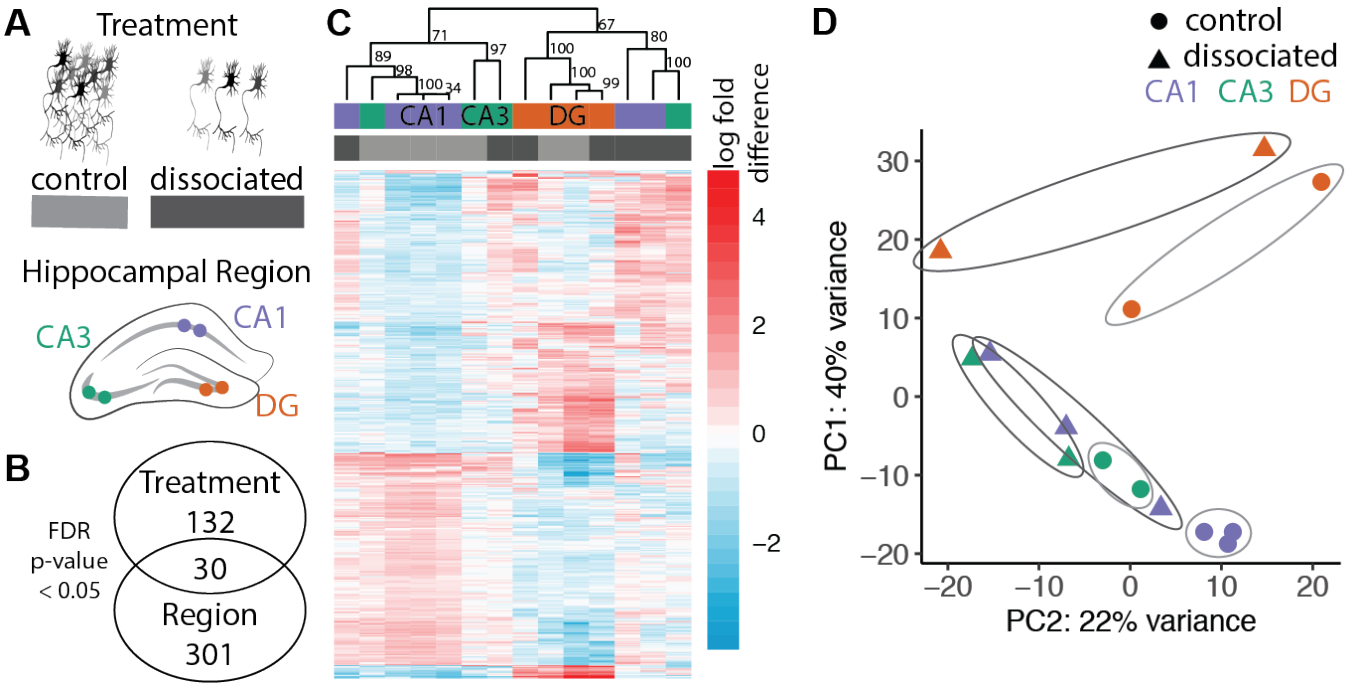

A
Treatment
control
dissociated
Hippocampal Region
CA3
CA1
DG
B
FDR
p-value
< 0.05
Treatment
132
30
Region
301
C
CA1
CA3
DG
log fold
difference
4
2
0
−2
D
control
dissociated
CA1 CA3 DG
PC1: 40% variance
PC2: 22% variance
30
20
10
0
−10
−20
−20
−10
0
10
20

Figure 2

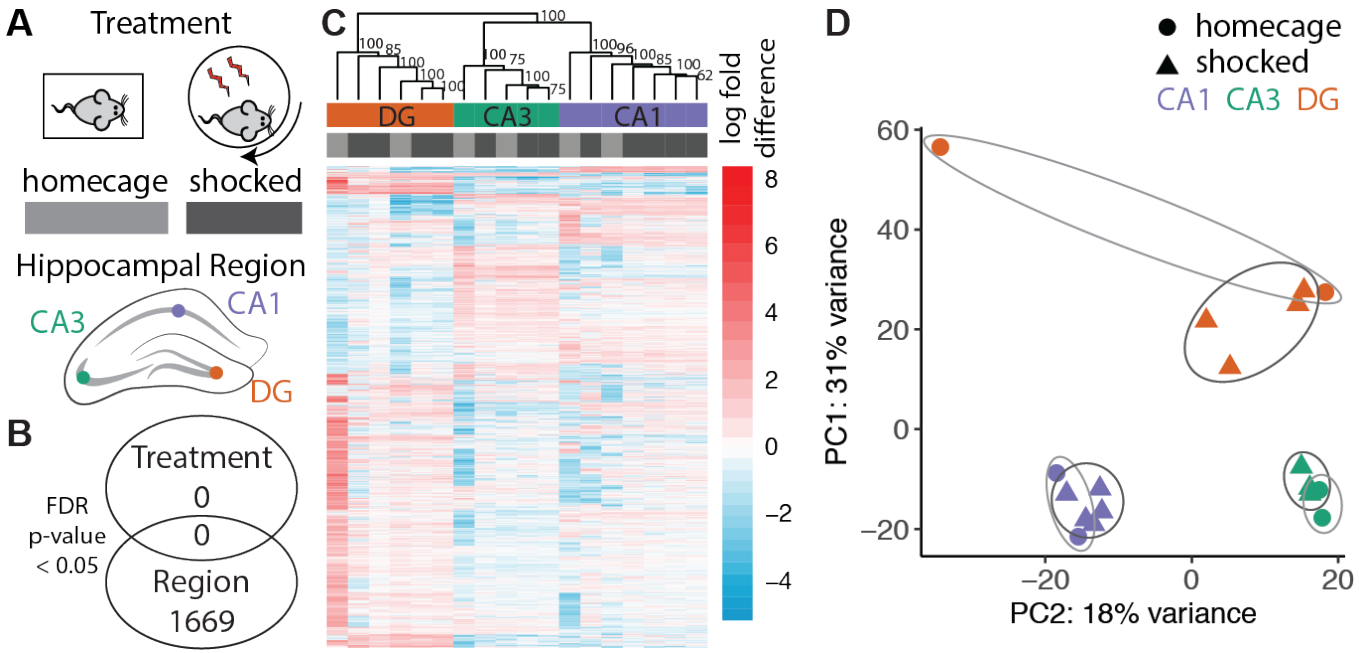

A
Treatment
homecage
shocked
Hippocampal Region
CA3
CA1
DG
B
Treatment
0
FDR
p-value
< 0.05
0
Region
1669
C
DG
CA3
CA1
log fold
difference
8
6
4
2
0
−2
−4
D
homecage
shocked
CA1 CA3 DG
PC1: 31% variance
PC2: 18% variance
60
40
20
0
−20
−20
0
20

Figure 3

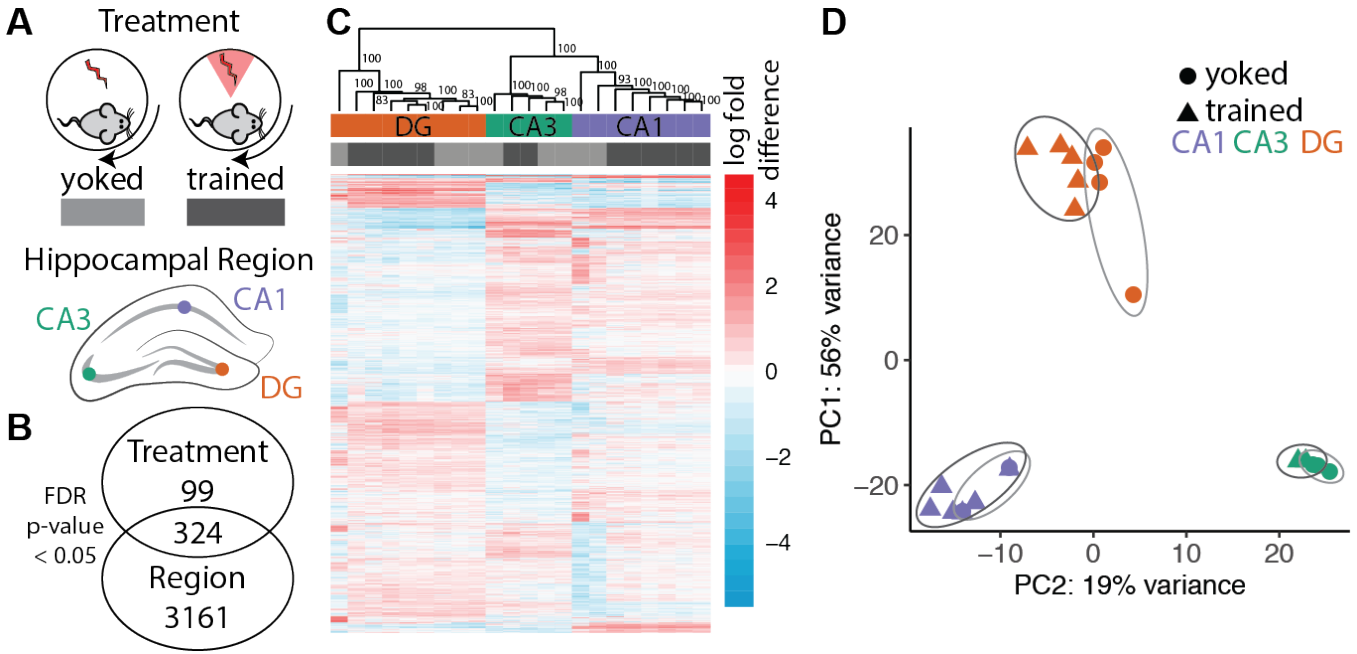
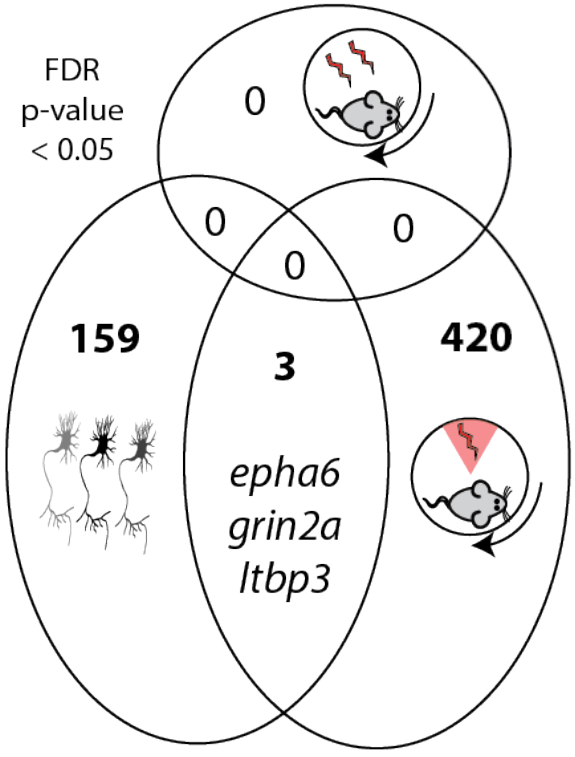

A
Treatment
yoked
trained
Hippocampal Region
CA3
CA1
DG
B
Treatment
99
FDR
p-value
< 0.05
324
Region
3161
C
DG
CA3
CA1
log fold
difference
4
2
0
−2
−4
D
yoked
trained
CA1 CA3 DG
PC1: 56% variance
PC2: 19% variance
20
0
−20
−10
0
10
20

Figure 4

A. Treatment-incuced gene expression changes



B. Dissociation-induced molecular functions

UP

74/325 structural molecule
42/88 structural constituent of ribosome
15/55 rRNA binding
8/128 helicase
19/245 ligase, forming carbon–nitrogen bonds
32/433 ligase
12/62 oxidoreductase, acting on NAD(P)H
50/596 oxidoreductase
10/36 oxidoreductase, acting on NAD(P)H, quinone or similar
11/66 hydrogen ion transmembrane transporter

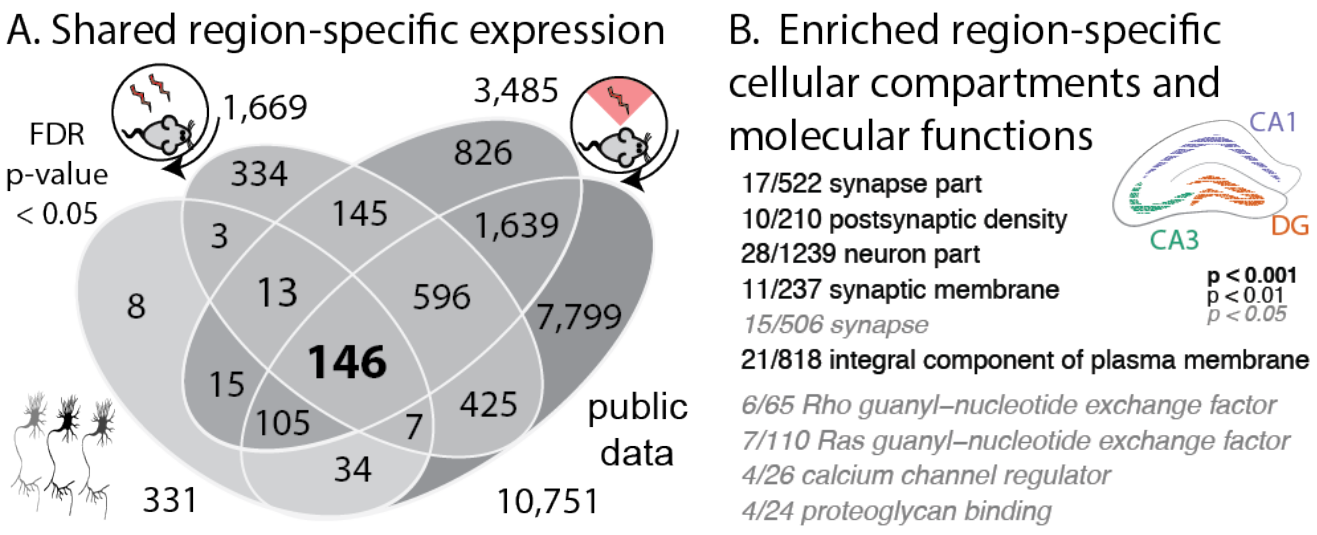
Down

p < 0.00001
p < 0.0001
p < 0.001

C. Cognitive training -induced molecular functions

180/801 poly(A) RNA binding
20/87 structural constituent of ribosome
10/36 oxidoreductase, acting on NAD(P)H, quinone or similar
11/25 glutamate receptor
128/801 signal transducer
105/678 receptor
13/66 hydrogen ion transmembrane transporter
143/735 transmembrane transporter
80/357 calcium ion binding

Figure 5


A. Shared region-specific expression
FDR
p-value
< 0.05
1,669
3,485
334
826
145
1,639
3
13
596
7,799
8
146
15
105
7
425
34
public
data
331
10,751
B. Enriched region-specific cellular compartments and molecular functions
CA1
CA3
DG
17/522 synapse part
10/210 postsynaptic density
28/1239 neuron part
11/237 synaptic membrane
15/506 synapse
p < 0.001
p < 0.01
p < 0.05
21/818 integral component of plasma membrane
6/65 Rho guanyl–nucleotide exchange factor
7/110 Ras guanyl–nucleotide exchange factor
4/26 calcium channel regulator
4/24 proteoglycan binding

Graphical Abstract