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(54) **OPTOGENETIC CONTROL OF INPUTS TO THE VENTRAL TEGMENTAL AREA**

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(52) **U.S. Cl.**

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(57) **ABSTRACT**

The present disclosure provides a method of inducing or modulating reward- or aversive-related behaviors in animals using light-responsive opsins. The present disclosure provides methods of identifying or screening compounds that may be used to treating mental disorders, or are relevant to disrupt or improve reward- or aversive related behaviors.

FIGURE 1

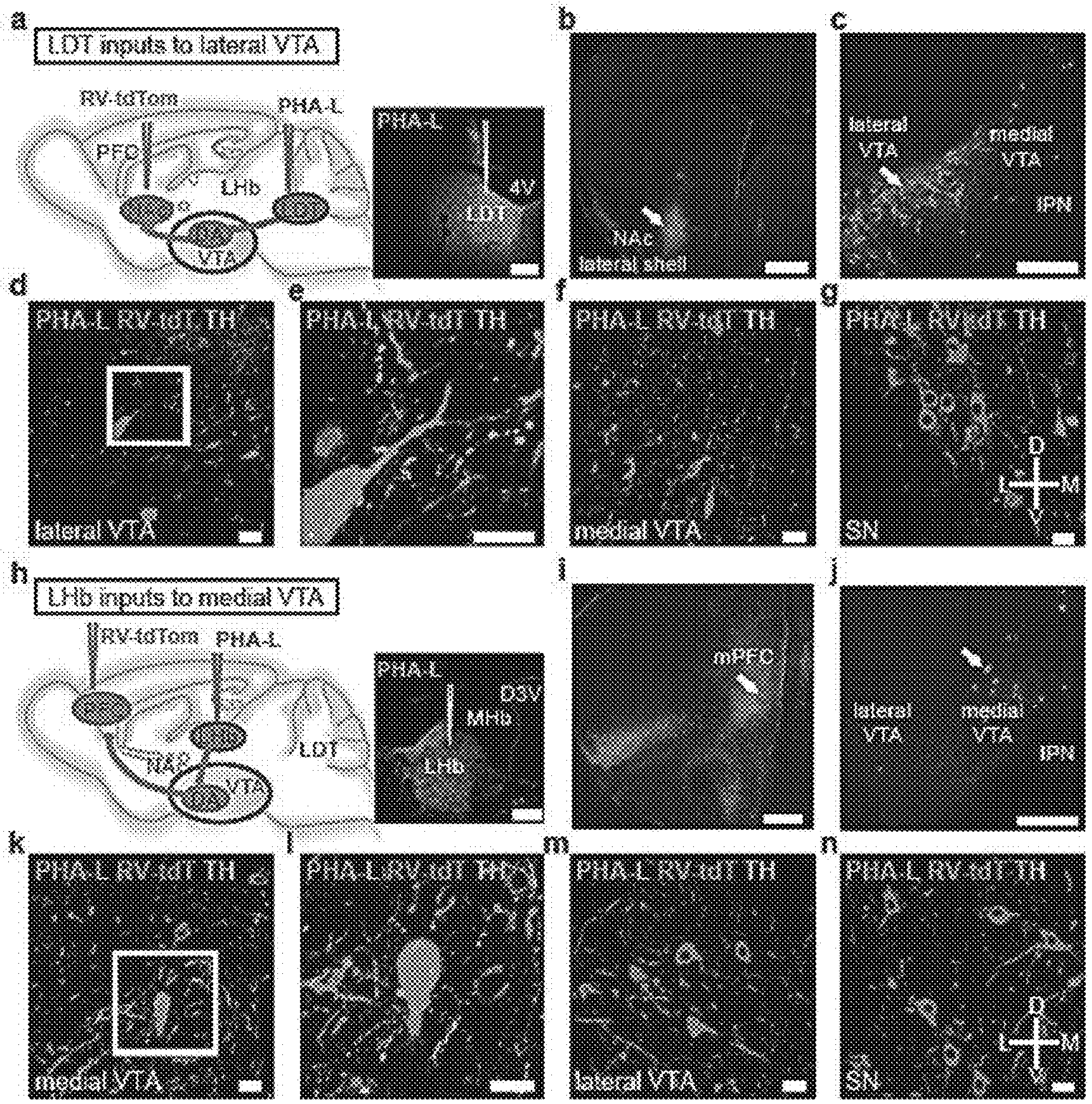

a
LDT inputs to lateral VTA
RV-tdTom
PHA-L
PFC
LHb
VTA
PHA-L
4V
LDT
b
NAc
lateral shell
c
lateral VTA
medial VTA
IPN
d
PHA-L RV-tdT TH
lateral VTA
e
PHA-L RV-tdT TH
f
PHA-L RV-tdT TH
medial VTA
g
PHA-L RV-tdT TH
D
L
M
V
SN
h
LHb inputs to medial VTA
RV-tdTom
PHA-L
NAc
VTA
LDT
PHA-L
D3V
MHb
LHb
i
mPFC
j
lateral VTA
medial VTA
IPN
k
PHA-L RV-tdT TH
medial VTA
l
PHA-L RV-tdT TH
m
PHA-L RV-tdT TH
lateral VTA
n
PHA-L RV-tdT TH
D
L
M
V
SN

FIGURE 2

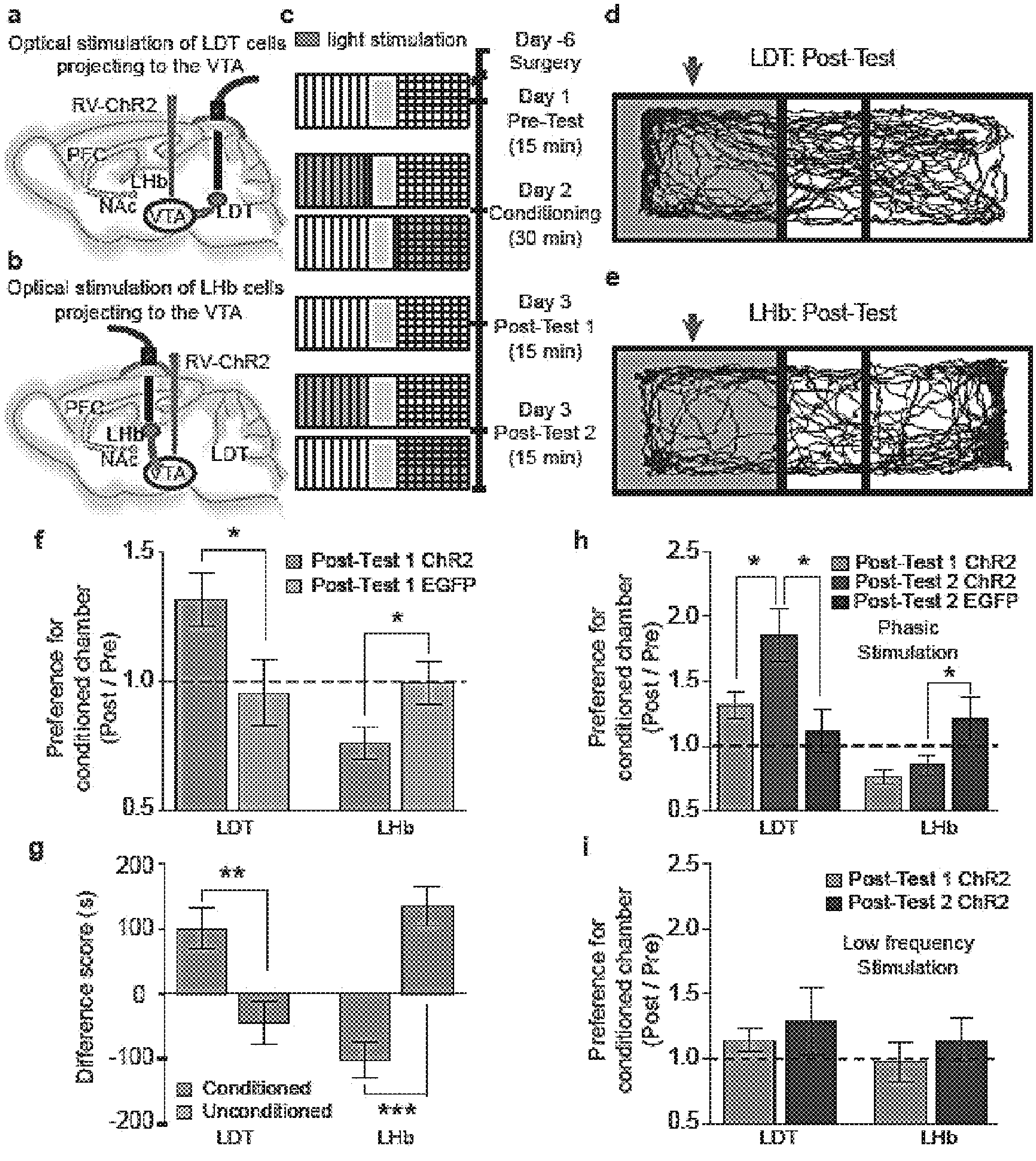

a
Optical stimulation of LDT cells projecting to the VTA
RV-ChR2
PFC
LHb
NAc
VTA
LDT
b
Optical stimulation of LHb cells projecting to the VTA
RV-ChR2
PFC
LHb
NAc
VTA
LDT
c
▨ light stimulation
Day -6 Surgery
Day 1 Pre-Test (15 min)
Day 2 Conditioning (30 min)
Day 3 Post-Test 1 (15 min)
Day 3 Post-Test 2 (15 min)
d
LDT: Post-Test
e
LHb: Post-Test
f
Preference for conditioned chamber (Post / Pre)
1.5
1.0
0.5
▨ Post-Test 1 ChR2
▨ Post-Test 1 EGFP
*
*
LDT
LHb
g
Difference score (s)
200
100
0
-100
-200
**
***
▨ Conditioned
▨ Unconditioned
LDT
LHb
h
Preference for conditioned chamber (Post / Pre)
2.5
2.0
1.5
1.0
0.5
▨ Post-Test 1 ChR2
▨ Post-Test 2 ChR2
■ Post-Test 2 EGFP
Phasic Stimulation
*
*
*
LDT
LHb
i
Preference for conditioned chamber (Post / Pre)
2.5
2.0
1.5
1.0
0.5
▨ Post-Test 1 ChR2
■ Post-Test 2 ChR2
Low frequency Stimulation
LDT
LHb

FIGURE 3

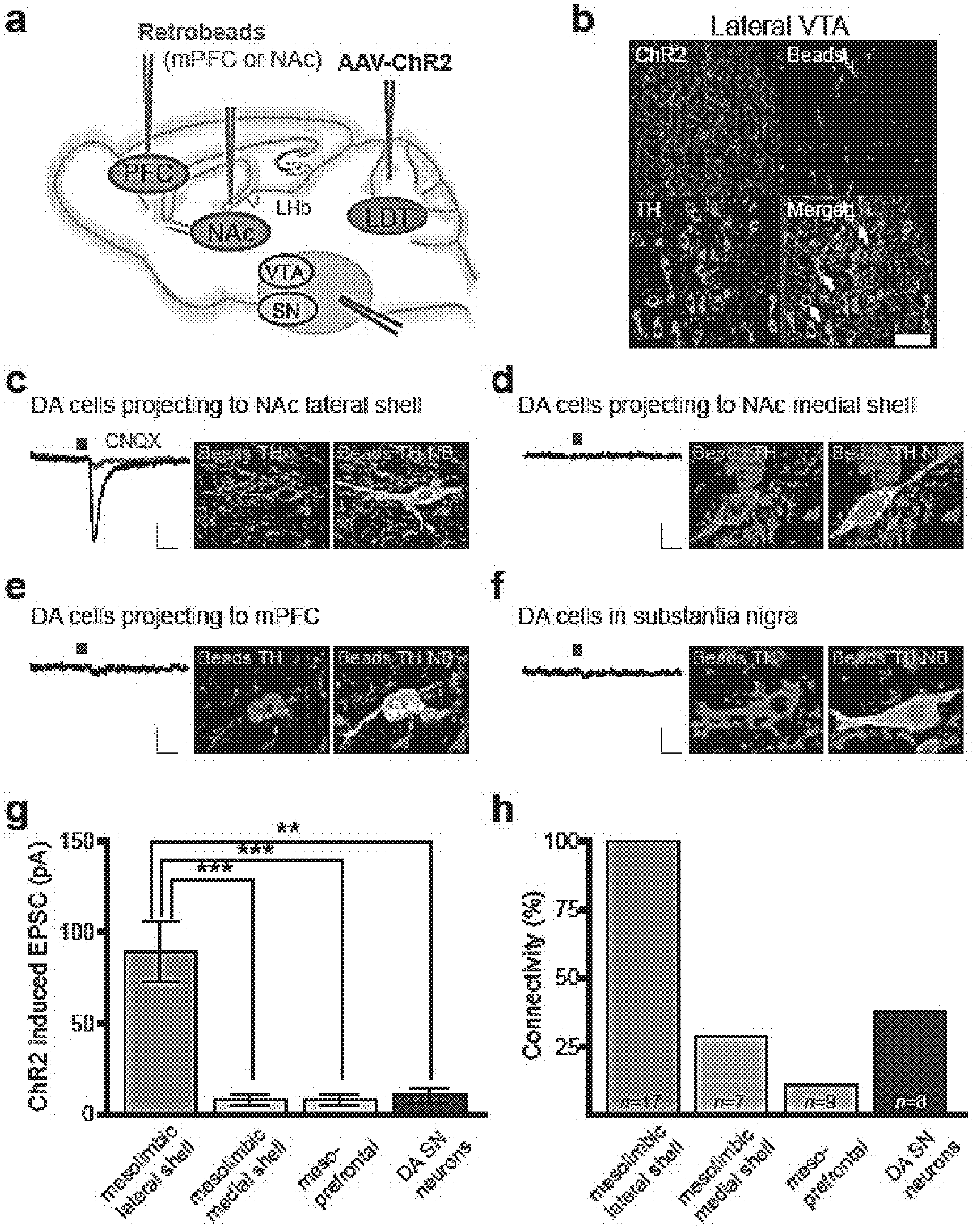

a
Retrobeads
(mPFC or NAc)
AAV-ChR2
PFC
NAc
LHb
LDT
VTA
SN
b
Lateral VTA
ChR2
Beads
TH
Merged
c
DA cells projecting to NAc lateral shell
CNQX
Beads TH
Beads TH NB
d
DA cells projecting to NAc medial shell
Beads TH
Beads TH NB
e
DA cells projecting to mPFC
Beads TH
Beads TH NB
f
DA cells in substantia nigra
Beads TH
Beads TH NB
g
ChR2 induced EPSC (pA)
150
100
50
0
**
***
***
mesolimbic lateral shell
mesolimbic medial shell
meso-prefrontal
DA SN neurons
h
Connectivity (%)
100
75
50
25
n=17
n=7
n=9
n=8
mesolimbic lateral shell
mesolimbic medial shell
meso-prefrontal
DA SN neurons

FIGURE 4

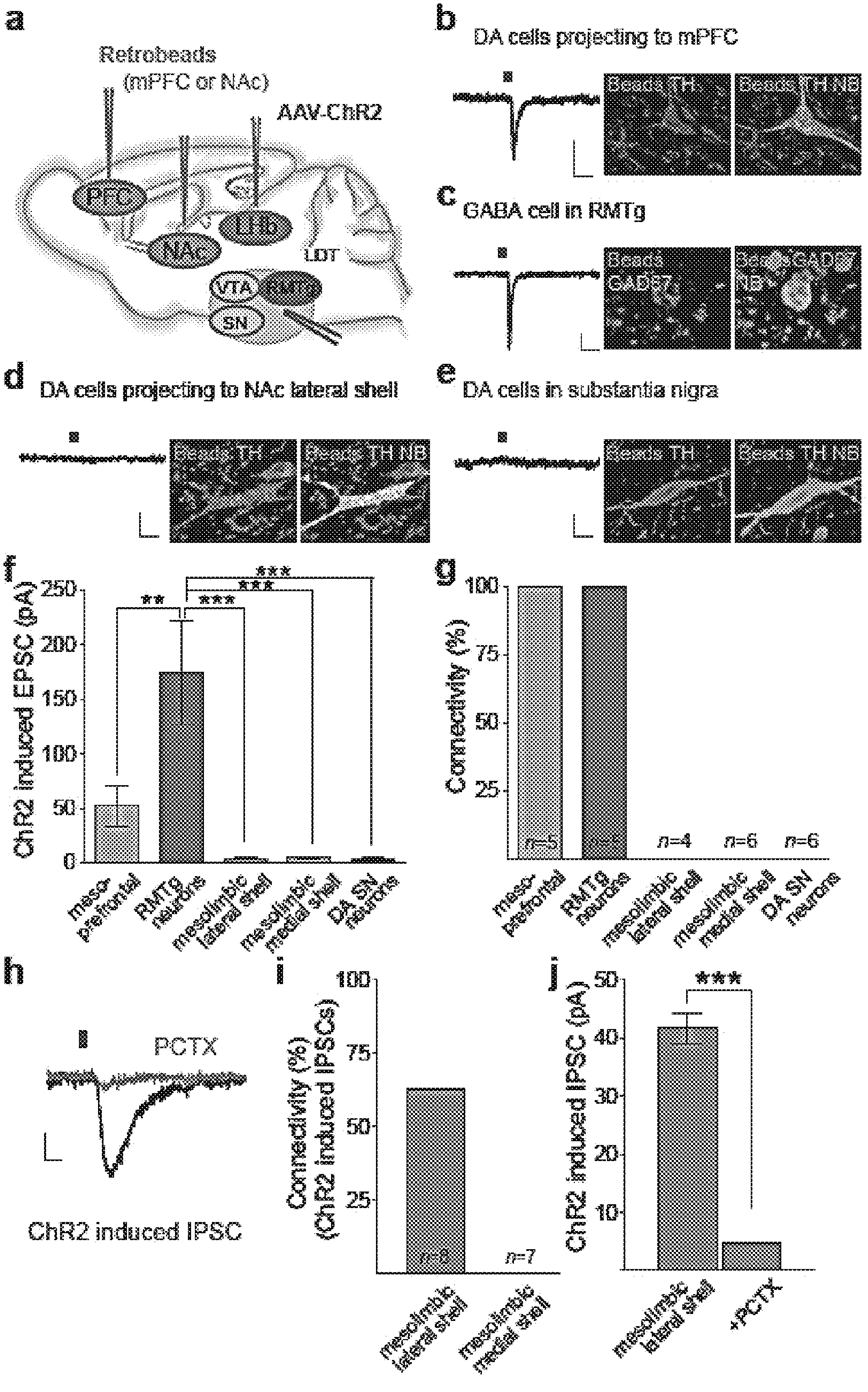

a
Retrobeads
(mPFC or NAc)
AAV-ChR2
PFC
NAc
LHb
LDT
VTA
RMTg
SN
b
DA cells projecting to mPFC
Beads TH
Beads TH NB
c
GABA cell in RMTg
Beads GAD67
Beads GAD67 NB
d
DA cells projecting to NAc lateral shell
Beads TH
Beads TH NB
e
DA cells in substantia nigra
Beads TH
Beads TH NB
f
ChR2 induced EPSC (pA)
0
50
100
150
200
250
**
***
***
***
meso-prefrontal
RMTg neurons
mesolimbic lateral shell
mesolimbic medial shell
DA SN neurons
g
Connectivity (%)
25
50
75
100
n=5
n=4
n=4
n=6
n=6
meso-prefrontal
RMTg neurons
mesolimbic lateral shell
mesolimbic medial shell
DA SN neurons
h
PCTX
ChR2 induced IPSC
i
Connectivity (%) (ChR2 induced IPSCs)
25
50
75
100
n=8
n=7
mesolimbic lateral shell
mesolimbic medial shell
j
ChR2 induced IPSC (pA)
10
20
30
40
50
***
mesolimbic lateral shell
+PCTX

FIGURE 5

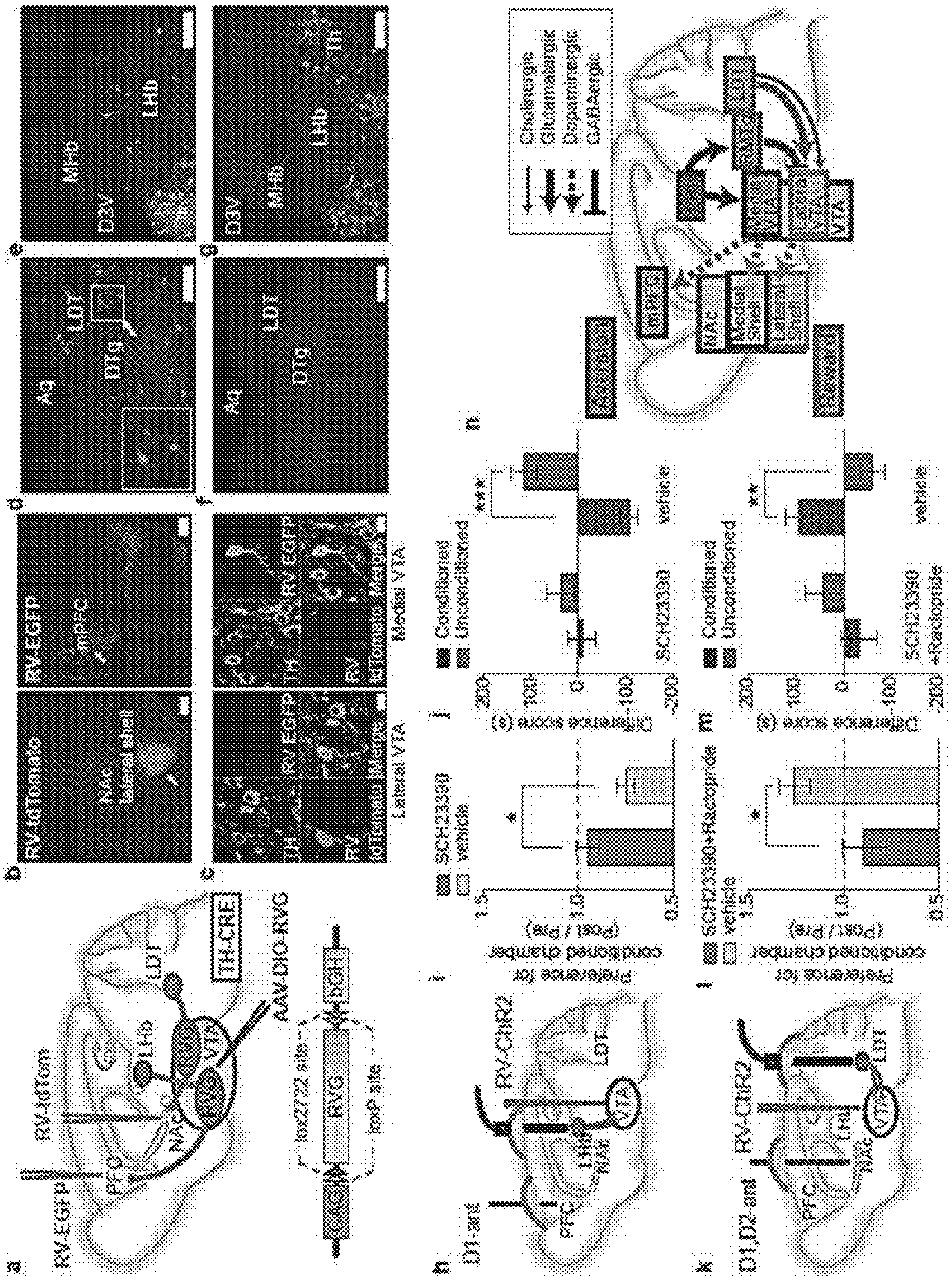

a
RV-EGFP
RV-tdTom
PFC
NAc
LHb
LDT
VTA
TH-CRE
AAV-DIO-RVG
lox2722 site
loxP site
RVG
bGH
b
RV-tdTomato
NAc
lateral shell
RV-EGFP
mPFC
c
TH
RV tdTomato
RV EGFP
Merge
Lateral VTA
Medial VTA
d
Aq
LDT
DTg
e
D3V
MHb
LHb
f
Aq
LDT
DTg
g
D3V
MHb
LHb
h
D1-ant
RV-ChR2
PFC
LHb
NAc
LDT
VTA
i
Preference for conditioned chamber (Post / Pre)
1.5
1.0
0.5
SCH23390
vehicle
*
j
Difference score (s)
200
100
0
-100
-200
Conditioned
Unconditioned
SCH23390
vehicle
***
k
D1,D2-ant
RV-ChR2
PFC
LHb
NAc
LDT
VTA
l
Preference for conditioned chamber (Post / Pre)
1.5
1.0
0.5
SCH23390+Raclopride
vehicle
*
m
Difference score (s)
200
100
0
-100
-200
Conditioned
Unconditioned
SCH23390
+Raclopride
vehicle
**
n
Cholinergic
Glutamatergic
Dopaminergic
GABAergic
Aversion
Reward
mPFC
NAc
Medial Shell
Lateral Shell
Medial VTA
Lateral VTA
VTA
LHb
RMTg
LDT

FIGURE 6A

INHIBITORY

The amino acid sequence of NpHR without the signal peptide:

VTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDDPRAKLIAVSTILVPVVSIAS
YTGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMWGRYLTWALSTPMILLALG
LLAGSNATKLFTAITFDIAMCVTGLAAALTTSSHLMRWFWYAISCACFLVVLYILLVEW
AQDAKAAGTADMFNTLKLLTVVMWLGYPIVWALGVEGIAVLPVGVTSWGYSFLDIVA
KYIFAFLLLNYLTSNESVVSGSILDVPSASGTPADD (SEQ ID NO:1).

The amino acid sequence of eYFP-NpHR3.0:

MTETLPPVTESAVALQAEVTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDD
PRAKLIAVSTILVPVVSIASYTGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMW
GRYLTWALSTPMILLALGLLAGSNATKLFTAITFDIAMCVTGLAAALTTSSHLMRWFWY
AISCACFLVVLYILLVEWAQDAKAAGTADMFNTLKLLTVVMWLGYPIVWALGVEGIAV
LPVGVTSWGYSFLDIVAKYIFAFLLLNYLTSNESVVSGSILDVPSASGTPADDAAKSRIT
SEGEYIPLDQIDINVVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKF
ICTTGKLPVPWPTLVTTFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN
YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVN
FKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEF
VTAAGITLGMDELYKFCYENEV (SEQ ID NO:2).

FIGURE 6B

INHIBITORY

The amino acid sequence of eYFP-NpHR3.1:

MVTQRELFEFVLNDPLLASSLYINIALAGLSILLFVFMTRGLDDPRAKLIAVSTILVPVVSI
ASYTGLASGLTISVLEMPAGHFAEGSSVMLGGEEVDGVVTMWGRYLTWALSTPMILLA
LGLLAGSNATKLFTAITFDIAMCVTGLAAALTTSSHLMRWFWYAISCACFLVVLYILLVE
WAQDAKAAGTADMFNTLKLLTVVMWLGYPIVWALGVEGIAVLPVGVTSWGYSFLDIV
AKYIFAFLLLNYLTSNESVVSGSILDVPSASGTPADDAAAKSRITSEGEYIPLDQIDINVVS
KGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
HYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKF
CYENEV (SEQ ID NO:3).

The amino acid sequence of GtR3:

ASSFGKALLEFVFIVFACITLLLGINAAKSKAASRVLFPATFVTGIASIAYFSMASGGGWVI
APDCRQLFVARYLDWLITTPLLLIDLGLVAGVSRWDIMALCLSDVLMIATGAFGSLTVG
NVKWVWWFFGMCWFLHIIFALGKSWAEAAKAKGGDSASVYSKIAGITVITWFCYPVV
WVFAEGFGNFSVTFEVLIYGVLDVISKAVFGLILMSGAATGYESI (SEQ ID NO:4).

FIGURE 6C

INHIBITORY

The amino acid sequence of eArch:

MDPIALQAGYDLLGDGRPETLWLGIGTLLMLIGTFYFLVRGWGVTDKDAREYYAVTIL
VPGIASAAYLSMFFGIGLTEVTVGGEMLDIYYARYADWLFTTPLLLLDLALLAKVDRV
TIGTLVGVDALMIVTGLIGALSHTAIARYSWWLFSTICMIVVLYFLATSLRSAAKERGPE
VASTFNTLTALVLVLWTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGFILLR
SRAILGDTEAPEPSAGADVSAAD (SEQ ID NO:12).

The amino acid sequence of eArch3.0-EYFP:
MDPIALQAGYDLLGDGRPETLWLGIGTLLMLIGTFYFLVRGWGVTDKDAREYYAVTIL
VPGIASAAYLSMFFGIGLTEVTVGGEMLDIYYARYADWLFTTPLLLLDLALLAKVDRV
TIGTLVGVDALMIVTGLIGALSHTAIARYSWWLFSTICMIVVLYFLATSLRSAAKERGPE
VASTFNTLTALVLVLWTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGFILLR
SRAILGDTEAPEPSAGADVSAADRPVVAVSKAAAKSRITSEGEYIPLDQIDINVVSKGEE
LFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTFG
YGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNR
IELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
HYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELY
KFCYENEV (SEQ ID NO:13).

FIGURE 6D

INHIBITORY

ArchT 3.0

MDPIALQAGYDLLGDGRPETLWLGIGTLLMLIGTFYFIVKGWGVTDKEAREYYSITILVP
GIASAAYLSMFFGIGLTEVTVAGEVLDIYYARYADWLFTTPLLLLDLALLAKVDRVSIGT
LVGVDALMIVTGLIGALSHTPLARYSWWLFSTICMIVVLYFLATSLRAAAKERGPEVAST
FNTLTALVLVLWTAYPILWIIGTEGAGVVGLGIETLLFMVLDVTAKVGFGFILLRSRAIL
GDTEAPEP (SEQ ID NO:14)

Mac 3.0

MIVDQFEEVLMKTSQLFPLPTATQSAQPTHVAPVPTVLPDTPIYETVGDSGSKTLWVVFV
LMLIASAAFTALSWKIPVNRRLYHVITTIITLTAALSYFAMATGHGVALNKIVIRTQHDH
VPDTYETVYRQVYYARYIDWAITTPLLLLDLGLLAGMSGAHIFMAIVADLIMVLTGLFAA
FGSEGTPQKWGWYTIACIAYIFVVWHLVLNGGANARVKGEKLRSFFVAIGAYTLILWTAY
PIVWGLADGARKIGVDGEIIAYAVLDVLAKGVFGAWLLVTHANLRESDVELNGFWANGLN
REGAIRIGEDDGARPVVAVSK (SEQ ID NO:15)

FIGURE 6E

EXCITATORY

The amino acid sequence of ChR2:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQW
LAAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQ
WLRYAEWLLTCPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIF
FCLGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEG
FGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVET
LVEDEAEAGAVP (SEQ ID NO:5).

The amino acid sequence of SFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQW
LAAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQ
WLRYAEWLLTSPVILIHLSNLTGLSNDYSRRTMGLLVSDIGTIVWGATSAMATGYVKVIF
FCLGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEG
FGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVET
LVEDEAEAGAVP (SEQ ID NO:6).

FIGURE 6F

EXCITATORY

The amino acid sequence of SSFO:

MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQTASNVLQWLAAGFSILLLMFYAYQTWKSTCGWEEIYVCAIEMVKVILEFFFEFKNPSMLYLATGHRVQWLRYAEWLLTSPVILIHLSNLTGLSNDYSRRTMGLLVSAIGTIVWGATSAMATGYVKVIFFCLGLCYGANTFFHAAKAYIEGYHTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGHTIIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLVEDEAEAGAVP (SEQ ID NO:7).

The amino acid sequence of C1V1:

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTLENNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKSTCGWEEIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVLLIHLSNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIEAFHTVPKGICRELVRVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKNMWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ ID NO:8).

FIGURE 6G

EXCITATORY

The amino acid sequence of C1V1 (E122T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSYTL
ENNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTWKST
CGWETIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVLLIHLSNLT
GLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHAAKVYIE
AFHTVPKGICRELVRVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSILDLIAKN
MWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ ID NO:9).

The amino acid sequence of C1V1 (E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSY
TLENNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTW
KSTCGWEEIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHL
SNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHA
AKVYIEAFHTVPKGICRELVRVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSI
LDLIAKNMWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ
ID NO:10).

FIGURE 6H

EXCITATORY

The amino acid sequence of C1V1 (E122T/E162T):

MSRRPWLLALALAVALAAGSAGASTGSDATVPVATQDGPDYVFHRAHERMLFQTSY
TLENNGSVICIPNNGQCFCLAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTW
KSTCGWETIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYATWLLTCPVLLIHL
SNLTGLKDDYSKRTMGLLVSDVGCIVWGATSAMCTGWTKILFFLISLSYGMYTYFHA
AKVYIEAFHTVPKGICRELVRVMAWTFFVAWGMFPVLFLLGTEGFGHISPYGSAIGHSI
LDLIAKNMWGVLGNYLRVKIHEHILLYGDIRKKQKITIAGQEMEVETLVAEEED (SEQ
ID NO:11).

FIGURE 7

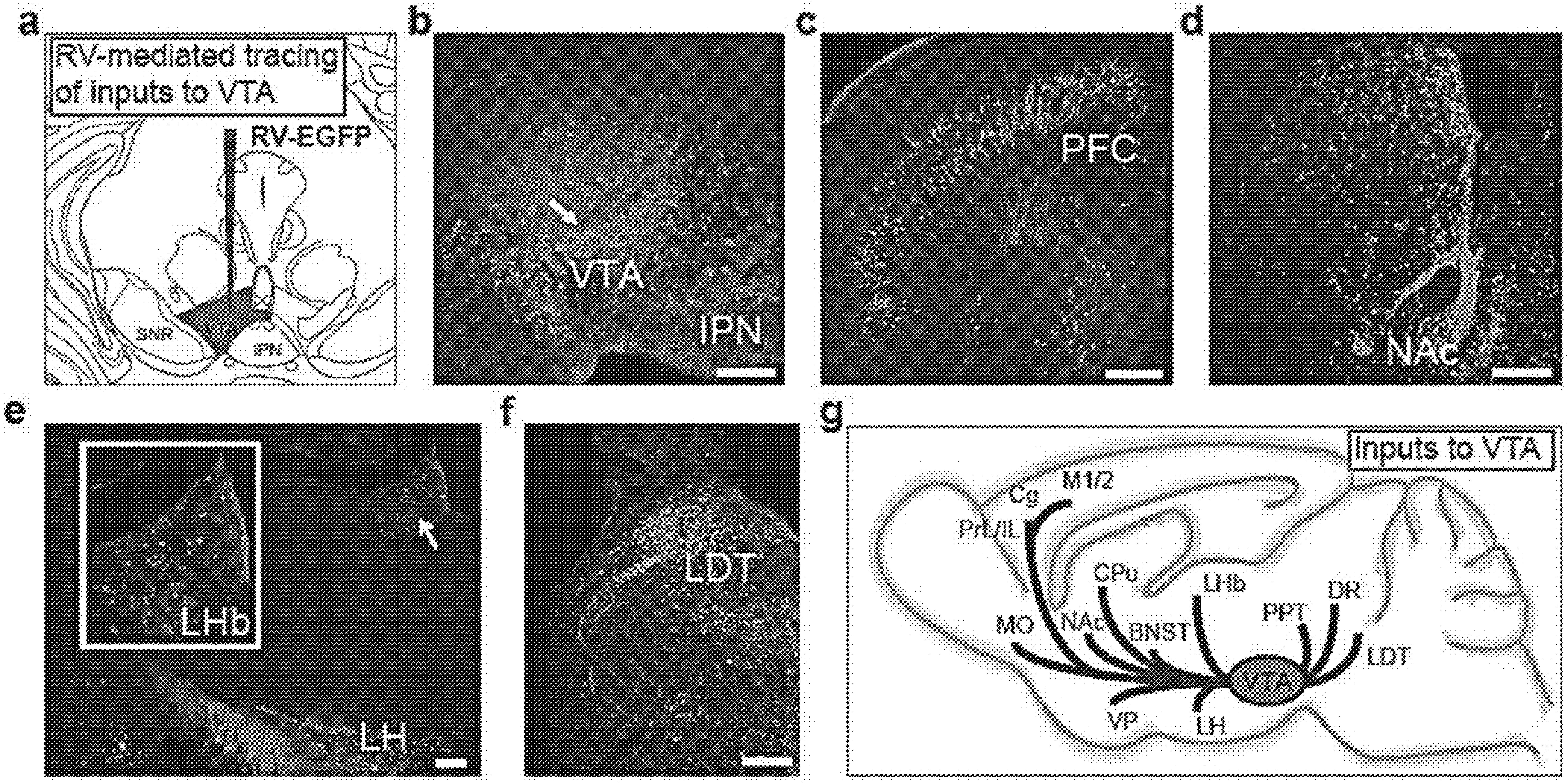

a
RV-mediated tracing of inputs to VTA
RV-EGFP
SNR
IPN
b
VTA
IPN
c
PFC
d
NAc
e
LHb
LH
f
LDT
g
Inputs to VTA
Cg
M1/2
PrL/IL
CPu
LHb
PPT
DR
MO
NAc
BNST
LDT
VTA
VP
LH

FIGURE 8

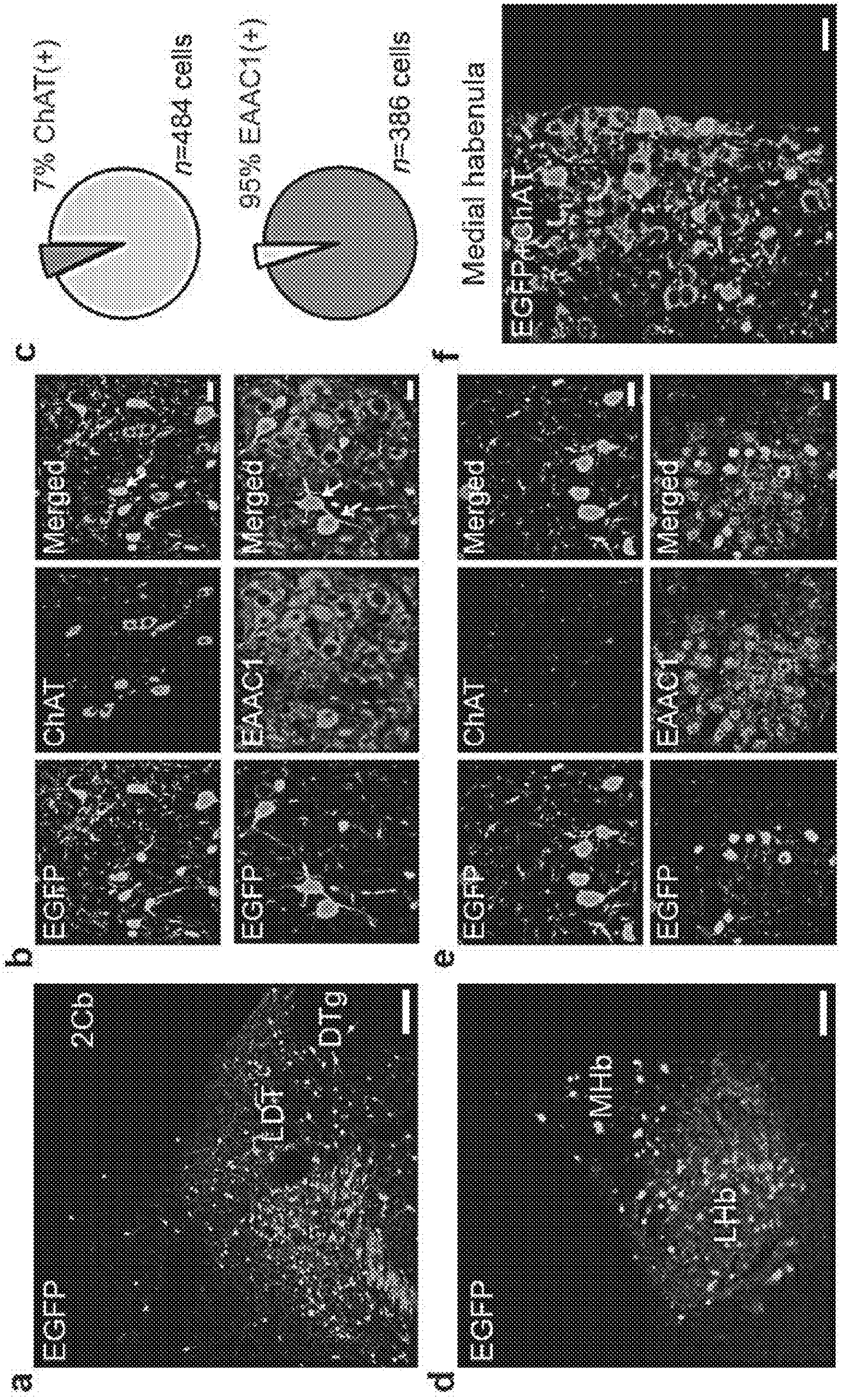

a
EGFP
2Cb
LDT
DTg
b
EGFP
ChAT
Merged
EGFP
EAAC1
Merged
c
7% ChAT(+)
n=484 cells
95% EAAC1(+)
n=386 cells
d
EGFP
MHb
LHb
e
EGFP
ChAT
Merged
EGFP
EAAC1
Merged
f
Medial habenula
EGFP/ChAT

FIGURE 9

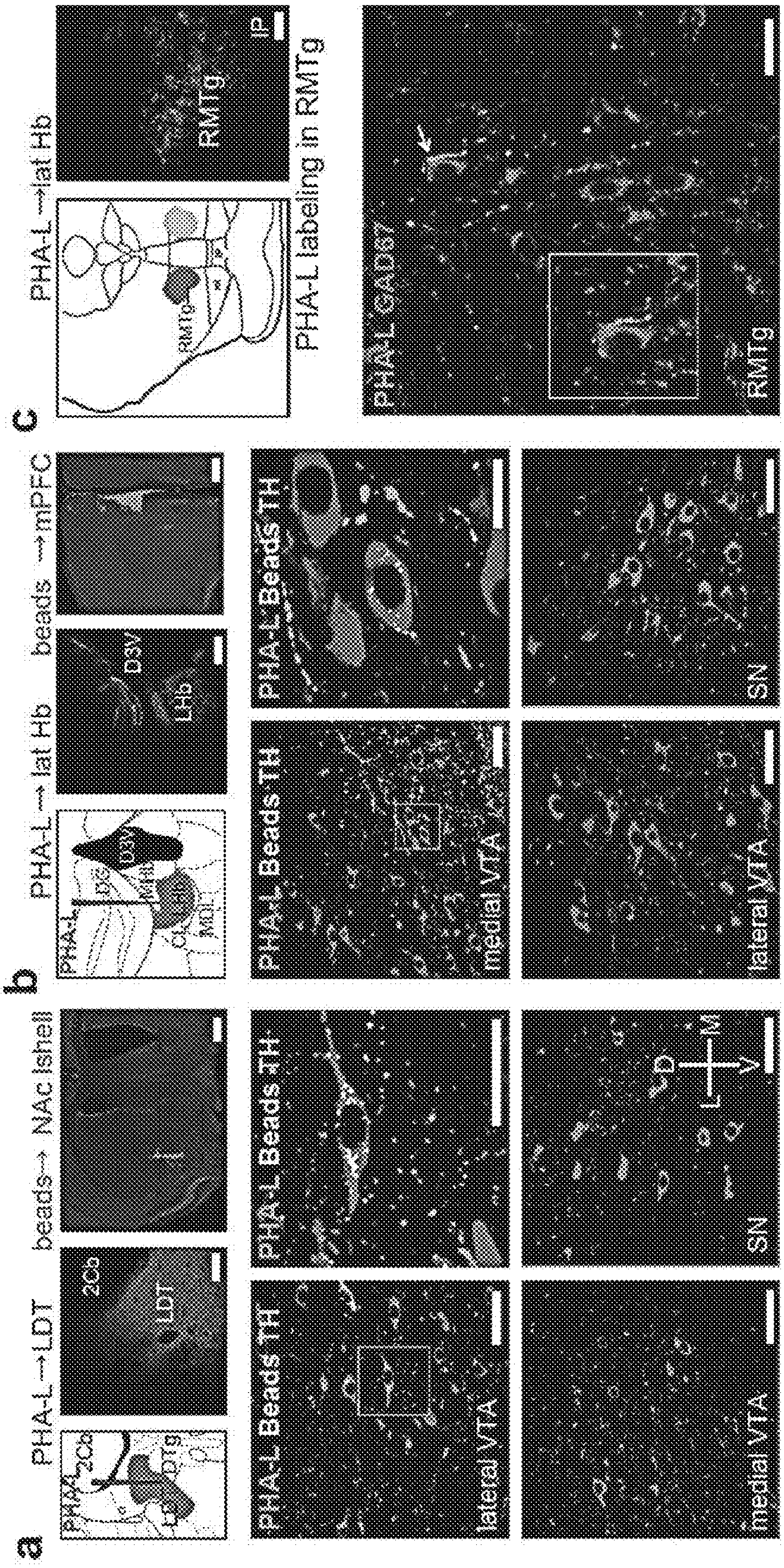

a
PHA-L→LDT
PHA-L
2Cb
LDTg
2Cb
LDT
beads→ NAc lshell
PHA-L Beads TH
lateral VTA
PHA-L Beads TH
medial VTA
SN
D
L
M
V
b
PHA-L → lat Hb
PHA-L
DG
D3V
MHb
LHb
CL
MDL
D3V
LHb
beads → mPFC
PHA-L Beads TH
medial VTA
PHA-L Beads TH
lateral VTA
SN
c
PHA-L → lat Hb
RMTg
IP
RMTg
IP
PHA-L labeling in RMTg
PHA-L GAD67
RMTg

FIGURE 10

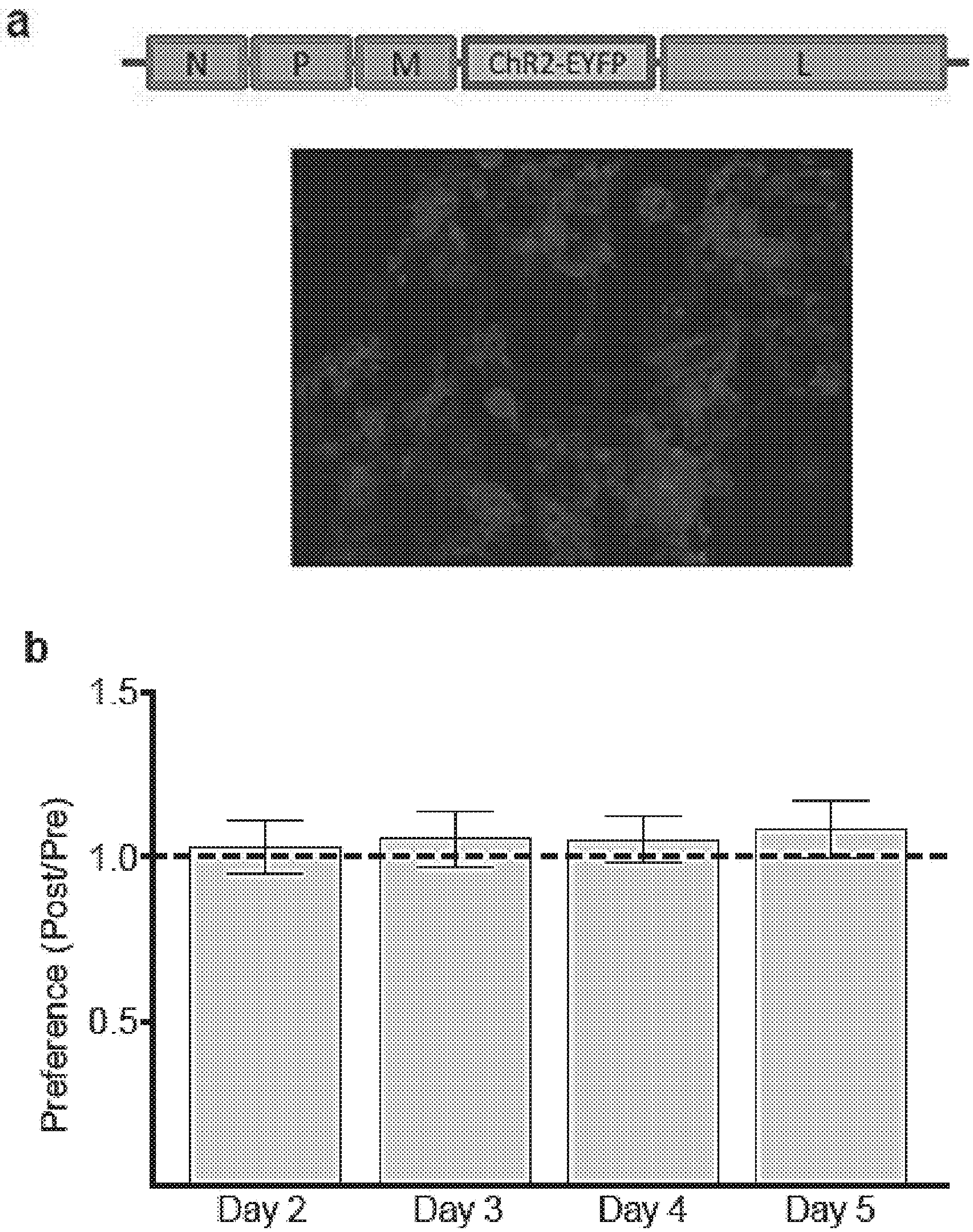
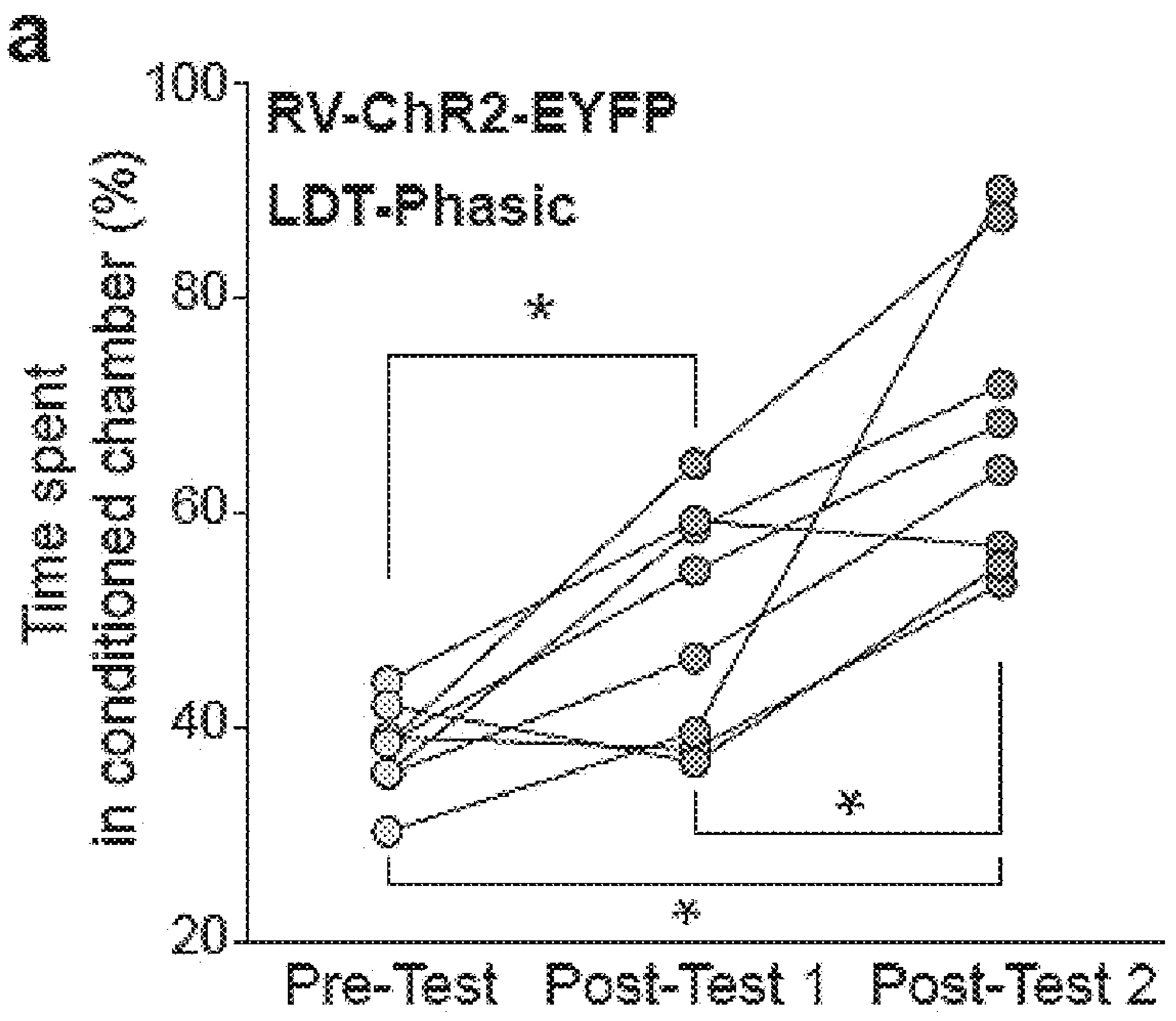

a
N
P
M
ChR2-EYFP
L
b
1.5
1.0
0.5
Preference (Post/Pre)
Day 2
Day 3
Day 4
Day 5

FIGURE 11A


a
RV-ChR2-EYFP
LDT-Phasic
Time spent in conditioned chamber (%)
100
80
60
40
20
*
*
*
Pre-Test
Post-Test 1
Post-Test 2

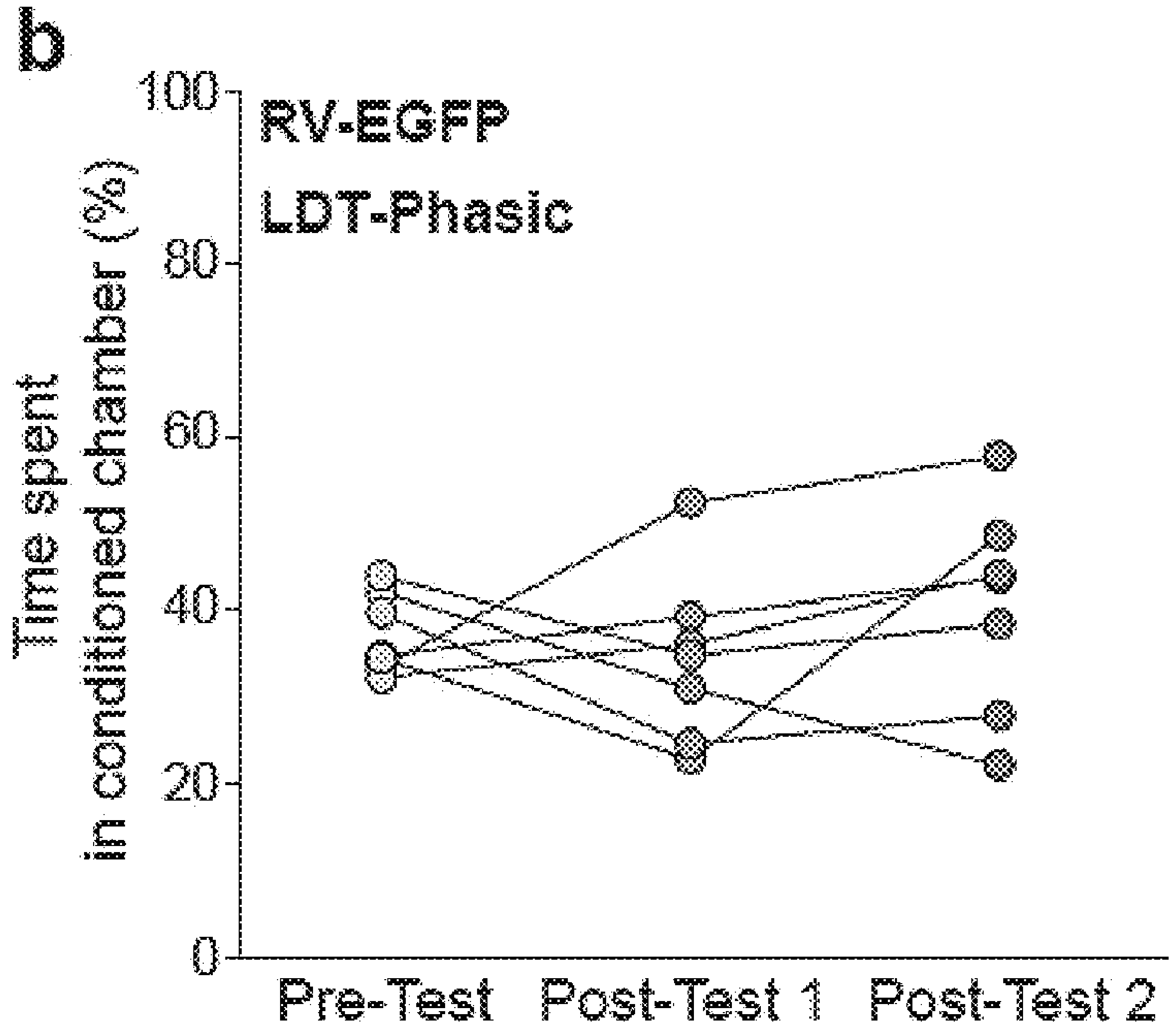
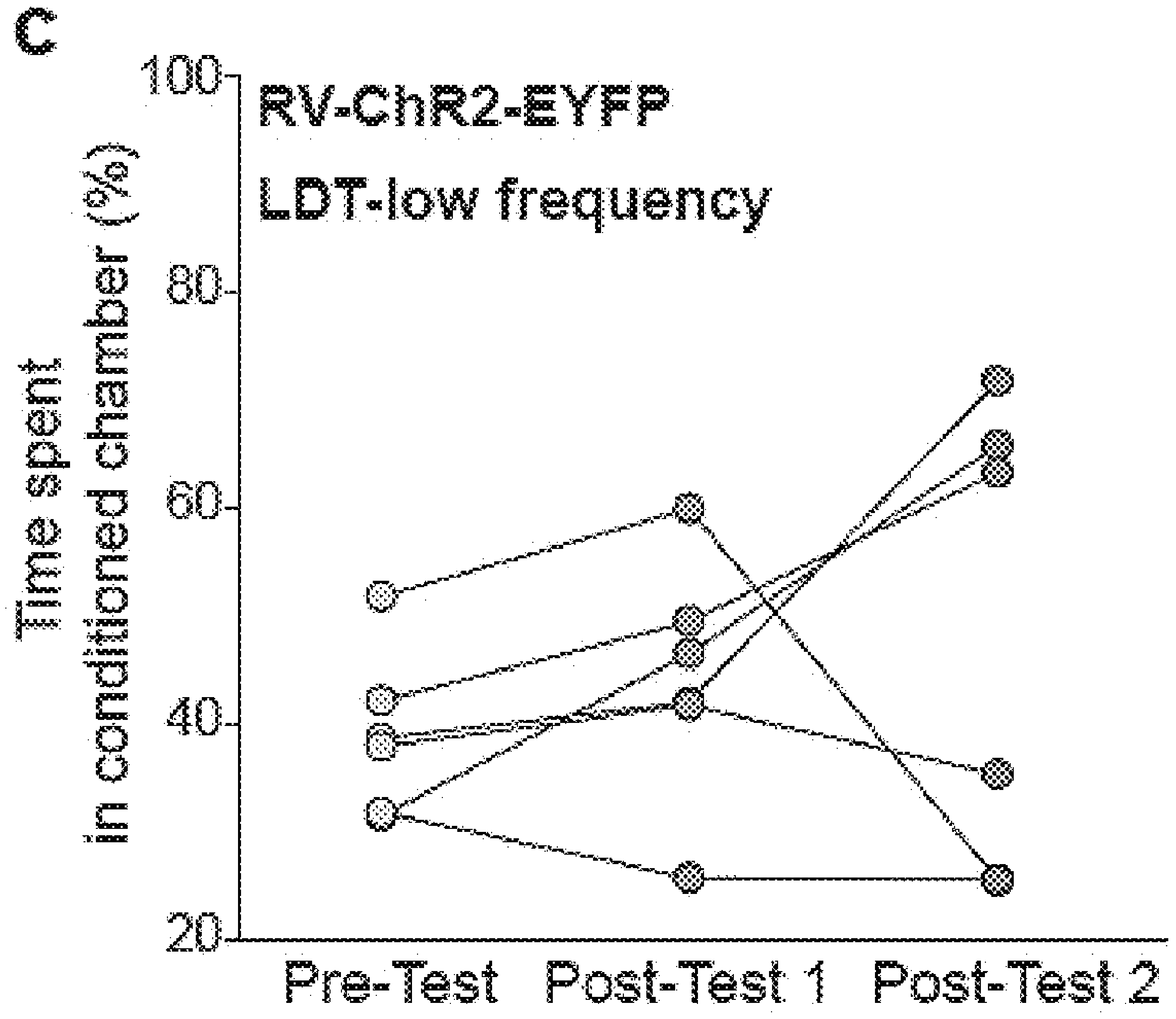

b
RV-EGFP
LDT-Phasic
Time spent in conditioned chamber (%)
100
80
60
40
20
0
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 11B


c
100
80
60
40
20
Time spent in conditioned chamber (%)
RV-ChR2-EYFP
LDT-low frequency
Pre-Test
Post-Test 1
Post-Test 2

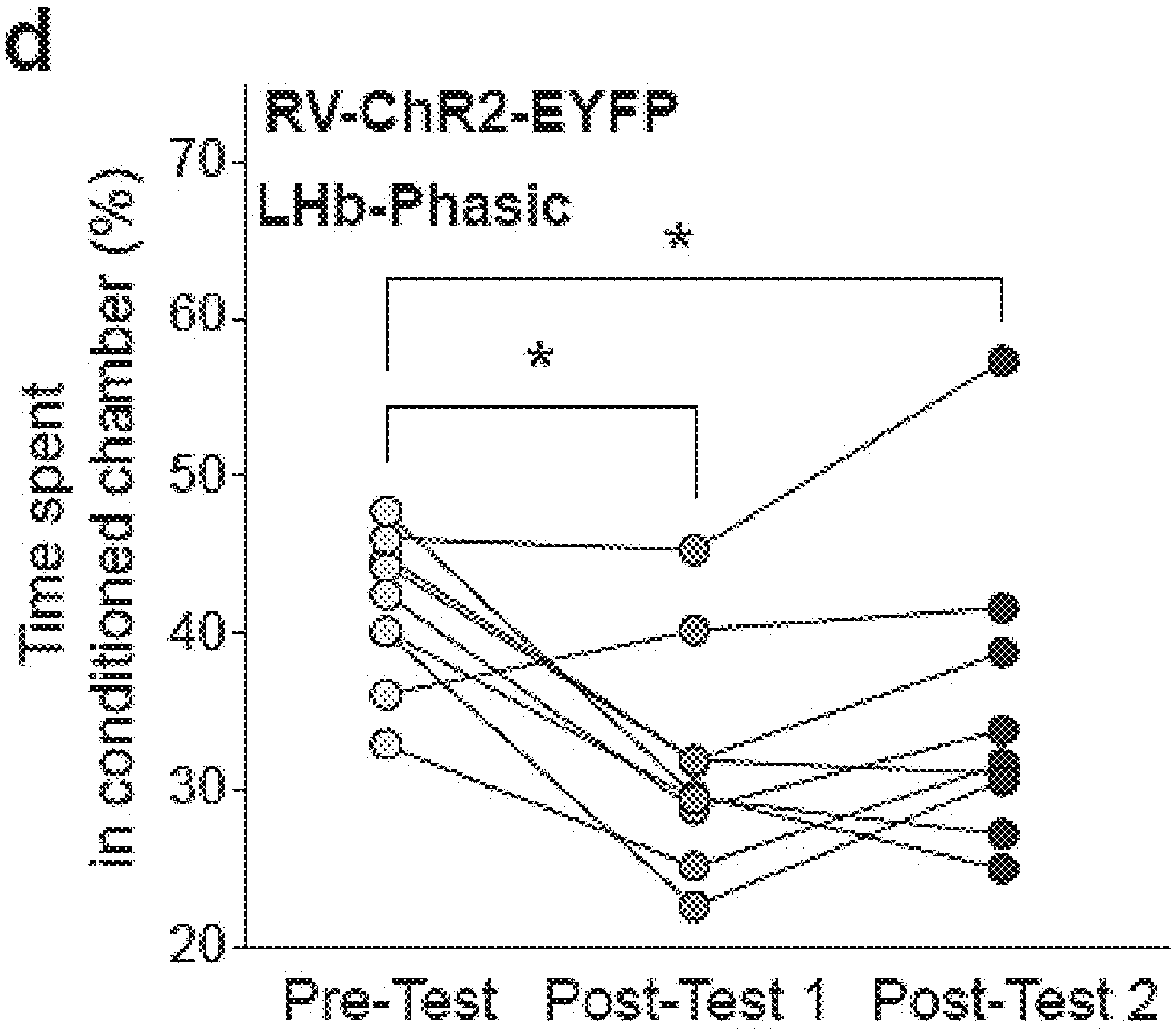
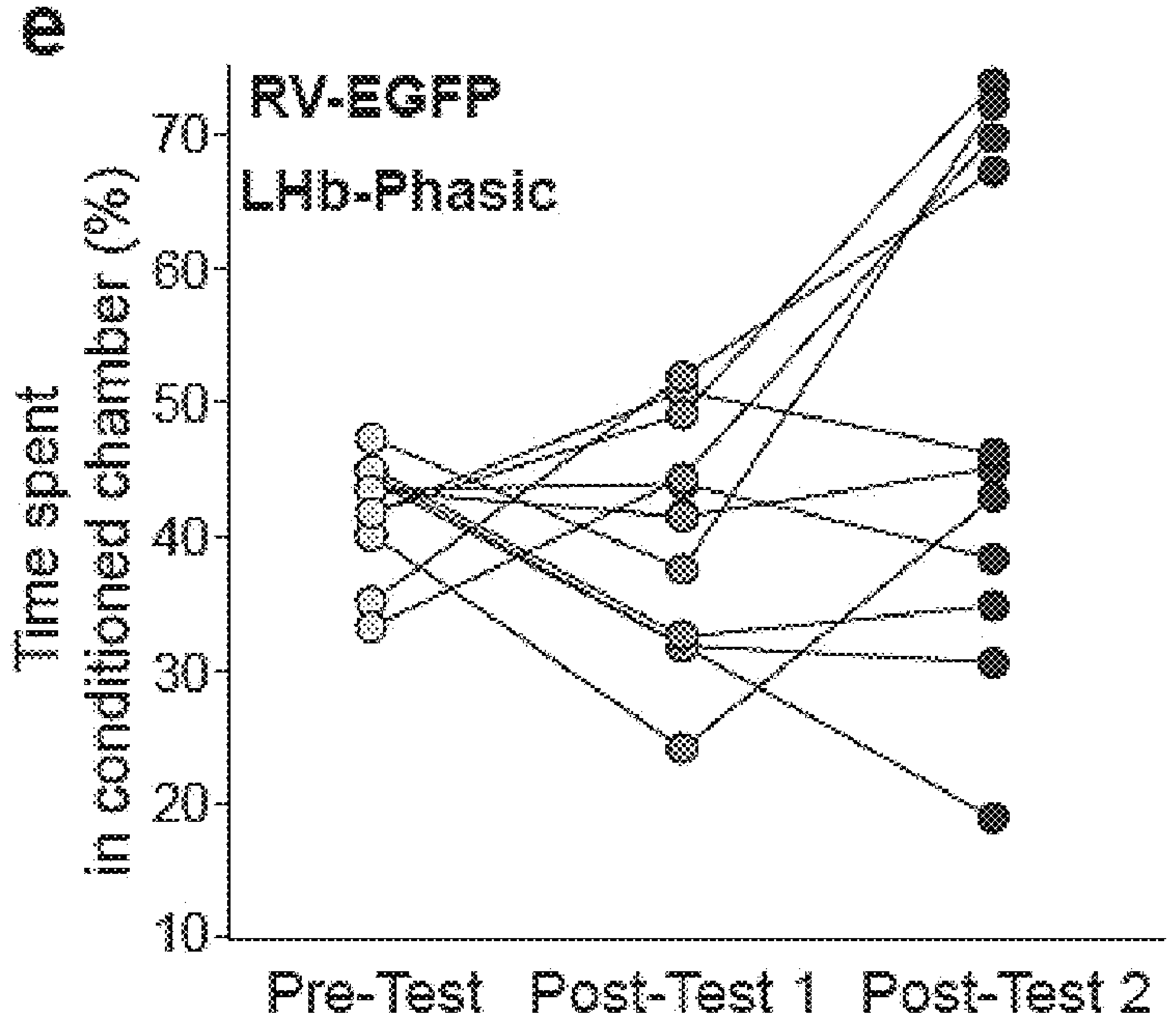

d
70
60
50
40
30
20
Time spent in conditioned chamber (%)
RV-ChR2-EYFP
LHb-Phasic
*
*
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 11C


e
RV-EGFP
LHb-Phasic
Time spent in conditioned chamber (%)
70
60
50
40
30
20
10
Pre-Test
Post-Test 1
Post-Test 2

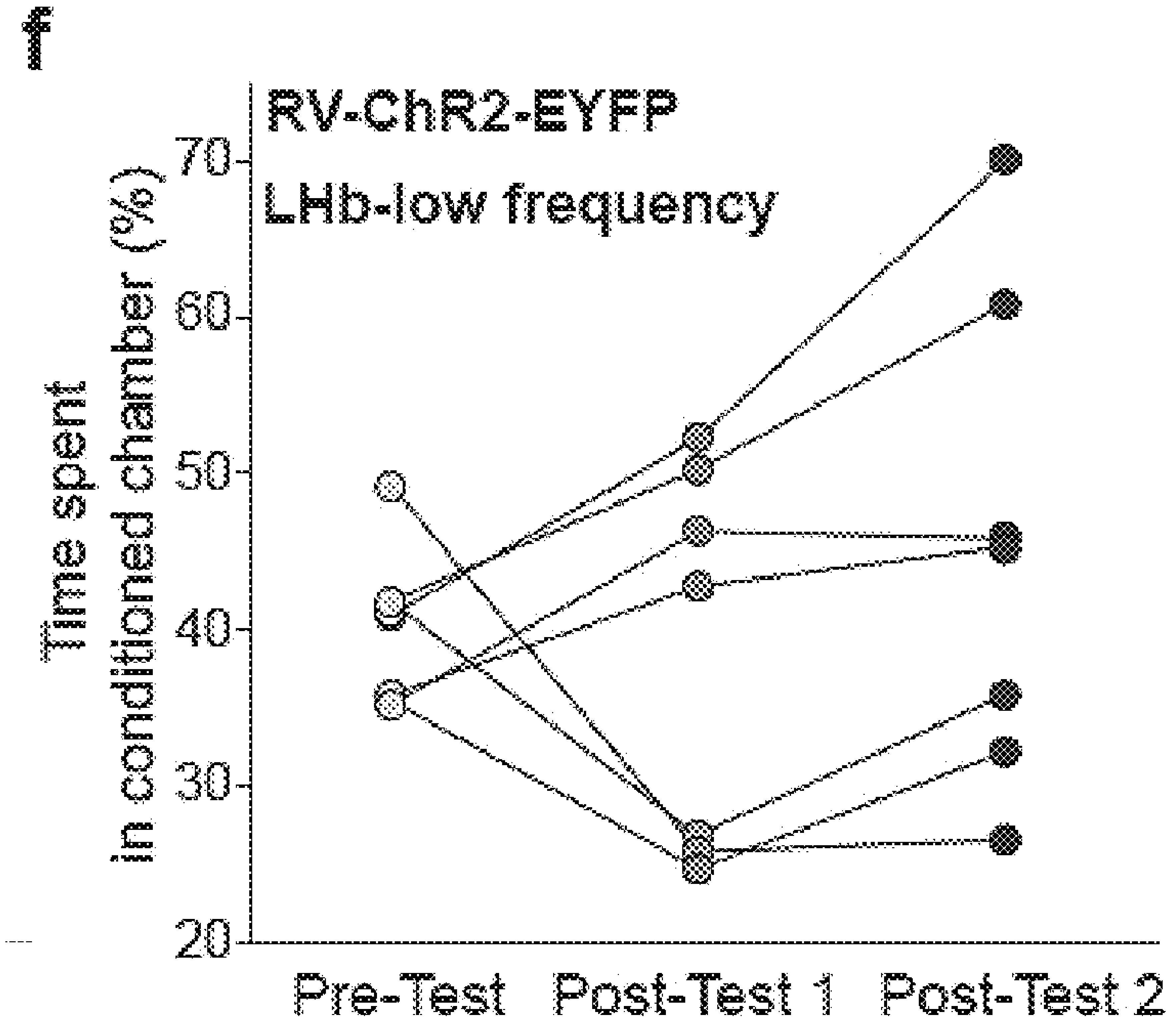

f
RV-ChR2-EYFP
LHb-low frequency
Time spent in conditioned chamber (%)
70
60
50
40
30
20
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 12A

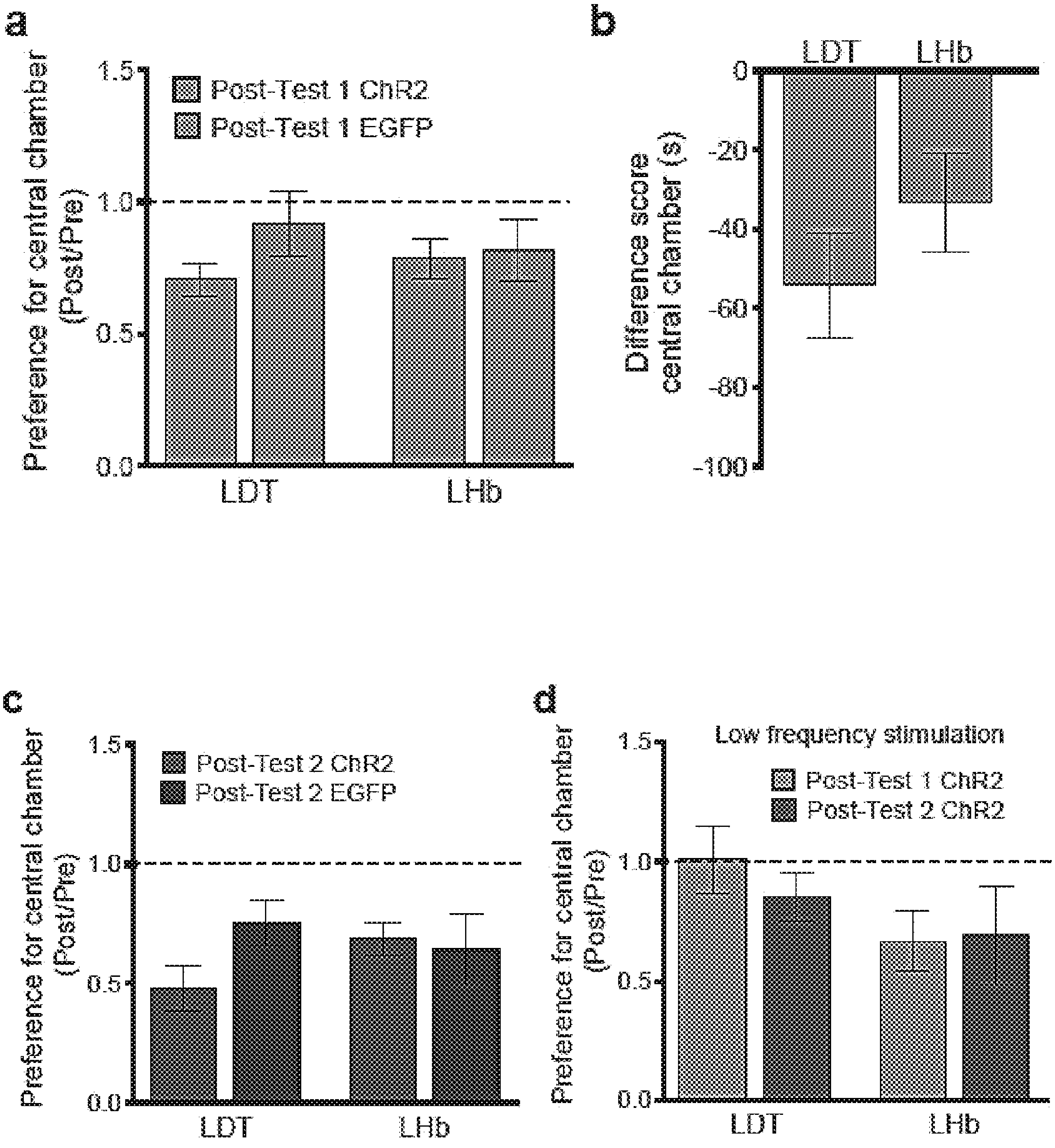

a
Preference for central chamber (Post/Pre)
1.5
1.0
0.5
0.0
Post-Test 1 ChR2
Post-Test 1 EGFP
LDT
LHb
b
Difference score central chamber (s)
0
-20
-40
-60
-80
-100
LDT
LHb
c
Preference for central chamber (Post/Pre)
1.5
1.0
0.5
0.0
Post-Test 2 ChR2
Post-Test 2 EGFP
LDT
LHb
d
Low frequency stimulation
Preference for central chamber (Post/Pre)
1.5
1.0
0.5
0.0
Post-Test 1 ChR2
Post-Test 2 ChR2
LDT
LHb

FIGURE 12B

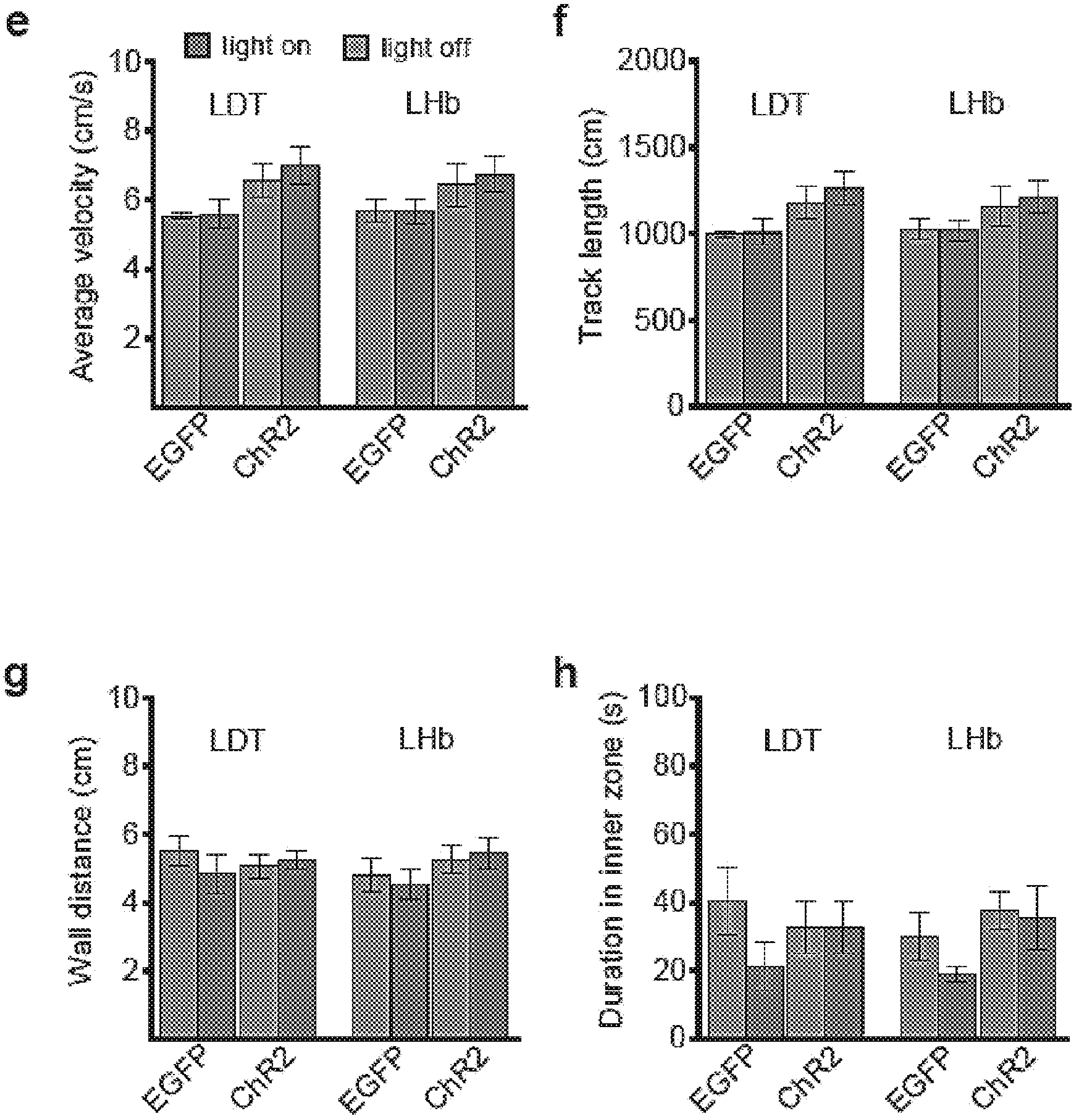

e
▨ light on
▨ light off
Average velocity (cm/s)
10
8
6
4
2
LDT
LHb
EGFP
ChR2
EGFP
ChR2
f
Track length (cm)
2000
1500
1000
500
0
LDT
LHb
EGFP
ChR2
EGFP
ChR2
g
Wall distance (cm)
10
8
6
4
2
LDT
LHb
EGFP
ChR2
EGFP
ChR2
h
Duration in inner zone (s)
100
80
60
40
20
0
LDT
LHb
EGFP
ChR2
EGFP
ChR2

FIGURE 13A

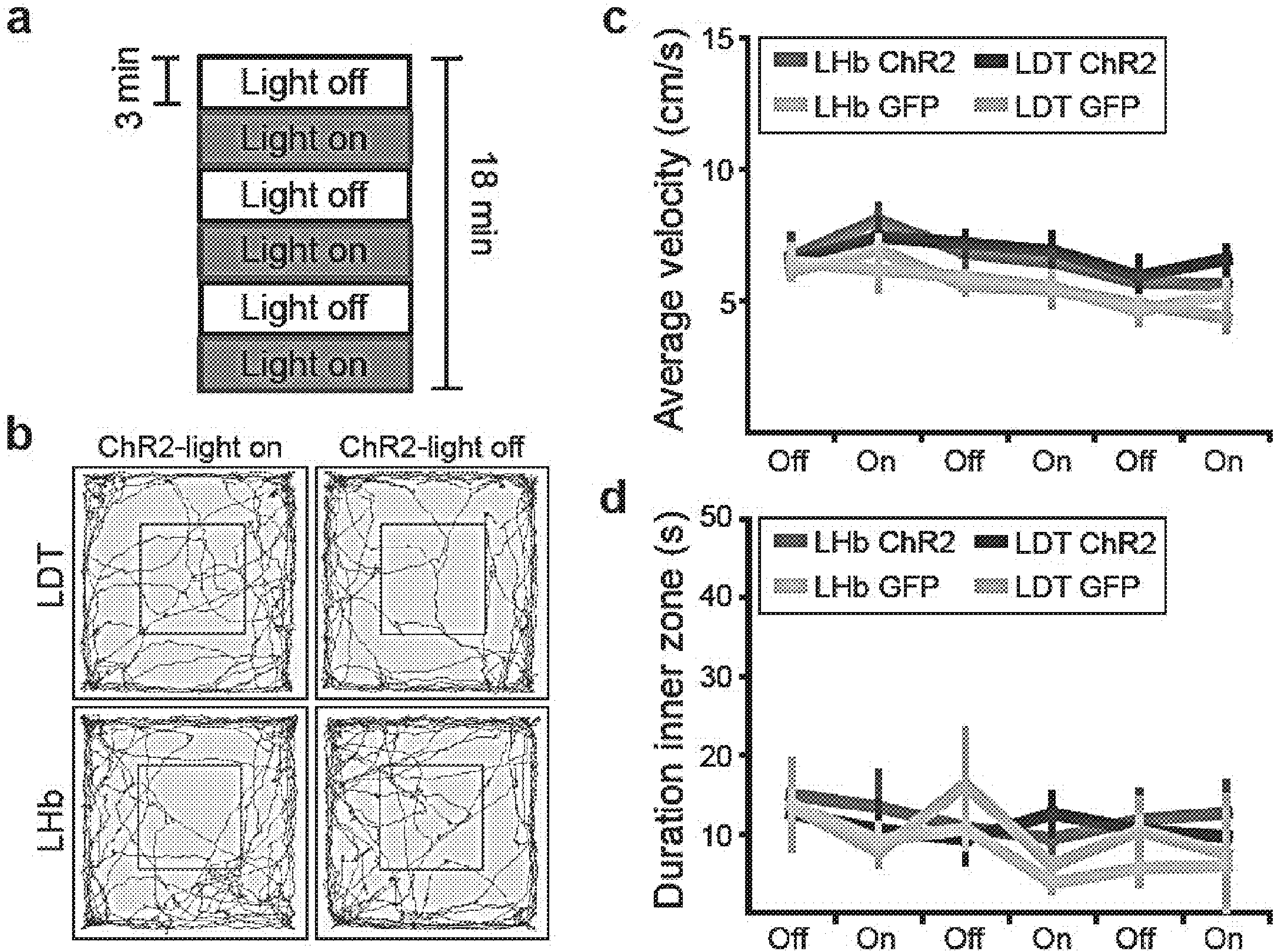

a
3 min
Light off
Light on
Light off
Light on
Light off
Light on
18 min
b
ChR2-light on
ChR2-light off
LDT
LHb
c
Average velocity (cm/s)
15
10
5
LHb ChR2
LDT ChR2
LHb GFP
LDT GFP
Off
On
Off
On
Off
On
d
Duration inner zone (s)
50
40
30
20
10
LHb ChR2
LDT ChR2
LHb GFP
LDT GFP
Off
On
Off
On
Off
On

FIGURE 13B

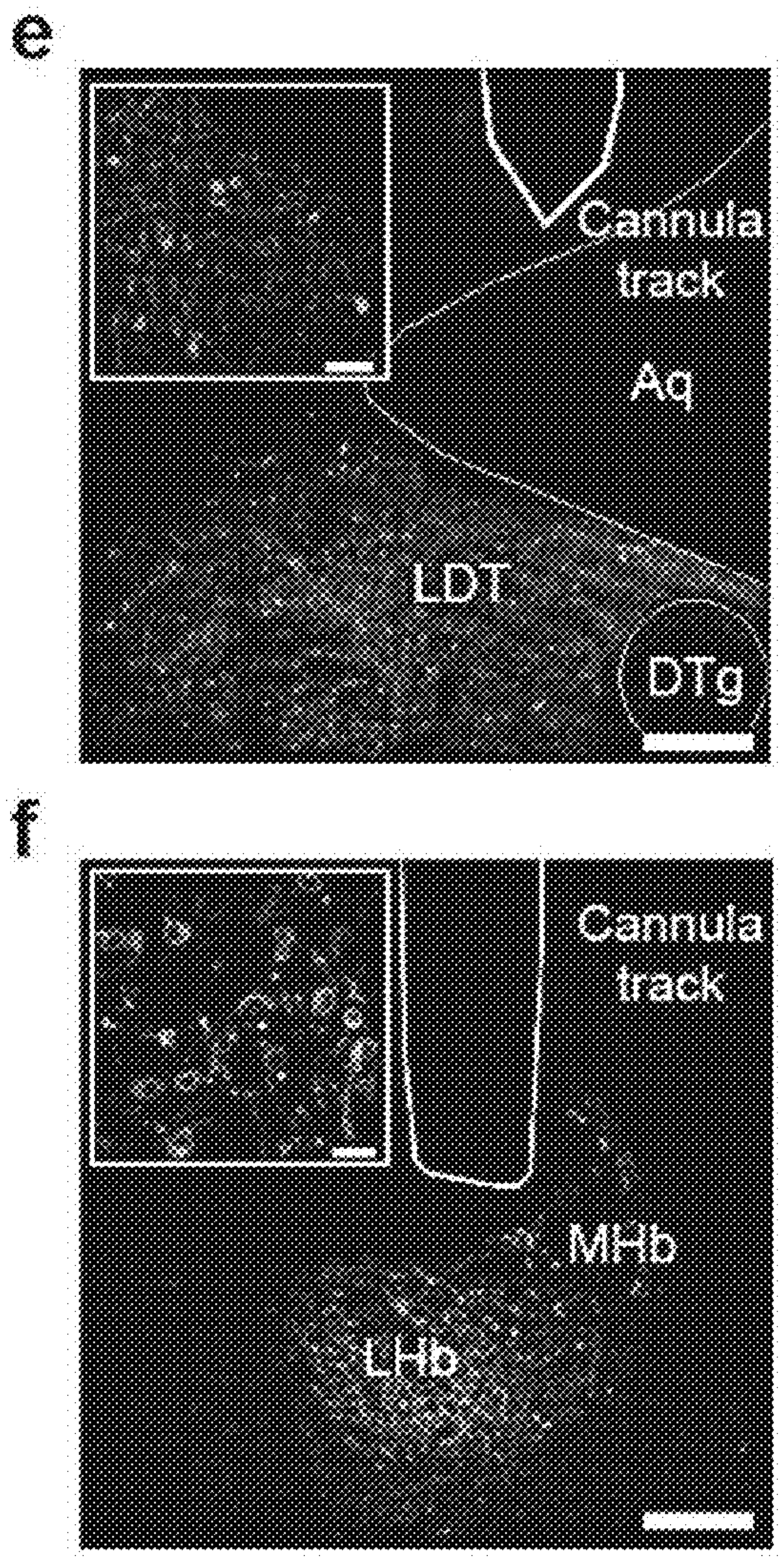


FIGURE 14A

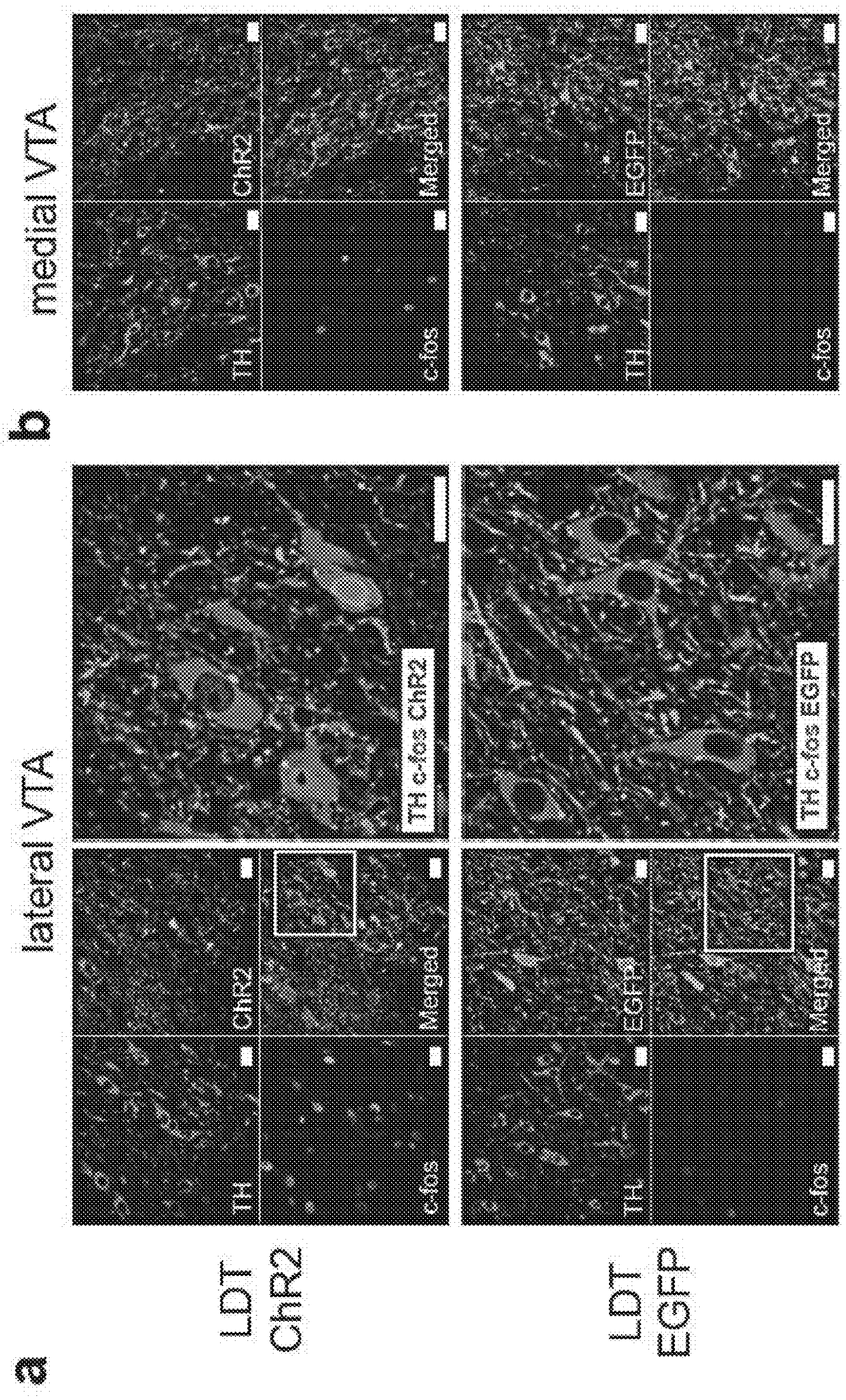

a
LDT ChR2
LDT EGFP
lateral VTA
TH
ChR2
c-fos
Merged
TH c-fos ChR2
TH
EGFP
c-fos
Merged
TH c-fos EGFP
b
medial VTA
TH
ChR2
c-fos
Merged
TH
EGFP
c-fos
Merged

FIGURE 14B

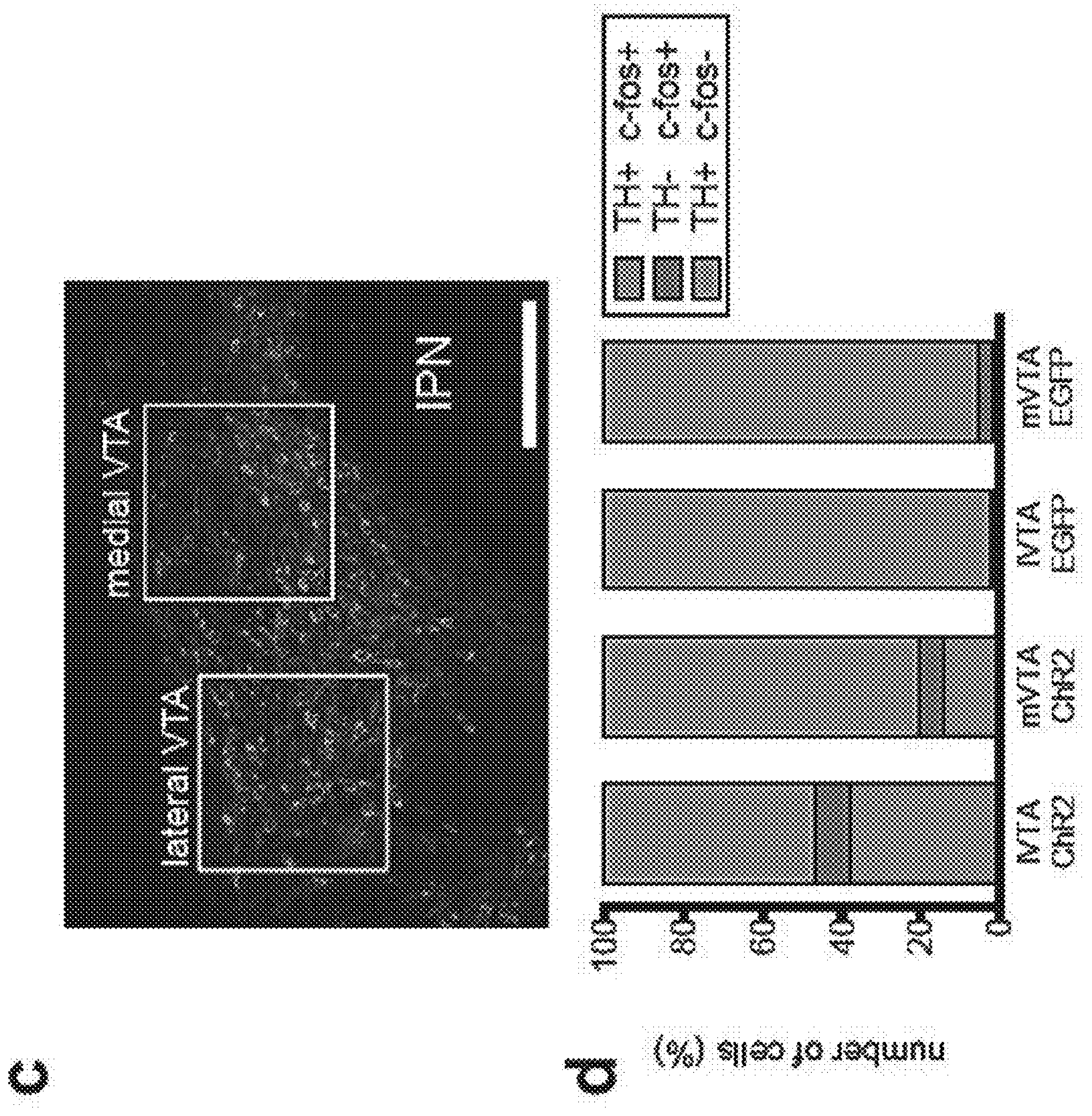

c
lateral VTA
medial VTA
IPN
d
number of cells (%)
100
80
60
40
20
0
lVTA ChR2
mVTA ChR2
lVTA EGFP
mVTA EGFP
TH+ c-fos+
TH- c-fos+
TH+ c-fos-

FIGURE 14C

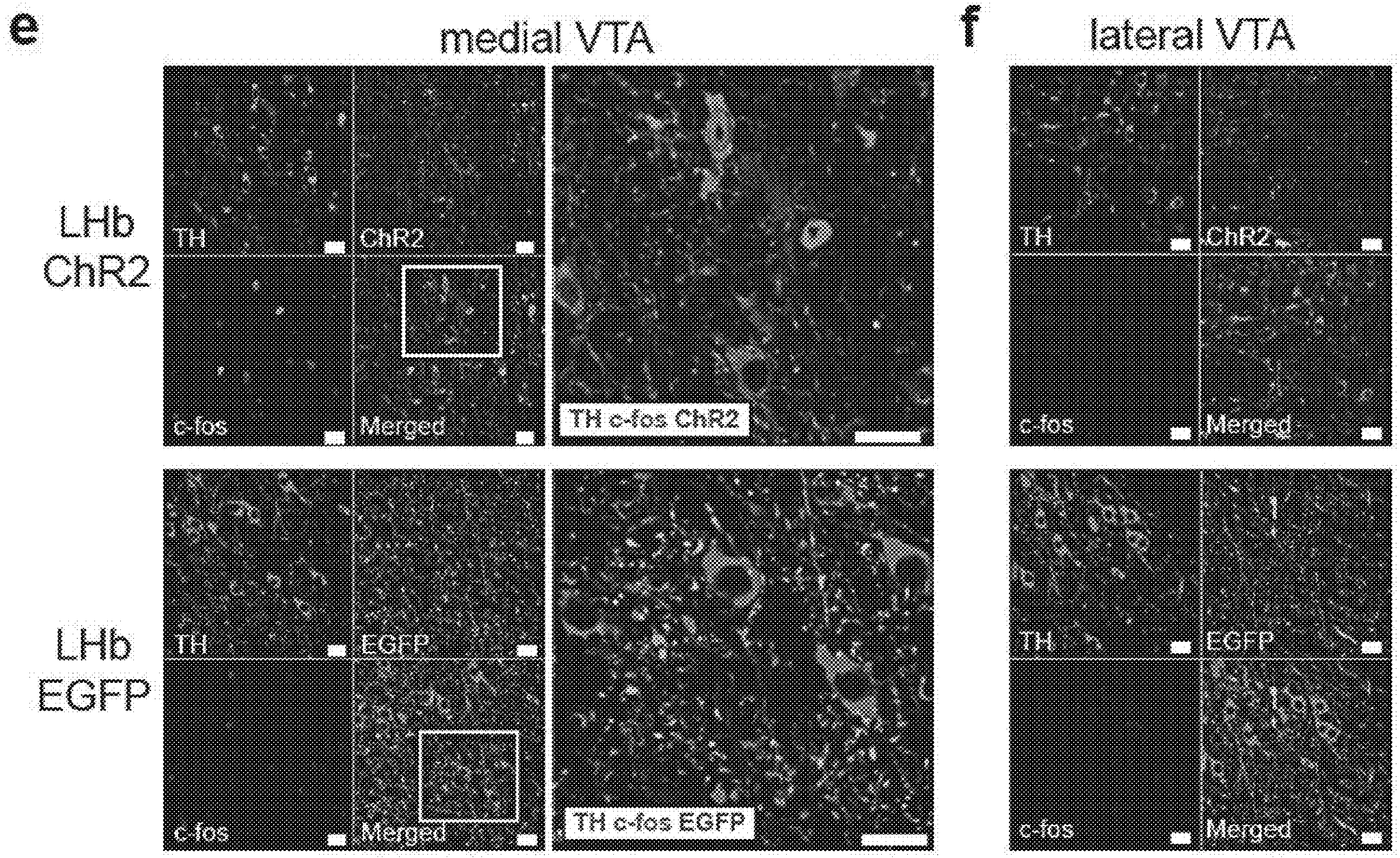

e
medial VTA
LHb
ChR2
TH
ChR2
c-fos
Merged
TH c-fos ChR2
LHb
EGFP
TH
EGFP
c-fos
Merged
TH c-fos EGFP
f
lateral VTA
TH
ChR2
c-fos
Merged
TH
EGFP
c-fos
Merged

FIGURE 14D

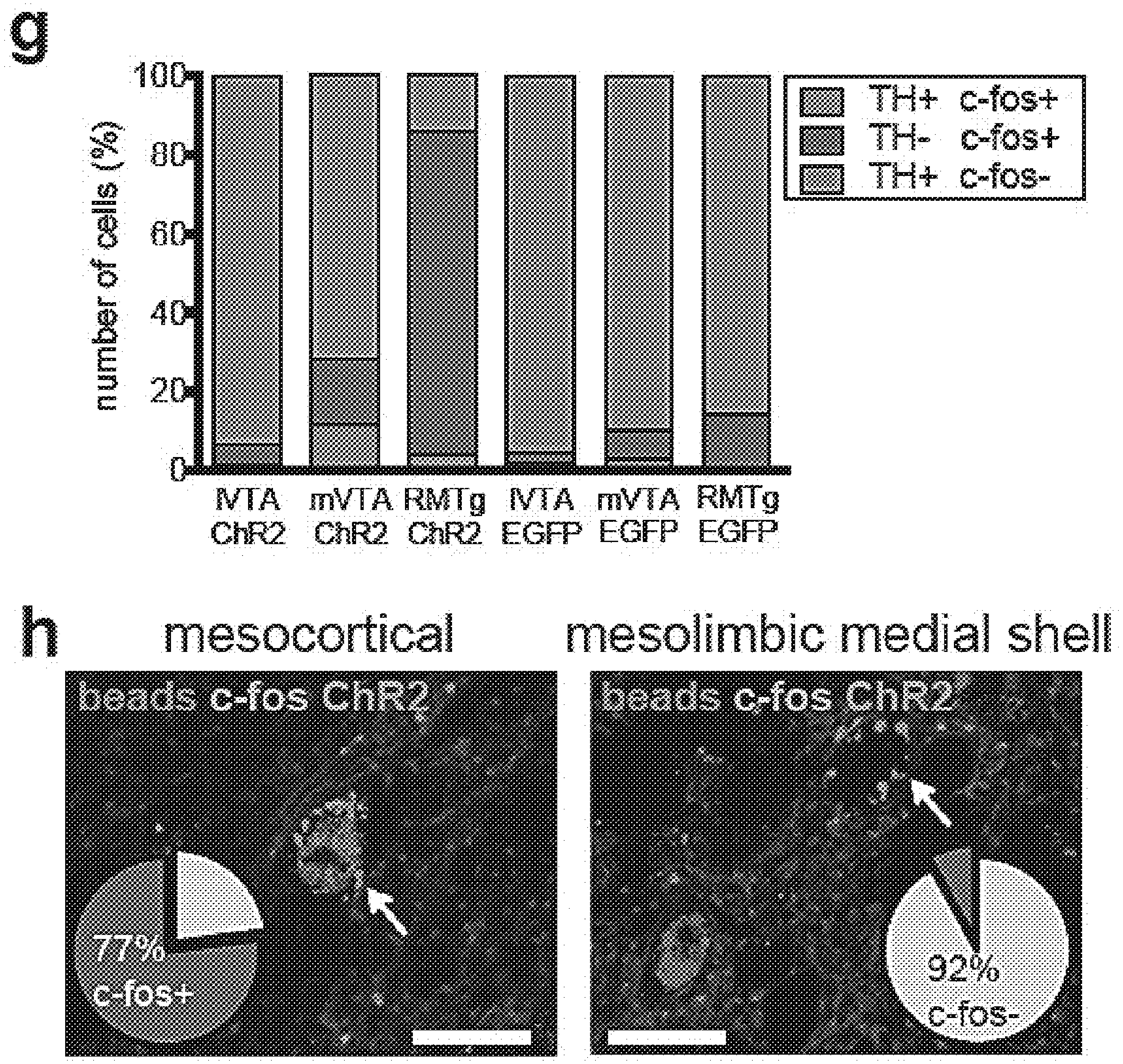

g
number of cells (%)
100
80
60
40
20
0
lVTA ChR2
mVTA ChR2
RMTg ChR2
lVTA EGFP
mVTA EGFP
RMTg EGFP
TH+ c-fos+
TH- c-fos+
TH+ c-fos-
h
mesocortical
mesolimbic medial shell
beads c-fos ChR2
77% c-fos+
beads c-fos ChR2
92% c-fos-

FIGURE 15

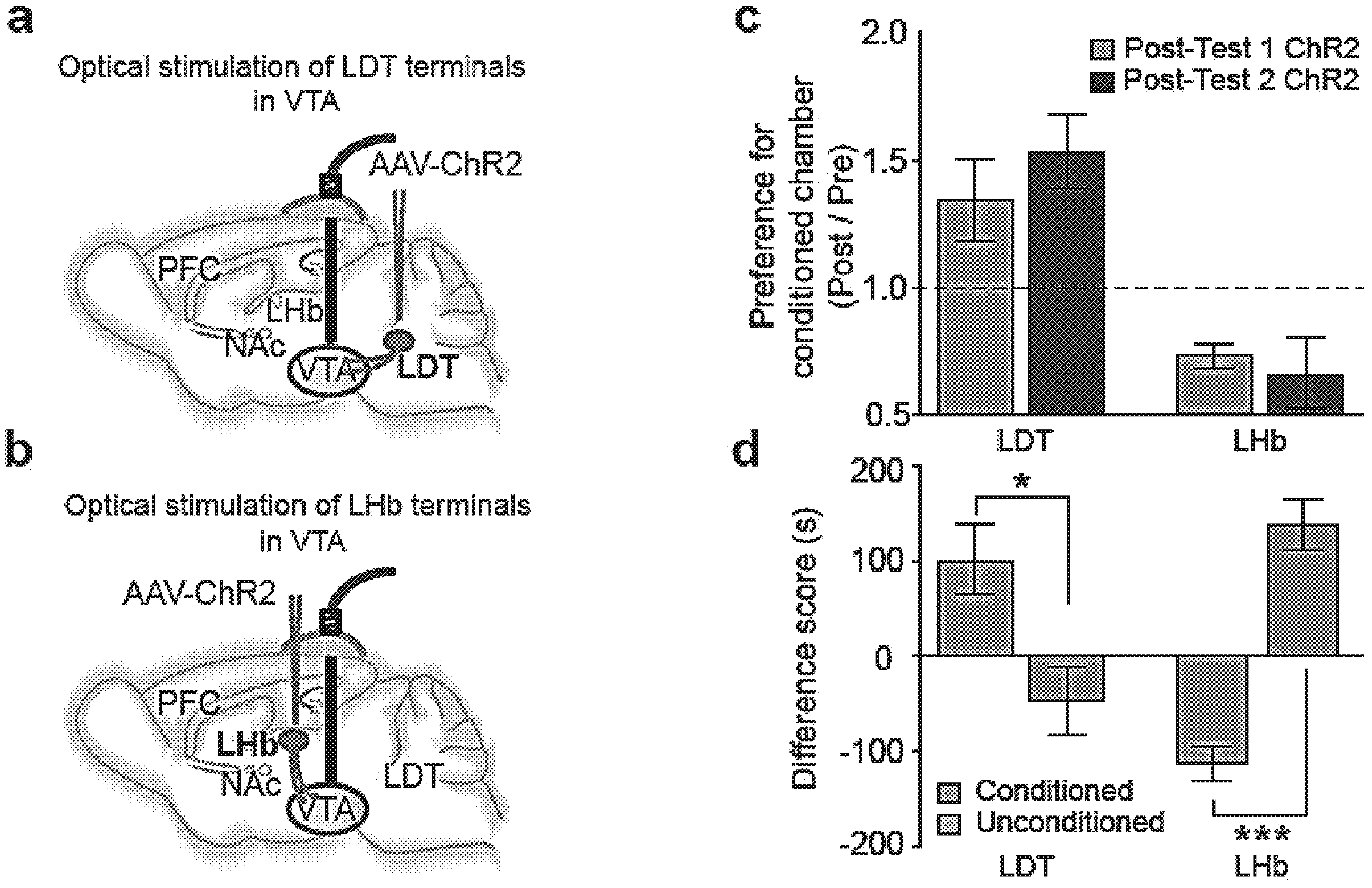
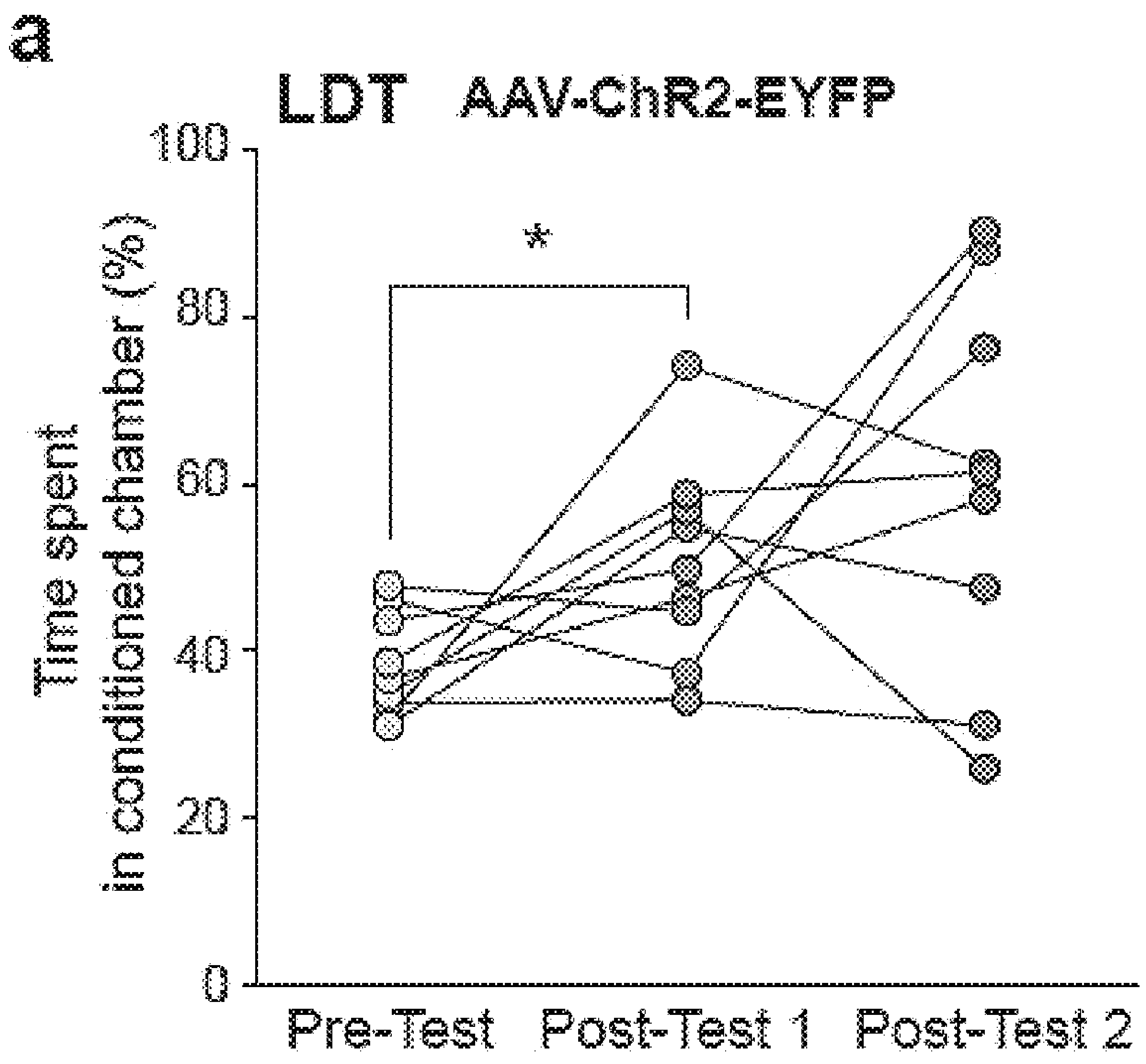

a
Optical stimulation of LDT terminals in VTA
AAV-ChR2
PFC
LHb
NAc
VTA
LDT
b
Optical stimulation of LHb terminals in VTA
AAV-ChR2
PFC
LHb
NAc
VTA
LDT
c
Preference for conditioned chamber (Post / Pre)
2.0
1.5
1.0
0.5
▨ Post-Test 1 ChR2
■ Post-Test 2 ChR2
LDT
LHb
d
Difference score (s)
200
100
0
-100
-200
*
***
▨ Conditioned
▨ Unconditioned
LDT
LHb

FIGURE 16


a
LDT AAV-ChR2-EYFP
Time spent in conditioned chamber (%)
100
80
60
40
20
0
*
Pre-Test
Post-Test 1
Post-Test 2

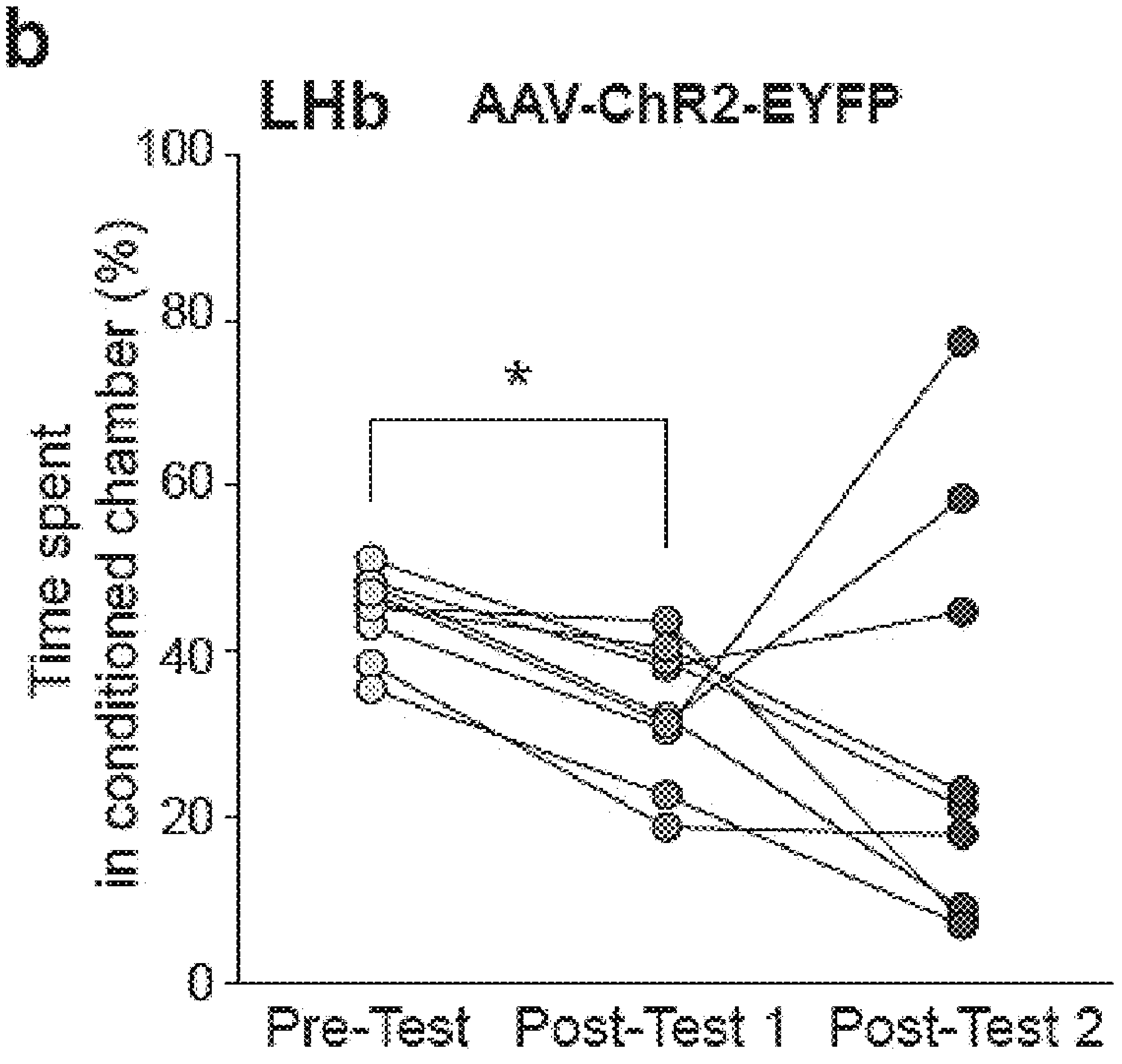

b
LHb AAV-ChR2-EYFP
Time spent in conditioned chamber (%)
100
80
60
40
20
0
*
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 17A

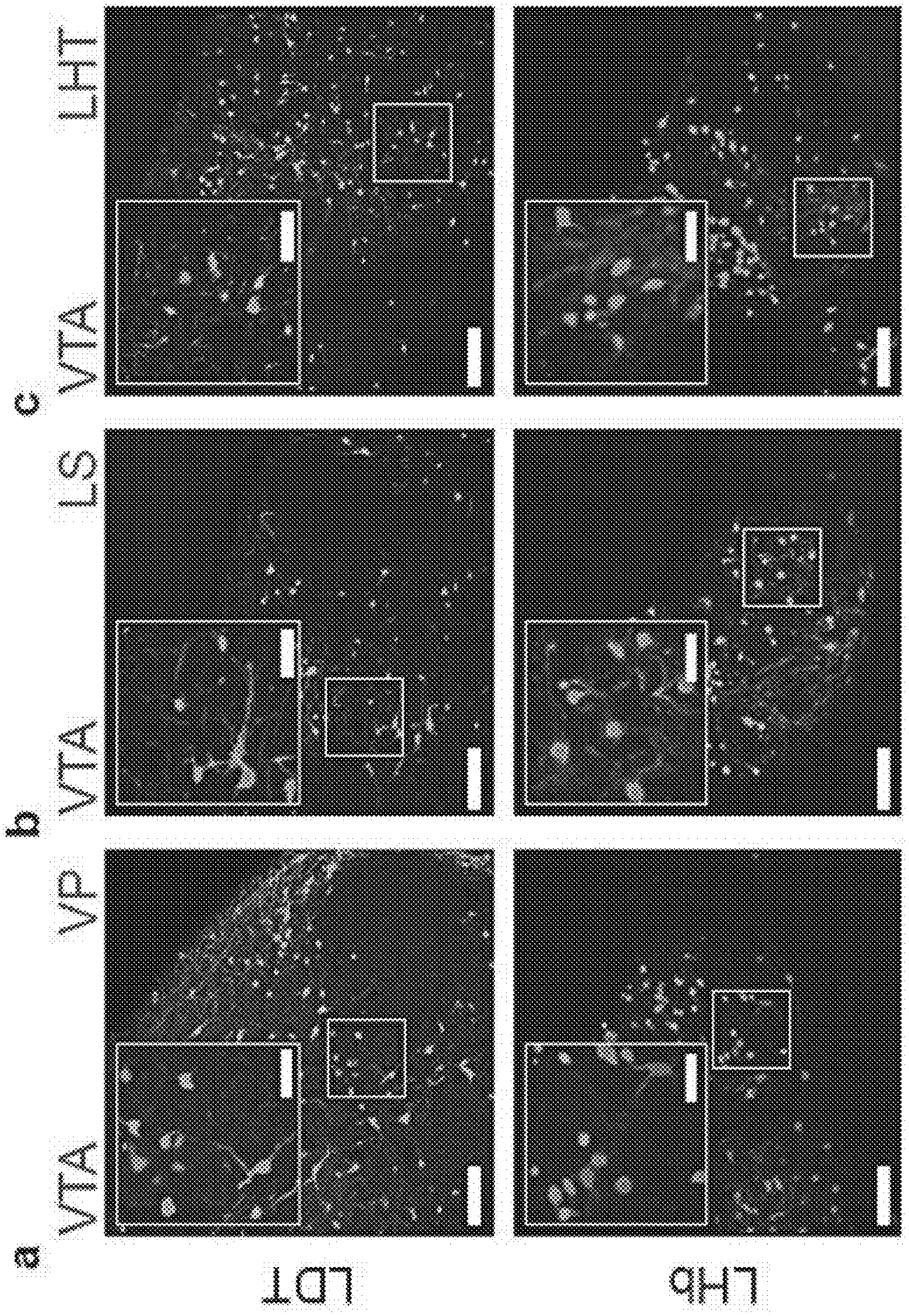


FIGURE 17B

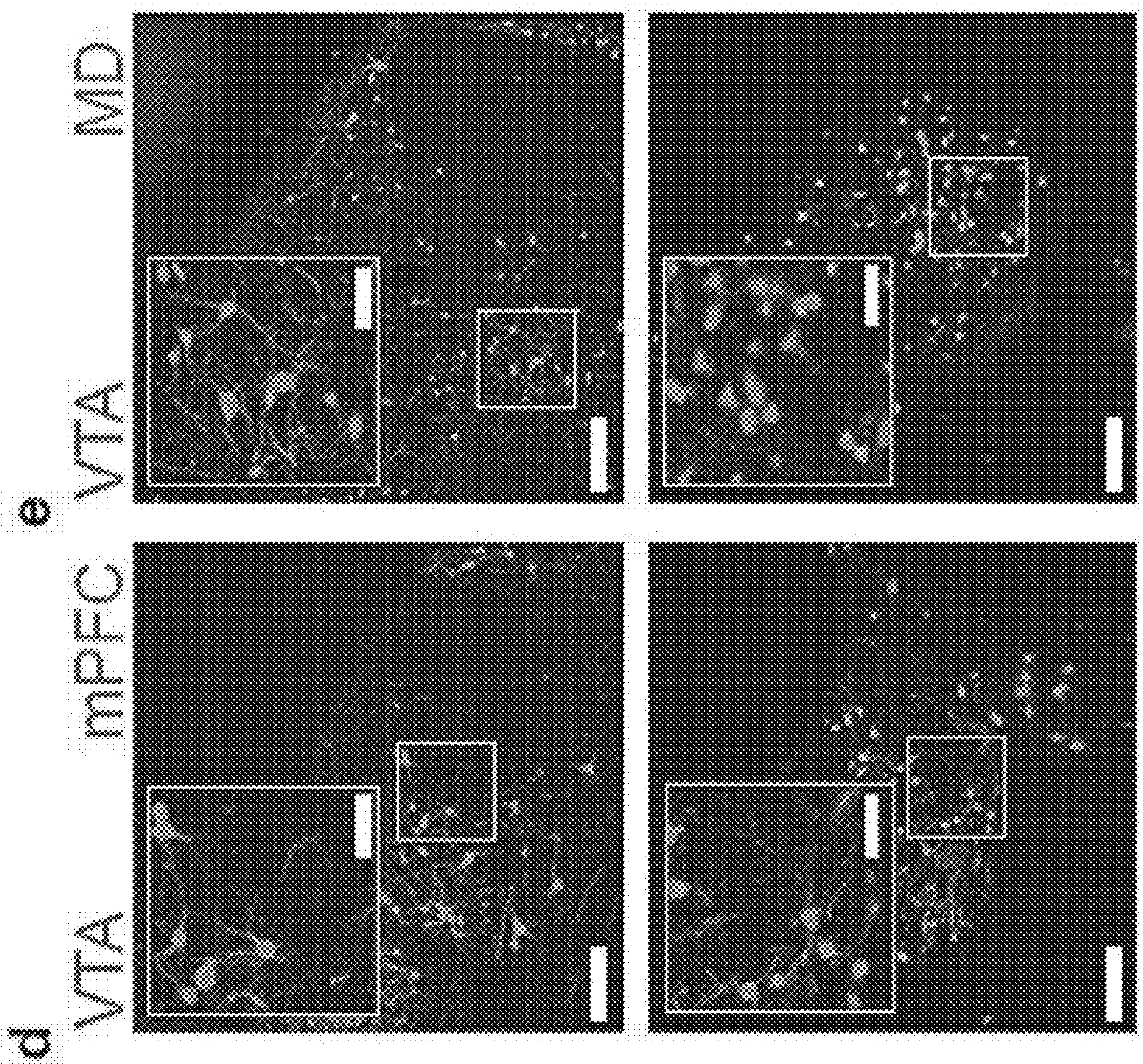


FIGURE 17C

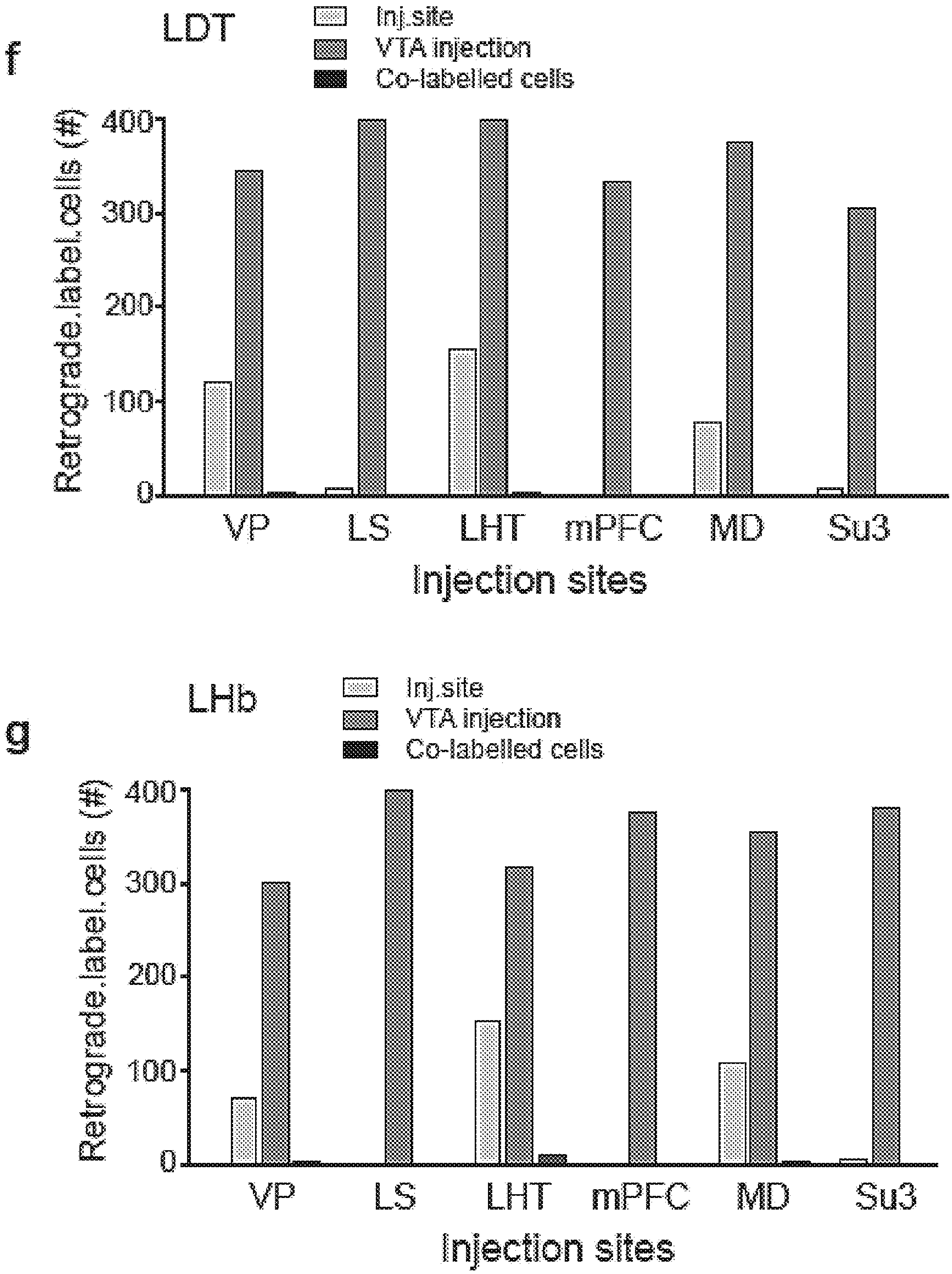

f
LDT
▨ Inj.site
▨ VTA injection
■ Co-labelled cells
Retrograde.label.cells (#)
0
100
200
300
400
VP
LS
LHT
mPFC
MD
Su3
Injection sites
g
LHb
▨ Inj.site
▨ VTA injection
■ Co-labelled cells
Retrograde.label.cells (#)
0
100
200
300
400
VP
LS
LHT
mPFC
MD
Su3
Injection sites

FIGURE 17D

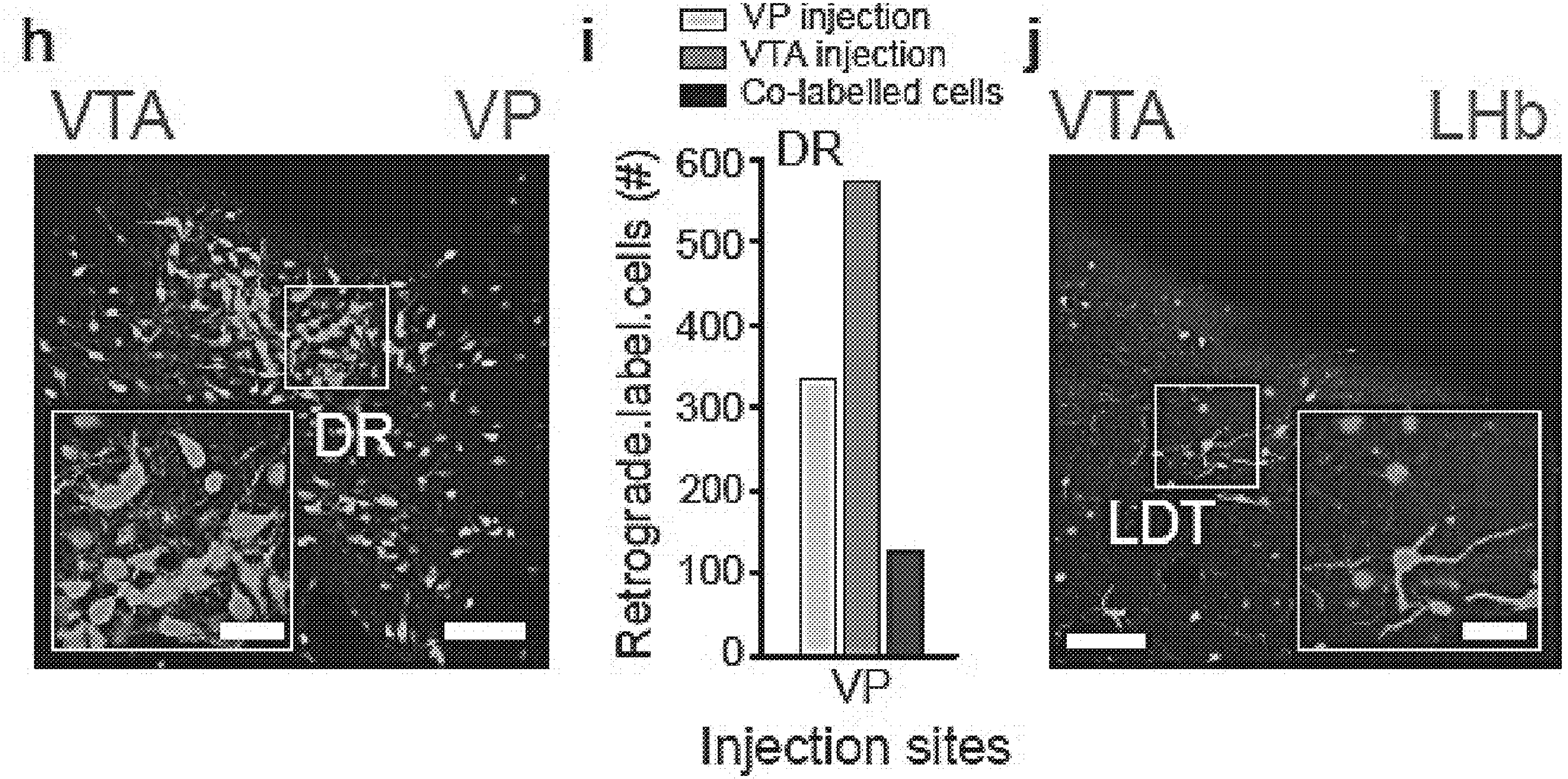

h
VTA
VP
DR
i
VP injection
VTA injection
Co-labelled cells
DR
Retrograde.label.cells (#)
600
500
400
300
200
100
0
VP
Injection sites
j
VTA
LHb
LDT

FIGURE 17E

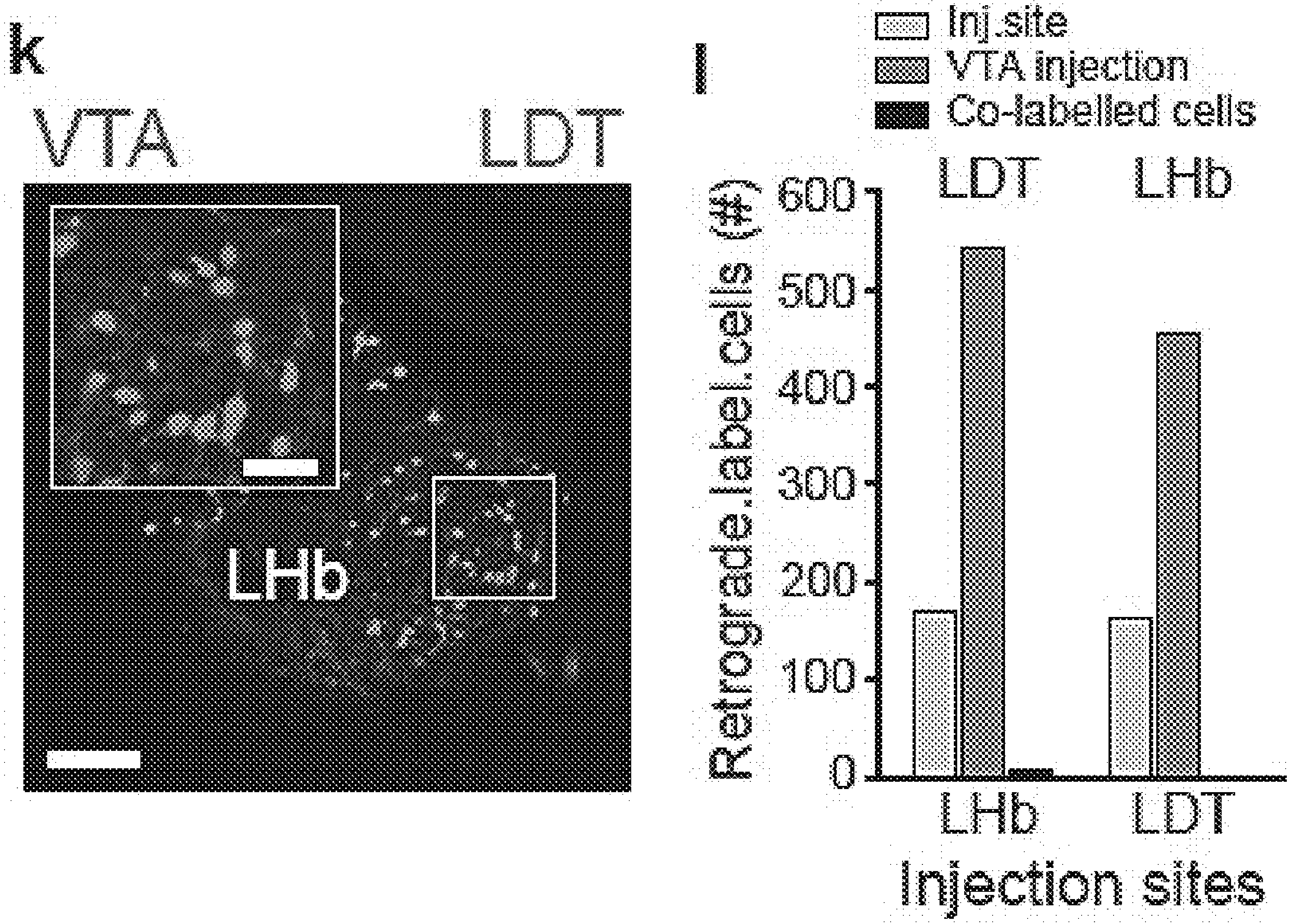

k
VTA
LDT
LHb
l
Inj.site
VTA injection
Co-labelled cells
LDT
LHb
Retrograde.label.cells (#)
600
500
400
300
200
100
0
LHb
LDT
Injection sites

FIGURE 18A

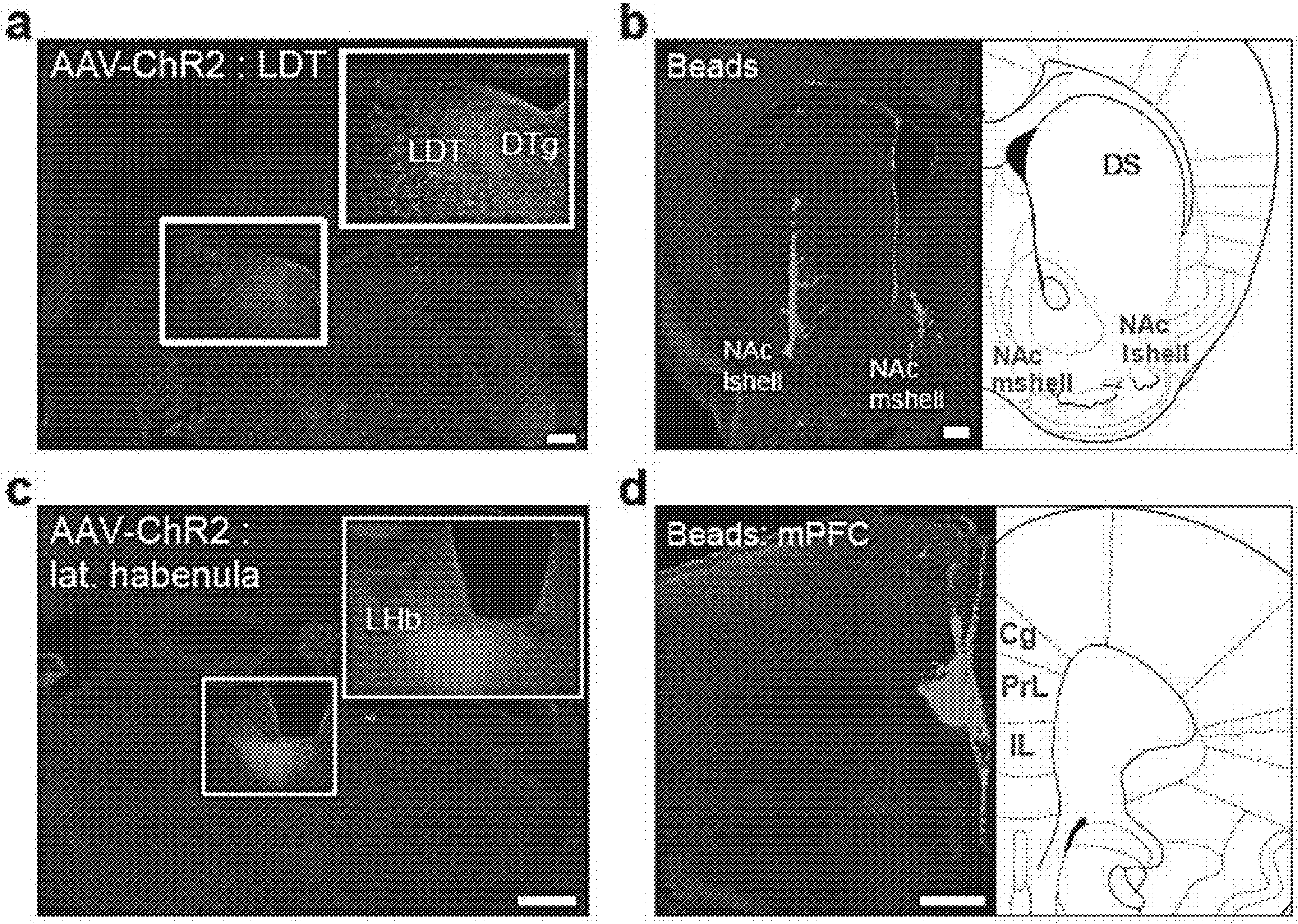

a
AAV-ChR2 : LDT
LDT
DTg
b
Beads
NAc lshell
NAc mshell
DS
NAc mshell
NAc lshell
c
AAV-ChR2 : lat. habenula
LHb
d
Beads: mPFC
Cg
PrL
IL

FIGURE 18B

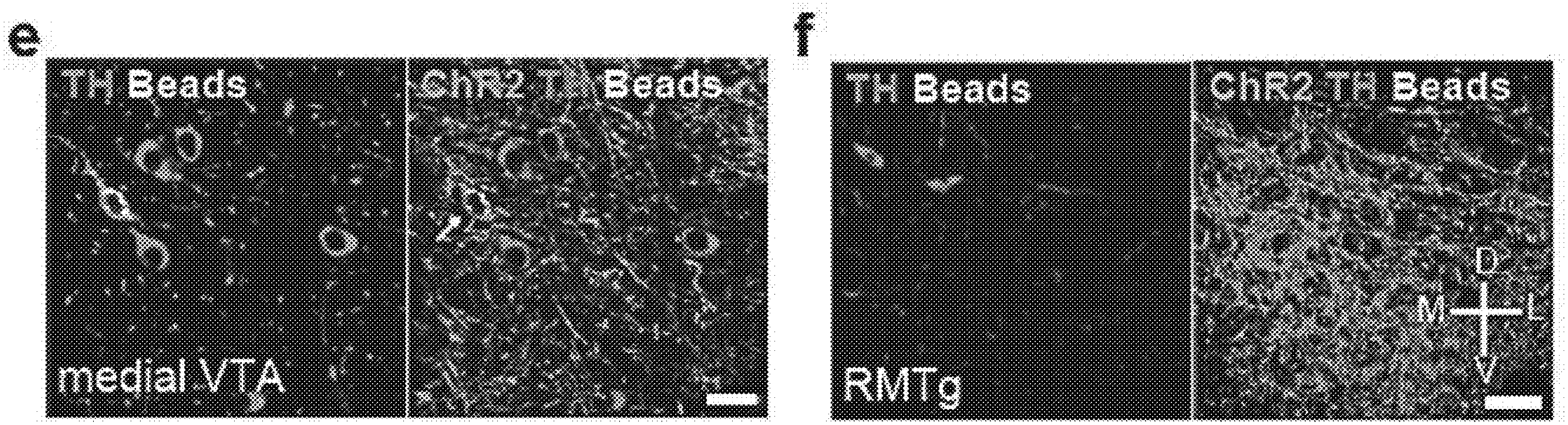

e
TH Beads
medial VTA
ChR2 TH Beads
f
TH Beads
RMTg
ChR2 TH Beads
D
M
L
V

FIGURE 19A

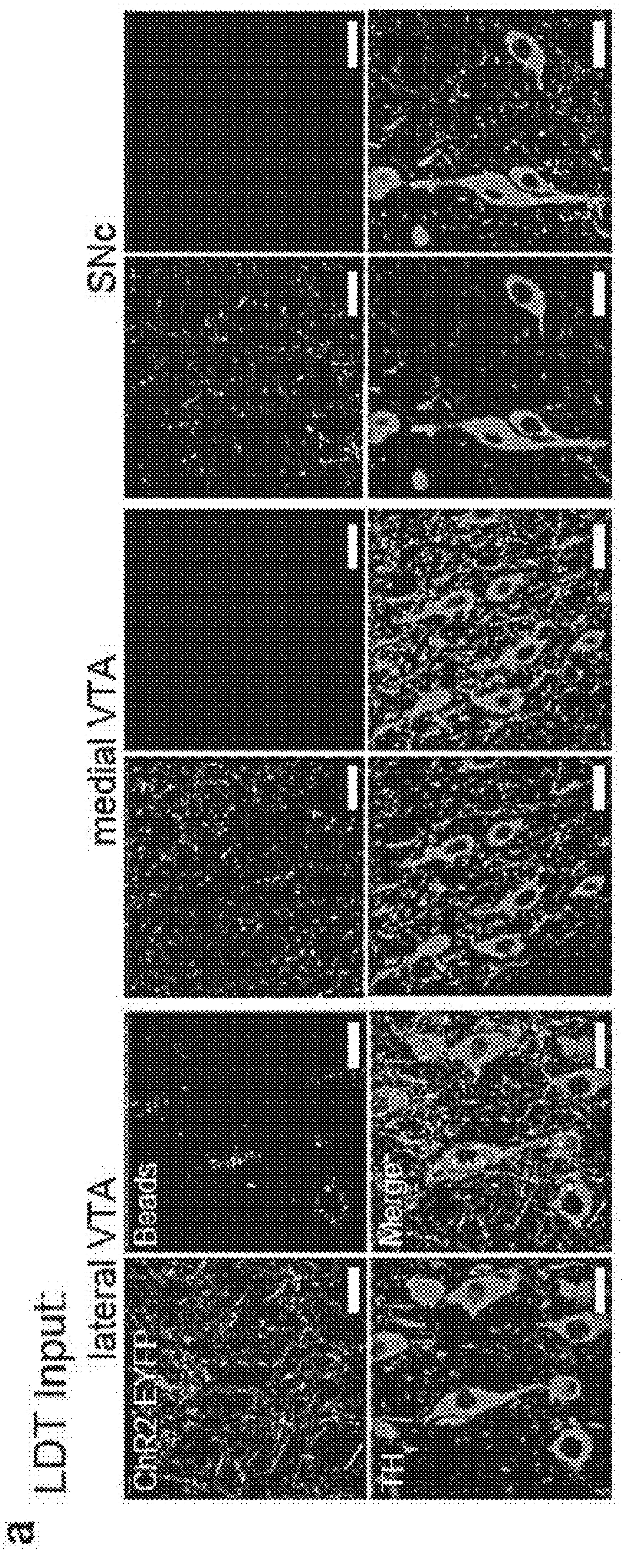

a
LDT Input:
lateral VTA
medial VTA
SNc
ChR2-EYFP
Beads
TH
Merge

FIGURE 19B

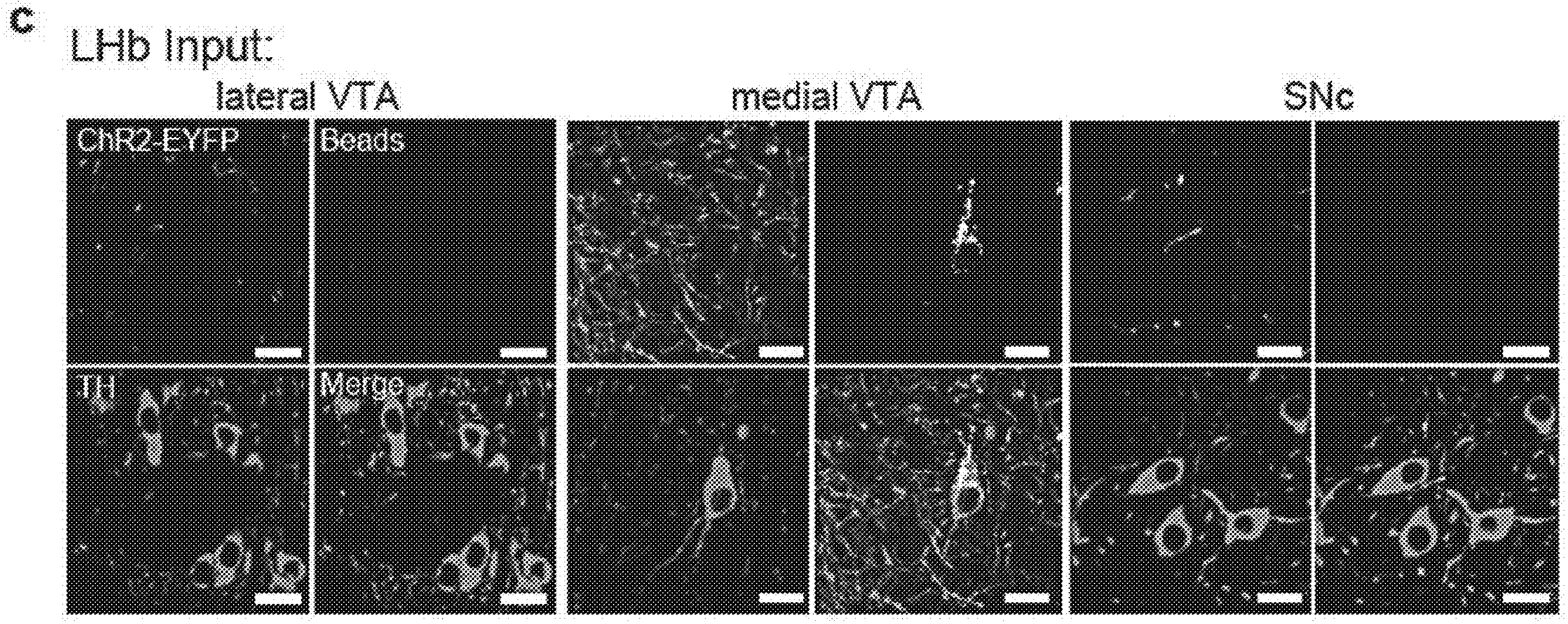

C
LHb Input:
lateral VTA
medial VTA
SNc
ChR2-EYFP
Beads
TH
Merge

FIGURE 19C

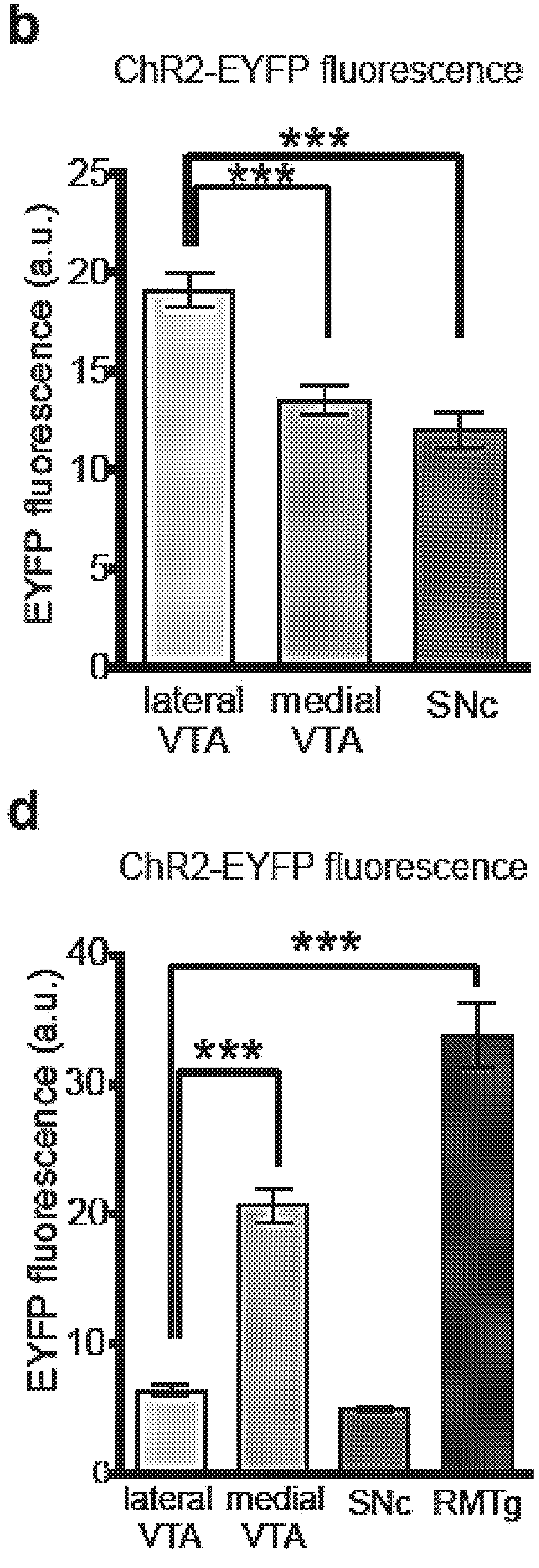

b
ChR2-EYFP fluorescence
***
***
EYFP fluorescence (a.u.)
25
20
15
10
5
0
lateral VTA
medial VTA
SNc
d
ChR2-EYFP fluorescence
***
***
EYFP fluorescence (a.u.)
40
30
20
10
0
lateral VTA
medial VTA
SNc
RMTg

FIGURE 20A

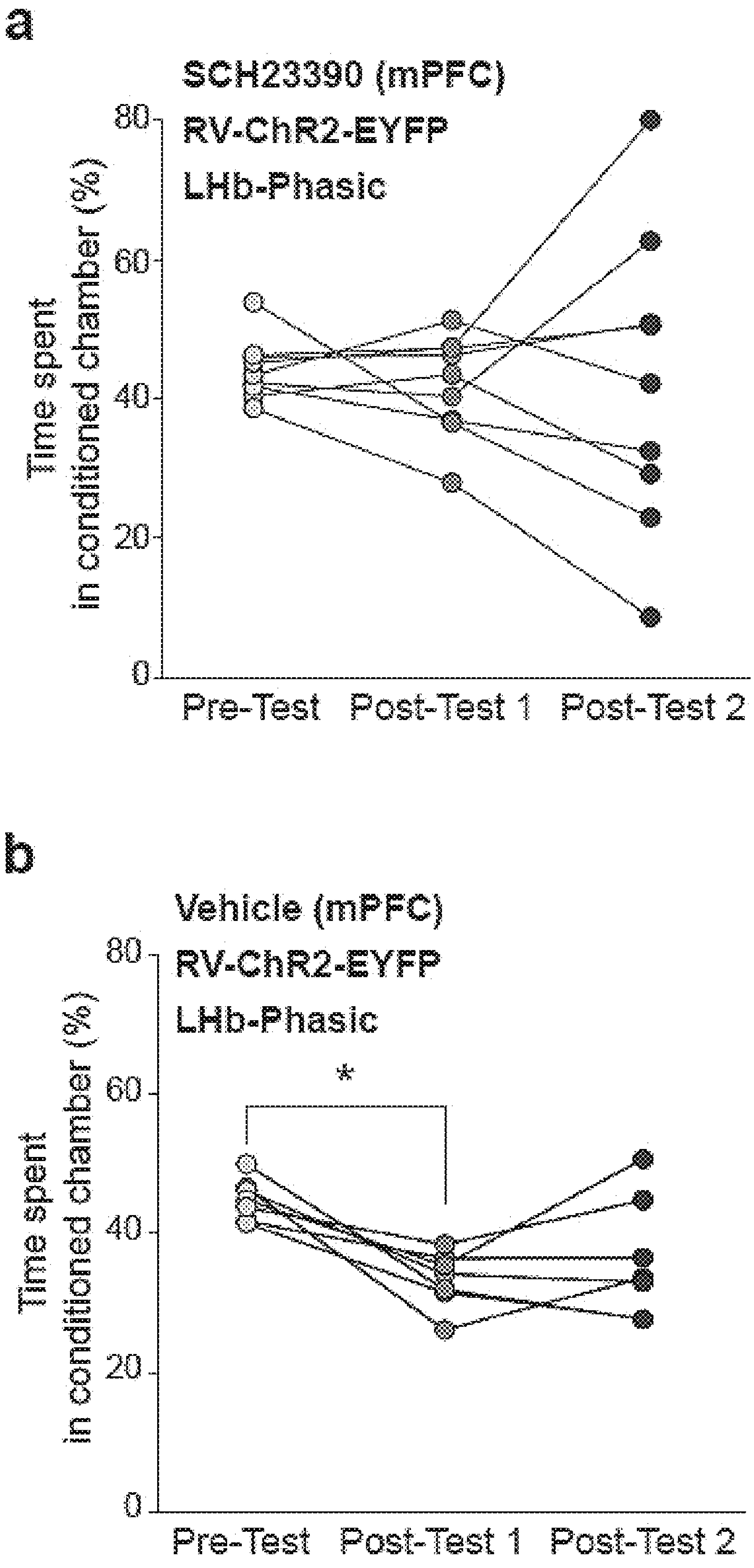
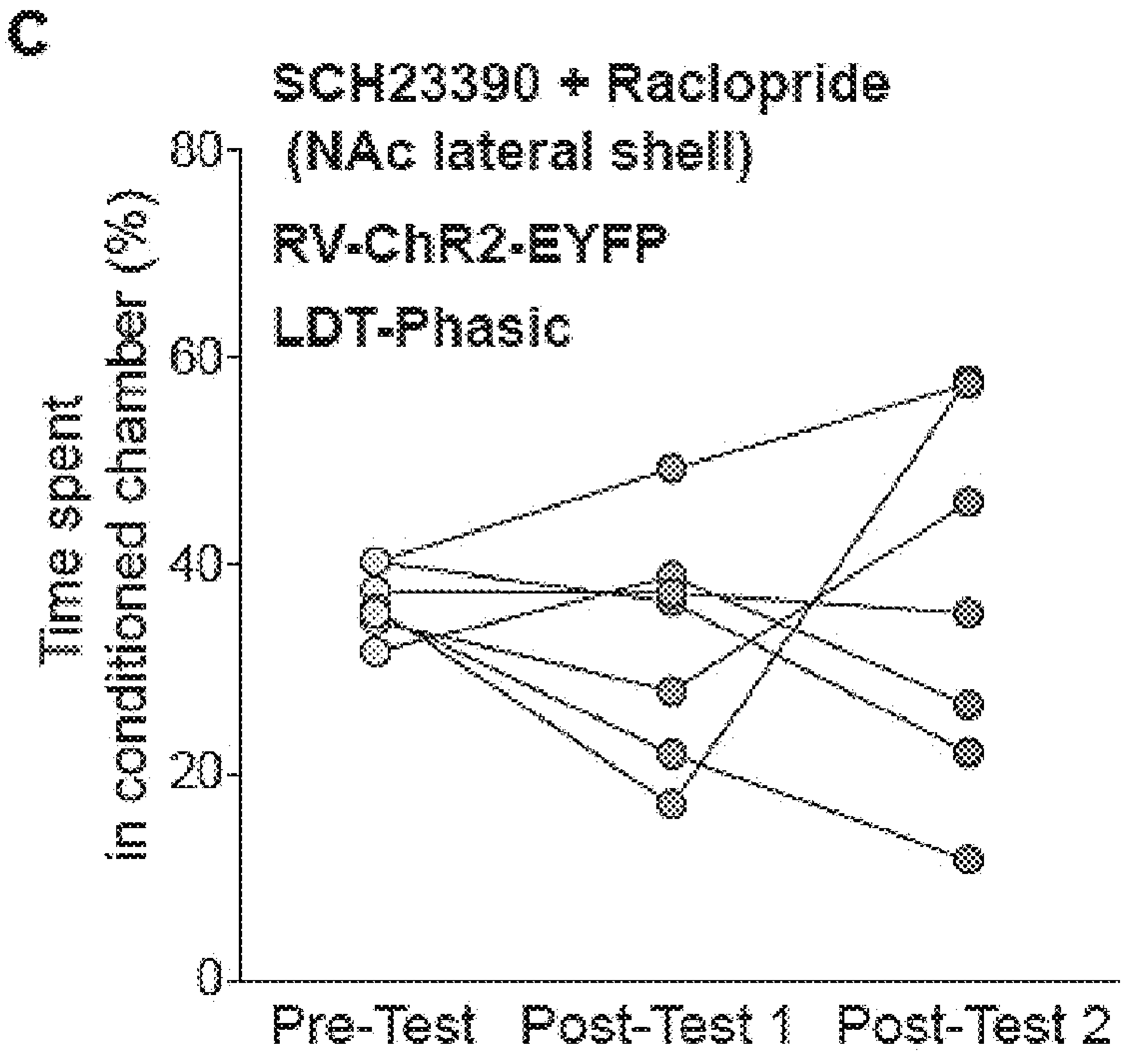

a
SCH23390 (mPFC)
RV-ChR2-EYFP
LHb-Phasic
Time spent in conditioned chamber (%)
80
60
40
20
0
Pre-Test
Post-Test 1
Post-Test 2
b
Vehicle (mPFC)
RV-ChR2-EYFP
LHb-Phasic
*
Time spent in conditioned chamber (%)
80
60
40
20
0
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 20B


c
SCH23390 + Raclopride
(NAc lateral shell)
RV-ChR2-EYFP
LDT-Phasic
Time spent
in conditioned chamber (%)
0
20
40
60
80
Pre-Test
Post-Test 1
Post-Test 2

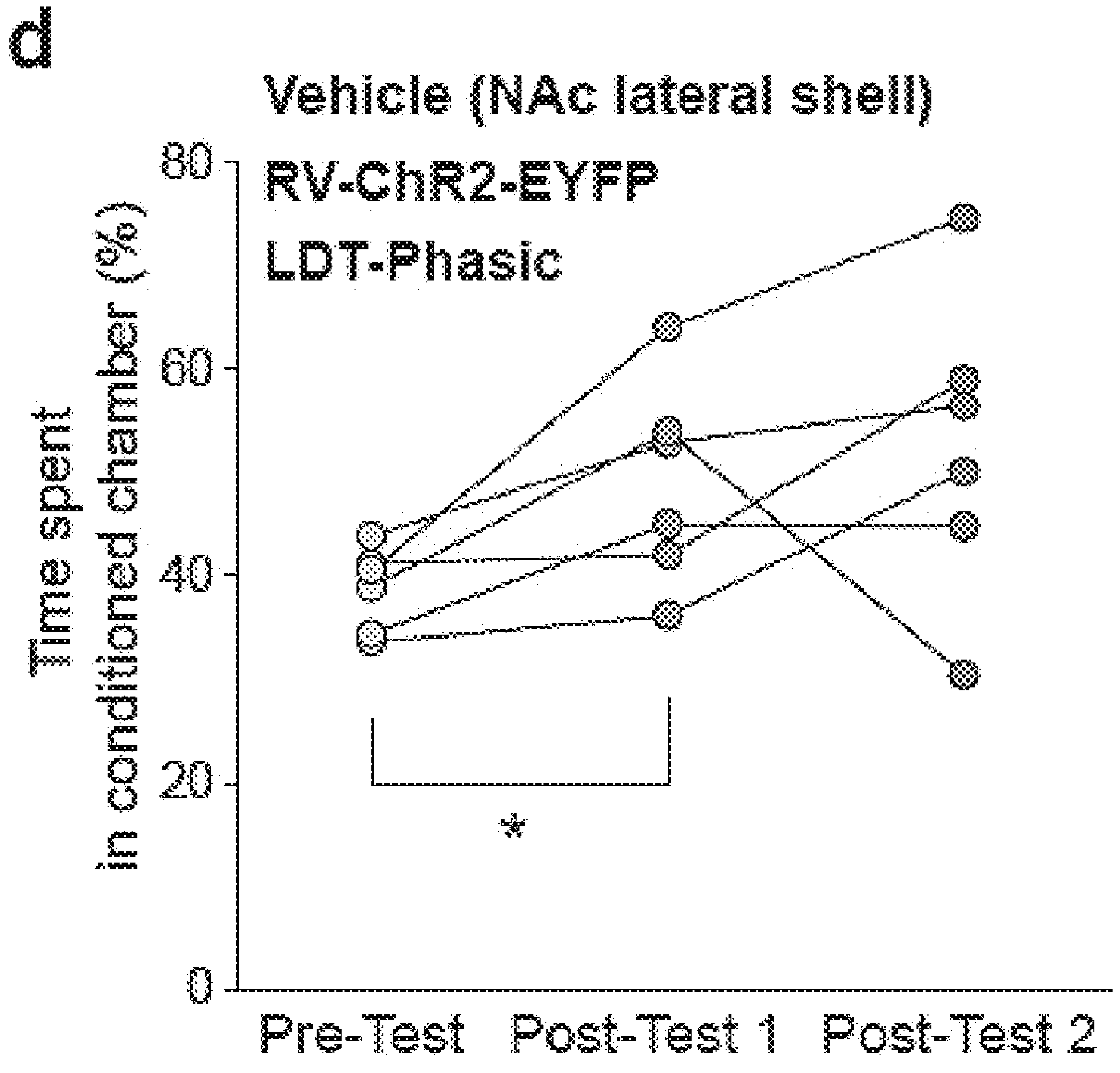

d
Vehicle (NAc lateral shell)
RV-ChR2-EYFP
LDT-Phasic
Time spent
in conditioned chamber (%)
0
20
40
60
80
*
Pre-Test
Post-Test 1
Post-Test 2

FIGURE 21A

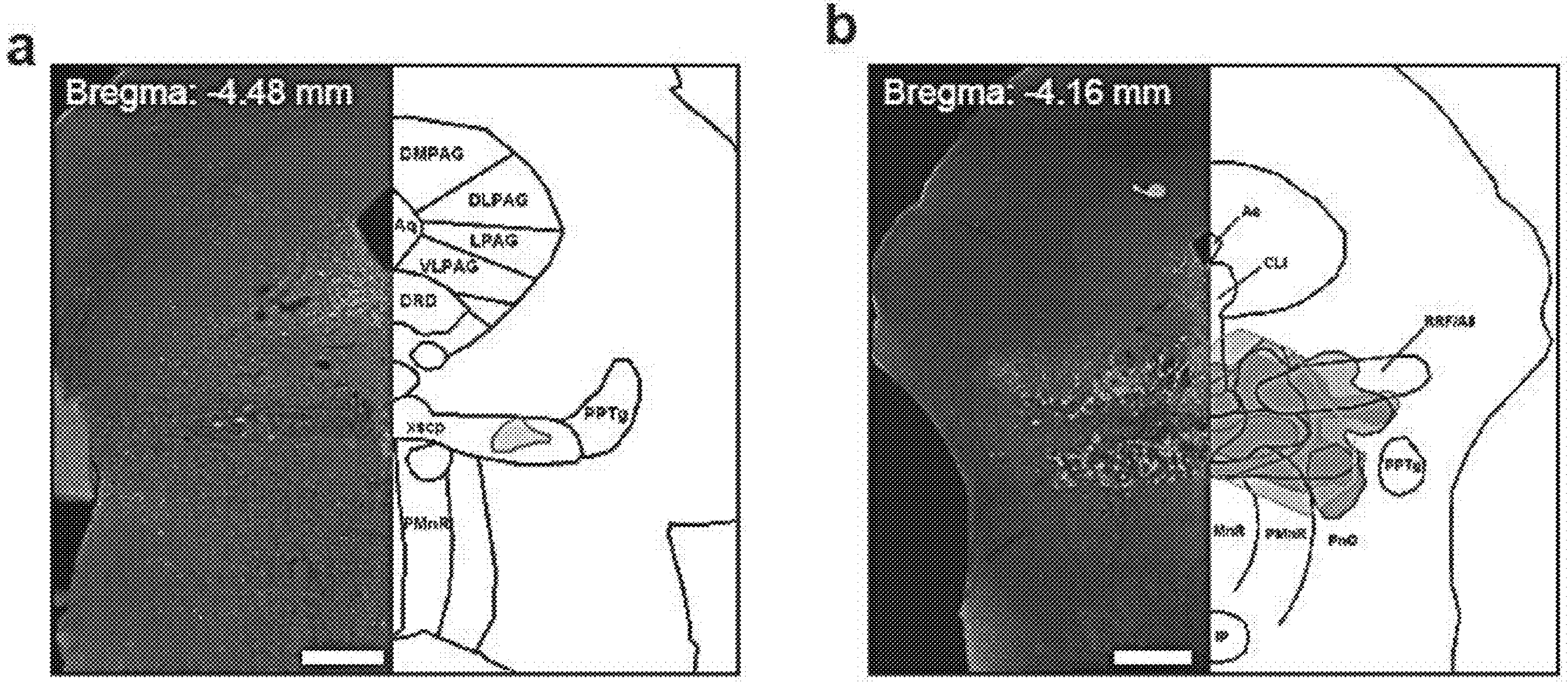

a
Bregma: -4.48 mm
DMPAG
DLPAG
Aq
LPAG
VLPAG
DRD
xscp
PPTg
PMnR
b
Bregma: -4.16 mm
Aq
CLi
RRF/A8
PPTg
MnR
PMnR
PnO
IP

FIGURE 21B

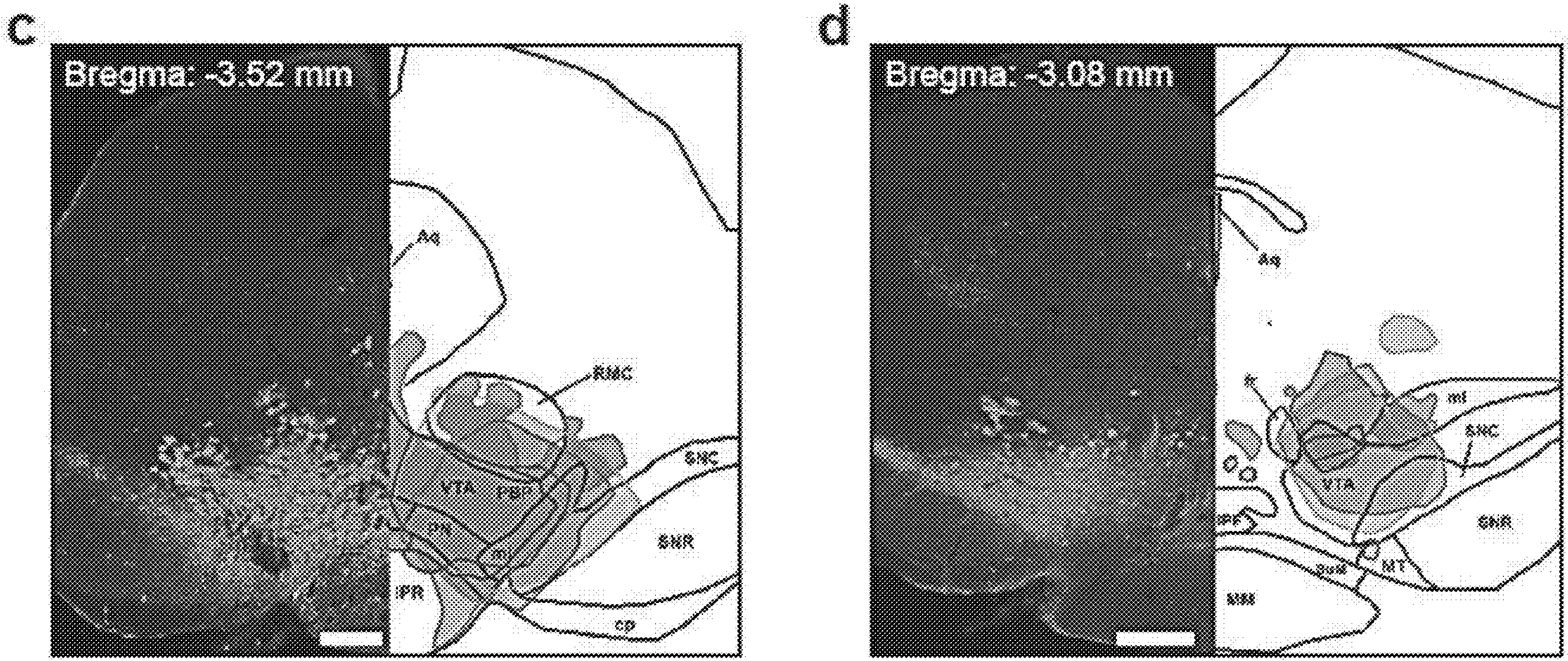

c
Bregma: -3.52 mm
Aq
RMC
VTA
PBP
PN
ml
SNC
SNR
IPR
cp
d
Bregma: -3.08 mm
Aq
fr
ml
SNC
VTA
SNR
IPF
SuM
MT
MM

FIGURE 21C

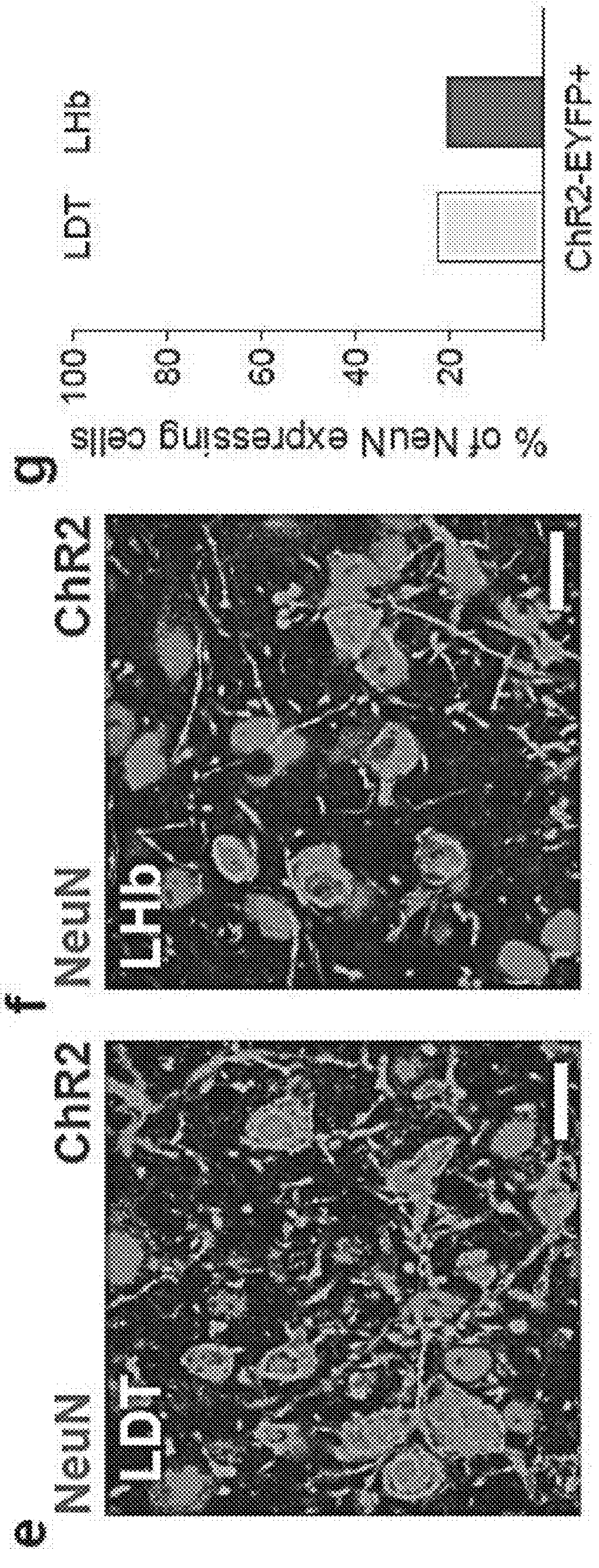

e
NeuN
ChR2
LDT
f
NeuN
ChR2
LHb
g
% of NeuN expressing cells
100
80
60
40
20
LDT
LHb
ChR2-EYFP+

FIGURE 22A

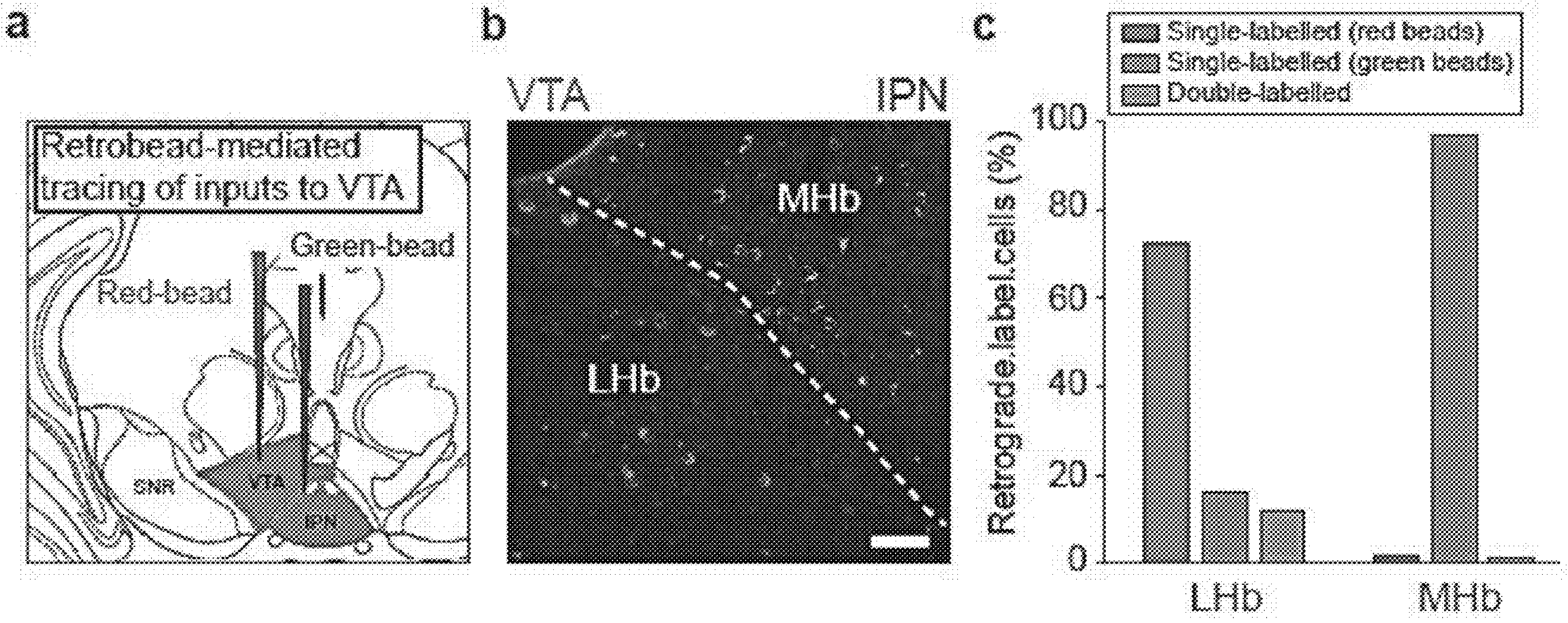
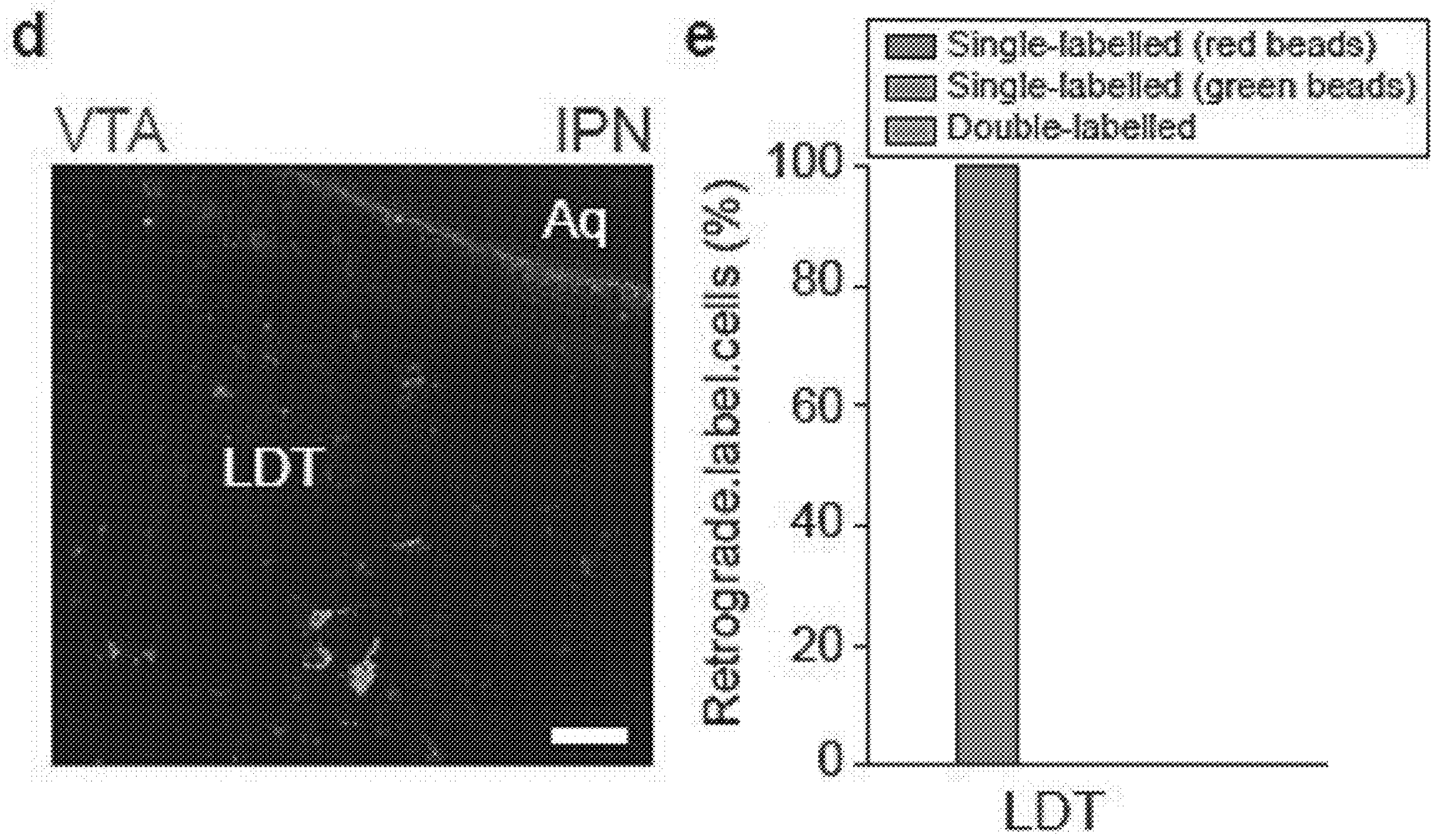

a
Retrobead-mediated tracing of inputs to VTA
Red-bead
Green-bead
SNR
VTA
IPN
b
VTA
IPN
MHb
LHb
c
Single-labelled (red beads)
Single-labelled (green beads)
Double-labelled
Retrograde.label.cells (%)
100
80
60
40
20
0
LHb
MHb

FIGURE 22B


d
VTA
IPN
Aq
LDT
e
Single-labelled (red beads)
Single-labelled (green beads)
Double-labelled
Retrograde.label.cells (%)
100
80
60
40
20
0
LDT

OPTOGENETIC CONTROL OF INPUTS TO THE VENTRAL TEGMENTAL AREA

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 61/789,486, filed Mar. 15, 2013, and 61/890,000, filed Oct. 11, 2013, which applications are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with Government support under contract MH086403 awarded by the National Institutes of Health. The Government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

[0003] A Sequence Listing is provided herewith as a text file, "STAN-1019 SeqList_ST25.txt" created on Mar. 12, 2014 and having a size of 50 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

[0004] The functional roles of VTA dopamine (DA) neurons have received great attention because they are the primary source of DA in target structures such as the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), which play important roles in a broad range of motivated behaviors and neuropsychiatric disorders[1-3]. Although DA neuron activity often correlates with a reward prediction error (i.e. the difference between expected and actual rewards) these cells also can signal aversion, saliency, uncertainty and novelty. They are heterogeneous in their anatomical location, targets to which they project, electrophysiological properties and several molecular features. In addition, the VTA receives both excitatory and inhibitory input from distributed brain areas. Thus different subpopulations of VTA DA and GABAergic neurons may subserve different functions but little is known about the afferent control of their activity and the circuits in which they are embedded.

SUMMARY

[0005] The present disclosure provides a method of inducing or modulating reward- or aversive-related behaviors in animals using light-responsive opsins. The present disclosure provides methods of identifying or screening compounds that may be used to treating mental disorders, or are relevant to disrupt or improve reward- or aversive related behaviors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. **1** depicts retrograde labeling of DA projection neurons and anterograde labeling of LDT or LHb fibers.

[0007] FIG. **2** depicts conditioned place preference/aversion following stimulation of LDT and LHb inputs to VTA.

[0008] FIG. **3** depicts various measures of connectivity and related quantification of AAV-ChR2 injected LDT and fluorescent retrobead injected target structures of VTA DA neurons.

[0009] FIG. **4** depicts various measures of connectivity and related quantification of AAV-ChR2 injected LHb and fluorescent retrobead injected NAc lateral and medial shells or mPFC.

[0010] FIG. **5** depicts neuronal connectivity between VTA neurons and mPFC and NAc lateral shell neurons and the effects of DA receptor antagonists on conditioned place preference/avoidance.

[0011] FIGS. **6**A-H provide amino acid sequences of various light-responsive proteins.

[0012] FIG. **7** depicts inputs to the VTA.

[0013] FIG. **8** depicts the mainly glutamatergic LDT and LHb neurons that project to the VTA.

[0014] FIG. **9** depicts the LDT and LHb terminals in the VTA.

[0015] FIG. **10** depicts a schematic of the genome of the recombinant rabies virus expressing ChR2-EYFP, cells expressing the ChR2-EYFP, and control preference/avoidance for chambers of a preference/aversion assay.

[0016] FIGS. **11**A-C depict various behavioral assays of mice injected with RV-ChR2-EYFP or RV-EGFP.

[0017] FIGS. **12**A-B depict assays of locomotor activity and anxiety related to stimulation of LDT and LHb.

[0018] FIGS. **13**A-B depict assays of locomotor activity and anxiety related to stimulation of LDT and LHb.

[0019] FIGS. **14**A-D depict c-fos expression in VTA subpopulations induced by optical activation of LDT and LHb neurons.

[0020] FIG. **15** depicts optical stimulation of axon terminals from LDT and LHb in VTA.

[0021] FIG. **16** depicts behavioral assays of mice in which AAV-ChR2 was injected into the LDT or Lhb and LDT or LHb axon terminals in the VTA were stimulated.

[0022] FIGS. **17**A-E depict retrograde labeling of various brain areas with two different fluorophores and related quantification.

[0023] FIGS. **18**A-B depict injection of retrobeads and AAV-ChR2 for studies of synaptic connectivity.

[0024] FIGS. **19**A-C depict fluorescent intensity of LDT and LHb terminals expressing ChR2-EYFP in VTA subregions.

[0025] FIGS. **20**A-B depict behavioral assays of mice in which dopamine receptor antagonists were injected into the mPFC or NAc lateral shell and LHb or LDT neurons that project to VTA were optically stimulated.

[0026] FIGS. **21**A-C depict expression of ChR2-EYFP in the midbrain, the LDT and the LHb following RV-ChR2 injections into the VTA.

[0027] FIGS. **22**A-B depict retrobead injections into the VTA or IPN and quantification of connectivity.

DEFINITIONS

[0028] An "individual" can be a mammal, including a human Mammals include, but are not limited to, ungulates, canines, felines, bovines, ovines, non-human primates, lagomorphs, and rodents (e.g., mice and rats). In one aspect, an individual is a human. In another aspect, an individual is a non-human mammal.

[0029] Amino acid substitutions in a native protein sequence may be "conservative" or "non-conservative" and such substituted amino acid residues may or may not be one encoded by the genetic code. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a chemically similar side chain (i.e., replacing an amino acid possessing a basic side chain with another amino acid with a basic side chain). A "non-conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a chemically different side chain (i.e., replacing an amino acid having a basic side chain with an amino acid having an aromatic side chain). The standard twenty amino acid "alphabet" is divided into chemical families based on chemical properties of their side chains. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and side chains having aromatic groups (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0030] As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0031] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of individuals.

[0032] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0033] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0035] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a light-responsive protein" includes a plurality of such proteins and reference to "the reward- or aversive-related behavior" includes reference to one or more reward- or aversive-related behaviors and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0036] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0037] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0038] The present disclosure provides a method of inducing or modulating reward- or aversive-related behaviors in animals using light-responsive opsins. The present disclosure provides methods of identifying or screening compounds that may be used to treating mental disorders, or are relevant to disrupt or improve reward- or aversive related behaviors.

Modulating a Reward- or Aversive-Related Behavior

[0039] The present disclosure provides a method of inducing or modulating reward- or aversive-related behaviors in animals using light-responsive opsins. The method generally involves expressing a light-responsive opsin protein in one or both of two main inputs to the ventral tegmental area (VTA) from the laterodorsal tegmentum (LDT) and the lateral habenula (LHb); and exposing the inputs to light of a wavelength to which the light-responsive opsin protein responds.

[0040] In some cases, an excitatory light-activated opsin protein is expressed in an LHb neuron projecting to the VTA. In some cases, an inhibitory light-activated opsin protein is expressed in an LHb neuron projecting to the VTA. In some cases, an excitatory light-activated opsin protein is expressed in an LDT neuron projecting to the VTA. In some cases, an inhibitory light-activated opsin protein is expressed in an LDT neuron projecting to the VTA.

[0041] For example, an excitatory light-responsive polypeptide is expressed in an LHb neuron projecting to the VTA or in an LDT neuron projecting to the VTA, where the excitatory light-responsive polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:5, 6, 7, 8,9, 10, and 11. In some cases, the excitatory light-responsive polypeptide comprises both ER export and membrane trafficking signals. For example, in some cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:5, an ER export signal, and a membrane trafficking signal. In other cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:5, a membrane trafficking signal, and a ER export signal. In some cases, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some cases, the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16). In some cases, the ER export signal comprises the sequence FCYENEV (SEQ ID NO:17).

[0042] For example, an inhibitory light-responsive polypeptide is expressed in an LHb neuron projecting to the VTA or in an LDT neuron projecting to the VTA, where the inhibitory light-responsive polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:1, 2, 3, 4, 12, 13, 14, and 15. In some cases, the excitatory light-responsive polypeptide comprises both ER export and membrane trafficking signals. For example, in some cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In other cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, a membrane trafficking signal, and a ER export signal. In some cases, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some cases, the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16). In some cases, the ER export signal comprises the sequence FCYENEV (SEQ ID NO:17).

Light-Responsive Opsin Proteins

[0043] Provided herein are optogenetic-based methods for selectively hyperpolarizing or depolarizing the neurons involved in reward- or aversive-related behaviors, using light-responsive opsin proteins to effectively modulate reward- or aversive-related behaviors, e.g., in individuals afflicted with disorders related to reward- or aversive-related behaviors. Optogenetics refers to the combination of genetic and optical methods used to control specific events in targeted cells of living tissue, even within freely moving mammals and other animals, with the temporal precision (millisecond-timescale) needed to keep pace with functioning intact biological systems. Optogenetics requires the introduction of fast light-responsive channel or pump proteins to the plasma membranes of target neuronal cells that allow temporally precise manipulation of neuronal membrane potential while maintaining cell-type resolution through the use of specific targeting mechanisms. Any microbial opsin that can be used to promote neural cell membrane hyperpolarization or depolarization in response to light may be used.

[0044] For example, the Halorhodopsin family of light-responsive chloride pumps (e.g., NpHR, NpHR2.0, NpHR3.0, NpHR3.1) and the GtR3 proton pump can be used to promote neural cell membrane hyperpolarization in response to light. As another example, eArch (a proton pump) can be used to promote neural cell membrane hyperpolarization in response to light. As another example, an ArchT opsin protein or a Mac opsin protein can be used to promote neural cell membrane hyperpolarization in response to light.

[0045] Additionally, members of the Channelrhodopsin family of light-responsive cation channel proteins (e.g., ChR2, SFOs, SSFOs, C1V1s) can be used to promote neural cell membrane depolarization or depolarization-induced synaptic depletion in response to a light stimulus.

Enhanced Intracellular Transport Amino Acid Motifs

[0046] The present disclosure provides for the modification of light-responsive opsin proteins expressed in a cell by the addition of one or more amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells. Light-responsive opsin proteins having components derived from evolutionarily simpler organisms may not be expressed or tolerated by mammalian cells or may exhibit impaired subcellular localization when expressed at high levels in mammalian cells. Consequently, in some embodiments, the light-responsive opsin proteins expressed in a cell can be fused to one or more amino acid sequence motifs selected from the group consisting of a signal peptide, an endoplasmic reticulum (ER) export signal, a membrane trafficking signal, and/or an N-terminal golgi export signal. The one or more amino acid sequence motifs which enhance light-responsive protein transport to the plasma membranes of mammalian cells can be fused to the N-terminus, the C-terminus, or to both the N- and C-terminal ends of the light-responsive protein. Optionally, the light-responsive protein and the one or more amino acid sequence motifs may be separated by a linker. In some embodiments, the light-responsive protein can be modified by the addition of a trafficking signal (ts) which enhances transport of the protein to the cell plasma membrane. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16).

[0047] Trafficking sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such a trafficking sequence of human inward rectifier potassium channel Kir2.1 (e.g., KSRITSEGEYIPLDQIDINV (SEQ ID NO:16)).

[0048] A trafficking sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[0049] Signal sequences that are suitable for use can comprise an amino acid sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, amino acid sequence identity to an amino acid sequence such as one of the following:

[0050] 1) the signal peptide of hChR2 (e.g., MDYGGALSAVGRELLFVTNPVVVNGS (SEQ ID NO:18))

[0051] 2) the β2 subunit signal peptide of the neuronal nicotinic acetylcholine receptor (e.g., MAGHSNSMALFSFSLLWLCSGVLGTEF (SEQ ID NO:19));

[0052] 3) a nicotinic acetylcholine receptor signal sequence (e.g., MGLRALMLWLLAAAGLVRESLQG (SEQ ID NO:20)); and

[0053] 4) a nicotinic acetylcholine receptor signal sequence (e.g., MRGTPLLLVVSLFSLLQD (SEQ ID NO:21)).

[0054] A signal sequence can have a length of from about 10 amino acids to about 50 amino acids, e.g., from about 10 amino acids to about 20 amino acids, from about 20 amino acids to about 30 amino acids, from about 30 amino acids to about 40 amino acids, or from about 40 amino acids to about 50 amino acids.

[0055] Endoplasmic reticulum (ER) export sequences that are suitable for use in a modified opsin of the present disclosure include, e.g., VXXSL (SEQ ID NO:22) (where X is any amino acid) (e.g., VKESL (SEQ ID NO:23); VLGSL (SEQ ID NO:24); etc.); NANSFCYENEVALTSK (SEQ ID NO:25); FXYENE (SEQ ID NO:26) (where X is any amino acid), e.g., FCYENEV (SEQ ID NO:17); and the like. An ER export sequence can have a length of from about 5 amino acids to about 25 amino acids, e.g., from about 5 amino acids to about 10 amino acids, from about 10 amino acids to about 15 amino acids, from about 15 amino acids to about 20 amino acids, or from about 20 amino acids to about 25 amino acids.

[0056] In some embodiments, the signal peptide sequence in the protein can be deleted or substituted with a signal peptide sequence from a different protein.

Inhibitory Light-Responsive Opsin Proteins

[0057] In some embodiments, a subject method for modulating a behavioral feature involves use of an inhibitory light-responsive opsin protein. Inhibitory light-responsive opsin proteins include polypeptides having sequence similarity (e.g., at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity) to one of SEQ ID NOs:1, 2, 3, 4, 12, 13, 14, and 15 (FIGS. **6**A-H).

Light-Responsive Chloride Pumps

[0058] In some aspects of the methods provided herein, one or more members of the Halorhodopsin family of light-responsive chloride pumps are expressed on the plasma membranes of one or both of two main inputs to the VTA from LDT and the lateral LHb.

[0059] In some aspects, said one or more light-responsive chloride pump proteins expressed on the plasma membranes of the neurons described above can be derived from *Natronomonas pharaonis*. In some embodiments, the light-responsive chloride pump proteins can be responsive to amber light as well as red light and can mediate a hyperpolarizing current in the neuron when the light-responsive chloride pump proteins are illuminated with amber or red light. The wavelength of light which can activate the light-responsive chloride pumps can be between about 580 and 630 nm. In some embodiments, the light can be at a wavelength of about 589 nm or the light can have a wavelength greater than about 630 nm (e.g. less than about 740 nm). In another embodiment, the light has a wavelength of around 630 nm. In some embodiments, the light-responsive chloride pump protein can hyperpolarize a neural membrane for at least about 90 minutes when exposed to a continuous pulse of light. In some embodiments, the light-responsive chloride pump protein can comprise an amino acid sequence at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1. Additionally, the light-responsive chloride pump protein can comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive protein to regulate the polarization state of the plasma membrane of the cell. In some embodiments, the light-responsive chloride pump protein contains one or more conservative amino acid substitutions. In some embodiments, the light-responsive protein contains one or more non-conservative amino acid substitutions. The light-responsive protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

[0060] Additionally, in other aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and an endoplasmic reticulum (ER) export signal. This ER export signal can be fused to the C-terminus of the core amino acid sequence or can be fused to the N-terminus of the core amino acid sequence. In some embodiments, the ER export signal is linked to the core amino acid sequence by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the ER export signal can comprise the amino acid sequence FXYENE (SEQ ID NO:26), where X can be any amino acid. In another embodiment, the ER export signal can comprise the amino acid sequence VXXSL (SEQ ID NO:22), where X can be any amino acid. In some embodiments, the ER export signal can comprise the amino acid sequence FCYENEV (SEQ ID NO:17).

[0061] Endoplasmic reticulum (ER) export sequences that are suitable for use in a modified opsin of the present disclosure include, e.g., VXXSL (SEQ ID NO:22) (where X is any amino acid) (e.g., VKESL (SEQ ID NO:23); VLGSL (SEQ ID NO:24); etc.); NANSFCYENEVALTSK (SEQ ID NO:25); FXYENE (SEQ ID NO:26) (where X is any amino acid), e.g., FCYENEV (SEQ ID NO:17); and the like. An ER export sequence can have a length of from about 5 amino acids to about 25 amino acids, e.g., from about 5 amino acids to about 10 amino acids, from about 10 amino acids to about 15 amino acids, from about 15 amino acids to about 20 amino acids, or from about 20 amino acids to about 25 amino acids.

[0062] In other aspects, the light-responsive chloride pump proteins described herein can comprise a light-responsive protein expressed on the cell membrane, wherein the protein comprises a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and a trafficking signal (e.g., which can enhance transport of the light-responsive chloride pump protein to the plasma membrane). The trafficking signal may be fused to the C-terminus of the core amino acid sequence or may be fused to the N-terminus of the core amino acid sequence. In some embodiments, the trafficking signal can be linked to the core amino acid sequence by a linker which can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments, the trafficking signal can be derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In other embodiments, the trafficking signal can comprise the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16).

[0063] In some aspects, the light-responsive chloride pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of an ER export signal, a signal peptide, and a membrane trafficking signal. In some embodiments, the light-responsive chloride pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal can be linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker can also further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal can be more C-terminally located than the trafficking signal. In other embodiments the trafficking signal is more C-terminally located than the ER Export signal. In some embodiments, the signal peptide comprises the amino acid sequence MTETLPPVTESAVALQAE (SEQ ID NO:27). In another embodiment, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:2.

[0064] Moreover, in other aspects, the light-responsive chloride pump proteins can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO: 1, wherein the N-terminal signal peptide of SEQ ID NO:1 is deleted or substituted. In some embodiments, other signal peptides (such as signal peptides from other opsins) can be used. The light-responsive protein can further comprise an ER transport signal and/or a membrane trafficking signal described herein. In some embodiments, the light-responsive chloride pump protein comprises an amino acid sequence at least 95% identical to SEQ ID NO:3.

[0065] In some embodiments, the light-responsive opsin protein is a NpHR opsin protein comprising an amino acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the sequence shown in SEQ ID NO:1. In some embodiments, the NpHR opsin protein further comprises an endoplasmic reticulum (ER) export signal and/or a membrane trafficking signal. For example, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 and an endoplasmic reticulum (ER) export signal. In some embodiments, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 is linked to the ER export signal through a linker. In some embodiments, the ER export signal comprises the amino acid sequence FXYENE (SEQ ID NO:26), where X can be any amino acid. In another embodiment, the ER export signal comprises the amino acid sequence VXXSL (SEQ ID NO:22), where X can be any amino acid. In some embodiments, the ER export signal comprises the amino acid sequence FCYENEV (SEQ ID NO:17). In some embodiments, the NpHR opsin protein comprises an amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the ER export signal, and the membrane trafficking signal. In other embodiments, the NpHR opsin protein comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, the membrane trafficking signal, and the ER export signal. In some embodiments, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some embodiments, the membrane trafficking signal comprises the amino acid sequence K S R I T S E G E Y I P L D Q I D I N V (SEQ ID NO:16). In some embodiments, the membrane trafficking signal is linked to the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1 by a linker. In some embodiments, the membrane trafficking signal is linked to the ER export signal through a linker. The linker may comprise any of 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein (GFP), or a cyan fluorescent protein. In some embodiments, the light-responsive opsin protein further comprises an N-terminal signal peptide. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:2. In some embodiments, the light-responsive opsin protein comprises the amino acid sequence of SEQ ID NO:3.

[0066] Also provided herein are polynucleotides encoding any of the light-responsive chloride ion pump proteins described herein, such as a light-responsive protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In another embodiment, the polynucleotides comprise a sequence which encodes an amino acid at least 95% identical to SEQ ID NO:2 and SEQ ID NO:3. The polynucleotides may be in an expression vector (such as, but not limited to, a viral vector described herein). The polynucleotides may be used for expression of the light-responsive chloride ion pump proteins.

[0067] Further disclosure related to light-responsive chloride pump proteins can be found in U.S. Patent Application Publication Nos: 2009/0093403 and 2010/0145418 as well as in International Patent Application No: PCT/US2011/028893, the disclosures of each of which are hereby incorporated by reference in their entireties.

Light-Responsive Proton Pumps

[0068] In some aspects of the methods provided herein, one or more light-responsive proton pumps are expressed on the plasma membranes of one or both of two main inputs to the VTA from LDT and the lateral LHb. In some embodiments, the light-responsive proton pump protein can be responsive to blue light and can be derived from *Guillardia theta*, wherein the proton pump protein can be capable of mediating a hyperpolarizing current in the cell when the cell is illuminated with blue light. The light can have a wavelength between about 450 and about 495 nm or can have a wavelength of about 490 nm. In another embodiment, the light-responsive proton pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The light-responsive proton pump protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive proton pump protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive proton pump protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

[0069] In other aspects of the methods disclosed herein, the light-responsive proton pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of a signal peptide, an ER export signal, and a membrane trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal ER export signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises a C-terminal ER Export signal and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal are linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal is more C-terminally located than the trafficking signal. In some embodiments the trafficking signal is more C-terminally located than the ER Export signal.

[0070] Also provided herein are isolated polynucleotides encoding any of the light-responsive proton pump proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. Also provided herein are expression vectors (such as a viral vector described herein) comprising a polynucleotide encoding the proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:4. The polynucleotides may be used for expression of the light-responsive protein in one or both of two main inputs to the VTA from LDT and the lateral LHb.

[0071] Further disclosure related to light-responsive proton pump proteins can be found in International Patent Application No. PCT/US2011/028893, the disclosure of which is hereby incorporated by reference in its entirety.

[0072] In some embodiments, the light-responsive proton pump protein can be responsive to green or yellow light and can be derived from *Halorubrum sodomense*, wherein the proton pump protein can be capable of mediating a hyperpolarizing current in the cell when the cell is illuminated with green or yellow light. The light can have a wavelength between about 560 and about 570 nm or can have a wavelength of about 566 nm. In another embodiment, the light-responsive proton pump protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:12. The light-responsive proton pump protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive proton pump protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive proton pump protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to hyperpolarize the plasma membrane of a neuronal cell in response to light.

[0073] In other aspects of the methods disclosed herein, the light-responsive proton pump protein can comprise a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:12 and at least one (such as one, two, three, or more) amino acid sequence motifs which enhance transport to the plasma membranes of mammalian cells selected from the group consisting of a signal peptide, an ER export signal, and a membrane trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal ER export signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises an N-terminal signal peptide, a C-terminal ER Export signal, and a C-terminal trafficking signal. In some embodiments, the light-responsive proton pump protein comprises a C-terminal ER Export signal and a C-terminal trafficking signal. In some embodiments, the C-terminal ER Export signal and the C-terminal trafficking signal are linked by a linker. The linker can comprise any of about 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, or 500 amino acids in length. The linker may further comprise a fluorescent protein, for example, but not limited to, a yellow fluorescent protein, a red fluorescent protein, a green fluorescent protein, or a cyan fluorescent protein. In some embodiments the ER Export signal is more C-terminally located than the trafficking signal. In some embodiments the trafficking signal is more C-terminally located than the ER Export signal.

[0074] Also provided herein are isolated polynucleotides encoding any of the light-responsive proton pump proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:12. Also provided herein are expression vectors (such as a viral vector described herein) comprising a polynucleotide encoding the proteins described herein, such as a light-responsive proton pump protein comprising a core amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:12. The polynucleotides may be used for expression of the light-responsive protein in neural cells (e.g. one or both of two main inputs to the VTA from LDT and the lateral LHb).

Excitatory Light-Responsive Opsin Proteins

[0075] In some embodiments, a subject method for modulating a behavioral feature involves use of an excitatory light-responsive opsin protein. Excitatory light-responsive opsin proteins include polypeptides having sequence similarity (e.g., at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity) to one of SEQ ID NOs:5, 6, 7, 8, 9, 10, and 11 (FIGS. **6**A-H).

Light-Responsive Cation Channel Proteins

[0076] In some aspects of the methods provided herein, one or more light-responsive cation channels can be expressed on the plasma membranes of one or both of two main inputs to the VTA from LDT and the lateral LHb.

[0077] In some aspects, the light-responsive cation channel protein can be derived from *Chlamydomonas reinhardtii*, wherein the cation channel protein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In another embodiment, the light-responsive cation channel protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:5. The light used to activate the light-responsive cation channel protein derived from *Chlamydomonas reinhardtii* can have a wavelength between about 460 and about 495 nm or can have a wavelength of about 480 nm. Additionally, the light can have an intensity of at least about 100 Hz. In some embodiments, activation of the light-responsive cation channel derived from *Chlamydomonas reinhardtii* with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the light-responsive cation channel. The light-responsive cation channel protein can additionally comprise substitutions, deletions, and/or insertions introduced into a native amino acid sequence to increase or decrease sensitivity to light, increase or decrease sensitivity to particular wavelengths of light, and/or increase or decrease the ability of the light-responsive cation channel protein to regulate the polarization state of the plasma membrane of the cell. Additionally, the light-responsive cation channel protein can contain one or more conservative amino acid substitutions and/or one or more non-conservative amino acid substitutions. The light-responsive proton pump protein comprising substitutions, deletions, and/or insertions introduced into the native amino acid sequence suitably retains the ability to depolarize the plasma membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0078] In some embodiments, the light-responsive cation channel comprises a T159C substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a L132C substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an E123A substitution of the amino acid sequence set forth in SEQ ID

NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution, an L132C substitution, and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises a T159C substitution, an L132C substitution, and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an L132C substitution and an E123T substitution of the amino acid sequence set forth in SEQ ID NO:5. In some embodiments, the light-responsive cation channel comprises an L132C substitution and an E123A substitution of the amino acid sequence set forth in SEQ ID NO:5.

[0079] Further disclosure related to light-responsive cation channel proteins can be found in U.S. Patent Application Publication No. 2007/0054319 and International Patent Application Publication Nos. WO 2009/131837 and WO 2007/024391, the disclosures of each of which are hereby incorporated by reference in their entireties.

[0080] Step Function Opsins and Stabilized Step Function Opsins

[0081] In other embodiments, the light-responsive cation channel protein can be a step function opsin (SFO) protein or a stabilized step function opsin (SSFO) protein that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the protein. In some embodiments, the SFO protein can have a mutation at amino acid residue C128 of SEQ ID NO:5. In other embodiments, the SFO protein has a C128A mutation in SEQ ID NO:5. In other embodiments, the SFO protein has a C128S mutation in SEQ ID NO:5. In another embodiment, the SFO protein has a C128T mutation in SEQ ID NO:5. In some embodiments, the SFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:6.

[0082] In some embodiments, the SSFO protein can have a mutation at amino acid residue D156 of SEQ ID NO:5. In other embodiments, the SSFO protein can have a mutation at both amino acid residues C128 and D156 of SEQ ID NO:5. In one embodiment, the SSFO protein has an C128S and a D156A mutation in SEQ ID NO:5. In another embodiment, the SSFO protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:7. In another embodiment, the SSFO protein can comprise a C128T mutation in SEQ ID NO:5. In some embodiments, the SSFO protein comprises C128T and D156A mutations in SEQ ID NO:5.

[0083] In some embodiments the SFO or SSFO proteins provided herein can be capable of mediating a depolarizing current in the cell when the cell is illuminated with blue light. In other embodiments, the light can have a wavelength of about 445 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the SFO or SSFO protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the SFO or SSFO protein. In some embodiments, each of the disclosed step function opsin and stabilized step function opsin proteins can have specific properties and characteristics for use in depolarizing the membrane of a neuronal cell in response to light.

[0084] Further disclosure related to SFO or SSFO proteins can be found in International Patent Application Publication No. WO 2010/056970 and U.S. Provisional Patent Application Nos. 61/410,704 and 61/511,905, the disclosures of each of which are hereby incorporated by reference in their entireties.

[0085] C1V1 Chimeric Cation Channels

[0086] In other embodiments, the light-responsive cation channel protein can be a C1V1 chimeric protein derived from the VChR1 protein of *Volvox carteri* and the ChR1 protein from *Chlamydomonas reinhardti*, wherein the protein comprises the amino acid sequence of VChR1 having at least the first and second transmembrane helices replaced by the first and second transmembrane helices of ChR1; is responsive to light; and is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments, the C1V1 protein can further comprise a replacement within the intracellular loop domain located between the second and third transmembrane helices of the chimeric light responsive protein, wherein at least a portion of the intracellular loop domain is replaced by the corresponding portion from ChR1. In another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1 extending to amino acid residue A145 of the ChR1. In other embodiments, the C1V1 chimeric protein can further comprise a replacement within the third transmembrane helix of the chimeric light responsive protein, wherein at least a portion of the third transmembrane helix is replaced by the corresponding sequence of ChR1. In yet another embodiment, the portion of the intracellular loop domain of the C1V1 chimeric protein can be replaced with the corresponding portion from ChR1 extending to amino acid residue W163 of the ChR1. In other embodiments, the C1V1 chimeric protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:8.

[0087] In some embodiments, the C1V1 protein can mediate a depolarizing current in the cell when the cell is illuminated with green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 542 nm. In some embodiments, the C1V1 chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein is not capable of mediating a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1 chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1 chimeric protein. In some embodiments, the disclosed C1V1 chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0088] C1V1 Chimeric Mutant Variants

[0089] In some aspects, the present disclosure provides polypeptides comprising substituted or mutated amino acid sequences, wherein the mutant polypeptide retains the characteristic light-activatable nature of the precursor C1V1 chimeric polypeptide but may also possess altered properties in some specific aspects. For example, the mutant light-responsive C1V1 chimeric proteins described herein can exhibit an increased level of expression both within an animal cell or on the animal cell plasma membrane; an altered responsiveness when exposed to different wavelengths of light, particularly red light; and/or a combination of traits whereby the chimeric C1V1 polypeptide possess the properties of low desensitization, fast deactivation, low violet-light activation for minimal cross-activation with other light-responsive cation channels, and/or strong expression in animal cells.

[0090] Accordingly, provided herein are C1V1 chimeric light-responsive opsin proteins that can have specific amino acid substitutions at key positions throughout the retinal binding pocket of the VChR1 portion of the chimeric polypeptide. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E122 of SEQ ID NO:7. In some embodiments, the C1V1 protein can have a mutation at amino acid residue E162 of SEQ ID NO:7. In other embodiments, the C1V1 protein can have a mutation at both amino acid residues E162 and E122 of SEQ ID NO:7. In other embodiments, the C1V1 protein can comprise an amino acid sequence at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence shown in SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11. In some embodiments, each of the disclosed mutant C1V1 chimeric proteins can have specific properties and characteristics for use in depolarizing the membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0091] In some aspects, the C1V1-E122 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In other embodiments, the C1V1-E122 mutant chimeric protein can mediate a depolarizing current in the cell when the cell is illuminated with red light. In some embodiments, the red light can have a wavelength of about 630 nm. In some embodiments, the C1V1-E122 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1-E122 mutant chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E122 mutant chimeric protein. In some embodiments, the disclosed C1V1-E122 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0092] In other aspects, the C1V1-E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 535 nm to about 540 nm. In some embodiments, the light can have a wavelength of about 542 nm. In other embodiments, the light can have a wavelength of about 530 nm. In some embodiments, the C1V1-E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1-E162 mutant chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E162 mutant chimeric protein. In some embodiments, the disclosed C1V1-E162 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0093] In yet other aspects, the C1V1-E122/E162 mutant chimeric protein is capable of mediating a depolarizing current in the cell when the cell is illuminated with light. In some embodiments the light can be green light. In other embodiments, the light can have a wavelength of between about 540 nm to about 560 nm. In some embodiments, the light can have a wavelength of about 546 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with violet light. In some embodiments, the chimeric protein does not mediate a depolarizing current in the cell when the cell is illuminated with light having a wavelength of about 405 nm. In some embodiments, the C1V1-E122/E162 mutant chimeric protein can exhibit less activation when exposed to violet light relative to C1V1 chimeric proteins lacking mutations at E122/E162 or relative to other light-responsive cation channel proteins. Additionally, the light can have an intensity of about 100 Hz. In some embodiments, activation of the C1V1-E122/E162 mutant chimeric protein with light having an intensity of 100 Hz can cause depolarization-induced synaptic depletion of the neurons expressing the C1V1-E122/E162 mutant chimeric protein. In some embodiments, the disclosed C1V1-E122/E162 mutant chimeric protein can have specific properties and characteristics for use in depolarizing the membrane of one or both of two main inputs to the VTA from LDT and the lateral LHb in response to light.

[0094] Further disclosure related to C1V1 chimeric cation channels as well as mutant variants of the same can be found in U.S. Provisional Patent Application Nos. 61/410,736, 61/410,744, and 61/511,912, the disclosures of each of which are hereby incorporated by reference in their entireties.

Polynucleotides

[0095] The disclosure also provides polynucleotides comprising a nucleotide sequence encoding a light-responsive protein described herein. In some embodiments, the polynucleotide comprises an expression cassette. In some embodiments, the polynucleotide is a vector comprising the above-described nucleic acid. In some embodiments, the nucleic acid encoding a light-responsive protein of the disclosure is operably linked to a promoter. Promoters are well known in the art. Any promoter that functions in the host cell can be used for expression of the light-responsive opsin proteins and/or any variant thereof of the present disclosure. In one embodiment, the promoter used to drive expression of the light-responsive opsin proteins can be a promoter that is specific to a particular neuron. Initiation control regions or promoters, which are useful to drive expression of the light-responsive opsin proteins or variant thereof in a specific animal cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these nucleic acids can be used. In some embodiments, the promoter used to drive expression of the light-responsive protein can be a ThyI promoter (See, e.g., Llewellyn, et al., 2010*, Nat. Med.,* 16(10):1161-1166). In other embodiments, the promoter used to drive expression of the light-responsive protein can be the EF1α promoter, a cytomegalovirus (CMV) promoter, the CAG promoter, a synapsin-I promoter (e.g., a human synapsin-I promoter), a human synuclein 1 promoter, a calcium/calmodulin-dependent kinase II alpha (CAMKIIα) promoter, or any other promoter capable of driving expression of the light-responsive opsin proteins in a neuron of mammals.

[0096] Also provided herein are vectors comprising a nucleotide sequence encoding a light-responsive protein or any variant thereof described herein. The vectors that can be administered according to the present disclosure also include vectors comprising a nucleotide sequence which encodes an RNA (e.g., an mRNA) that when transcribed from the polynucleotides of the vector will result in the accumulation of light-responsive opsin proteins on the plasma membranes of target animal cells. Vectors which may be used include, without limitation, lentiviral, HSV, adenoviral, and adeno-associated viral (AAV) vectors. Lentiviruses include, but are not limited to HIV-1, HIV-2, SIV, FIV and EIAV. Lentiviruses may be pseudotyped with the envelope proteins of other viruses, including, but not limited to VSV, rabies, Mo-MLV, baculovirus and Ebola. Such vectors may be prepared using standard methods in the art.

[0097] In some embodiments, the vector is a recombinant AAV vector. AAV vectors are DNA viruses of relatively small size that can integrate, in a stable and site-specific manner, into the genome of the cells that they infect. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies. The AAV genome has been cloned, sequenced and characterized. It encompasses approximately 4700 bases and contains an inverted terminal repeat (ITR) region of approximately 145 bases at each end, which serves as an origin of replication for the virus. The remainder of the genome is divided into two essential regions that carry the encapsidation functions: the left-hand part of the genome, that contains the rep gene involved in viral replication and expression of the viral genes; and the right-hand part of the genome, that contains the cap gene encoding the capsid proteins of the virus.

[0098] AAV vectors may be prepared using standard methods in the art. Adeno-associated viruses of any serotype are suitable (see, e.g., Blacklow, pp. 165-174 of "*Parvoviruses and Human Disease*" J. R. Pattison, ed. (1988); Rose, *Comprehensive Virology* 3:1, 1974; P. Tattersall "The Evolution of Parvovirus Taxonomy" In *Parvoviruses* (JR Kerr, S F Cotmore. M E Bloom, R M Linden, C R Parrish, Eds.) p5-14, Hudder Arnold, London, U K (2006); and DE Bowles, JE Rabinowitz, R J Samulski "*The Genus Dependovirus*" (J R Kerr, S F Cotmore. M E Bloom, R M Linden, C R Parrish, Eds.) p15-23, Hudder Arnold, London, UK (2006), the disclosures of each of which are hereby incorporated by reference herein in their entireties). Methods for purifying for vectors may be found in, for example, U.S. Pat. Nos. 6,566,118, 6,989,264, and 6,995,006 and WO/1999/011764 titled "Methods for Generating High Titer Helper-free Preparation of Recombinant AAV Vectors", the disclosures of which are herein incorporated by reference in their entirety. Methods of preparing AAV vectors in a baculovirus system are described in, e.g., WO 2008/024998. AAV vectors can be self-complementary or single-stranded. Preparation of hybrid vectors is described in, for example, PCT Application No. PCT/US2005/027091, the disclosure of which is herein incorporated by reference in its entirety. The use of vectors derived from the AAVs for transferring genes in vitro and in vivo has been described (See e.g., International Patent Application Publication Nos.: 91/18088 and WO 93/09239; U.S. Pat. Nos. 4,797,368, 6,596,535, and 5,139,941; and European Patent No.: 0488528, all of which are hereby incorporated by reference herein in their entireties). These publications describe various AAV-derived constructs in which the rep and/or cap genes are deleted and replaced by a gene of interest, and the use of these constructs for transferring the gene of interest in vitro (into cultured cells) or in vivo (directly into an organism). The replication defective recombinant AAVs according to the present disclosure can be prepared by co-transfecting a plasmid containing the nucleic acid sequence of interest flanked by two AAV inverted terminal repeat (ITR) regions, and a plasmid carrying the AAV encapsidation genes (rep and cap genes), into a cell line that is infected with a human helper virus (for example an adenovirus). The AAV recombinants that are produced are then purified by standard techniques.

[0099] In some embodiments, the vector(s) for use in the methods of the present disclosure are encapsidated into a virus particle (e.g. AAV virus particle including, but not limited to, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAV14, AAV15, and AAV16). Accordingly, the present disclosure includes a recombinant virus particle (recombinant because it contains a recombinant polynucleotide) comprising any of the vectors described herein. Methods of producing such particles are known in the art and are described in U.S. Pat. No. 6,596,535, the disclosure of which is hereby incorporated by reference in its entirety.

Delivery of Light-Responsive Opsin Proteins

[0100] In some aspects, polynucleotides encoding the light-responsive opsin proteins disclosed herein (for example, an AAV vector) can be delivered directly to one or both of two main inputs to the VTA from LDT and the lateral LHb with a needle, catheter, or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (See, e.g., Stein et al., *J. Virol,* 73:34243429, 1999; Davidson et al., *PNAS,* 97:3428-3432, 2000; Davidson et al., *Nat. Genet.* 3:219-223, 1993; and Alisky & Davidson, *Hum. Gene Ther.* 11:2315-2329, 2000, the contents of each of which are hereby incorporated by reference herein in their entireties) or fluoroscopy.

[0101] Other methods to deliver the light-responsive opsin proteins to the neurons of interest can also be used, such as, but not limited to, transfection with ionic lipids or polymers, electroporation, optical transfection, impalefection, or via gene gun.

Light and Electrical Sources

[0102] In some aspects of the present disclosure, the light-responsive opsin proteins disclosed herein can be activated by an implantable light source (such as a light cuff) or an implantable electrode placed around or near neurons expressing the light-responsive opsin proteins. Electrode cuffs and electrodes surgically placed around or near neurons for use in electrical stimulation of those neurons are well known in the art (See, for example, U.S. Pat. Nos. 4,602,624, 7,142,925 and 6,600,956 as well as U.S. Patent Publication Nos. 2008/0172116 and 2010/0094372, the disclosures of each of which are hereby incorporated by reference in their entireties). The light sources (such as a light cuff) or electrodes of the present invention can be comprised of any useful composition or mixture of compositions, such as platinum or stainless steel, as are known in the art, and may be of any useful configuration for stimulating the light-responsive opsin proteins disclosed herein.

[0103] The electrodes or implantable light source (such as a light cuff) may be placed around or near a neuron expressing a light-responsive protein.

[0104] In some embodiments, the implantable light source (such as a light cuff) does not completely surround the a region containing a neuron expressing a light-responsive protein, but, rather, can have a U-shape. In another embodiment, the implantable light source can have an attachment arm that can be used to guide the implantable light source (such as a light cuff) to the neuronal region to be exposed to light. The attachment arm can be removed following implantation of the light source or can be left in place to fix the position of the light source in proximity to the neurons of interest.

[0105] The implantable light source (such as a light cuff) can comprise an inner body, the inner body having at least one means for generating light which is configured to a power source. In some embodiments, the power source can be an internal battery for powering the light-generating means. In another embodiment, the implantable light source can comprise an external antenna for receiving wirelessly transmitted electromagnetic energy from an external source for powering the light-generating means. The wirelessly transmitted electromagnetic energy can be a radio wave, a microwave, or any other electromagnetic energy source that can be transmitted from an external source to power the light-generating means of the implantable light source (such as a light cuff). In one embodiment, the light-generating means is controlled by an integrated circuit produced using semiconductor or other processes known in the art.

[0106] In some aspects, the light means can be a light emitting diode (LED). In some embodiments, the LED can generate blue and/or green light. In other embodiments, the LED can generate amber and/or yellow light. In some embodiments, several micro LEDs are embedded into the inner body of the implantable light source (such as a light cuff). In other embodiments, the light-generating means is a solid state laser diode or any other means capable of generating light. The light generating means can generate light having an intensity sufficient to activate the light-responsive opsin proteins expressed on the plasma membrane of the nerves in proximity to the light source (such as a light cuff). In some embodiments, the light-generating means produces light having an intensity of any of about 0.05 mW/mm^2, 0.1 mW/mm^2, 0.2 mW/mm^2, 0.3 mW/mm^2, 0.4 mW/mm^2, 0.5 mW/mm^2, about 0.6 mW/mm^2, about 0.7 mW/mm^2, about 0.8 mW/mm^2, about 0.9 mW/mm^2, about 1.0 mW/mm^2, about 1.1 mW/mm^2, about 1.2 mW/mm^2, about 1.3 mW/mm^2, about 1.4 mW/mm^2, about 1.5 mW/mm^2, about 1.6 mW/mm^2, about 1.7 mW/mm^2, about 1.8 mW/mm^2, about 1.9 mW/mm^2, about 2.0 mW/mm^2, about 2.1 mW/mm^2, about 2.2 mW/mm^2, about 2.3 mW/mm^2, about 2.4 mW/mm^2, about 2.5 mW/mm^2, about 3 mW/mm^2, about 3.5 mW/mm^2, about 4 mW/mm^2, about 4.5 mW/mm^2, about 5 mW/mm^2, about 5.5 mW/mm^2, about 6 mW/mm^2, about 7 mW/mm^2, about 8 mW/mm^2, about 9 mW/mm^2, or about 10 mW/mm^2, inclusive, including values in between these numbers. In other embodiments, the light-generating means produces light having an intensity of at least about 100 Hz.

[0107] In some aspects, the light-generating means can be externally activated by an external controller. The external controller can comprise a power generator which can be mounted to a transmitting coil. In some embodiments of the external controller, a battery can be connected to the power generator, for providing power thereto. A switch can be connected to the power generator, allowing an individual to manually activate or deactivate the power generator. In some embodiments, upon activation of the switch, the power generator can provide power to the light-generating means on the light source through electromagnetic coupling between the transmitting coil on the external controller and the external antenna of the implantable light source (such as a light cuff). The transmitting coil can establish an electromagnetic coupling with the external antenna of the implantable light source when in proximity thereof, for supplying power to the light-generating means and for transmitting one or more control signals to the implantable light source. In some embodiments, the electromagnetic coupling between the transmitting coil of the external controller and the external antenna of the implantable light source (such as a light cuff) can be radio-frequency magnetic inductance coupling. When radio-frequency magnetic inductance coupling is used, the operational frequency of the radio wave can be between about 1 and 20 MHz, inclusive, including any values in between these numbers (for example, about 1 MHz, about 2 MHz, about 3 MHz, about 4 MHz, about 5 MHz, about 6 MHz, about 7 MHz, about 8 MHz, about 9 MHz, about 10 MHz, about 11 MHz, about 12 MHz, about 13 MHz, about 14 MHz, about 15 MHz, about 16 MHz, about 17 MHz, about 18 MHz, about 19 MHz, or about 20 MHz). However, other coupling techniques may be used, such as an optical receiver, infrared, or a biomedical telemetry system (See, e.g., Kiourti, "Biomedical Telemetry: Communication between Implanted Devices and the External World, *Opticon* 1826, (8): Spring, 2010).

Non-Human Animal Model of Reward- or Aversive-Related Behavior

[0108] The present disclosure provides a non-human animal model of reward- or aversive-related behavior disorder, which non-human animal model expresses a light-activated opsin protein in one or both of two main inputs to the VTA from the LDT and the LHb. Exposure of the light-activated opsin protein to light of a wavelength to which the light-activated opsin protein responds results in an adverse reward- or aversive-related behavior. A subject non-human animal model is in some embodiments a mouse. A subject non-human animal model is in some embodiments a rat. A subject non-human animal model is in some embodiments a non-human primate. A subject non-human animal model is useful in screening methods to identify agents that modulate pathological reward- or aversive related behaviors, where such behaviors include addiction, eating disorders, anxiety disorders, and the like.

[0109] In some cases, a subject non-human animal model expresses an excitatory light-activated opsin protein in an LHb neuron projecting to the VTA. In some cases, a subject non-human animal model expresses an inhibitory light-activated opsin protein in an LHb neuron projecting to the VTA. In some cases, a subject non-human animal model expresses an excitatory light-activated opsin protein in an LDT neuron projecting to the VTA. In some cases, a subject non-human animal model expresses an inhibitory light-activated opsin protein in an LDT neuron projecting to the VTA.

[0110] For example, in some cases, a subject non-human animal model expresses an excitatory light-activated opsin protein in an LDT neuron projecting to the VTA, such that, when the LDT neuron is exposed to light of a wavelength to which the excitatory light-activated opsin protein responds, the non-human animal model exhibits strong conditioned place preference (CPP).

[0111] In other cases, a subject non-human animal model expresses an excitatory light-activated opsin protein in an LHb neuron projecting to the VTA, such that, when the LHb neuron is exposed to light of a wavelength to which the excitatory light-activated opsin protein responds, the non-human animal model exhibits strong conditioned place aversion.

[0112] For example, in some embodiments, a subject non-human animal model comprises an excitatory light-responsive polypeptide comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:5, 6, 7, 8,9, 10, and 11, where the polypeptide is expressed in an LDT neuron projecting to the VTA, and wherein, exposure of the LDT neuron projecting to the VTA to light of a wavelength to which the light-responsive protein responds results in strong CPP. In some cases, the excitatory light-responsive polypeptide comprises both ER export and membrane trafficking signals. For example, in some cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:5, an ER export signal, and a membrane trafficking signal. In other cases, the excitatory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:5, a membrane trafficking signal, and a ER export signal. In some cases, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some cases, the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16). In some cases, the ER export signal comprises the sequence FCYENEV (SEQ ID NO:17).

[0113] As another example, in some embodiments, a subject non-human animal model comprises an excitatory light-responsive polypeptide comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:5, 6, 7, 8,9, 10, and 11, where the polypeptide is expressed in an LHb neuron projecting to the VTA, and wherein, exposure of the LHb neuron projecting to the VTA to light of a wavelength to which the light-responsive protein responds results in strong CPA.

[0114] As another example, in some embodiments, a subject non-human animal model comprises an inhibitory light-responsive polypeptide comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:1, 2, 3, 4, 12, 13, 14, and 15, where the polypeptide is expressed in an LDT neuron projecting to the VTA. For example, in some cases, the inhibitory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In other cases, the inhibitory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, a membrane trafficking signal, and a ER export signal. In some cases, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some cases, the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16). In some cases, the ER export signal comprises the sequence FCYENEV (SEQ ID NO:17).

[0115] As another example, in some embodiments, a subject non-human animal model comprises an inhibitory light-responsive polypeptide comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to one of SEQ ID NOs:1, 2, 3, 4, 12, 13, 14, and 15, where the polypeptide is expressed in an LHb neuron projecting to the VTA. For example, in some cases, the inhibitory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, an ER export signal, and a membrane trafficking signal. In other cases, the inhibitory light-responsive polypeptide comprises, from the N-terminus to the C-terminus, the amino acid sequence at least 95% identical to the sequence shown in SEQ ID NO:1, a membrane trafficking signal, and a ER export signal. In some cases, the membrane trafficking signal is derived from the amino acid sequence of the human inward rectifier potassium channel Kir2.1. In some cases, the membrane trafficking signal comprises the amino acid sequence KSRITSEGEYIPLDQIDINV (SEQ ID NO:16). In some cases, the ER export signal comprises the sequence FCYENEV (SEQ ID NO:17).

[0116] A nucleic acid (e.g., an expression vector) comprising a nucleotide sequence encoding a light-responsive protein can be introduced into a non-human animal (e.g., a rodent such as a rat or a mouse) by any convenient means. For example, a nucleic acid (e.g., an expression vector) comprising a nucleotide sequence encoding a light-responsive protein can be injected stereotactically into the VTA, into an LDT neuron projecting into the VTA, or into an LHb neuron projecting into the VTA.

[0117] Suitable expression vectors include, but are not limited to, lentiviral, HSV, adenoviral, rabies virus vectors, and adeno-associated viral (AAV) vectors. Lentiviruses include, but are not limited to HIV-1, HIV-2, SIV, FIV and EIAV. Lentiviruses may be pseudotyped with the envelope proteins of other viruses, including, but not limited to VSV, rabies, Mo-MLV, baculovirus and Ebola. Such vectors may be prepared using standard methods in the art. Suitable expression vectors are described above, and in the Examples.

Screening Methods

[0118] Provided herein are two different methods to induce reward- or aversive related behaviors. First, by injection of a vector, e.g., an adeno-associated virus vector, that expresses a light sensitive opsin into a defined target region and optical stimulation of terminals that express this opsin in another brain region. Second, by injection of a viral vector, e.g., a retrogradely transported virus (e.g. rabies virus) vector, which expresses a light-sensitive (light-responsive) opsin into a defined brain area and optical stimulation of cell bodies that project to this brain area and express the opsin.

[0119] A subject screening method generally involves contacting a non-human animal model of a reward- or aversive-related behavior disorder with a test agent; and determining the effect of the test agent on a reward- or aversive-related behavior, e.g., when a neuron expressing a light-activated opsin is exposed to light of a wavelength that activates the opsin. A test agent that reduces an adverse reward- or aversive-related behavior is considered a candidate agent for treating a reward- or aversive-related behavior disorder.

[0120] For example, a test agent that reduces a reward- or aversive-related behavior, exhibited by a subject non-human animal model, by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or more than 25% (e.g., 25% to 50%; 50% to 75%; etc.) can be considered a candidate agent for ameliorating (treating) a pathological reward- or aversive-related behavior. Test agents identified using a subject method can be considered candidate agents for treating any of a variety of pathological reward- or aversive-related behaviors, e.g., addiction, anxiety disorders, and the like.

[0121] As described above, a non-human animal model of reward- or aversive-related behavior disorder expresses a light-activated opsin protein in one or both of two main inputs to the VTA from the LDT and the LHb. Exposure of the light-activated opsin protein to light of a wavelength to which the light-activated opsin protein responds results in an adverse reward- or aversive-related behavior. A test agent that reduces the adverse reward- or aversive-related behavior is considered a candidate agent for treating a reward- or aversive-related behavior disorder.

[0122] A light-responsive protein expressed in a subject non-human animal model can be activated by an implantable light source, where suitable light sources are described above and in the Examples. Suitable wavelengths for activating an inhibitory or an excitatory opsin protein are described above.

[0123] Various symptoms can be analyzed in a subject non-human animal model. Suitable tests include the conditioned place preference (CPP) test; and the conditioned place aversion (CPA) test. Other tests that may be used include, e.g., the forced swim test (FST) (see, e.g., Porsolt et al. (1977) *Nature* 266:730; and Petit-Demouliere, et al. (2005) *Psychopharmacology* 177: 245); the tail suspension test (see, e.g., Cryan et al. (2005) *Neurosci. Behav. Rev.* 29:571; and Li et al. (2001) *Neuropharmacol.* 40:1028); conditioned place aversion (see, e.g., Bechtholt-Gompf et al. (2010) *Neuropsychopharmacol.* 35:2049); the novelty hypophagia test (Dulawa, et al. (2005) *Neurosci. Biobehav. Rev.* 29:771); the social defeat stress test (see, e.g., Blanchard et al. (2001) *Physiol Behav.* 73:261-271; and Kudryavtseva et al. (1991) *Pharmacol. Biochem. Behav.* 38: 315); the sucrose preference test (see, e.g., Kurre Nielsen, et al. (2000) *Behavioural Brain Research* 107:21-33); the open field test (see, e.g., Holmes (2001) *Neurosci. Biobehav. Rev.* 25:261-273); the elevated plus maze test (see, e.g., Holmes (2001) supra); and the like. Any such test can be used in a subject screening method.

[0124] As used herein, the term "determining" refers to both quantitative and qualitative determinations and as such, the term "determining" is used interchangeably herein with "assaying," "measuring," and the like.

[0125] The terms "candidate agent," "test agent," "agent", "substance" and "compound" are used interchangeably herein. Candidate agents encompass numerous chemical classes, typically synthetic, semi-synthetic, or naturally occurring inorganic or organic molecules. Candidate agents include those found in large libraries of synthetic or natural compounds. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ComGenex (South San Francisco, Calif.), and MicroSource (New Milford, Conn.). A rare chemical library is available from Aldrich (Milwaukee, Wis.) and can also be used. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from Pan Labs (Bothell, Wash.) or are readily producible.

[0126] Candidate agents can be small organic or inorganic compounds having a molecular weight of more than 50 daltons and less than about 2,500 daltons. Candidate agents can comprise functional groups necessary for structural interaction with proteins, e.g., hydrogen bonding, and may include at least an amine, carbonyl, hydroxyl or carboxyl group, and may contain at least two of the functional chemical groups. The candidate agents may comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, and derivatives, structural analogs or combinations thereof.

[0127] Assays of the present disclosure include controls, where suitable controls include a subject non-human animal model that has been exposed to activating light, but has not been administered the test agent.

EXAMPLES

[0128] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Input-Specific Control of Reward and Aversion in the VTA

Materials and Methods

[0129] Animals.

[0130] Male adult (10-12 weeks of age) C57Bl6 (Charles River) or TH-Cre [B6.Cg-Tg(Th-cre)1 Tmd/J; Jackson Laboratory] mice were used for all experiments. All procedures complied with the animal care standards set forth by the National Institutes of Health and were approved by Stanford University's Administrative Panel on Laboratory Animal Care.

[0131] Virus Generation.

[0132] The adeno-associated viruses (AAVs) used in this study were generated as previously described' either by the Deisseroth lab (AAV-ChR2) or the Stanford Neuroscience Gene Vector and Virus Core (AAV-DIO-RVG). Rabies virus (RV) was generated from a full length cDNA plasmid containing all components of RV (SAD L16)[39]. We replaced the rabies virus glycoprotein with enhanced green fluorescent protein (EGFP), tdTomato or ChR2-H134R fused to enhanced yellow fluorescent protein (EYFP) to generate RV expressing EGFP (RV-EGFP), tdTomato (RV-tdTomato), or ChR2-H134R (RV-ChR2). To harvest RV from this cDNA we used a modified version of published protocols[39, 40]. Briefly, HEK293T cells were transfected with a total of 6 plasmids; 4 plasmids expressing the RV components pTIT-N, pTIT-P, pTIT-G, and pTIT-L; one plasmid expressing T7 RNA polymerase (pCAGGS-T7), and the aforementioned glycoprotein-deleted RV cDNA plasmid expressing EGFP, tdTomato, or ChR2. For the amplification of RV, the media bathing these HEK293T cells was collected 3-4 days post-transfection and moved to baby hamster kidney (BHK) cells stably expressing RV glycoprotein (BHK-B19G)[40]. After three days, the media from BHK-B19G cells was collected, centrifuged for 5 min at 3,000×g to remove cell debris, and concentrated by ultracentrifugation (55,000×g for 2 hr). Pellets were suspended in DPBS, aliquoted and stored at −80° C. The titer of concentrated RV was measured by infecting HEK293 cells and monitoring fluorescence.

[0133] Stereotaxic Injections and Optic Fiber/Cannula Implantations.

[0134] As previously described[4, 5], all stereotaxic injections were performed under general ketamine-medetomidine anesthesia and using a stereotaxic instrument (Kopf Instruments). Adult (10-12 weeks; 25-30 g) male C57BL/6 and TH-Cre mice were group-housed until surgery. Mice were maintained on a 12:12 light cycle (lights on at 07:00). For retrobead labeling (100 nl; LumaFluor Inc., Naples, Fla.) mice were injected unilaterally with fluorescent retrobeads in the nucleus accumbens (NAc) lateral shell (bregma 1.45 mm; lateral 1.75 mm; ventral 4.0 mm), NAc medial shell (bregma 1.78 mm; lateral 0.5 mm; ventral 4.1 mm), or medial prefrontal cortex (mPFC) (two injections at four different sites: bregma 1.95 mm, 2.05 mm, 2.15 mm, and 2.25 mm; lateral 0.27 mm; ventral 2.1 mm and 1.6 mm; injected total volume in mPFC: 400 nl; the target area was the prelimbic and infralimbic cortex) using a 1 μl Hamilton syringe (Hamilton, Reno, Nev.). Note that these empirically derived stereotaxic coordinates do not precisely match those given in the mouse brain atlas (Franklin and Paxinos, 2001), which we used as references for the injection-site images. On average, the caudo-rostral axis appeared to be approximately shifted caudally by 400 μm. Little labeling was observed in the pipette tract (i.e. cingulate and motor cortices for mPFC injections or in the dorsal striatum for NAc lateral shell injections). To allow adequate time for retrograde transport of the Retrobeads into the somas of midbrain DA neurons, minimal survival periods prior to sacrifice depended on the respective injection areas: NAc lateral shell, 3 days; NAc medial shell, 14 days; and mPFC, 21 days. For viral infections a small amount of concentrated rabies virus (RV) solution (0.5-1 μl of RV-EGFP, RV-tdTomato or RV-ChR2) or AAV-DIO-RVG or AAV-ChR2-EYFP was injected unilaterally in the LDT (bregma −5.0 mm; lateral 0.5 mm; ventral 3.0 mm) or LHb (bregma −1.58 mm; lateral 0.4 mm; ventral 2.65 mm) or into the VTA (bregma −3.4 mm; lateral 0.35 mm; ventral 4.0 mm) or mPFC or NAc lateral shell (same coordinates as for retrobead injections) using a syringe pump (Harvard Apparatus, MA) at a slow rate (100-150 nl/min). The injection needle was withdrawn 5 min after the end of the infusion.

[0135] For the dual RV injections (FIG. **17**), one virus (RV-EGFP or RV-tdTomato) was injected into the VTA and the other was injected into either the ventral pallidum (bregma 0.62 mm; lateral 1.10 mm; ventral 4.75 mm), lateral septum (bregma 0.62 mm; lateral 0.35 mm; ventral 3.0 mm), lateral hypothalamus (bregma −0.94 mm; lateral 1.00 mm; ventral 4.75 mm), mPFC (two injections at four different sites: bregma 1.95 mm, 2.05 mm, 2.15 mm, and 2.25 mm; lateral 0.27 mm; ventral 2.1 mm and 1.6 mm), mediodorsal thalamic nucleus (bregma −1.22 mm; lateral 0.25 mm; ventral 3.25 mm) or supraoculomotor central grey (bregma −4.04 mm; lateral 0.3 mm; ventral 2.7 mm). For anterograde labeling of LDT and LHb terminals in the VTA the anterograde tracer *Phaseolus vulgaris leucoagglutinin* (PHA-L; 50 nl; 2.5% in 0.01 M phosphate buffer; Vector, Burlingame, Calif.) was injected into the LDT or LHb (same coordinates as for virus injections). The survival period for the PHA-L injected animals was 3 weeks and for the AAV-ChR2 injected animals 8-12 weeks.

[0136] For behavioral experiments mice that were injected with RV-EGFP or RV-ChR2 in the VTA received unilateral implantation of a doric patch-cord chronically implantable fiber (NA=0.22; Doric lenses, Quebec, Canada) over the LDT (bregma −5.0 mm, lateral 0.5 mm, ventral 2.0 mm) or LHb (bregma −1.58 mm, lateral 0.4 mm, ventral 2.0 mm). One layer of adhesive cement (C&B metabond; Parkell, Edgewood, N.Y.) followed by cranioplastic cement (Dental cement; Stoelting, Wood Dale, Ill.) was used to secure the fiber guide system to the skull. After 20 min, the incision was closed with a suture and tissue adhesive (Vetbond; Fisher, Pittsburgh, Pa.). The animal was kept on a heating pad until it recovered from anesthesia. For intra-VTA stimulation of LHb or LDT axon terminals, AAV-ChR2 was injected into LHb or LDT, respectively. 10 weeks following the virus injection a doric optic fiber was implanted unilaterally over the caudal medial VTA for stimulation of LHb axon terminals (bregma −3.4 mm; lateral 0.35 mm; ventral 3.6 mm) and over the lateral VTA for stimulation of LDT axon terminals (bregma −3.4 mm; lateral 0.5 mm; ventral 3.6 mm). Behavioral experiments were performed 2 weeks after the implantation. For microinjection of DA receptor antagonists into the mPFC or NAc lateral shell a guide cannula (PlasticOne, Roanoke, Va.) was implanted directly over the ipsilateral mPFC (bregma: 2.2 mm; lateral 0.3 mm; ventral −2.0 mm) or NAc lateral shell (bregma: 1.45 mm; lateral 1.75 mm; ventral 4.0 mm) of the mice in which RV-ChR2 injection into the VTA and the implantation of doric optic fibers were made. Optical fiber and cannula placements were confirmed in all animals Although placements varied slightly from mouse to mouse, behavioral data from all mice were included in the study.

[0137] Electrophysiology.

[0138] Mice were deeply anaesthetized with pentobarbital (200 mg/kg ip; Ovation Pharmaceuticals, Deerfield, Ill.). Coronal midbrain slices (250 μm) were prepared after intracardial perfusion with ice-cold artificial cerebrospinal fluid (ACSF) containing elevated sucrose (in mM): 50 sucrose, 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 0.1 $CaCl_2$, 4.9 $MgCl_2$, and 2.5 glucose (oxygenated with 95% O_2/5% CO_2). After 90 min of recovery, slices were transferred to a recording chamber and perfused continuously at 2-4 ml/min with oxygenated ACSF (125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 11 glucose, 1.3 $MgCl_2$, and 2.5 $CaCl_2$) at ~30° C. For recording of excitatory postsynaptic currents (EPSCs) picrotoxin (50 μM, Sigma) was added to block inhibitory currents mediated by $GABA_A$ receptors. The internal solution contained (in mM): 117 $CsCH_3SO_3$, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA, 4 MgATP, 0.3 NaGTP, 5 QX314, 0.1 Spermine, and 0.1% neurobiotin. For recording of inhibitory postsynaptic currents (IPSCs) the internal solution contained (in mM): 130 CsCl, 1 EGTA, 10 HEPES, 2 MgATP, 0.2 NaGTP, and 0.1% neurobiotin (for both internal solutions pH 7.35, 270-285 mOsm). Patch pipettes (3.8-4.4 MΩ) were pulled from borosilicate glass (G150TF-4; Warner Instruments).

[0139] Labeled DA neurons were visualized with a 40× water-immersion objective on an upright fluorescent microscope (BX51WI, Olympus USA) equipped with infrared-differential interference contrast (IR-DIC) video microscopy and epifluorescence (Olympus USA) for detection of retrobeads. ChR2 was stimulated by flashing 473 nm light (5 ms pulses; 0.1 Hz; 1-2 mW) through the light path of the microscope using a ultrahigh-powered LED powered by an LED driver (Prizmatix, Modiin Ilite, Israel) under computer control. The light intensity of the LED was not changed during the experiments and the whole slice was illuminated. A dual lamp house adapter (Olympus USA) was used to switch between fluorescence lamp and LED light source. Excitatory postsynaptic currents (EPSCs) were recorded in whole-cell voltage clamp (Multiclamp 700B, Molecular Devices, CA, USA), filtered at 2 KHz, digitized at 10 KHz (ITC-18 interface, HEKA) and collected on-line using custom IgorPro software (Wavemetrics, Lake Oswego, Oreg., USA). Series resistance (15-25 MΩ) and input resistance were monitored on-line with a 4 mV hyperpolarizing step (50 ms) given with each afferent stimulus. VTA/SN and RMTg neurons were voltage-clamped at ~70 mV and EPSC or IPSC amplitudes were calculated by measuring the peak current from the average EPSC or IPSC response from 10-15 consecutive sweeps.

[0140] For pharmacological characterization light-evoked EPSCs or IPSCs were recorded for 5 min followed by bath perfusion of 10 μM CNQX (Tocris Bioscience, Ellisville, Mich., USA) or 50 picrotoxin (Sigma) for an additional 10 min, respectively. 10-15 consecutive sweeps pre- and post-drug were averaged and peak EPSCs or IPSCs amplitudes were then measured. For detection of IPSCs, DA cells were recorded from the caudal VTA in slices that contained the RMTg. For determination of DA or GABAergic phenotype, neurons were filled with neurobiotin (Vector, Burlingame, Calif., USA) during the patch clamp experiment, then fixed in 4% PFA and 24 h later immunostained for TH or GAD67. Approximately 80% of all whole-cell patch clamped neurons could be successfully recovered. The DA phenotype or GABAergic phenotype (in the RMTg) was confirmed in all of these neurons.

[0141] Immunohistochemistry.

[0142] Immunohistochemistry and confocal microscopy were performed as described previously[4, 5]. Briefly, after intracardial perfusion with 4% paraformaldehyde in PBS, pH 7.4, the brains were post-fixed overnight and coronal midbrain slices (50 or 100 μm) were prepared. The primary antibody used were mouse anti-tyrosine hydroxylase (TH) (1:1000; Millipore, Temecula, Calif., USA), rabbit anti-tyrosine hydroxylase (TH) (1:1000; Calbiochem, San Diego, Calif., USA), rabbit anti-PHA-L (1:1000; Vector, Burlingame, Calif., USA), goat anti-glutamate transporter (EAAC1; 1:1000; Millipore), rabbit anti-ChAT (1:200; Millipore), mouse anti-GAD67 (clone 1G10.2; 1:500; Millipore), rabbit anti-c-fos (1:500, Calbiochem) and rabbit anti-NeuN (1:1000; Millipore). The secondary antibodies used were Alexa Fluor488 anti-rabbit, AlexaFluor546 anti-goat, AlexaFluor546 anti-rabbit, AlexaFluor546 anti-mouse, Alexa Fluor647 anti-rabbit, Alexa Fluor647 anti-mouse (all 1:750), AlexaFluor488 streptavidin (1:1000) (all Molecular Probes, Eugene, Oreg.). Image acquisition was performed with a confocal system (Zeiss LSM510) using 10×, 40× or 63× objectives and on a Zeiss AxioImager M1 upright widefield fluorescence/DIC microscope with CCD camera using 2.5× and 10× objectives. Images were analyzed using the Zeiss LSM Image Browser software and ImageJ software.

[0143] For quantification of ChR2-EYFP fluorescence intensity and quantification of c-fos-positive cells, confocal images were acquired using identical pinhole, gain, and laser settings. Images in the medial and lateral VTA as well as the SN from the same tissue sections were acquired at the same focus level. The medial and lateral VTA was defined as the area that corresponds to the anatomical location of distinct DA subpopulations[4, 5]. The medial VTA was defined as the region comprising the medial paranigral nucleus (PN) and medial parabrachial pigmented nucleus (PBP), while the lateral VTA was defined as the lateral parabrachial pigmented nucleus (FIG. **14**). No additional post-processing was performed on any of the collected images. ChR2 fluorescence intensity was then quantified using a scale from 0-255 in ImageJ to determine the mean intensity across the entire image. For retrobead, AAV and PHA-L injections as well as RV injections in the mPFC and NAc lateral shell the injection-sites were confirmed in all animals by preparing coronal sections (100 μm). Counterstaining of injection sites was performed with green or red Nissl (NeuroTrace 500/525 or 530/615, Molecular Probes, Eugene, Oreg.).

[0144] We routinely carried out complete serial analyses of the injection sites. Animals with significant contaminations outside target areas were discarded (see Lammel et al., 2008[4] for serial analysis of retrobead injection-sites and definition of DA target areas). For RV injections into the VTA we confirmed that all animals had the center of the viral injection located in the caudal VTA (Bregma −3.4 mm). However, quantification of the "spread" of the RV-ChR2 injected into the VTA is difficult because for expression of the transgene, the RV must be taken up by terminals and the transgene must be synthesized in the cytosol and then transported within the axons. Any EYFP within the VTA and adjacent structures will represent axons/terminals of cells that project to the VTA and adjacent structures as well as the cell bodies of neurons (i.e. RMTg) that have local connectivity within the VTA and adjacent structures. Thus transgene expression in structures adjacent to the VTA does not indicate that LHb or LDT neurons project to these structures. Nevertheless, in FIG. **21** we present a serial reconstruction for the caudo-rostral extent of the midbrain showing the expression of ChR2-EYFP one week after injection of RV-ChR2 into the VTA (n=5 mice). TH-stained coronal midbrain sections (100 μM) were prepared from the injected mice and reconstructed using Neurolucida software (MicroBrightfield, Colchester, Vt.). Sections were labeled relative to bregma using landmarks and neuroanatomical nomenclature as described in the Franklin and Paxinos mouse brain atlas (2001). We report all brain areas in which detectable EYFP was observed. The strongest transgene expression was observed in the caudal VTA and several of its distinct subnuclei, most commonly in the interpeduncular nucleus (IPN). We also always detected high transgene expression in the RMTg. Thus when referred to in the text, the VTA includes the RMTg, which was originally termed the "tail of the VTA"[22].

[0145] Because the IPN expressed ChR2-EYFP following intra-VTA injections, we conducted additional double retrograde tracing experiments in which we injected small amounts of green Retrobeads (20 nl; LumaFlauor Inc., Naples, Fla.) into the IPN (bregma −3.9 mm; lateral 0 mm; ventral 4.55 mm) and red Retrobeads (60 nl; LumaFlauor Inc., Naples, Fla.) into the VTA (bregma −3.4 mm; lateral 0.35 mm; ventral 4.0 mm). Fluorescently-labeled latex Retrobeads were used in these experiments (n=2 mice) because they show very limited diffusion from the injection site even after several weeks in vivo and thus can be highly localized. While a large number of cells in the lateral habenula contained red beads (~84%, 79/94 cells), confirming a projection from this structure to the VTA, only a small proportion of these cells (~12%, 11/94 cells) also contained green beads (FIG. **22**). In contrast, a large number of medial habenula cells contained green beads (~98%, 214/218 cells) and less than 2% (3/218 cells) of these also contained red beads (FIG. **22**), demonstrating that the medial habenula preferentially projects to the IPN. In the LDT, many cells (>100) contained red beads and none of these cells contained green beads (FIG. **22**). These results suggest that LDT cells likely only project to VTA and not the IPN while the proportion of LHb neurons that project to the IPN in addition to the VTA is small.

[0146] For quantification of the expression of RV-ChR2-EYFP in the LDT and LHb 50 μm coronal sections from mice which had been injected with RV-ChR2-EYFP in the VTA were stained for NeuN. 66 confocal images from the LDT and 55 confocal images from the LHb were obtained using a 40× objective (n=3 mice). The percent of ChR2-EYFP-positive cells relative to the number of NeuN-positive cells in a 125 μm×125 μm area was analyzed using the ImageJ software. Approximately 20% of all NeuN-positive LDT and LHb neurons expressed ChR2-EYFP following RV-ChR2 injection into the VTA (FIG. **21**).

[0147] Behavioral Assays.

[0148] All behavioral tests were conducted during the same circadian period (13:00-19:00). The conditioned place preference (CPP) and aversion (CPA) protocols were performed in a rectangular cage with a left chamber measuring 28 cm×24 cm with black and white stripes on the walls and a metal grill floor, a center chamber measuring 11.5 cm×24 cm with white walls and a smooth plastic floor; and a right chamber measuring 28 cm×24 cm with black and white squares on the walls and a punched metal floor. The apparatus was designed so that mice did not have any consistent bias for a particular chamber (FIG. **10**). The CPP/CPA test consisted of 3 sessions over 3 days. On day 1 (1 week after infusion of RV-EGFP or RV-ChR2 into the VTA), individual mice were placed in the center chamber and allowed to freely explore the entire apparatus for 15 min (pre-test). On day 2 mice were confined to one of the side chambers for 30 min during optical stimulation. Stimulation in left or right chambers was counter-balanced across mice. For stimulation the optical fiber was connected to a 473 nm laser diode (OEM Laser Systems, East Lansing, Mich.) through an FC/PC adapter. Laser output was controlled using a Master-8 pulse stimulator (A.M.P.I., Jerusalem, Israel) which delivered 8 pulses of 5 ms light flashes at 30 Hz every 5 s (phasic stimulation) or 5 ms light flashes delivered at 1 Hz (low frequency stimulation). For stimulation of LDT and LHb axon terminals in the VTA 15 pulses of 5 ms light flashes at 30 Hz every 2 s were delivered. Light output through the optical fibers was adjusted to 20 mW using a digital power meter console (Thorlabs, Newton, N.J.) and was checked before and after stimulation of each mouse. On day 3, similar to day 1, mice were placed in the center chamber and allowed to freely explore the entire apparatus for 15 min (Post-Test 1). After Post-Test 1 the blue light laser was switched on and the mouse received phasic or low frequency stimulation for whenever it was in the chamber in which it had been conditioned on day 2 for a total duration of 15 min (Post-Test 2). There was no interruption between Post-Test 1 and Post-Test 2. A video tracking system (BiObserve, Fort Lee, N.J.) recorded all animal movements. To calculate preference or aversion during Post-Test 1, we divided the relative time (in %) the mouse spent during Post-Test 1 in the conditioned chamber (i.e. the chamber in which it received either phasic or low frequency light stimulation of LDT or LHb inputs to the VTA) by the relative time (in %) the mouse spent in this chamber during the Pre-test (Post-Test 1/Pre ratio). During Post-Test 2, preference or aversion was calculated by dividing the relative time (in %) the mouse spent during Post-Test 2 in the conditioned chamber by the relative time (in %) the mouse spent in this chamber during the Pre-test (Post-Test 2/Pre ratio).

[0149] For microinjection of the D1 dopamine receptor antagonist SCH23390 into mPFC and the microinjection of the D1 and D2 dopamine receptor antagonists SCH23390 and raclopride into the NAc lateral shell a 33-gauge injector cannula connected to a syringe pump (Harvard Apparatus, MA) was inserted into the guide cannula which had been implanted in the mPFC or NAc lateral shell. All microinjections were delivered at a rate of 100 nl/min. Injector cannulas remained in place for an additional minute before being removed. Drugs were infused 5 min before the beginning of the light stimulation on day 2, For the pharmacological control experiments, the animals were treated identically except no optical stimulation was provided, Doses of drugs used for microinjections were: 50 ng SCH23390 in 0.2 μl saline (mPFC); 300 ng SCH23390 and 3 μg raclopride in 0.3 μl saline/DMSO (NAc lateral shell).

[0150] The open field test was conducted on different cohorts of mice to measure the effect of optogenetic stimulation on anxiety-like responses and general locomotor ability. The mice were placed in the chamber (50×50 cm) and their movement was recorded and analyzed for 18 min using the same video-tracking software that was used in the CPP/CPA tests (BiObserve, Fort Lee, N.J.). After three minutes without optical stimulation, phasic stimulation was turned on for 3, three min epochs interspersed with 3 min epochs of no stimulation. For all analyses and graphs where total "off" and "on" conditions are displayed, the 3 "off" epochs were pooled and the 3 "on" epochs were pooled. The inner zone of the open field chamber was defined as the 23×23 cm central square area.

[0151] For quantification of c-fos immunreactivity, LDT and LHb inputs to the VTA were stimulated for 30 min using the phasic light stimulation protocol. During this time the mice remained in their home cage. The mice were perfused with 4% PFA 60 min after the in vivo light stimulation and 24 h later immunohistochemistry was performed.

[0152] Statistics.

[0153] Student's t tests, Mann-Whitney U-tests or one-way ANOVA tests were used to determine statistical differences using GraphPad prism 5 (Graphpad Software, San Diego, Calif.). Bonferroni post hoc analysis was applied, when necessary, to compare means. Statistical significance was set at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). All data values are presented as means±SEM.

[0154] 38. Zhang, F., et al. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat. Protoc.* 5, 439-456 (2010).

[0155] 39. Mebatsion, T., Konig, M. & Conzelmann, K. K. Budding of rabies virus particles in the absence of the spike glycoprotein. *Cell* 84, 941-951 (1996).

[0156] 40. Wickersham, I. R., Sullivan, H. A. & Seung, H. S. Production of glycoprotein-deleted rabies viruses for monosynaptic tracing and high-level gene expression in neurons. *Nat. Protoc.* 5, 595-606 (2010).

Results

[0157] Inputs to the VTA from LDT and LHb

[0158] To identify unambiguously the afferent inputs to the VTA, we used a rabies virus in which the glycoprotein is replaced by EGFP (RV-EGFP)[16]. Consistent with recent results[17], injection of RV-EGFP into the VTA resulted in expression of EGFP in diverse brain areas with large clusters of EGFP-expressing cells in the PFC, NAc, lateral hypothalamus, LHb and LDT (FIG. **7**). We focused on inputs to the VTA from the LDT and LHb because both play roles in motivated behaviors by influencing VTA neuronal activity and the consequent release of DA in target structures[2, 7, 18]. EGFP-positive LDT neurons expressed markers for both glutamatergic neurons (the glutamate transporter EAAC1) and cholinergic neurons (choline acetyltransferase; ChAT) (FIG. **8**)[19, 20]. However, while 95% of LDT neurons projecting to VTA expressed EAAC1, only ~7% expressed ChAT. LHb neurons are excited by the absence of an expected reward[18] and likely send direct inputs to GABAergic cells in the tail of the VTA, the rostromedial tegmental nucleus (RMTg)[21, 22], that inhibit VTA DA neurons[23, 26]. EGFP-positive LHb neurons were immunopositive for EAAC1 but not for ChAT (FIG. **8**) indicating that LHb neurons projecting to VTA are glutamatergic[8].

[0159] To visualize fibers within the VTA from LDT and LHb we injected the anterograde tracer *Phaseolus vulgaris leucoagglutinin* (PHA-L). It was apparent that the density of LDT and LHb inputs differed between VTA subregions in which different subpopulations of DA neurons reside[4, 5]. To test this conclusion, we simultaneously retrogradely labeled DA projection neurons and anterogradely labeled LDT or LHb fibers (FIG. **1**). Injection of PHA-L into LDT and RV expressing tdTomato (RV-tdTomato) into NAc lateral shell (FIG. **1**) revealed that RV-tdTomato cells were predominantly located in lateral VTA (FIG. **1**) that in close proximity contained LDT terminals as well as TH-immunopositive processes (FIG. **1**). More modest PHA-L labeling was observed in medial VTA (FIG. **1**) and substantia nigra (SN; FIG. **1**). In contrast, injection of PHA-L into LHb and RV-tdTomato into mPFC (FIG. **1**) revealed RV-tdTomato cells mainly in medial VTA (FIG. **1**) in close proximity to LHb terminals and TH-immunopositive processes (FIG. **1**, **1**). There was minimal PHA-L labeling of LHb inputs in the lateral VTA (FIG. **1**) or SN (FIG. **1**) but as expected[21, 22] PHA-L terminals were present in RMTg adjacent to GABAergic neurons (FIG. **9**). In additional experiments, we injected fluorescent retrobeads into NAc lateral shell or mPFC and labeled LDT or LHb inputs with PHA-L, respectively. A similar anatomical distribution of pre- and post-synaptic elements was observed (FIG. **9**).

Input Specific Control of Reward and Aversion

[0160] These anatomical results suggest that LDT and LHb inputs preferentially terminate in different VTA subregions adjacent to DA neuron subpopulations that project to different target structures (NAc lateral shell versus mPFC) and may subserve different behavioral functions[5, 6]. To address functional differences in these inputs, we generated a RV expressing the light-activated ion channel ChR2 fused to enhanced yellow fluorescent protein (EYFP, RV-ChR2) (FIG. **10**) and tested the consequences of activation of LDT-VTA and LHb-VTA pathways in a conditioned place preference (CPP) assay by injecting RV-ChR2 or RV-EGFP into VTA and implanting an optical fiber over LDT or LHb (FIG. **2**). Using a three day protocol (FIG. **2**), phasic stimulation of LDT neurons projecting to VTA on day 2 caused a strong CPP on day 3 (FIG. **2**), while phasic stimulation of LHb neurons projecting to VTA caused a strong conditioned place aversion (CPA) (FIG. **2**). Moreover, after the day 3 testing procedure (Post-Test 1), stimulating LDT neurons whenever animals were in the chamber in which they were conditioned on day 2 (Day 3, Post-Test 2) caused a further increase in CPP (FIG. **2**) whereas stimulating LHb neurons did not further enhance CPA (FIG. **2**). (See FIG. **11** for, non-normalized behavioral results.)

[0161] Additional results indicate that the effects of stimulating LDT and LHb neurons projecting to VTA were specific and due to driving activity in distinct populations of VTA neurons. First, animals that received intra-VTA injections of RV-EGFP exhibited no behavioral effects of phasic optical stimulation in LDT and LHb (FIG. **2**; FIG. **11**). Furthermore, low frequency stimulation of ChR2 in LDT and LHb had no effects in CPP/CPA assays (FIG. **2**; FIG. **11**). Second, non-stimulated animals showed no preference for either chamber (FIG. **10**) and there was no effect of the optogenetic manipulations on time spent in the central chamber (FIG. **12**). Third, stimulation of LDT and LHb neurons projecting to VTA had no effects on open field assays of anxiety or locomotor activity (FIG. **12**, FIG. **13**). Fourth, the placement of optical fiber in LDT and LHb was confirmed in all animals (FIG. **13**). Fifth, VTA DA neuron activation following LDT and LHb stimulation was quantified by assaying the proportion of TH-immunopositive and TH-immunonegative neurons that expressed the activity-dependent immediate early gene c-fos (FIG. **14**). Following LDT stimulation, ~40% of DA neurons in lateral VTA expressed c-fos whereas in medial VTA three-fold less DA neurons expressed c-fos. Activation of LHb inputs to the VTA caused an opposite pattern of c-fos expression: ~12% of DA neurons in medial VTA were c-fos-positive whereas <2% of DA neurons in lateral VTA expressed c-fos. Importantly, ~80% of non-DA neurons in the RMTg were c-fos-positive following LHb stimulation (FIG. **14**).

[0162] Based on these results we hypothesized that LHb inputs drive DA neurons in the medial posterior VTA that project to mPFC[4-6]. To test this prediction, we activated LHb inputs to VTA in animals in which medial VTA neuron subpopulations that project to different targets were identified by the presence of fluorescent retrobeads (FIG. **14**). In medial VTA, ~80% of neurons projecting to mPFC were c-fos-positive following LHb stimulation. In contrast, <10% of neurons projecting to NAc medial shell that are located in medial VTA[4-6] expressed c-fos following LHb stimulation.

[0163] Although the c-fos results confirm that stimulation of LDT and LHb neurons activated neurons in the VTA, axon collaterals of LDT and LHb neurons may project to other brain regions, activation of which mediated the observed CPP and CPA. To address this possibility, we injected adeno-associated viruses expressing ChR2-EYFP (AAV-ChR2) into LDT or LHb and stimulated axons of infected neurons using light application directly in the caudal VTA and RMTg (FIG. **15**). This produced robust CPP following intra-VTA LDT axonal stimulation and robust CPA following intra-VTA LHb axonal stimulation (FIG. **15**, FIG. **16**). A limitation of these experiments is that intra-VTA activation of LDT and LHb axons may cause antidromic activation of axon collaterals projecting to other brain regions. To address this possibility, we injected RV-EGFP or RV-tdTomato into VTA and the other virus into brain regions that receive inputs from LDT or LHb[27, 28]. If single LDT or LHb neurons projecting to VTA send collaterals to these other brain regions, the neurons will express both fluorophores. An extremely small number of LDT and LHb neurons projecting to other structures (i.e. ventral pallidum, lateral septum, lateral hypothalamus, mPFC, mediodorsal thalamic nucleus, and supraoculomotor central grey) expressed both fluorophores (FIG. **17**), suggesting that almost all of these neurons project solely to VTA/RMTg. As a positive control we injected one RV into VTA and the other into ventral pallidum and found dorsal raphe neurons (~20%), which are known to project to these two structures[29], expressed both EGFP and tdTomato (FIG. **17**). We also injected RVs into VTA and either LDT or LHb and examined labeling of cells in the other structure. Our results confirm that LDT and LHb have reciprocal anatomical connections[28] but the cells providing these projections do not project to VTA (FIG. **17**).

Synaptic Connectivity of LDT and LHb Inputs

[0164] The results thus far suggest that LDT and LHb inputs activate distinct populations of VTA and RMTg neurons and that this leads to reward and aversion, respectively. To address the specific synaptic connectivity of these inputs, we injected AAV-ChR2 into LDT and fluorescent retrobeads into target structures of VTA DA neurons (FIG. **3**; FIG. **18**). 812 weeks following these injections, ChR2-EYFP was expressed adjacent to VIA DA neurons projecting to NAc lateral shell (FIG. **3**) and its levels were significantly higher in the lateral VTA (FIG. **19**). To determine the DA neuron populations upon which LDT inputs directly synapse, we made whole-cell recordings from retrogradely labeled DA neurons projecting to the NAc lateral and NAc medial shell as well as non-labeled DA SN neurons (FIG. **3**). On average, optical stimulation of LDT fibers generated larger excitatory postsynaptic currents (EPSCs) in DA neurons projecting to NAc lateral shell than in DA neurons projecting to medial shell or DA neurons in SN (FIG. **3**), all recorded in the same sets of slices. The EPSCs in DA neurons projecting to NAc lateral shell were blocked by an AMPA receptor antagonist (CNQX, 10 μM; FIG. **3**) indicating that LDT fibers released glutamate. Importantly, stimulation of LDT inputs generated EPSCs (>10 pA) in 100% of DA neurons projecting to NAc lateral shell but only in ~30-40% of DA neurons projecting to NAc medial shell or in SN (FIG. **3**). Furthermore, only ~10% of DA neurons projecting to mPFC yielded EPSCs (FIG. **3**).

[0165] The same methodology (FIG. **4**; FIG. **18**) revealed that LHb inputs synapse on a different subpopulation of VTA DA neurons as well as on GABAergic cells in the RMTg. ChR2-EYFP expressing fibers from the LHb were found in medial posterior VTA in close proximity to DA neurons projecting to mPFC as well as in the RMTg (FIG. **18**, FIG. **19**). Importantly, light-evoked EPSCs were generated in 100% of DA neurons projecting to mPFC as well as GABAergic RMTg neurons whereas detectable EPSCs were not generated in DA neurons projecting to NAc medial shell or NAc lateral shell nor in SN neurons (FIG. **4**). Since LHb inputs preferentially synapse on NAc DA neurons projecting to mPFC and RMTg GABAergic cells, we predicted that LHb inputs may inhibit DA neurons projecting to NAc lateral shell via feed-forward inhibition. Indeed, in ~60% of DA neurons projecting to NAc lateral shell stimulation of LHb inputs evoked IPSCs (FIG. **4**). In contrast, stimulation of LHb axons did not generate detectable IPSCs in DA neurons projecting to NAc medial shell (FIG. **4**).

[0166] These results suggest that LDT and LHb inputs to VTA preferentially activate distinct populations of DA neurons that project to different target structures and that in addition, LHb inputs activate GABAergic cells in RMTg and perhaps within the VTA itself. Such differences in connectivity can explain the different behavioral consequences of LDT and LHb stimulation (FIG. **2**). To further test these conclusions, we generated AAVs expressing a double floxed RV glycoprotein (AAV-DIO-RVG) and infected the VTA in TH-Cre mice so that glycoprotein was only expressed in DA neurons (FIG. **5**). Two weeks later, RV-EGFP and RV-tdTomato were injected into mPFC and NAc lateral shell, respectively (FIG. **5**). Because RV-EGFP and RV-tdTomato lack RV glycoprotein, expression of EGFP and tdTomato is restricted to initially infected cells[16]. However, in VTA DA neurons projecting to these targets (FIG. **5**), transcomplementation with RV glycoproteins occurs and allows RV-EGFP and RV-tdTomato to spread retrogradely, thus labeling cells that synaptically contact the DA neurons. After RV injections, cells in LDT were clearly labeled with tdTomato with almost no cells expressing EGFP (tdTomato, n=18.75±7.12 cells per animal, EGFP, n=1.25±0.75, n=4 mice; FIG. **5**) while LHb cells were clearly labeled with EGFP with almost no cells expressing tdTomato (EGFP, 8.25±3.44 cells; tdTomato, 0.5±0.22, n=4 mice; FIG. **5**). When AAV-DIO-RVG was not injected into VTA prior to RV injections, no tdTomato-positive or EGFP-positive cells in LDT or LHb, respectively, were observed (n=3 mice) (FIG. **5**). These results confirm that LDT neurons preferentially synapse on VTA DA neurons projecting to NAc lateral shell and LHb neurons preferentially synapse on VTA DA cells projecting to mPFC.

Effects of DA Receptor Antagonists in mPFC and NAc Lateral Shell

[0167] Activation of VTA GABAergic cells alone can elicit CPA[13] and disrupt reward consummatory behavior[15]. These results raise the question of whether activation of DA neurons projecting to mPFC is necessary for the CPA elicited by activation of LHb inputs to VTA and RMTg. To address this question, we infused the D1 dopamine receptor antagonist SCH23390 into mPFC immediately prior to stimulating LHb neurons projecting to VTA and RMTg (FIG. **5**). This manipulation, which does not impair cocaine CPP[30], prevented the occurrence of CPA, which was elicited in control animals that received vehicle injections into mPFC (FIG. **5**; FIG. **20**). Similarly, infusion of D1 and D2 receptor antagonists into NAc lateral shell, but not infusion of vehicle, prevented the CPP elicited by activation of LDT neurons projecting to VTA (FIG. **5**, FIG. **20**). Control experiments revealed that infusion of DA receptor antagonists alone into either the mPFC or NAc lateral shell did not elicit CPP or CPA compared to animals that received vehicle infusions (n=4 mice in each group; p>0.05 Mann-Whitney U-tests). These results provide further evidence that activation of different subpopulations of VTA DA neurons and the consequent release of DA in different target structures are necessary for mediating the reward and aversion generated by activation of LDT and LHb inputs, respectively.

[0168] Ventral tegmental area (VTA) dopamine neurons play important roles in adaptive and pathological brain functions related to reward and motivation. It is unknown, however, if subpopulations of VTA dopamine neurons participate in distinct circuits that encode different motivational signatures and whether inputs to the VTA differentially modulate such circuits. Here we show that because of differences in synaptic connectivity activation of inputs to the VTA from the laterodorsal tegmentum and the lateral habenula elicit reward and aversion in mice, respectively. Laterodorsal tegmentum neurons preferentially synapse on dopamine neurons projecting to nucleus accumbens lateral shell while lateral habenula neurons synapse primarily on dopamine neurons projecting to medial prefrontal cortex as well as on GABAergic neurons in the VTA tail. These results establish that distinct VTA circuits generate reward and aversion and thereby provide a novel framework for understanding the circuit basis of adaptive and pathological motivated behaviors.

[0169] FIG. **1**. LDT and LHb preferentially project to distinct VTA subregions. a, Injection sites for RV-tdTomato in NAc and PHA-L in LDT. Image shows PHA-L staining in LDT (4V: fourth ventricle). b, RV-tdTomato in NAc lateral shell. c, VTA neurons projecting to NAc lateral shell are mainly located in lateral VTA (IPN: interpeduncular nucleus) (a-c scale bars, 200 μm). d,e, PHA-L labeled terminals (green) from LDT are adjacent to cells projecting to NAc lateral shell (red) as well as TH-immunopositive processes (blue). f, g, Few PHA-L labeled terminals were detected in medial VTA (f) and in SN (g) (d-g scale bars, 20 μm). h, Injection sites for RV-tdTomato in mPFC and PHA-L in LHb. Image shows PHA-L staining in LHb (MHb: medial habenula; D3V: dorsal third ventricle). i, RV-tdTomato in mPFC. j, VTA neurons projecting to mPFC are mainly located in medial VTA (h-j scale bars, 200 μm). k, 1, PHA-L labeled terminals (green) from LHb are found adjacent to cells projecting to mPFC (red) as well as TH-immunopositive processes (blue). m, n, Few PHA-L labeled terminals were detected in lateral VTA (m) and in SN (n) (k-n scale bars, 20 μm).

[0170] FIG. **2**. Stimulation of LDT and LHb inputs to VTA elicits CPP and CPA. a,b, RV-ChR2 injection into VTA and optical stimulation of (a) LDT- and (b) LHb projection neurons. c, Procedure to elicit and test CPP and CPA. d,e, Example day 3 mouse tracks, Post-Test 1. Arrow indicates chamber in which (d) LDT or (e) LHb projection neurons were stimulated on Day 2. f, Ratio from Post-Test 1/Pre-Test of time spent in conditioned chamber was higher in LDT-ChR2 mice compared to LDT-EGFP mice (LDT-ChR2: 1.32±0.1, n=8; LDT-EGFP: 0.96±0.13, n=7) but lower in LHb-ChR2 mice (LHb-ChR2: 0.76±0.06, n=9; LHb-EGFP: 0.99±0.08, n=11). g, Differences between Post-Test 1 and Pre-Test in time mice spent in conditioned or unconditioned chambers. (LDT-ChR2 mice: conditioned chamber: 105.4±34.38, n=8; unconditioned chamber: −51.1±26.76, n=8) (LHb-ChR2 mice: cond. chamber: −90.87±22.59, n=9; unconditioned chamber: 124.3±26.27, n=9). h, Stimulation of LDT-ChR2 mice during Post-Test 2 enhanced preference for conditioned chamber (LDT-ChR2 Post-Test 1, 1.32±0.1, n=8; Post-Test 2, 1.85±0.2, n=8; Post-Test 2 LDT-EGFP mice 1.13±0.16, n=7). Stimulation of LHb-Chr2 mice during Post-Test 2 did not cause further aversion (LHb-ChR2 Post-Test 1, 0.76±0.06, n=9; Post-Test 2, 0.85±0.08, n=9) which was still present (LHb-EGFP Post-Test 2, 1.22±0.16, n=11). (Post-test 1 results are same as in f). i, Low frequency stimulation of LDT-ChR2 and LHb-ChR2 cells did not elicit CPP or CPA (Post-Test 1, LDT-ChR2, 1.13±0.09, n=6; Post-Test 2, LDT-ChR2, 1.28±0.26, n=6; Post-Test 1, LHb-ChR2, 0.97±0.14, n=7; Post-Test 2, LHb-ChR2, 1.14±0.17, n=6). Error bars denote s.e.m. *p<0.05; **p<0.01; ***p<0.001, Mann-Whitney U-test.

[0171] FIG. **3**. LDT neurons preferentially synapse on DA neurons projecting to NAc lateral shell. a, AAV-ChR2-EYFP injected into LDT and retrobeads injected into NAc lateral shell and NAc medial shell or in mPFC. b, ChR2-EYFP expression in close proximity to retrogradely labeled (beads) TH-immunopositive neurons in lateral VTA (scale bar, 50 μm). c-f, Traces from whole-cell recordings at −70 mV showing EPSCs generated by stimulation of LTD inputs in retrogradely labeled VTA neurons (beads) projecting to (c) NAc lateral shell, (d) NAc medial shell, (e) mPFC or (f) SN neurons. All cells were filled with neurobiotin (NB, green) and are TH-immunopositive (blue). Scale bars: 20 pA/20 ms. g, Summary of average EPSCs generated by optical stimulation of LDT inputs in the four cell populations (**p<0.01, ***p<0.001, 1 way ANOVA with Bonferroni post-hoc test; Error bars denote s.e.m.). h, Percentage of cells in which optical stimulation generated EPSCs >10 pA. N's shown within each bar also apply to g.

[0172] FIG. **4**. LHb neurons preferentially synapse on DA neurons projecting to mPFC and RMTg GABAergic neurons. a, AAV-ChR2-EYFP injected into LHb and retrobeads injected either into NAc lateral shell and NAc medial shell or in mPFC. b-e, Traces from whole-cell recordings at −70 mV showing EPSCs generated by optical stimulation of LHb inputs in retrogradely labeled VTA neurons (beads, red) projecting to (b) mPFC or (c) NAc lateral shell or (d) an RMTg cell and (e) SN cell. All cells were filled with neurobiotin (NB, green) and are either TH-immunopositive (blue) (b, c, e) or GAD67-immunopositive (blue, d). Scale bars: 20 pA/20 ms. f, Summary of average EPSCs generated by optical stimulation of LHb inputs in five cell populations (**p<0.01, ***p<0.001, 1 way ANOVA with Bonferroni post-hoc test, error bars denote s.e.m.). g, Percentage of cells in which optical stimulation generated EPSCs >10 pA. N's shown in this graph also apply to f. h, Optical stimulation of LHb inputs generates IPSC in DA cell projecting to NAc lateral shell (PCTX, picrotoxin) (scale bars, 20 pA/20 ms). Graph shows percentage of DA cells projecting to NAc lateral shell or medial shell in which IPSCs were generated by LHb input stimulation. i, Average IPSC size from DA cells projecting to NAc lateral shell, IPSCs were blocked by picrotoxin (n=3; ***p<0.0001, unpaired Student's t-test).

[0173] FIG. **5**. Rabies virus reveals distinct VTA circuits and effects of DA receptor antagonists on CPP/CPA. a, AAV expressing rabies glycoprotein (RVG) in a Cre-dependent manner was injected into VTA of TH-Cre mice. RV-EGFP and RV-tdTomato, injected subsequently into mPFC and NAc, respectively, are retrogradely transported to subpopulations of DA neuron in which transcomplementation occurs, allowing RV to spread retrogradely and label cells that synaptically contact infected DA neurons. b, Injection sites in NAc lateral shell (RV-tdTomato) and mPFC (RV-EGFP) (scale bars, 200 μm). c, TH-immunoreactive neurons in VTA retrogradely labeled by RV-tdTomato or RV-EGFP (scale bars, 20 μm). d,e tdTomato and EGFP labeling in LDT (d) and LHb (e) neurons, respectively, when injection of AAV-DIO-RVG into VTA of TH-Cre mice was performed prior to RV injections (DTg, dorsal tegmental nucleus; Aq, aqueduct; MHb, medial habenula; D3V, dorsal third ventricle; Th, thalamus) (d, e scale bars, 100 μm). f,g, Lack of tdTomato expression in LDT (f) and lack of EGFP expression in LHb (g) following RV injections in TH-Cre mice that were not injected with AAV-DIO-RVG (f, g scale bars, 100 μm). h, Placements of drug infusion cannula into mPFC and optic fiber into LHb as well as injection of RV-ChR2 into VTA. i, Ratio of Post-Test/Pre-Test time spent in conditioned chamber when SCH23390 (SCH) or vehicle was infused into mPFC prior to LHb optical stimulation (SCH: 0.95±0.05, n=9; vehicle: 0.75±0.04, n=7). j, Difference between Post-Test and Pre-Test in time mice spent in conditioned or unconditioned chambers following LHb stimulation (SCH: conditioned chamber, −7.24±28.79, unconditioned chamber: 36.83±30.74, n=9; vehicle: conditioned chamber, −106.88±18.82, unconditioned chamber, 112.61±26.48, n=7). k, Placements of drug infusion cannula into NAc lateral shell and optic fiber into LDT as well as injection of RV-ChR2 into VTA. 1, Ratio of Post-Test/Pre-Test time spent in conditioned chamber when SCH23390 and raclopride (rac) or vehicle were infused into NAc lateral shell prior to LDT optical stimulation (SCH/rac: 0.89±0.1, n=7; vehicle: 1.26±0.08, n=6). m, Difference between Post-Test and Pre-Test in time mice spent in conditioned or unconditioned chamber following LDT stimulation (SCH/rac: conditioned chamber: −30.17±37.38, unconditioned chamber: 42.22±34.68, n=7; vehicle: conditioned chamber: 94.58±27.77, unconditioned chamber, −59.38±26.44, n=6) *p<0.05, **p<0.01, ***p<0.001, Mann-Whitney U-test. Error bars denote s.e.m. n, Hypothesized circuits driven by LDT and LHb inputs into the VTA. Green shading indicates circuit involved in aversion; red/pink shading indicates circuit involved in reward and salience.

[0174] FIG. **7**. Identification of inputs to VTA. a, Schematic showing rabies virus (RV)-EGFP injected into the VTA. b, Image showing EGFP expression in VTA (IPN: interpeduncular nucleus). c-f, Images of clusters of EGFP-positive VTA projection neurons in (c) prefrontal cortex (PFC), (d) nucleus accumbens (NAc), (e) lateral hypothalamus (LH) and lateral habenula (LHb, inset), (f) laterodorsal tegmentum (LDT) (scale bars, 200 μm). g, Summary schematic of all labeled inputs to the VTA (M1/2, primary and secondary motor cortex; Cg, cingulate cortex; PrL/IL, prelimbic/infralimbic cortex; MO, medial orbital cortex; NAc, nucleus accumbens; CPu, caudate-putamen; VP, ventral pallidum; BNST, bed nucleus of the stria terminalis; LHb, lateral habenula; LH, lateral hypothalamus; PPT, pedunculopontine tegmental nucleus; DR, dorsal raphe; LDT, laterodorsal tegmentum).

[0175] FIG. **8**. LDT and LHb neurons that project to the VTA are mainly glutamatergic. a, EGFP-positive neurons (green) in the LDT following injection of RV-EGFP in the VTA (DTg, dorsal tegmental nucleus; 2cb, 2nd cerebellar lobule) (Scale bar, 100 μm). b, Upper row: confocal images showing an EGFP-positive neuron in the LDT that is immunopositive for choline acetyltransferase (ChAT, red, arrow). Note, that many ChAT-positive neurons do not co-localize with EGFP. Lower row: confocal images showing an EGFP-positive neuron in the LDT that is immunopositive for the glutamate transporter (EAAC1, red, left arrow) and another EGFP-positive neuron that is immunonegative for EAAC1 (right arrow) (Scale bars, 20 μm). c, ChAT was coexpressed in 7% of EGFP-positive neurons in the LDT (n=484 cells analyzed from 2 mice). EAAC1 was coexpressed in 95% of EGFP positive neurons in the LDT (n=386 cells analyzed from 2 mice). d, EGFP-positive neurons (green) in the LHb following injection of RV-EGFP in the VTA (DTg, dorsal tegmental nucleus; 2cb, 2nd cerebellar lobule) (Scale bar, 100 μm). Note, that some EGFP-positive neurons are located in the medial habenula (MHb) due to small virus contaminations in the dorsal interpeduncular nucleus (IPN). Control experiments in which retrobeads were injected into the VTA with no contamination in the IPN did not show labeling of MHb neurons indicating that LHb, but not MHb, neurons project to the VTA (data not shown). e, Upper row: confocal images showing that EGFP-positive neurons in the LHb are ChAT (red)immunonegative. Lower row: Confocal images showing EGFP-positive neurons are EAAC1 (red)immunopositive. All analyzed EGFP-positive cells (n=499 cells from 3 mice) were immunopositive for EAAC1 (Scale bars, 20 μm). f, Confocal image showing that ChAT-positive neurons (red) could be found in the medial habenula.

[0176] FIG. **9**. LDT and LHb terminals in the VTA are immediately adjacent to DA neurons projecting to different target structures. a, Upper row: schematic (left) and representative image of the injection-site of the anterograde tracer *Phaseolus vulgaris leucoagglutinin* (PHA-L, green) in the LDT (middle) (DTg, dorsal tegmental nucleus; 2cb, 2nd cerebellar lobule). Retrobead injection-site in the NAc lateral shell from the same animal (right image) (Scale bars, 200 μm). Middle row: Confocal images showing that PHA-L immunopositive terminals from the LDT are mainly found in the lateral VTA in close proximity to TH-positive (blue) neurons which are retrogradely labeled (beads, white), indicating that they project to the NAc lateral shell (Scale bars, left/right, 50/20 μm). Lower row: Confocal images showing very sparse PHA-L labeling in the medial VTA and SN (Scale bars, 50 μm). b, Upper row: schematic (left) and representative image of the PHA-L injection site (green) in the LHb (middle) (DG, dentate gyrus; MHb, medial habenula; CL, centrolateral thalamic nucleus; MDL, mediodorsal thalamic nucleus, lateral part). Retrobead injection-site in the mPFC from the same animal (right) (Scale bars, 200 μm). Middle row: confocal images showing that PHA-L immunopositive terminals from the LHb are mainly found in the medial VTA in close proximity to TH-positive (blue) neurons which are retrogradely labeled (beads, white), indicating that they project to the mPFC (Scale bars, left/right, 50/20 μm). Lower row: confocal images showing very sparse PHA-L labeling in the lateral VTA and SN (Scale bars, 50 μm). c, Following injection of PHA-L into the LHb, intense PHA-L labeling could be found in the RMTg. Schematic of the anatomical location of the RMTg (upper left) and representative image showing PHA-L immunopositive terminals in the RMTg (upper right; Scale bar 200 μm) (IP, caudal interpeduncular nucleus). Lower picture: confocal image showing PHA-L-positive LHb terminals in the RMTg are adjacent to GAD67-immunopositive neurons (red; Scale bar, 20 μm).

[0177] FIG. **10**. Establishing a behavioral assay for examining the consequences of activating LDT and LHb neurons that project to the VTA. To investigate the in vivo function of LDT and LHb inputs to the VTA we developed a rabies virus (RV) encoding channelrhodopsin 2 (ChR2) and used a well-validated behavioral assay: conditioned place preference/aversion. a, Schematic diagram of the genome of recombinant rabies virus expressing ChR2-EYFP and an image of the expression of ChR2-EYFP by rabies virus in HEK293 cells. The open reading frame of the glycoprotein is replaced with that of ChR2-EYFP. N, P, M and L denote the open reading frames of remaining viral genes that express nucleoprotein, phosphoprotein, matrix protein, and viral polymerase, respectively. b, The conditioned place preference/aversion chamber was designed so that mice did not have a consistent bias for a particular chamber. To test this, we used male, 12 week old C57Bl/6 mice (n=9) and let them freely explore the chamber for 15 min over a period of 5 days. There was no significant change in the Post/Pre ratio over the 5 days (Post/Pre ratio day 2: 1.03±0.08, day 3: 1.05±0.09, day 4: 1.05±0.07, day 5: 1.08±0.09, $p>0.05$ Tway ANOVA, Bonferroni post-hoc test).

[0178] FIG. **11**. Pre- and Post-Test times spent in conditioned chamber for the behavioral assays of individual mice that were injected with RV-ChR2-EYFP or RV-EGFP into the VTA. a-c, For LDT stimulation mice received phasic simulation after expression of ChR2-EYFP (a) or EGFP (b) as well as tonic stimulation after expression of ChR2-EYFP (c). d-e, Similarly, for LHb stimulation mice received phasic simulation after expression of ChR2-EYFP (d) or EGFP (e) as well as tonic stimulation after expression of ChR2-EYFP (f). Note that there are no significant differences in the Pre-Test times between mice injected with RV-ChR2-EYFP and mice injected with RV-EGFP as well as between mice in which the LDT or LHb was optically stimulated (*$p<0.05$, Mann-Whitney U-Test).

[0179] FIG. **12**. Stimulation of LDT and LHb neurons projecting to VTA does not influence locomotor activity nor anxiety. a, Bar graph showing that there was no significant difference in the Post-Test 1/Pre-Test ratio for time spent in the central chamber following stimulation of LDT and LHb inputs to the VTA between RV-ChR2 and RV-EGFP injected mice (LDT-ChR2: 0.71±0.06, n=8; LDT-EGFP: 0.92±0.12, n=7, $p>0.05$ Mann-Whitney U-Test; LHb-ChR2: 0.79±0.08, n=9; LHb-EGFP: 0.82±0.12, n=11, $p>0.05$ Mann-Whitney U-Test). b, Bar graph showing that the difference score for the central chamber was not significantly different between LDT-ChR2 and LHb-ChR2 mice (LDT-ChR2: −54.25±13.33, n=8; LHb-ChR2: −33.42±12.33, n=9, p=0.236; $p>0.05$ Mann-Whitney U-Test). c, Bar graph showing that there was no significant difference in the Post-Test 2/Pre-Test ratio for the central chamber between RV-ChR2 and RV-EGFP injected mice (LDT-ChR2: 0.48±0.1, n=8, LDT-EGFP: 0.76±0.1, n=7, $p>0.05$ Mann-Whitney U-Test; LHb-ChR2: 0.69±0.07, n=9, LHb-EGFP: 0.64±0.15, n=11, $p>0.05$ Mann-Whitney U-Test). d, Bar graph showing that a low frequency stimulation protocol did not significantly change the post/pre ratio of the central chamber in RV-ChR2 and RV-EGFP injected mice (Post-Test 1: LDT-ChR2: 1±0.14, n=6, Post-Test 2: LDT-ChR2: 0.85±0.1, n=6, $p>0.05$ Mann-Whitney U-Test; Post-Test 1: LHb-ChR2: 0.67±0.13, n=7, Post-Test 2: LHb-ChR2: 0.69±0.2, n=7; $p>0.05$ Mann-Whitney U-Test). e-h, Optical stimulation of LDT and LHb inputs to the VTA did not significantly influence general locomotor activity nor anxiety in an open-field-test. The bar graphs show the mean total time for the light on and light off period (9 min each) for: (e) average velocity (LDT-EGFP, light off, 5.5±0.1, light on, 5.6±0.4; LDT-ChR2, light off, 6.6±0.5, light on, 7±0.5; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test: LHb-EGFP, light off, 5.7±0.3, light on, 5.7±0.3; LHb-ChR2, light off, 6.4±0.6, light on, 6.7±0.5; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test). (f) track length (LDT-EGFP, light off, 997.5±18.4, light on, 1007±76; LDT-ChR2, light off, 1181±89, light on, 1260±97.8; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test: LHb-EGFP, light off, 1026±58.8, light on, 1020±62.1; LHb-ChR2, light off, 1157±114.9, light on, 1214±97.5; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test). (g) wall distance (LDT-EGFP, light off, 5.5±0.5, light on, 4.9±0.6; LDT-ChR2, light off, 5.1±0.3, light on, 5.2±0.3; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test: LHb-EGFP, light off, 4.8±0.5, light on, 4.6±0.4; LHb-ChR2, light off, 5.3±0.4, light on, 5.4±0.5; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test). (h) duration in inner zone (LDT-EGFP, light off, 40.2±10, light on, 21.3±7; LDT-ChR2, light off, 32.5±7.6, light on, 32.8±7.8; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test: LHb-

EGFP, light off, 30.1±7.2, light on, 19±2.2; LHb-ChR2 light off, 37.6±5.5, light on, 35.6±9.3; $p>0.05$ Tway ANOVA, Bonferroni post-hoc test). (LDT-EGFP, n=5 mice; LDT-ChR2, n=7 mice; LHb-EGFP, n=5 mice; LHb-ChR2, n=6 mice.)

[0180] FIG. **13**. Further evidence that stimulation of LDT and LHb neurons projecting to VTA does not influence locomotor activity or measures of anxiety in the open field. a, b, Diagram of experimental procedure (a) and examples of individual tracks in the open field (b) during optical stimulation of LDT or LHb neurons that project to VTA. c, d, Summary of the effects of LDT and LHb stimulation in the open field on locomotor velocity (c) and duration of time spent in the inner zone, a measure of anxiety (d; marked by red boxes in b). e, f, Confirmation of placement of optical fibers (cannula track) and ChR2 expression in LDT (e) and LHb (f) (e, f,

[0181] FIG. **14**. In vivo optical activation of LDT and LHb neurons projecting to the VTA induces c-fos expression in distinct VTA subpopulations. a,b, Confocal images showing TH-immunopositive (i.e. DA) neurons (red) in the (a) lateral VTA and (b) medial VTA. c-fos immunoreactivity (blue) and RV-ChR2 expression (green) or EGFP expression (green) are also shown following stimulation of LDT projection neurons for 30 min using the phasic stimulation protocol. Note lack of c-fos immunoreactivity following stimulation of LDT cells expressing EGFP and in images of medial VTA (Scale bars, 20 μm). c, TH-immunostaining showing the areas that have been analyzed in the lateral and medial VTA (Bregma −3.4 mm; Scale bar, 200 μm). d, Bar graph showing a high proportion of c-fos-positive DA neurons in the lateral VTA following phasic optical stimulation of LDT neurons projecting to VTA in mice injected with RV-ChR2 in VTA (1VTA: TH+cfos+, 38.1%; TH−cfos+, 8.5%; TH+cfos−, 53.4%; n=189 cells from 2 mice) with nearly 3-fold less c-fos-positive DA neurons in the medial VTA (mVTA: TH+cfos+, 14.3%; TH−cfos+, 5.8%; TH+c−fos−, 79.9%; n=224 cells from 2 mice). Extremely low c-fos expression was observed in both lateral and medial VTA following phasic optical stimulation of LDT in mice injected with RV-EGFP in VTA (lateral VTA: TH+cfos+, 0.6%; TH−cfos+, 1.2%; TH+cfos−, 98.2%; n=170 cells from 2 mice; medial VTA: TH+cfos+, 1.7%; TH−cfos+, 3.4%; TH+cfos−, 94.9%; n=177 cells from 2 mice). e, f, Same experimental design as in a,b, but for phasic optical stimulation of LHb neurons projecting to the VTA. Confocal images showing medial (e) and lateral (f) VTA (Scale bars, 20 μm). Note lack of c-fos staining in lateral VTA. g, Bar graphs showing that optical activation of LHb neurons projecting to VTA induced the highest proportion of c-fos-positive DA neurons in the medial VTA (TH+cfos+, 11.9%; TH-cfos+, 16.2%; TH+cfos−, 71.9%; n=210 cells from 2 mice) with 9-fold less c-fos-positive DA neurons in the lateral VTA (TH+cfos+, 1.3%; TH−cfos+, 5.2%; TH+cfos−, 93.5%; n=155 cells from 2 mice). There was a high proportion of c-fos-positive non-DA neurons in the RMTg (TH+ cfos+, 4.1%; TH−cfos+, 81.6%; TH+cfos, 14.3%; n=49 cells from 2 mice). There was sparse c-fos expression in any VTA subregion following optical stimulation of LHb in mice injected with RV-EGFP in the VTA (lateral VTA: TH+cfos+, 2.1%; TH−cfos+, 2.1%; TH+cfos−, 95.8%; n=94 cells from 2 mice; medial VTA: TH+cfos+, 3%; TH−cfos+, 7%; TH+cfos−, 90%; n=100 cells from 2 mice; RMTg: TH+cfos+, 0%; TH−cfos+, 14.3%; TH+cfos−, 85.7%; n=7 cells from 2 mice). h, Confocal images showing that retrogradely labeled (beads, red) neurons projecting to the mPFC (left) or NAc medial shell (right), both located in the medial VTA, show differences in c-fos induction following in vivo phasic stimulation of LHb neurons projecting to the VTA (Scale bars, 20 μm). 77% of the mesoprefrontal neurons (n=10/13 cells from 2 mice) were c-fos-positive. In contrast, only 8% of the neurons projecting to the NAc medial shell were c-fos-positive (n=2/25 cells from 2 mice). Note that even though we do not demonstrate that the retrogradely labeled neurons in this experiment are TH-immunopositive, we previously found that the majority of retrogradely labeled neurons in the posterior VTA were DAergic (Lammel et al., 2011).

[0182] FIG. **15**. Optical stimulation of axon terminals from LDT and LHb in VTA. a,b, AAV-ChR2 injection into LDT (a) and LHb (b) and optical stimulation of axon terminals in VTA. c, Ratios from Post-Test 1,2/Pre-Test of time spent in conditioned chamber in mice in which LDT axons in VTA were stimulated (LDT Post-Test 1, 1.34±0.2, n=9; Post-Test 2, 1.53±0.1, n=9) or LHb axons in VTA were stimulated (LHb Post-Test 1, 0.79±0.05, n=9; Post-Test 2, 0.65±0.2, n=9). d, Differences between Post-Test 1 and Pre-Test in time mice spent in the conditioned or unconditioned chamber (LDT axon stimulation: conditioned chamber: 100.9±49.07, n=9, unconditioned chamber: −50.05±46.22, n=9) (LHb axon stimulation: conditioned chamber: −105.12±16.92, n=9, unconditioned chamber: 130.4±26.96, n=9). Error bars denote s.e.m. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$, Mann-Whitney U-test.

[0183] FIG. **16**. Pre- and Post-Test times spent in conditioned chamber for the behavioral assays of individual mice in which AAV-ChR2 was injected into the LDT or Lhb and LDT or LHb axon terminals in the VTA were stimulated a, b, Both LDT (a) and LHb (b) axon terminals received light stimulation. Note that there are no significant differences in the Pre-Test times for the two populations of animals. ($^{*}p<0.05$, Mann-Whitney U-Test).

[0184] FIG. **17**. Double injections of RVs expressing two different fluorophores show LDT and LHb neurons projecting to VTA have very few if any axon collaterals. a-e, Representative images of LDT (top) and LHb (bottom) neurons that were retrogradely labeled by the simultaneous injection of RV-EGFP into VTA and RV-tdTomato into various brain areas including ventral pallidum (VP; a), lateral septum (LS; b), lateral hypothalamus (LHT; c), medial prefrontal cortex (mPFC; d) or mediodorsal nucleus of thalamus (MD, e). Scale bars 150 μm, 50 μm (insets). f-g, Summary bar graphs of the numbers of LDT (f) and LHb (g) neurons that were; (i) retrogradely labeled by the RV injection into the named brain areas, (ii) retrogradely labeled by the RV injection into the VTA, and (iii) retrogradely labeled by both RV's. Almost no cells in either the LDT or the LHb were double labeled. (Su3: supraoculomotor central grey; n=2 mice for each brain area). The bars that reach 400 for the number of LDT or LHb cells retrogradely labeled by the RV injected into the VTA indicate that greater than 400 cells were labeled. h, Confocal image of dorsal raphe-containing brain section from mouse in which RV-EGFP and RV-tdTomato were injected into VTA and VP, respectively. Scale bars, 150 μm, 50 μm (inset). i, Summary bar graphs of the number of DR neurons that were; (i) retrogradely labeled with tdTomato 7 days following RV-tdTomato injection into the VP, (ii) retrogradely labeled with EGFP following RV-

EGFP injection into the VTA, and (iii) retrogradely labeled by both RV's (n=2 mice). j, k, Confocal images of retrogradely labeled neurons in LDT (j) and LHb (k) following double injection of RV-EGFP into VTA and RV-tdTomato into LHb (j) or LDT (k), respectively. Scale bar 150 μm, 50 μm (inset). 1, Summary bar graphs of the numbers of LDT and LHb neurons that were; (i) retrogradely labeled by the RV injected into the opposite brain area, (ii) retrogradely labeled by the RV injected into the VTA, and (iii) retrogradely labeled by both RVs (n=2 mice). Almost no cells expressed both fluorophores.

[0185] FIG. **18**. Injection of retrobeads and AAV-ChR2 for studies of synaptic connectivity. a, Images showing injection-site of AAV-ChR2-EYFP in the LDT(scale bar, 200 μm). Inset shows higher magnification view (DTg, dorsal tegmental nucleus). b, Image showing injection sites of retrobeads in NAc medial shell (mshell) and NAc lateral shell (lshell) (scale bar, 200 μm) (DS, dorsal striatum). c, Image showing injection site of AAV-ChR2-EYFP in the LHb (scale bar, 200 μm). Inset shows higher magnification view. d, Image showing injection site of retrobeads in mPFC. (Cg, cingulate cortex; PrL, prelimbic cortex; IL, infralimbic cortex). e, Images showing strong ChR2-EYFP (green) expression from LHb axons in close proximity to retrogradely labeled (beads, white) TH-immunopositive (red) neurons in the medial VTA (scale bar, 20 μm). f, Images showing strong ChR2-EYFP (green) expression from LHb axons in RMTg with minimal TH-immunoreactivity and no retrogradely labeled neurons (scale bar, 50 Vim).

[0186] FIG. **19**. Fluorescence intensity of LDT and LHb terminals expressing ChR2-EYFP differs in VTA subregions. a, Confocal images of ChR2-EYFP immunopositive (green) terminals from the LDT in subregions of ventral midbrain also stained for TH (red). Neurons in the lateral VTA were retrogradely labeled by injection of retrobeads (white) in the NAc lateral shell. (Scale bars, 20μ). b, Bar graph showing that the average fluorescence intensity, a marker for the density of LDT terminals, is significant higher in the medial VTA, compared to the lateral VTA and SN (lateral VTA, 19.1±0.9; medial VTA, 13.5±0.7; SNc, 12±0.9; n=12 confocal images per brain region from 2 mice). c, Same experimental design and color code as in (a) but confocal images show ChR2-EYFP expressing terminals from the lateral habenula. (Scale bars, 20 μm). d, The average fluorescence intensity of LHb terminals in the lateral VTA is significantly lower than in the medial VTA and RMTg (confocal image of ChR2-EYFP expression in the RMTg is shown in FIG. **4**), but not significantly different from that in the SNc (lateral VTA, 6.4±0.5; medial VTA, 20.7±1.3; SNc, 5±0.2; RMTg, 33.8±2.5; n=12 confocal images per brain region from 2 mice). (***$p<0.001$ Tway ANOVA with Bonferroni post-hoc test.)

[0187] FIG. **20**. Pre- and Post-Test times spent in the conditioned chamber for the behavioral assays of individual mice in which dopamine receptor antagonists were injected into the mPFC or NAc lateral shell immediately prior to optical stimulation of LHb or LDT neurons that project to VTA. a,b, Infusion of SCH23390 into the mPFC (a) but not vehicle (b) prevented the CPA elicited by phasic stimulation of LHb neurons that project to VTA. c, d, Infusion of SCH23390 and raclopride (c) but not vehicle (d) prevented the CPP elicited by phasic stimulation of LDT neurons that project to VTA. Note that there are no significant differences in the Pre-Test times for the two populations of animals (*$p<0.05$, Mann-Whitney U-Test).

[0188] FIG. **21**. Expression of ChR2-EYFP in the midbrain, the LDT and the LHb following RV-ChR2 injections into the VTA. a-d, Left panels show images of RVChR2-EYFP (green) expression following TH-immunohistochemistry (red) across the caudorostral extent of the midbrain [bregma: −4.48 mm (a), −4.16 mm (b) −3.52 mm (c), and −3.08 mm (d)]. Right panels show schematic drawings of the corresponding brain regions and use different colors to outline the brains regions in which ChR2-EYFP was detected with each color representing the expression profile from a single mouse (n=5). Scale bars, 200 μm. e, f, Example confocal images of LDT and LHb neurons expressing ChR2-EYFP 7 days following RV-ChR2 injection into the VTA injections. NeuN immunoreactivity identifies all cells in the image. Scale bar, 15 μm. g, Summary graph (n=3 mice) showing the proportion of LDT and LHb cells (identified by NeuN) that expressed ChR2-EYFP. More than 20% of NeuN-positive LDT neurons (22.8%, n=218 of 956) and LHb (20.9%, n=209 of 997) neurons expressed ChR2-EYFP.

[0189] FIG. **22**. LHb and LDT neurons projecting to VTA send very few axon collaterals to the interpeduncular nucleus (IPN). a, Schematic showing red and green retrobead injections into VTA or IPN, respectively. b, Confocal image of medial habenula (MHb) and LHb neurons that project to VTA (red beads) and IPN (green beads). Scale bar, 50 μm. c, Summary of all labeled cells (n=2 mice) showing the percentage of LHb and MHb cells that projected to VTA (red beads), IPN (green beads) or both areas (double-labeled). Note that neurons projecting to IPN are predominantly located in the MHb while neurons projecting to VTA are almost exclusively in the LHb. d, e, Confocal image (d; Scale bar, 50 μm; Aq, aqueduct) and summary bar graphs (e) of LDT neurons from the same animals showing that 100% of labeled cells contained red beads and therefore LDT neurons send projections to the VTA but not the IPN.

REFERENCES

[0190] 1. Bjorklund, A. & Dunnett, S. B. Dopamine neuron systems in the brain: an update. *Tr. Neurosci.* 30, 194-202 (2007).

[0191] 2. Bromberg-Martin, E. S., Matsumoto, M. & Hikosaka, O. Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron* 68, 815-834 (2010).

[0192] 3. Schultz, W. Multiple dopamine functions at different time courses. *Annu. Rev.*

[0193] Neurosci. 30, 259-288 (2007).

[0194] 4. Lammel, S., et al. Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* 57, 760-773 (2008).

[0195] 5. Lammel, S., Ion, D.I., Roeper, J. & Malenka, R. C. Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. *Neuron* 70, 855-862 (2011).

[0196] 6. Margolis, E. B., Mitchell, J.M., Ishikawa, J., Hjelmstad, G. O. & Fields, H. L. Midbrain dopamine neurons: projection target determines action potential duration and dopamine D(2) receptor inhibition. *J. Neurosci.* 28, 8908-8913 (2008).

[0197] 7. Sesack, S. R. & Grace, A. A. Cortico-Basal Ganglia reward network: microcircuitry. *Neuropsychopharmacol.* 35, 27-47 (2010).

[0198] 8. Geisler, S., Derst, C., Veh, R. W. & Zahm, D. S. Glutamatergic afferents of the ventral tegmental area in the rat. *J. Neurosci.* 27, 5730-5743 (2007).

[0199] 9. Berridge, K. C., Robinson, T. E. & Aldridge, J. W. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr. Opin. Pharmacol.* 9, 65-73 (2009).

[0200] 10. Cohen, J. Y., Haesler, S., Vong, L., Lowell, B. B. & Uchida, N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature* 482, 85-88 (2012).

[0201] 11. Guarraci, F. A. & Kapp, B. S. An electrophysiological characterization of ventral tegmental area dopaminergic neurons during differential pavlovian fear conditioning in the awake rabbit. *Behav. Brain Res.* 99, 169-179 (1999).

[0202] 12. Kim, Y, Wood, J. & Moghaddam, B. Coordinated activity of ventral tegmental neurons adapts to appetitive and aversive learning. *PLoS One* 7, e29766 (2012).

[0203] 13. Tan, K. R., et al. GABA neurons of the VTA drive conditioned place aversion. *Neuron* 73, 1173-1183 (2012).

[0204] 14. Matsumoto, M. & Hikosaka, O. Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* 459, 837-841 (2009).

[0205] 15. van Zessen, R., Phillips, J. L., Budygin, E. A. & Stuber, G. D. Activation of VTA GABA neurons disrupts reward consumption. *Neuron* 73, 1184-1194 (2012).

[0206] 16. Wickersham, I. R., Finke, S., Conzelmann, K K & Callaway, E.M. Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat. Methods.* 4, 47-49 (2007).

[0207] 17. Watabe-Uchida, M., Zhu, L., Ogawa, S. K., Vamanrao, A. & Uchida, N. Whole-brain mapping of direct inputs to midbrain dopamine neurons. *Neuron* 74, 858-873 (2012).

[0208] 18. Hikosaka, O. The habenula: from stress evasion to value-based decision-making *Nat. Rev. Neurosci.* 11, 503-513 (2010).

[0209] 19. Forster, G. L. & Blaha, C. D. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur. J. Neurosci.* 12, 3596-3604 (2000).

[0210] 20. Lodge, D. J. & Grace, A. A. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *Proc. Natl. Acad. Sci. USA* 103, 5167-5172 (2006).

[0211] 21. Jhou, T. C., Geisler, S., Marinelli, M., Degarmo, B. A. & Zahm, D S The mesopontine rostromedial tegmental nucleus: A structure targeted by the lateral habenula that projects to the ventral tegmental area of Tsai and substantia nigra compacta. *J. Comp. Neurol.* 513, 566-596 (2009).

[0212] 22. Kaufling, J., Veinante, P., Pawlowski, S. A., Freund-Mercier, M. J. & Barrot, M. Afferents to the GABAergic tail of the ventral tegmental area in the rat. *J. Comp. Neurol.* 513, 597-621 (2009).

[0213] 23. Christoph, G. R., Leonzio, R. J. & Wilcox, K. S. Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. *J. Neurosci.* 6, 613-619 (1986).

[0214] 24. Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B. & Holland, P. C. The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron* 61, 786-800 (2009).

[0215] 25. Ji, H. & Shepard, P. D. Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *J. Neurosci.* 27, 6923-6930 (2007).

[0216] 26. Omelchenko, N., Bell, R. & Sesack, S. R. Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. *Eur. J. Neurosci.* 30, 1239-1250 (2009).

[0217] 27. Araki, M., McGeer, P. L. & Kimura, H. The efferent projections of the rat lateral habenular nucleus revealed by the PHA-L anterograde tracing method. *Brain Res.* 441, 319-330 (1988).

[0218] 28. Cornwall, J., Cooper, J. D. & Phillipson, O. T. Afferent and efferent connections of the laterodorsal tegmental nucleus in the rat. *Brain Res. Bull.* 25, 271-284 (1990).

[0219] 29. Vertes, R.P., Fortin, W. J. & Crane, A.M. Projections of the median raphe nucleus in the rat. *J. Comp. Neurol.* 407, 555-582 (1999).

[0220] 30. Sanchez, C. J., Bailie, T.M., Wu, W. R., Li, N. & Sorg, B. A. Manipulation of dopamine dl-like receptor activation in the rat medial prefrontal cortex alters stress- and cocaine-induced reinstatement of conditioned place preference behavior. *Neuroscience* 119, 497-505 (2003).

[0221] 31. Tsai, H. C., et al. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* 324, 1080-1084 (2009).

[0222] 32. Witten, I. B., et al. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron* 72, 721-733 (2011).

[0223] 33. Robbins, T. W. & Arnsten, A. F. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annu. Rev. Neurosci.* 32, 267-287 (2009).

[0224] 34. Lecourtier, L., Defrancesco, A. & Moghaddam, B. Differential tonic influence of lateral habenula on prefrontal cortex and nucleus accumbens dopamine release. *Eur. J. Neurosci.* 27, 1755-1762 (2008).

[0225] 35. Li, B., et al. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* 470, 535-539 (2011).

[0226] 36. Lecourtier, L. & Kelly, P. H. Bilateral lesions of the habenula induce attentional disturbances in rats. *Neuropsychopharmacol.* 30, 484-496 (2005).

[0227] 37. Shepard, P. D., Holcomb, H. H. & Gold, J.M. Schizophrenia in translation: the presence of absence: habenular regulation of dopamine neurons and the encoding of negative outcomes. *Schizophr Bull* 32, 417-421 (2006).

[0228] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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            260                 265                 270

Val Val Ser Gly Ser Ile Leu Asp Val Pro Ser Ala Ser Gly Thr Pro
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            340                 345                 350

Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
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Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr
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    450                 455                 460

Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln
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Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
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Leu Leu Leu Met Phe Tyr Ala Tyr Gln Thr Trp Lys Ser Thr Cys Gly
65                  70                  75                  80

Trp Glu Glu Ile Tyr Val Cys Ala Ile Glu Met Val Lys Val Ile Leu
                85                  90                  95

Glu Phe Phe Phe Glu Phe Lys Asn Pro Ser Met Leu Tyr Leu Ala Thr
            100                 105                 110

Gly His Arg Val Gln Trp Leu Arg Tyr Ala Glu Trp Leu Leu Thr Ser
        115                 120                 125

Pro Val Ile Leu Ile His Leu Ser Asn Leu Thr Gly Leu Ser Asn Asp
    130                 135                 140

Tyr Ser Arg Arg Thr Met Gly Leu Leu Val Ser Asp Ile Gly Thr Ile
145                 150                 155                 160

Val Trp Gly Ala Thr Ser Ala Met Ala Thr Gly Tyr Val Lys Val Ile
                165                 170                 175

Phe Phe Cys Leu Gly Leu Cys Tyr Gly Ala Asn Thr Phe Phe His Ala
            180                 185                 190

Ala Lys Ala Tyr Ile Glu Gly Tyr His Thr Val Pro Lys Gly Arg Cys
        195                 200                 205
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Arg Gln Val Val Thr Gly Met Ala Trp Leu Phe Phe Val Ser Trp Gly
    210   215   220

Met Phe Pro Ile Leu Phe Ile Leu Gly Pro Glu Gly Phe Gly Val Leu
225   230   235   240

Ser Val Tyr Gly Ser Thr Val Gly His Thr Ile Ile Asp Leu Met Ser
                245   250   255

Lys Asn Cys Trp Gly Leu Leu Gly His Tyr Leu Arg Val Leu Ile His
            260   265   270

Glu His Ile Leu Ile His Gly Asp Ile Arg Lys Thr Thr Lys Leu Asn
        275   280   285

Ile Gly Gly Thr Glu Ile Glu Val Glu Thr Leu Val Glu Asp Glu Ala
    290   295   300

Glu Ala Gly Ala Val Pro
305   310

<210> SEQ ID NO 7
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 7

Met Asp Tyr Gly Gly Ala Leu Ser Ala Val Gly Arg Glu Leu Leu Phe
1   5   10   15

Val Thr Asn Pro Val Val Val Asn Gly Ser Val Leu Val Pro Glu Asp
            20   25   30

Gln Cys Tyr Cys Ala Gly Trp Ile Glu Ser Arg Gly Thr Asn Gly Ala
        35   40   45

Gln Thr Ala Ser Asn Val Leu Gln Trp Leu Ala Ala Gly Phe Ser Ile
    50   55   60

Leu Leu Leu Met Phe Tyr Ala Tyr Gln Thr Trp Lys Ser Thr Cys Gly
65   70   75   80

Trp Glu Glu Ile Tyr Val Cys Ala Ile Glu Met Val Lys Val Ile Leu
                85   90   95

Glu Phe Phe Phe Glu Phe Lys Asn Pro Ser Met Leu Tyr Leu Ala Thr
            100   105   110

Gly His Arg Val Gln Trp Leu Arg Tyr Ala Glu Trp Leu Leu Thr Ser
        115   120   125

Pro Val Ile Leu Ile His Leu Ser Asn Leu Thr Gly Leu Ser Asn Asp
    130   135   140

Tyr Ser Arg Arg Thr Met Gly Leu Leu Val Ser Ala Ile Gly Thr Ile
145   150   155   160

Val Trp Gly Ala Thr Ser Ala Met Ala Thr Gly Tyr Val Lys Val Ile
                165   170   175

Phe Phe Cys Leu Gly Leu Cys Tyr Gly Ala Asn Thr Phe Phe His Ala
            180   185   190

Ala Lys Ala Tyr Ile Glu Gly Tyr His Thr Val Pro Lys Gly Arg Cys
        195   200   205

Arg Gln Val Val Thr Gly Met Ala Trp Leu Phe Phe Val Ser Trp Gly
    210   215   220

Met Phe Pro Ile Leu Phe Ile Leu Gly Pro Glu Gly Phe Gly Val Leu
225   230   235   240

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Ser Val Tyr Gly Ser Thr Val Gly His Thr Ile Ile Asp Leu Met Ser
                245                 250                 255

Lys Asn Cys Trp Gly Leu Leu Gly His Tyr Leu Arg Val Leu Ile His
            260                 265                 270

Glu His Ile Leu Ile His Gly Asp Ile Arg Lys Thr Thr Lys Leu Asn
    275                 280                 285

Ile Gly Gly Thr Glu Ile Glu Val Glu Thr Leu Val Glu Asp Glu Ala
    290                 295                 300

Glu Ala Gly Ala Val Pro
305                 310


<210> SEQ ID NO 8
<211> LENGTH: 349
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 8

Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                   10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
    35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Glu Ile Tyr Val Ala Thr Ile
    115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Glu Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
    195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270
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```
Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp Ser Glu Gln Ile Asp
            340                 345


<210> SEQ ID NO 9
<211> LENGTH: 344
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 9

Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                   10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Thr Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Glu Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270
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```
Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp
            340


<210> SEQ ID NO 10
<211> LENGTH: 349
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 10

Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                   10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Glu Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Thr Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270
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```
Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp Ser Glu Gln Ile Asp
            340                 345


<210> SEQ ID NO 11
<211> LENGTH: 349
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 11

Met Ser Arg Arg Pro Trp Leu Leu Ala Leu Ala Leu Ala Val Ala Leu
1               5                   10                  15

Ala Ala Gly Ser Ala Gly Ala Ser Thr Gly Ser Asp Ala Thr Val Pro
            20                  25                  30

Val Ala Thr Gln Asp Gly Pro Asp Tyr Val Phe His Arg Ala His Glu
        35                  40                  45

Arg Met Leu Phe Gln Thr Ser Tyr Thr Leu Glu Asn Asn Gly Ser Val
    50                  55                  60

Ile Cys Ile Pro Asn Asn Gly Gln Cys Phe Cys Leu Ala Trp Leu Lys
65                  70                  75                  80

Ser Asn Gly Thr Asn Ala Glu Lys Leu Ala Ala Asn Ile Leu Gln Trp
                85                  90                  95

Ile Thr Phe Ala Leu Ser Ala Leu Cys Leu Met Phe Tyr Gly Tyr Gln
            100                 105                 110

Thr Trp Lys Ser Thr Cys Gly Trp Glu Thr Ile Tyr Val Ala Thr Ile
        115                 120                 125

Glu Met Ile Lys Phe Ile Ile Glu Tyr Phe His Glu Phe Asp Glu Pro
    130                 135                 140

Ala Val Ile Tyr Ser Ser Asn Gly Asn Lys Thr Val Trp Leu Arg Tyr
145                 150                 155                 160

Ala Thr Trp Leu Leu Thr Cys Pro Val Leu Leu Ile His Leu Ser Asn
                165                 170                 175

Leu Thr Gly Leu Lys Asp Asp Tyr Ser Lys Arg Thr Met Gly Leu Leu
            180                 185                 190

Val Ser Asp Val Gly Cys Ile Val Trp Gly Ala Thr Ser Ala Met Cys
        195                 200                 205

Thr Gly Trp Thr Lys Ile Leu Phe Phe Leu Ile Ser Leu Ser Tyr Gly
    210                 215                 220

Met Tyr Thr Tyr Phe His Ala Ala Lys Val Tyr Ile Glu Ala Phe His
225                 230                 235                 240

Thr Val Pro Lys Gly Ile Cys Arg Glu Leu Val Arg Val Met Ala Trp
                245                 250                 255

Thr Phe Phe Val Ala Trp Gly Met Phe Pro Val Leu Phe Leu Leu Gly
            260                 265                 270
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```
Thr Glu Gly Phe Gly His Ile Ser Pro Tyr Gly Ser Ala Ile Gly His
        275                 280                 285

Ser Ile Leu Asp Leu Ile Ala Lys Asn Met Trp Gly Val Leu Gly Asn
    290                 295                 300

Tyr Leu Arg Val Lys Ile His Glu His Ile Leu Leu Tyr Gly Asp Ile
305                 310                 315                 320

Arg Lys Lys Gln Lys Ile Thr Ile Ala Gly Gln Glu Met Glu Val Glu
                325                 330                 335

Thr Leu Val Ala Glu Glu Glu Asp Ser Glu Gln Ile Asp
            340                 345


<210> SEQ ID NO 12
<211> LENGTH: 258
<212> TYPE: PRT
<213> ORGANISM: Halorubrum sodomense

<400> SEQUENCE: 12

Met Asp Pro Ile Ala Leu Gln Ala Gly Tyr Asp Leu Leu Gly Asp Gly
1               5                   10                  15

Arg Pro Glu Thr Leu Trp Leu Gly Ile Gly Thr Leu Leu Met Leu Ile
            20                  25                  30

Gly Thr Phe Tyr Phe Leu Val Arg Gly Trp Gly Val Thr Asp Lys Asp
        35                  40                  45

Ala Arg Glu Tyr Tyr Ala Val Thr Ile Leu Val Pro Gly Ile Ala Ser
    50                  55                  60

Ala Ala Tyr Leu Ser Met Phe Phe Gly Ile Gly Leu Thr Glu Val Thr
65                  70                  75                  80

Val Gly Gly Glu Met Leu Asp Ile Tyr Tyr Ala Arg Tyr Ala Asp Trp
                85                  90                  95

Leu Phe Thr Thr Pro Leu Leu Leu Leu Asp Leu Ala Leu Leu Ala Lys
            100                 105                 110

Val Asp Arg Val Thr Ile Gly Thr Leu Val Gly Val Asp Ala Leu Met
        115                 120                 125

Ile Val Thr Gly Leu Ile Gly Ala Leu Ser His Thr Ala Ile Ala Arg
    130                 135                 140

Tyr Ser Trp Trp Leu Phe Ser Thr Ile Cys Met Ile Val Val Leu Tyr
145                 150                 155                 160

Phe Leu Ala Thr Ser Leu Arg Ser Ala Ala Lys Glu Arg Gly Pro Glu
                165                 170                 175

Val Ala Ser Thr Phe Asn Thr Leu Thr Ala Leu Val Leu Val Leu Trp
            180                 185                 190

Thr Ala Tyr Pro Ile Leu Trp Ile Ile Gly Thr Glu Gly Ala Gly Val
        195                 200                 205

Val Gly Leu Gly Ile Glu Thr Leu Leu Phe Met Val Leu Asp Val Thr
    210                 215                 220

Ala Lys Val Gly Phe Gly Phe Ile Leu Leu Arg Ser Arg Ala Ile Leu
225                 230                 235                 240

Gly Asp Thr Glu Ala Pro Glu Pro Ser Ala Gly Ala Asp Val Ser Ala
                245                 250                 255

Ala Asp


<210> SEQ ID NO 13
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<211> LENGTH: 534
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 13

Met Asp Pro Ile Ala Leu Gln Ala Gly Tyr Asp Leu Leu Gly Asp Gly
1               5                   10                  15

Arg Pro Glu Thr Leu Trp Leu Gly Ile Gly Thr Leu Leu Met Leu Ile
            20                  25                  30

Gly Thr Phe Tyr Phe Leu Val Arg Gly Trp Gly Val Thr Asp Lys Asp
        35                  40                  45

Ala Arg Glu Tyr Tyr Ala Val Thr Ile Leu Val Pro Gly Ile Ala Ser
    50                  55                  60

Ala Ala Tyr Leu Ser Met Phe Phe Gly Ile Gly Leu Thr Glu Val Thr
65                  70                  75                  80

Val Gly Gly Glu Met Leu Asp Ile Tyr Tyr Ala Arg Tyr Ala Asp Trp
                85                  90                  95

Leu Phe Thr Thr Pro Leu Leu Leu Leu Asp Leu Ala Leu Leu Ala Lys
            100                 105                 110

Val Asp Arg Val Thr Ile Gly Thr Leu Val Gly Val Asp Ala Leu Met
        115                 120                 125

Ile Val Thr Gly Leu Ile Gly Ala Leu Ser His Thr Ala Ile Ala Arg
    130                 135                 140

Tyr Ser Trp Trp Leu Phe Ser Thr Ile Cys Met Ile Val Val Leu Tyr
145                 150                 155                 160

Phe Leu Ala Thr Ser Leu Arg Ser Ala Ala Lys Glu Arg Gly Pro Glu
                165                 170                 175

Val Ala Ser Thr Phe Asn Thr Leu Thr Ala Leu Val Leu Val Leu Trp
            180                 185                 190

Thr Ala Tyr Pro Ile Leu Trp Ile Ile Gly Thr Glu Gly Ala Gly Val
        195                 200                 205

Val Gly Leu Gly Ile Glu Thr Leu Leu Phe Met Val Leu Asp Val Thr
    210                 215                 220

Ala Lys Val Gly Phe Gly Phe Ile Leu Leu Arg Ser Arg Ala Ile Leu
225                 230                 235                 240

Gly Asp Thr Glu Ala Pro Glu Pro Ser Ala Gly Ala Asp Val Ser Ala
                245                 250                 255

Ala Asp Arg Pro Val Val Ala Val Ser Lys Ala Ala Ala Lys Ser Arg
            260                 265                 270

Ile Thr Ser Glu Gly Glu Tyr Ile Pro Leu Asp Gln Ile Asp Ile Asn
        275                 280                 285

Val Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
    290                 295                 300

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
305                 310                 315                 320

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
                325                 330                 335

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            340                 345                 350

Phe Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
        355                 360                 365
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Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
    370                 375                 380

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
385                 390                 395                 400

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
                405                 410                 415

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            420                 425                 430

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
        435                 440                 445

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
    450                 455                 460

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
465                 470                 475                 480

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
                485                 490                 495

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
            500                 505                 510

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Phe
        515                 520                 525

Cys Tyr Glu Asn Glu Val
    530


<210> SEQ ID NO 14
<211> LENGTH: 248
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 14

Met Asp Pro Ile Ala Leu Gln Ala Gly Tyr Asp Leu Leu Gly Asp Gly
1               5                   10                  15

Arg Pro Glu Thr Leu Trp Leu Gly Ile Gly Thr Leu Leu Met Leu Ile
            20                  25                  30

Gly Thr Phe Tyr Phe Ile Val Lys Gly Trp Gly Val Thr Asp Lys Glu
        35                  40                  45

Ala Arg Glu Tyr Tyr Ser Ile Thr Ile Leu Val Pro Gly Ile Ala Ser
    50                  55                  60

Ala Ala Tyr Leu Ser Met Phe Phe Gly Ile Gly Leu Thr Glu Val Thr
65                  70                  75                  80

Val Ala Gly Glu Val Leu Asp Ile Tyr Tyr Ala Arg Tyr Ala Asp Trp
                85                  90                  95

Leu Phe Thr Thr Pro Leu Leu Leu Leu Asp Leu Ala Leu Leu Ala Lys
            100                 105                 110

Val Asp Arg Val Ser Ile Gly Thr Leu Val Gly Val Asp Ala Leu Met
        115                 120                 125

Ile Val Thr Gly Leu Ile Gly Ala Leu Ser His Thr Pro Leu Ala Arg
    130                 135                 140

Tyr Ser Trp Trp Leu Phe Ser Thr Ile Cys Met Ile Val Val Leu Tyr
145                 150                 155                 160

Phe Leu Ala Thr Ser Leu Arg Ala Ala Ala Lys Glu Arg Gly Pro Glu
                165                 170                 175
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Val Ala Ser Thr Phe Asn Thr Leu Thr Ala Leu Val Leu Val Leu Trp
            180                 185                 190

Thr Ala Tyr Pro Ile Leu Trp Ile Ile Gly Thr Glu Gly Ala Gly Val
        195                 200                 205

Val Gly Leu Gly Ile Glu Thr Leu Leu Phe Met Val Leu Asp Val Thr
    210                 215                 220

Ala Lys Val Gly Phe Gly Phe Ile Leu Leu Arg Ser Arg Ala Ile Leu
225                 230                 235                 240

Gly Asp Thr Glu Ala Pro Glu Pro
                245


<210> SEQ ID NO 15
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 15

Met Ile Val Asp Gln Phe Glu Glu Val Leu Met Lys Thr Ser Gln Leu
1               5                   10                  15

Phe Pro Leu Pro Thr Ala Thr Gln Ser Ala Gln Pro Thr His Val Ala
            20                  25                  30

Pro Val Pro Thr Val Leu Pro Asp Thr Pro Ile Tyr Glu Thr Val Gly
        35                  40                  45

Asp Ser Gly Ser Lys Thr Leu Trp Val Val Phe Val Leu Met Leu Ile
    50                  55                  60

Ala Ser Ala Ala Phe Thr Ala Leu Ser Trp Lys Ile Pro Val Asn Arg
65                  70                  75                  80

Arg Leu Tyr His Val Ile Thr Thr Ile Ile Thr Leu Thr Ala Ala Leu
                85                  90                  95

Ser Tyr Phe Ala Met Ala Thr Gly His Gly Val Ala Leu Asn Lys Ile
            100                 105                 110

Val Ile Arg Thr Gln His Asp His Val Pro Asp Thr Tyr Glu Thr Val
        115                 120                 125

Tyr Arg Gln Val Tyr Tyr Ala Arg Tyr Ile Asp Trp Ala Ile Thr Thr
    130                 135                 140

Pro Leu Leu Leu Leu Asp Leu Gly Leu Leu Ala Gly Met Ser Gly Ala
145                 150                 155                 160

His Ile Phe Met Ala Ile Val Ala Asp Leu Ile Met Val Leu Thr Gly
                165                 170                 175

Leu Phe Ala Ala Phe Gly Ser Glu Gly Thr Pro Gln Lys Trp Gly Trp
            180                 185                 190

Tyr Thr Ile Ala Cys Ile Ala Tyr Ile Phe Val Val Trp His Leu Val
        195                 200                 205

Leu Asn Gly Gly Ala Asn Ala Arg Val Lys Gly Glu Lys Leu Arg Ser
    210                 215                 220

Phe Phe Val Ala Ile Gly Ala Tyr Thr Leu Ile Leu Trp Thr Ala Tyr
225                 230                 235                 240

Pro Ile Val Trp Gly Leu Ala Asp Gly Ala Arg Lys Ile Gly Val Asp
                245                 250                 255

Gly Glu Ile Ile Ala Tyr Ala Val Leu Asp Val Leu Ala Lys Gly Val
            260                 265                 270
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```
Phe Gly Ala Trp Leu Leu Val Thr His Ala Asn Leu Arg Glu Ser Asp
        275                 280                 285

Val Glu Leu Asn Gly Phe Trp Ala Asn Gly Leu Asn Arg Glu Gly Ala
    290                 295                 300

Ile Arg Ile Gly Glu Asp Asp Gly Ala Arg Pro Val Val Ala Val Ser
305                 310                 315                 320

Lys


<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 16

Lys Ser Arg Ile Thr Ser Glu Gly Glu Tyr Ile Pro Leu Asp Gln Ile
1               5                   10                  15

Asp Ile Asn Val
            20


<210> SEQ ID NO 17
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 17

Phe Cys Tyr Glu Asn Glu Val
1               5


<210> SEQ ID NO 18
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 18

Met Asp Tyr Gly Gly Ala Leu Ser Ala Val Gly Arg Glu Leu Leu Phe
1               5                   10                  15

Val Thr Asn Pro Val Val Val Asn Gly Ser
            20                  25


<210> SEQ ID NO 19
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 19

Met Ala Gly His Ser Asn Ser Met Ala Leu Phe Ser Phe Ser Leu Leu
1               5                   10                  15

Trp Leu Cys Ser Gly Val Leu Gly Thr Glu Phe
            20                  25


<210> SEQ ID NO 20
<211> LENGTH: 23
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 20

Met Gly Leu Arg Ala Leu Met Leu Trp Leu Leu Ala Ala Ala Gly Leu
1               5                   10                  15

Val Arg Glu Ser Leu Gln Gly
            20


<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 21

Met Arg Gly Thr Pro Leu Leu Leu Val Val Ser Leu Phe Ser Leu Leu
1               5                   10                  15

Gln Asp


<210> SEQ ID NO 22
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: the amino acid in this position may be any
      amino acid

<400> SEQUENCE: 22

Val Xaa Xaa Ser Leu
1               5


<210> SEQ ID NO 23
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic amino acid sequence

<400> SEQUENCE: 23

Val Lys Glu Ser Leu
1               5


<210> SEQ ID NO 24
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: the amino acid in this position may be any
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<400> SEQUENCE: 26

Phe Xaa Tyr Glu Asn Glu
1               5


<210> SEQ ID NO 27
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
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Ala Glu
```

What is claimed is:

1. A method of modulating a reward- or aversive-related behavior in a mammal, the method comprising:

a) expressing in one or both of two inputs to the ventral tegmental area (VTA) from the laterodorsal tegmentum and the lateral habenula a light-responsive opsin polypeptide; and

b) exposing the input to light,

wherein said exposing modulates a reward- or aversive-related behavior in the mammal.

2. The method of claim **1**, wherein said light-responsive opsin polypeptide comprises an amino acid sequence having at least about 90% amino acid sequence identity to an amino acid depicted in FIGS. **6**A-H.

3. A non-human animal model of a reward- or aversive-related behavioral disorder, wherein said non-human animal expresses a light-responsive opsin polypeptide in one or both of two inputs to the ventral tegmental area (VTA) from the laterodorsal tegmentum (LDT) and the lateral habenula (LHb).

4. A method of identifying an agent that modulates a reward- or aversive-related behavior in an individual, the method comprising:

a) administering a test agent to a non-human animal of claim **3**; and

b) determining the effect of the test agent on a reward- or aversive-related behavior exhibited by said non-human animal when the light-responsive opsin polypeptide is activated by light, wherein a test agent that reduces an adverse reward- or aversive-related behavior is considered a candidate agent for treating a reward- or aversive-related behavioral disorder.

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