

Supplementary Materials for
**Sex differences in neural projections of fear memory processing in mice
and humans**

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Other Supplementary Material for this manuscript includes the following:

Data S1
Movie S1

Abbreviation	Full name
Cg	cingulate cortex
PrL	prelimbic cortex
IL	infralimbic cortex
aca	anterior commissure, anterior part
cc	corpus callosum
LSV	lateral septal nucleus, ventral part
STMV	medial nucleus of the stria terminalis, medial division, ventral part
STLV	medial nucleus of the stria terminalis, lateral division, ventral part
STMA	medial nucleus of the stria terminalis, medial division, anterior part
STMAL	medial nucleus of the stria terminalis, medial division, anterolateral part
STMPM	medial nucleus of the stria terminalis, medial division, posteromedial part
STMPI	medial nucleus of the stria terminalis, medial division posterointermediate part
STMPL	medial nucleus of the stria terminalis, medial division, posterolateral part
STPL (or BNSTpl)	medial nucleus of the stria terminalis, lateral division, posterior part
CPU	caudate putamen (striatum)
VP	ventral pallidum
lc	internal capsule
BLA	basolateral amygdaloid nucleus, anterior part
BMA	basomedial amygdaloid nucleus, anterior part
CeC	central amygdaloid nucleus, capsular part
CeL	central amygdaloid nucleus, lateral division
CeM	central amygdaloid nucleus, medial division or centro-medial amygdala
MeA	medial amygdaloid nucleus, anterior part
VEn	ventral endopiriform claustrum,
ArcL	arcuate hypothalamic nucleus, lateral part
IPAC	interstitial nucleus of the posterior limb of the anterior commissure
ArcM	arcuate hypothalamic nucleus, medial part
MoDG	molecular layer of the dentate gyrus
ArcDM	arcuate hypothalamic nucleus, dorsomedial part
ArcLP	arcuate hypothalamic nucleus, lateral posterior part
ArcMP	arcuate hypothalamic nucleus, medial posterior part

ME	median eminence
SHy	septohypothalamic nucleus
LH	lateral hypothalamic area
PLH	peduncular part of lateral hypothalamus
CA1	field CA1 of the hippocampus
VMH	ventromedial hypothalamic nucleus
CA2	field CA2 of the hippocampus
CA3	field CA3 of the hippocampus
GrDG	granule cell layer of the dentate gyrus
LMol	lacunosum moleculare layer of the hippocampus
Py	pyramidal cell layer of the hippocampus
alv	alveus of the hippocampus
DMPAG	dorsomedial periaqueductal gray
DLPAG	dorsolateral periaqueductal gray
LPAG	lateral periaqueductal gray
VLPAG	ventrolateral periaqueductal gray

Table S1. Abbreviations for brain structures in the mouse brain according to Paxinos brain atlas.

This table contains all the abbreviations of anatomical brain regions in the mouse brain used in the text.

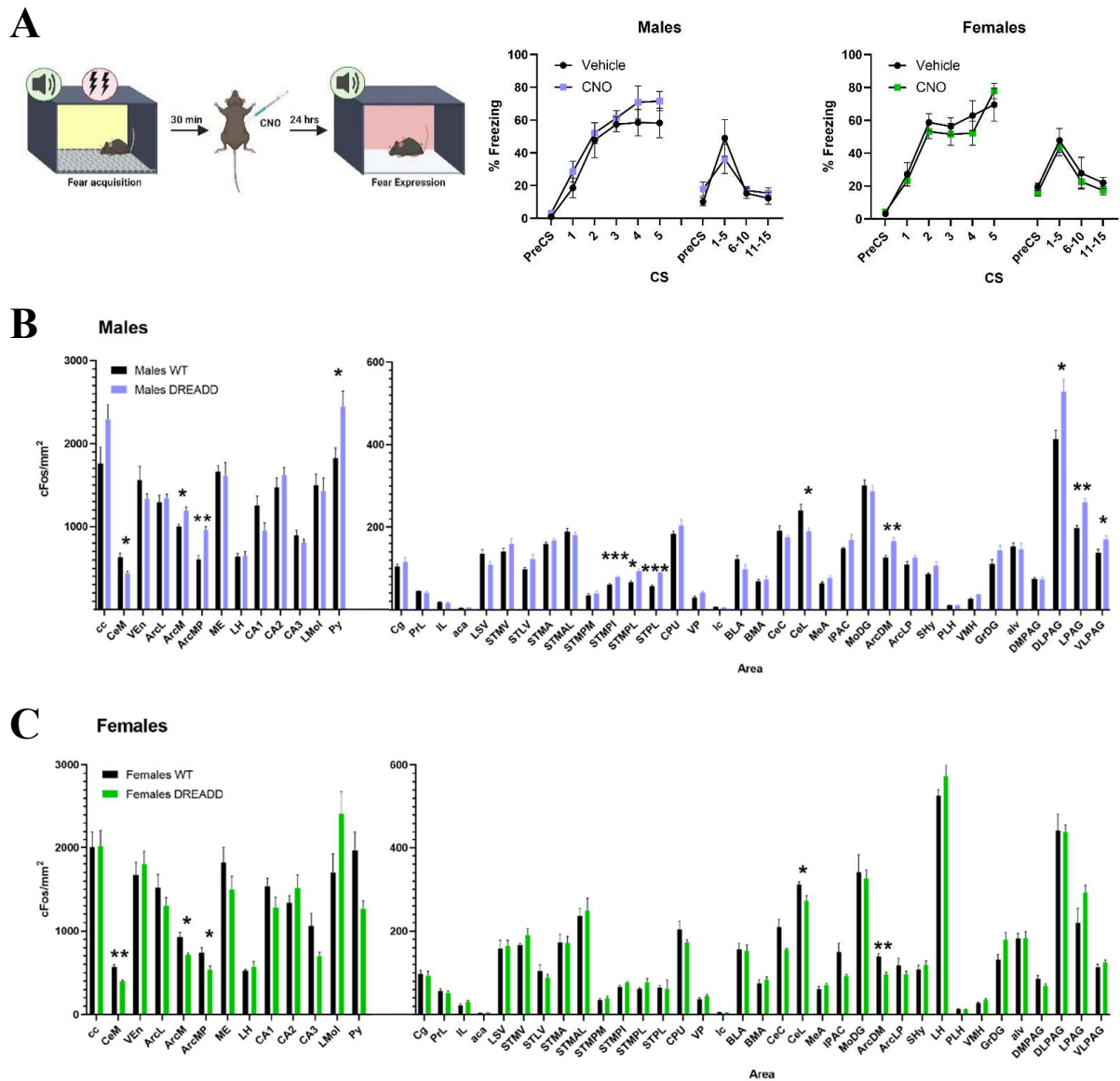


Fig. S1. Regulation of cFos density across the brain after chemogenetic silencing of CeM-*Tac2* neurons and CNO controls.

(A) Control experiments with wild-type mice show that CNO given systemically does not change fear memory consolidation in males or females. Images representing the experiment were created with BioRender.com. (B) cFos positive neurons by mm² in wild-type male mice and heterozygous *Tac2*-cre mice expressing hM4Di(Gq) in CeM-*Tac2* neurons and receiving CNO 30 minutes after fear conditioning; (C) wild-type female mice and heterozygous *Tac2*-cre female mice expression hM4Di(Gq) in CeM-*Tac2* neurons and receiving CNO 30 minutes after fear conditioning. Data are shown as Mean \pm SEM and analyzed using ANOVA repeated measures or two-tailed t-tests. **p* < 0.05; ** *p* < 0.01; *** *p* < 0.001.

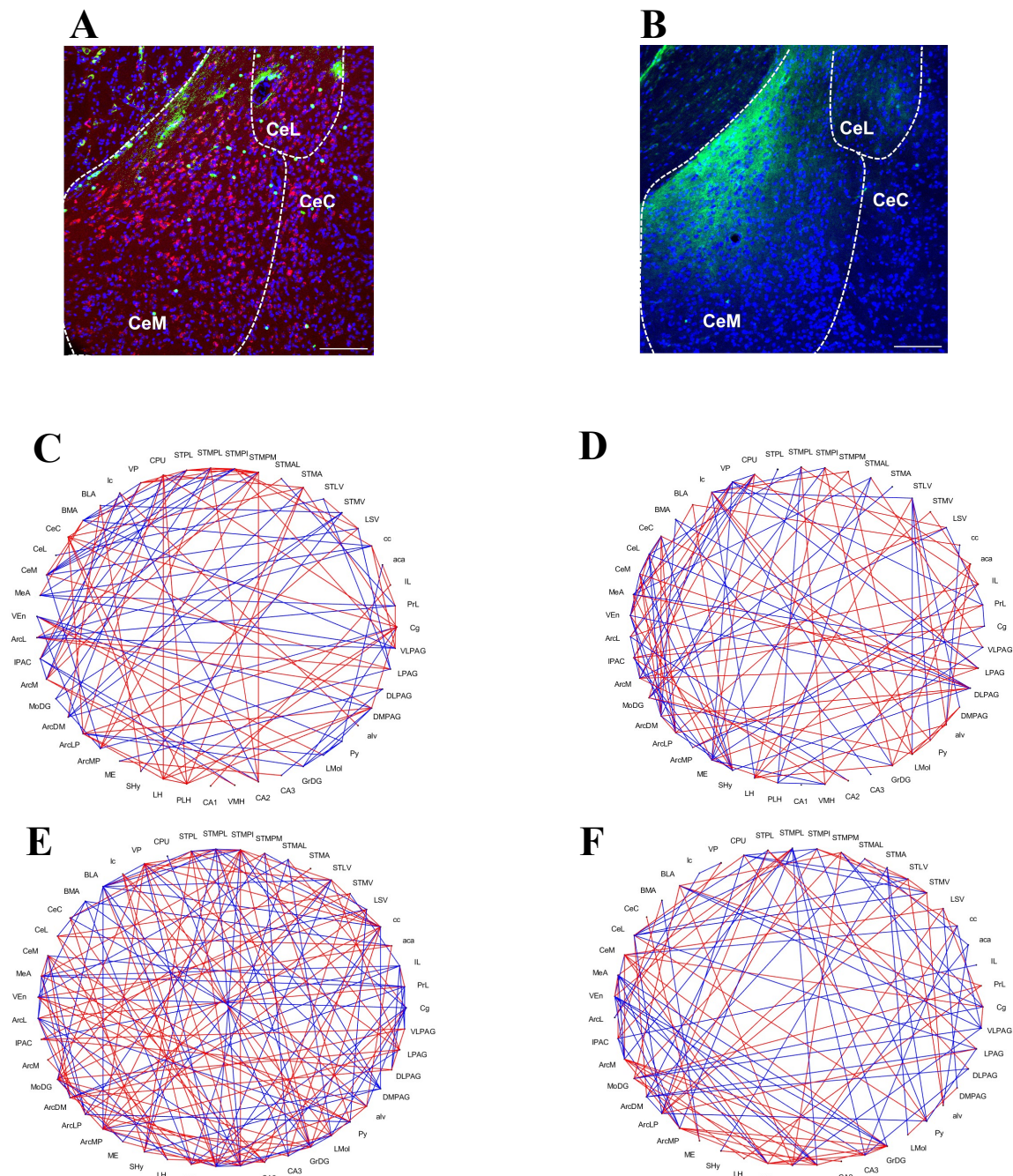


Fig. S2. Correlational regional relationships in cFos expression during fear memory consolidation after CeM-*Tac2* chemogenetic silencing.

(A) representative confocal image of cFos expression in the CeM during fear memory consolidation in wild-type mouse not expressing hM4Di; (B) representative confocal image of cFos expression in a CeM-expressing hM4Di(Gq) mouse during fear memory consolidation; (C-F), nodes and edges plots representing significant Pearson's correlation coefficients over 0.9 (red) and under -0.9 (blue) between cFos expressing regions (C: wild-type males; D: hM4Di(Gq) males; E: wild-type females; F: hM4Di(Gq) females).

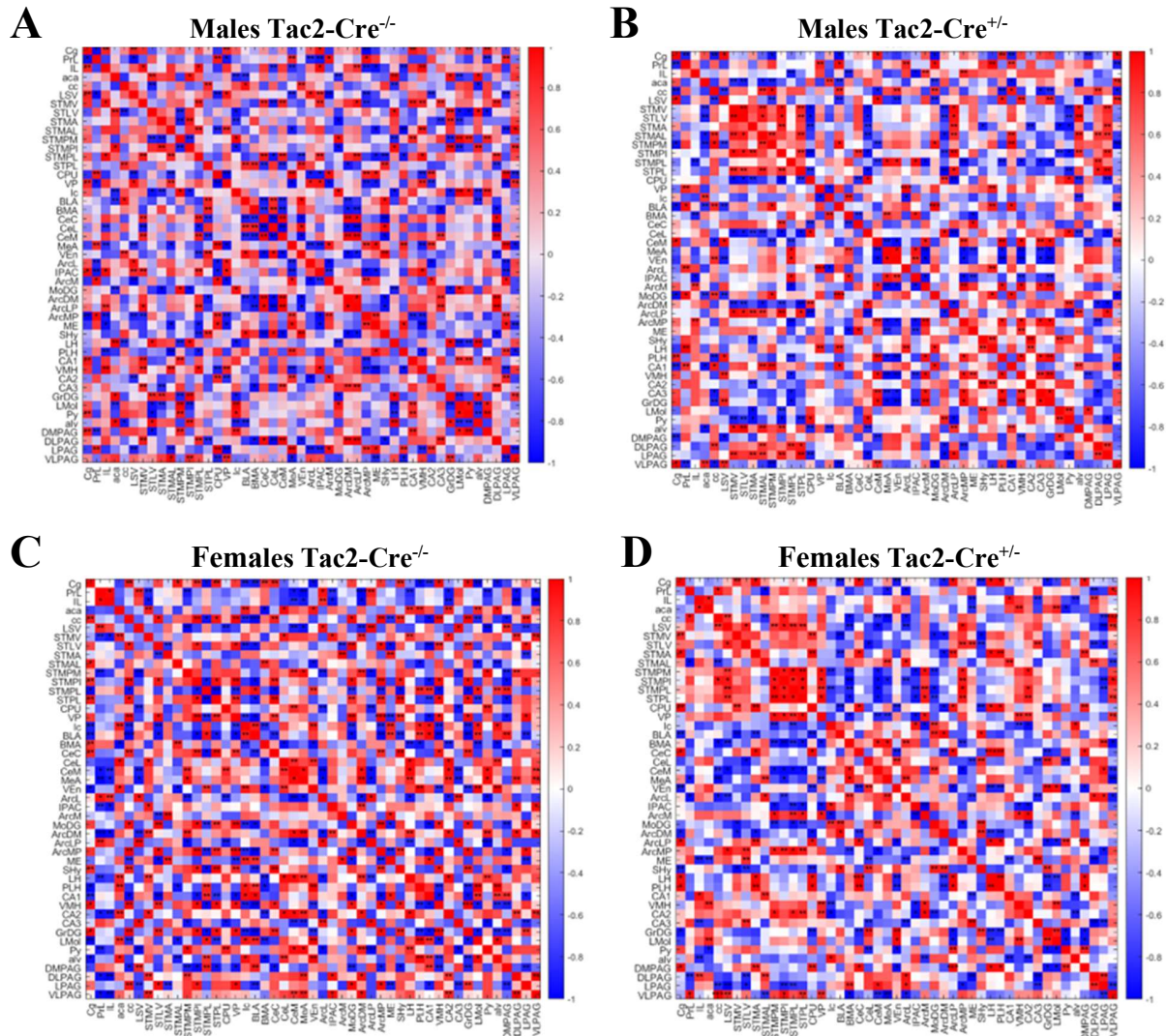


Fig. S3. Network connectivity analyses of cFos expression in the mouse brain during fear memory consolidation after chemogenetic silencing of CeM-*Tac2* neurons.

Heatmaps showing correlation coefficients of cFos expression between regions 2 hours after CeM-*Tac2* silencing in (A) wild-type male mice (B) heterozygous *Tac2*-cre male mice expressing hM4Di(Gq) in CeM-*Tac2* neurons and receiving CNO 30 minutes after fear conditioning; (C), wild-type female mice and (D), heterozygous *Tac2*-cre female mice expressing hM4Di(Gq) in CeM-*Tac2* neurons and receiving CNO 30 minutes after fear conditioning. Data were analyzed using Pearson's R coefficient. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

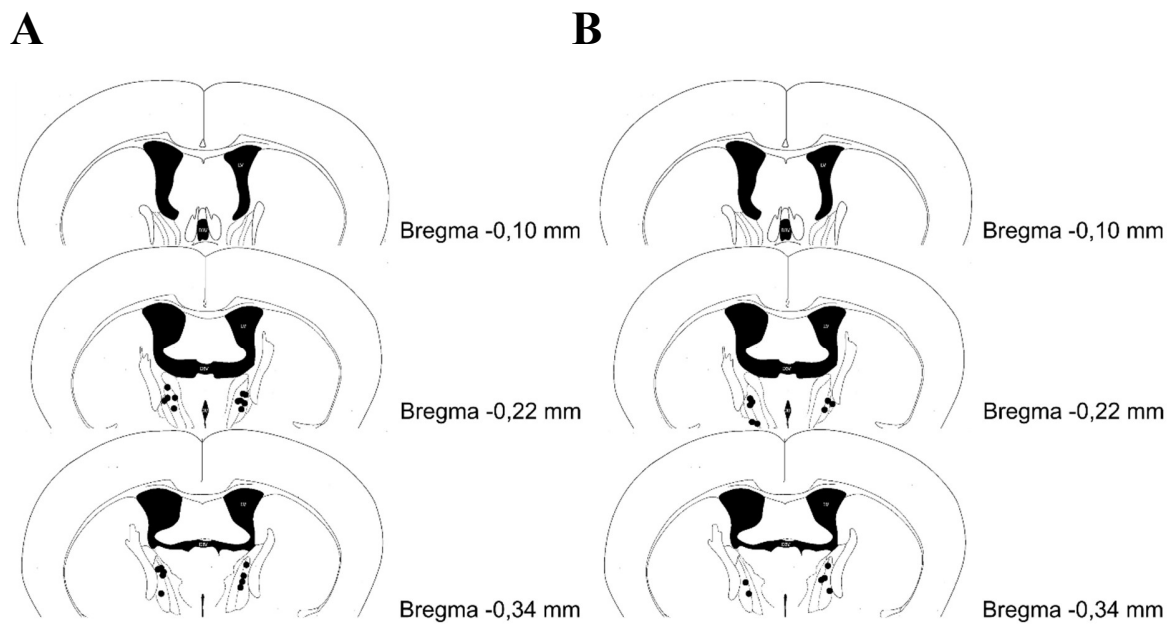


Fig. S4. Histological reconstruction of cannula placement.

The black dots represent CNO injection sites in males (A) and females (B).

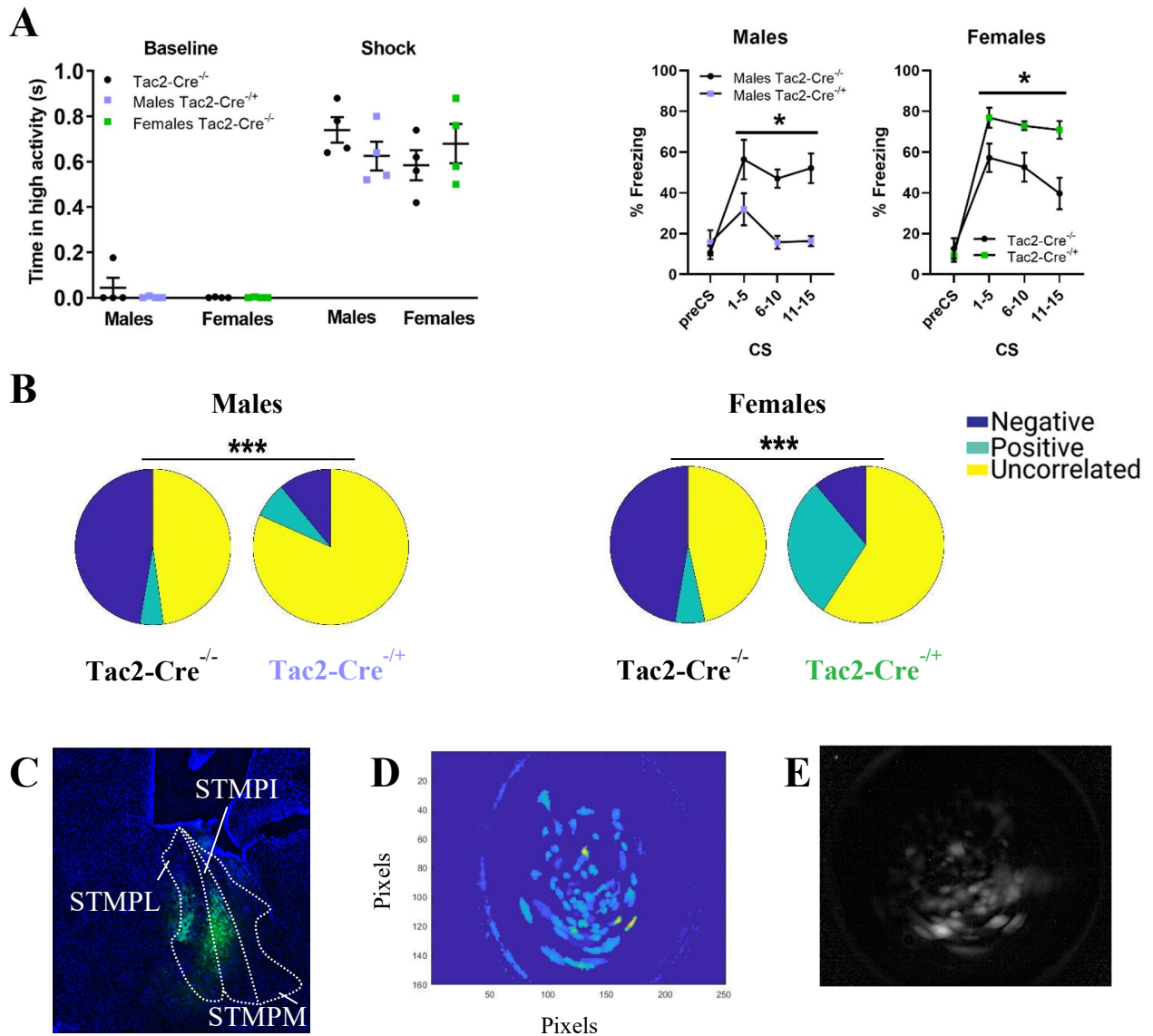


Fig. S5. *In vivo* calcium imaging in freely moving mice (Miniscopes), and freezing behavior.

(A) (Left) shock reactivity shown as duration of high activity peaks during the 5 seconds prior to the first shock (baseline) and during the first second after foot shock onset. No significant differences were observed in shock reactivity between groups in fear acquisition. (Right) binned data for the fear expression test. Data are shown as Mean \pm SEM. (B) The proportion of neurons correlating with freezing in Tac2-Cre^{-/-} and Tac2-Cre^{+/-} females during the PreCS. (C) green pixels represent GCaMP6f reporter expression in the BNST. Blue pixels represent DAPI. (D) Representative image of the spatial footprints of detected neurons in an animal during fear expression. (E) Maximum intensity projection of fluorescence detected across fear expression. Data were analyzed using One-way ANOVA, repeated measures ANOVA or Pearson's R coefficient. ***p < 0.001; * p < 0.05.

<i>TAC3R</i> rs2765 genotype			
	n	AA	G carriers
Total	1099	306	666
Men	459	183	276
Women	640	205	390

Table S2. *TAC3R* SNP rs 2765 genotype frequency for the human connectome project.

Distribution of genotype frequencies for the SNP rs2765 in the *TAC3R* in the sample among men and women in the Human Connectome Project data.

VARIABLES	WOMEN			MEN		
	AA	GA/GG	<i>p</i> -values	AA	GA/GG	<i>p</i> -values
SOCIODEMOGRAPHIC VARIABLES						
Age (years)	29,53 ± 3,63	29,52 ± 3,56	1	27,49 ± 3,42	28,15 ± 3,74	0.201
Race	76% White	73% White	0.956	84% White	75% White	0.206
Years of education	14,97 ± 1,83	15,11 ± 1,82	1	14,78 ± 1,7	14,86 ± 1,79	0.654
SSAGA Income	5,13 ± 2,08	5,22 ± 2,15	1	5,05 ± 2,18	5 ± 2,19	0.813
NEUROPSYCHOLOGICAL AND MENTAL HEALTH VARIABLES						
Picture Sequence Memory Test†	106,54 ± 15,95	108,53 ± 15,55	1	102,93 ± 16,38	100,98 ± 17,29	0.706
Dimensional Change Card Sort Test†	101,72 ± 10,07	102,2 ± 9,52	1	103,49 ± 9,99	101,88 ± 9,62	0.426
Flanker Task†	100,11 ± 9,51	100,81 ± 9,61	1	102,72 ± 10,04	101,91 ± 10,59	0.796
Penn Progressive Matrices	16,39 ± 4,79	16,49 ± 4,68	1	17,38 ± 4,38	17,57 ± 4,83	0.426
Oral Reading Recognition Test†	105,17 ± 14,25	105,57 ± 14,71	1	107,68 ± 14,58	109,69 ± 14,85	0.276

Picture Vocabulary Test†	107,55 ± 15,48	107,62 ± 14,5	1	110,41 ± 13,62	112,19 ± 15,7	0.310
Pattern Comparison Processing Speed Test†	104,75 ± 17,83	102,89 ± 19,97	1	104,15 ± 20,93	102,41 ± 20,79	0.775
Variable Short Penn Line Orientation Test	14,45 ± 4,53	13,78 ± 4,35	1	16,45 ± 4,32	15,8 ± 4,19	0.279
SCPT Sensitivity	0,95 ± 0,09	0,95 ± 0,08	1	0,95 ± 0,09	0,96 ± 0,06	0.426
SCPT Specificity	0,96 ± 0,02	0,96 ± 0,04	0.617	0,95 ± 0,03	0,95 ± 0,04	0.850
PWMT Correct responses	35,96 ± 3,05	35,87 ± 2,79	1	35,21 ± 2,95	35,3 ± 2,96	0.654
PWMT Reaction time	1561,81 ± 268,06	1571,95 ± 302,94	1	1553,4 ± 305,37	1572,7 ± 329,38	0.796
ER40 Correct responses	35,58 ± 2,43	35,83 ± 2,45	0.996	35,13 ± 2,62	35,48 ± 2,64	0.544
ER40 Reaction time	1776,71 ± 287,83	1819,74 ± 297,31	1	1847,3 ± 352,42	1904,48 ± 386,5	0.242
Perceived Stress Survey	48,68 ± 9,26	48,85 ± 8,85	1	45,72 ± 9,16	49,29 ± 9,35	0.009 **
Self-Efficacy Survey	50,43 ± 8,36	49,85 ± 8,01	1	53,4 ± 8,47	51,12 ± 8,55	0.052
Five Factor Model - Neuroticism	17,84 ± 6,92	17,23 ± 7,27	1	14,5 ± 7,47	16,61 ± 7,7	0.044 *
Friendship Survey	50,97 ± 9,03	50,25 ± 9,37	1	51,33 ± 8,8	49,67 ± 8,88	0.201

Loneliness Survey	50,81 ± 8,03	51,4 ± 8,32	1	49,87 ± 8,48	51,61 ± 9,22	0.109
Perceived Hostility Survey	47,29 ± 8,45	47,59 ± 8,77	1	48,37 ± 8,26	50,51 ± 8,2	0.044 *
Emotional Support Survey	53,49 ± 8,76	51,85 ± 9,64	0.956	51,43 ± 9,72	49,57 ± 9,89	0.097
Instrumental Support Survey	48,45 ± 8,65	48,52 ± 8,59	1	48,05 ± 9,02	47,99 ± 9,54	0.826
Anger-Affect Survey	47,11 ± 8,5	47,75 ± 7,73	1	47,07 ± 8,62	48,85 ± 8,53	0.178
Anger-Hostility Survey	50,75 ± 8,63	49,73 ± 8,35	1	50,25 ± 8,81	51,72 ± 8,76	0.201
Anger-Physical Aggression Survey	49,54 ± 7,52	49,83 ± 8,36	1	54,16 ± 9,32	54,33 ± 9,01	0.826
Fear-Affect Survey	51,33 ± 7,92	50,81 ± 8,2	1	48,78 ± 7,75	49,64 ± 7,85	0.426
Fear-Somatic Arousal Survey	52,07 ± 8,63	51,81 ± 8,16	1	51,83 ± 7,54	50,88 ± 8,21	0.775
Sadness Survey	45,87 ± 7,84	46,4 ± 7,94	1	44,96 ± 7,89	46,83 ± 7,99	0.062
General Life Satisfaction Survey	54,99 ± 9,36	54,94 ± 9,66	1	55,16 ± 8,64	53,6 ± 8,99	0.201
Meaning and Purpose Survey	53,35 ± 8,19	52,23 ± 9,08	1	51,61 ± 9,19	51,07 ± 8,38	0.414
Positive Affect Survey	50,64 ± 7,73	50,3 ± 8,07	1	50,47 ± 7,89	49,65 ± 8,02	0.426
ASR Anxiety and Depression‡	53,75 ± 5,7	53,61 ± 6,47	1	53,19 ± 5,29	54,71 ± 6,93	0.044 *

ASR Somatic Complaints‡	53,66 ± 5,53	54 ± 6,6	1	53,37 ± 5,55	54,08 ± 5,78	0.206
ASR Thought Problems‡	53,28 ± 5,39	53,02 ± 5,5	1	53,22 ± 5,16	54,55 ± 5,93	0.044 *
ASR Attention Problems‡	54,4 ± 5,94	53,98 ± 5,64	1	54,91 ± 4,69	56,14 ± 5,53	0.044 *
ASR Aggressive Behavior‡	52,29 ± 3,61	52,08 ± 4,12	1	52,33 ± 3,98	53,18 ± 4,61	0.109
ASR Rule Breaking Behavior‡	52,95 ± 4,29	53,24 ± 4,75	1	54,49 ± 5,46	54,47 ± 5,87	0.845
ASR Intrusive‡	53,35 ± 5	53,29 ± 5,01	1	53,81 ± 5,28	54,81 ± 6,01	0.279
ASR Internalizing‡	48,05 ± 9,91	48,24 ± 10,7	1	47,16 ± 10,47	49,94 ± 11,29	0.044 *
ASR Externalizing‡	47,05 ± 8,98	47,23 ± 9,05	1	49,48 ± 8,18	50,62 ± 8,54	0.213
ASR Total Problems‡	46,69 ± 8,77	46,64 ± 9,23	1	47,82 ± 8,03	49,79 ± 8,84	0.044 *
DSM Depressive Problems	53,75 ± 5,35	53,75 ± 5,81	1	53,23 ± 5,59	54,29 ± 6,1	0.124
DSM Anxiety Problems	53,23 ± 4,96	53,41 ± 5,83	1	52,57 ± 4,33	53,43 ± 5,28	0.109
DSM Somatic Problems	53,86 ± 5,22	53,97 ± 6,27	1	53,51 ± 5,62	54,14 ± 5,92	0.244
DSM Avoidant Personality Problems	53,81 ± 5,79	53,96 ± 5,65	1	54,1 ± 5,4	55,56 ± 7,1	0.052
DSM AD/H Problems	54,19 ± 5,92	53,64 ± 5,77	1	54,95 ± 4,98	56,25 ± 6,13	0.044 *

DSM Antisocial Personality Problems	52,4 ± 3,9	52,45 ± 4,34	1	53,17 ± 5,06	53,5 ± 5,29	0.650
SSAGA Childhood Conduct	0,37 ± 0,62	0,34 ± 0,61	1	0,7 ± 0,87	0,79 ± 0,89	0.706
SSAGA Panic Disorder	7%	9%	1	8%	7%	0.612
SSAGA Agoraphobia	8%	7%	1	7%	6%	0.853
SSAGA Past Depressive episode	9%	10%	1	6%	10%	0.317
SSAGA Past Depressive symptoms	1,55 ± 2,68	1,44 ± 2,68	1	0,73 ± 1,97	1,23 ± 2,57	0.290
Mother with Anxiety	6%	4%	1	2%	5%	0.426
Father with Anxiety	2%	4%	1	2%	4%	0.593
Mother with Depression	13%	10%	1	7%	8%	0.808
Father with Depression	8%	4%	1	8%	5%	0.706

TASK PERFORMANCE

Accuracy to Fearful Faces	98,6 ± 4,33	98,47 ± 2,53	1	98,09 ± 3,03	98,74 ± 4,5	0.414
Reaction time to Fearful Faces	811,21 ± 128,2	798,91 ± 139,01	1	767,23 ± 127,48	775,07 ± 125,76	0.865
Accuracy to Shapes	97,34 ± 4,31	96,76 ± 3,96	1	96,4 ± 3,35	96,16 ± 5,92	0.593
Reaction time to Shapes	785,21 ± 109,07	789,57 ± 120,72	1	737,39 ± 105,58	759,59 ± 112,36	0.212

Accuracy (Faces vs. Shapes)	1,26 ± 3,59	1,7 ± 4,3	1	1,69 ± 3,98	2,58 ± 4,55	0.192
Reaction time (Faces vs. Shapes)	26,01 ± 79,74	9,34 ± 86,61	1	29,85 ± 84,4	15,48 ± 77,83	0.165

Table S3. Descriptive statistics (Mean ± SD; %) and between-group comparisons of sociodemographic, neuropsychological, mental health and task performance variables.

Groups defined by rs2765 genotype (AA or GA/GG) and analyses performed independently for each sex. Between-group comparisons were performed by means of t-tests or χ^2 tests when appropriate. p values were corrected for multiple comparisons using the false discovery rate (FDR) method. SSAGA: Semi Structured Assessment For The Genetics Of Alcoholism; SCPT: Short Penn Continuous Performance Test; PWMT: Penn Word Memory Test; ER-40: Penn Emotion Recognition Test; ASR: Achenbach Adult Self-Report - Syndrome Scales; DSM: Achenbach DSM-Oriented Scale. Data were analyzed using t-test. †Adjusted by age. ‡Adjusted by age and gender. *pFDR < 0.05. **pFDR < 0.01.

SNP rs2765 *TAC3R*

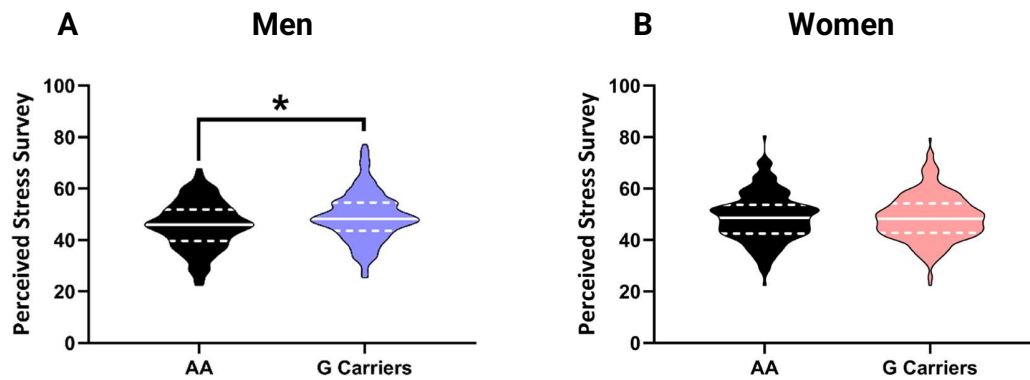


Fig. S6. Perceived stress survey results in men and women with the SNP rs2765 *TAC3R*.

(A) Men with the AA allele scored lower than men G carriers in the perceived stress survey of the human connectome project database. (B) There were no differences between women AA and G carriers in the perceived stress survey. Data are expressed as mean, first quartile (bottom dashed line) and third quartile (top dashed line) and analyzed using t-test. * pFDR <0.05.

Subject	Age (years)	Postmortem delay (hours)	RIN	Mechanism of death	Drugs in blood (mg/l) and ethanol (g/l)
Men subjects					
Men subjects included in both studies (n=89)					
1	63	48	6.9	Accidental	ETH (0.76)
2	18	25	6.3	Traffic	
3	74	46	6.5	Traffic	
4	81	41	3.1	Traffic	
5	16	30	4.3	Traffic	
6	20	48	5.2	Traffic	
7	31	59	4.9	Traffic	ETH (0.61)
8	41	19	7.4	Accidental	
9	19	22	4.3	traffic	
10	47	63	5.2	Traffic	ETH (1.16), LID (therapeutic range)
11	18	44	2.2	Traffic	
12	29	36.5	6.2	Traffic	ETH (1.71)
13	42	27	5.2	Traffic	ATR
14	22	32	8.5	Traffic	ETH (1.04)
15	26	33	6.3	Traffic	
16	16	28	5.9	Traffic	
17	18	48	4.4	Traffic	
18	19	20	7.6	Traffic	THC
19	30	23.5	6.9	Accidental electrocution	ETH (0.74)
20	29	31	8.2	Traffic	ETH (1.48)
21	19	29	6.4	Traffic	ETH (1.15)
22	80	96	6.1	Traffic	
23	26	27	6.1	Traffic	
24	42	41	6.7	Accidental drowning	ETH (1.34)
25	62	41	6.0	Accidental drowning	ETH (0.29)
26	59	41	7.5	Traffic	
27	73	19	7.5	Traffic	
28	43	43	5.2	Traffic	
29	19	61	7.9	Traffic	CQN (3.4)
30	43	59	5.9	Traffic	
31	37	55	3.7	Traffic	ETH (0.17)
32	19	54	5.1	Traffic	
33	42	9	6.2	Traffic	
34	41	20	5.7	Traffic	ATR, LID, MOR
35	58	27	2.4	Accidental jumping	
36	23	32	5.4	Traffic	
37	71	17.5	5.4	Traffic	ETH (0.35)
38	4	46	4.1	Accidental	
39	19	18	4.7	Traffic	
40	18	18	7.3	Traffic	
41	8	23	7.3	Accidental gunshot	
42	60	51	7.0	Accidental bullfighting	ETH (1.02)
43	78	39	6.6	Traffic	
44	60	26.5	6.4	Traffic	TCP

45	49	35.5	6.4	Accidental fall	ATR (4100), ETH (3.75)
46	70	14.5	8.1	Traffic	ETH (0.29), MID
47	49	48	5.5	Accidental fall	N/A
48	77	44	4.8	Traffic	ETH (0.3)
49	16	36	4.4	Traffic	ETH (1.77)
50	62	47.5	5.9	Traffic	N/A
51	65	49.5	7.6	Traffic	
52	66	20	6.5	Accidental jumping	ETH (0.84)
53	51	47	7.6	Crushed	ATR
54	73	49	5.4	Traffic	
55	22	63	4.5	Traffic	
56	47	26	5.7	Accident	
57	66	50	4.6	Traffic	
58	13	22.5	7.7	Traffic	
59	22	25	4.5	Traffic	
60	55	60	5.2	Traffic	MMZ
61	20	34	7.1	Traffic	
62	39	21	5.1	Traffic	ATR, THC
63	25	20.5	7.5	Accident	THC
64	21	16	6.5	Traffic	
65	34	27	8.4	Traffic	N/A
66	84	21.5	4.8	Polytrauma by train	
67	17	16	6.4	Traffic	
68	76	28	6.5	Traffic	
69	42	27	5.3	Crushed	ATR
70	63	44	4.7	Traffic	ETH (0.44), PCT
71	16	22.5	4.6	Accidental jumping	
72	56	38	4.5	Traffic	ETH (2.2), SAL
73	69	23	6.5	Traffic	
74	15	5.5	3.3	Accidental jumping	
75	20	21	5.9	Traffic	
76	71	14	7.3	Crushed	
77	48	9.5	8.1	Accidental fall	CLO, FUR, NAP, PCT, TIA
78	22	4	6.7	Traffic	
79	48	7	5.9	Traffic	
80	58	16	7.7	Traffic	ETH (0.77)
81	22	20	3.7	Traffic	NDZP (0.38)
82	61	23	5.9	Traffic	ETH (0.72)
83	18	9	8.1	Traffic	ETH (0.14)
84	19	12	5.8	Traffic	AMP (0.18), ETH (0.88)
85	30	11	8.2	Accidental electrocution	THC
86	32	28	5.9	Traffic	AMP, ETH (0.68)
87	89	33	5.5	Accidental jumping	N/A
88	18	3.5	7.5	Traffic	
89	56	4	4.6	Accidental hyperthermia	ETH (2.26)
Men subjects not included in genotyping study					
90	21	31	5.8	Traffic	AMP, ETH (0.24), THC
91	20	16	7.4	Traffic	AMP, EPH
Men subjects included in mRNA study (n=91)					

Mean±SEM	41±2	31±2	6.0±0.1		
Men subjects not included in mRNA study (n=42)					
92	45	30		Traffic	ETH (3.09)
93	36	32.5		Traffic	ETH (1)
94	37	54		Traffic	ETH (2.2)
95	79	66		Traffic	
96	36	14		Traffic	ETH (0.3)
97	28	15		Traffic	ETH (2.71)
98	47	20.5		Accidental jumping	MID, ATR
99	19	28		Traffic	
100	62	44		Traffic	LID (1.8)
101	43	15		Traffic	
102	37	21		Accidental	
103	49	17		Traffic	
104	41	24		Paragliding accident	
105	20	37		Traffic	
106	38	33		Traffic	ETH (1.62)
107	39	66.5		Traffic	ETH (1.23)
108	70	41		Traffic	ETH (0.23)
109	43	45		Accidental jumping	
110	58	19		Accidental	ETH (0.99)
111	23	27		Traffic	
112	28	30		Traffic	
113	59	38		Traffic	ETH (0.13)
114	52	14		Traffic	ETH (0.83)
115	33	41		Traffic	
116	78	22		Traffic	
117	37	38		Traffic	ETH (0.3), LID (5)
118	40	60		Traffic	
119	54	26		Accident	
120	N/A	N/A		Accident	
121	55	15.5		Traffic	
122	29	58		Traffic	ETH (0.37)
123	34	60		Traffic	ETH (0.74), LID, TPL
124	25	42.5		Crushed	ATR
125	48	20		Traffic	
126	26	52		Traffic	ETH (1.23)
127	29	19		Accident	PCT
128	36	18		Accident	BLG (0.08), ETH (1.69)
129	40	18		Accident	ETH (0.56)
130	31	19		Traffic	
131	51	33		Accidental ethylic intoxication	ETH (4.93)
132	43	10		Traffic	ETH (2.5)
133	52	18.5		Accident	EPH, pseudoEPH, LAU, MID, MOR, MND
All men subjects in the study (n=133)					
Mean±SEM	41±2	31±1			

Women subjects					
Women subjects included in both studies (n=33)					
134	59	25	7.7	Traffic	AMI, ATR, NTI
135	20	53.5	6.9	Traffic	
136	73	38.5	4.2	Choke on food	
137	28	55	7.2	Traffic	
138	23	15	4.1	Traffic	ETH (0.72)
139	23	17.5	7.5	Traffic	
140	51	62	5.4	Traffic	ATR
141	35	24.5	5.5	Traffic	
142	23	60	4.8	Traffic	ACU, MID
143	52	26	5.6	Traffic	
144	25	25	7.3	Traffic	
145	26	30	5.1	Traffic	
146	81	40	7.8	Traffic	N/A
147	16	15	7.7	Traffic	
148	29	31	6.1	Traffic	
149	19	56.5	7.0	Traffic	ETH (0.16)
150	42	61.5	6.9	Traffic	
151	85	58	6.6	Traffic	PXC
152	16	38	5.3	Train accident	
153	79	39	6.7	Traffic	
154	74	21	4.7	Traffic	
155	20	40	3.3	Traffic	
156	52	64	6.4	Traffic	
157	79	28	6.7	Traffic	
158	43	16	4.4	Traffic	
159	77	13.5	6.2	Traffic	PBB, PRI
160	35	22	8.2	Traffic	
161	69	19	7.7	Traffic	NVER (<0.1), VER (0.1)
162	32	10	5.5	Traffic	AMP (0.04), COC (2.1), THC (0.03)
163	66	16	7.4	Traffic	
164	81	13	9.0	Traffic	
165	70	14	8.2	Accidental suffocation	CIT, TFS
166	80	17	6.9	Traffic	ATR, PCT
Women subjects included in mRNA study (n=33)					
Mean±SEM	48±4	32±3	6.4±0.2		
Women subjects not included in mRNA study (n=6)					
167	88	39		Traffic	
168	41	22		Traffic	
169	33	68		Traffic	
170	50	31		Traffic	
171	75	39		Traffic	
172	70	6.5		Traffic	
All women subjects in the study (n=39)					
Mean±SEM	50±4	33±3			

Table S4. Subjects in the post-mortem analyses.

Age, post-mortem delay, RIN, mechanism of death and drugs in blood are reported in this table. ACU, atracurium; AMI, amitriptyline; AMP, amphetamine; ATR, atropine; BLG, benzoylecgonine; CIT, citalopram; COC, cocaine; CLO, clomethiazole; CQN, cloroquine; EPH, ephedrine; ETH, ethanol; FUR, furosemide; LAU, laudanosine; LID, lidocaine; MID, midazolam; MMZ, metamizol; MND, metronidazole; MOR, morphine; NAP, naproxen; NDZP, nordiazepam; NTI, nortriptyline; NVER, norverapamil; PBB, phenobarbital; PCT, paracetamol; PRI, primidone; PXC, piroxicam; SAL, salicylic acid; TIA, tiapride; TCP, ticlopidine; THC, cannabinoids; TFS, triflusal; TPL, sodium thiopental; VER, verapamil. N/A: Not Available.

Gene	Gene name	Taqman assay ID
TACR3	Tachykinin receptor 3	Hs00357277_m1
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Hs99999905_m1
RPS13	Ribosomal protein S13	Hs01945436_u1

Table S5. Taqman® assays used in qPCR experiments in postmortem human brain.

The following table contains the Taqman® probes used to target the above-mentioned human genes.

<i>TAC3R</i> rs2765 genotype			
	n	AA	G carriers
Total	130	49	81
Men	33	10	23
Women	97	39	58

Table S6. *TAC3R* SNP rs2765 genotype frequency for the human fear conditioning experiment.

Distribution of genotype frequencies for the SNP rs2765 in the *TAC3R* in the sample among men and women.

	AA (n = 49)			G carriers (n = 81)			P
Age (years)	23.00	±	4.00	23.60	±	4.90	0.455
Sex (n, %)	39 (79.6)			58 (71.6)			0.406
STAI-T	21.10	±	9.50	21.90	±	9.20	0.656
US calibration trials (n)	4.00	±	1.40	4.40	±	1.60	0.202
US intensity (mA)	3.90	±	1.30	3.90	±	1.40	0.361
US discomfort (0-9)	7.20	±	0.70	7.00	±	0.70	0.273
Startle probe discomfort (0-9)	7.00	±	1.50	7.00	±	1.70	0.837
Contingency aware individuals (n, %)	46 (93.9)			78(96.3)			0.672

Table S7. Participant's characteristics in the human fear conditioning experiment.

Data are presented as mean \pm SD or number of subjects and percentage (n, %). STAI-T: trait section from the Spanish Version of the Trait Anxiety Inventory (range 0 to 60), US: unconditioned stimulus. Discomfort ratings ranged from 0 (no discomfort) to 9 (maximum discomfort). t-tests, Mann-Whitney's U test or χ^2 test were used when appropriate.

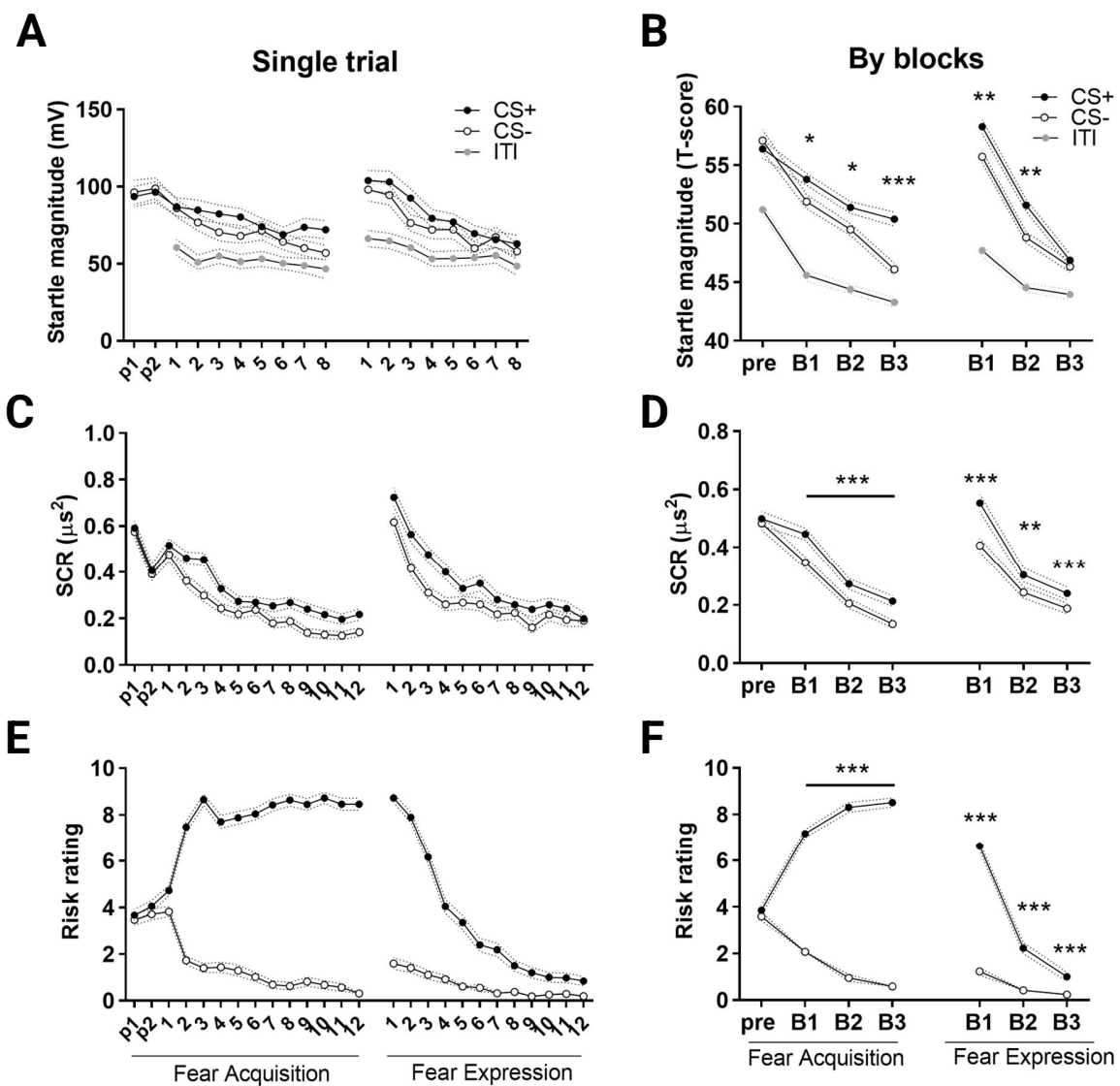


Fig. S7. Fear acquisition and fear expression in the human sample.

(A,B) Depicted are the FPS magnitudes (mV and T-score), (C,D) SCR (μs^2) and subjective risk ratings (E-F) elicited during the FC-FE task for all participants. Results are shown for all trials (left panels) and by blocks (right panels). B1,B2,B3: block, CS+: reinforced CS, CS-: non-reinforced CS, ITI: startle probe during inter-trial interval, p1: pre-acquisition trial 1, p2: pre-acquisition trial 2, pre: acquisition trials. Data were analyzed using Repeated measures ANOVA. Data are shown as Mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

SNP rs2765 *TAC3R*

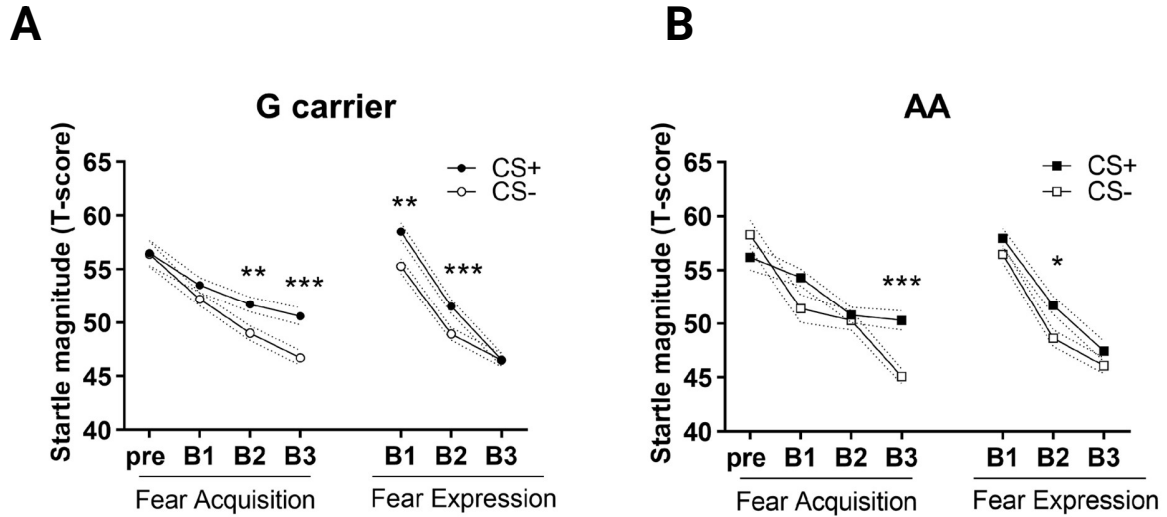


Fig. S8. Fear potentiated startle: fear acquisition and expression according to genotype.

G carrier subjects are shown in the left panel (A) and AA subjects in the right panel (B). B1,B2,B3: block, CS+: reinforced CS, CS-: non-reinforced CS, pre: pre-acquisition phase. Asterisks above a bar indicate main effect stimulus. * Indicates CS discrimination (i.e., higher responses to CS+>CS-). Data were analyzed using Repeated measures ANOVA. Data are shown as Mean \pm SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

SNP rs2765 *TAC3R*

SCR

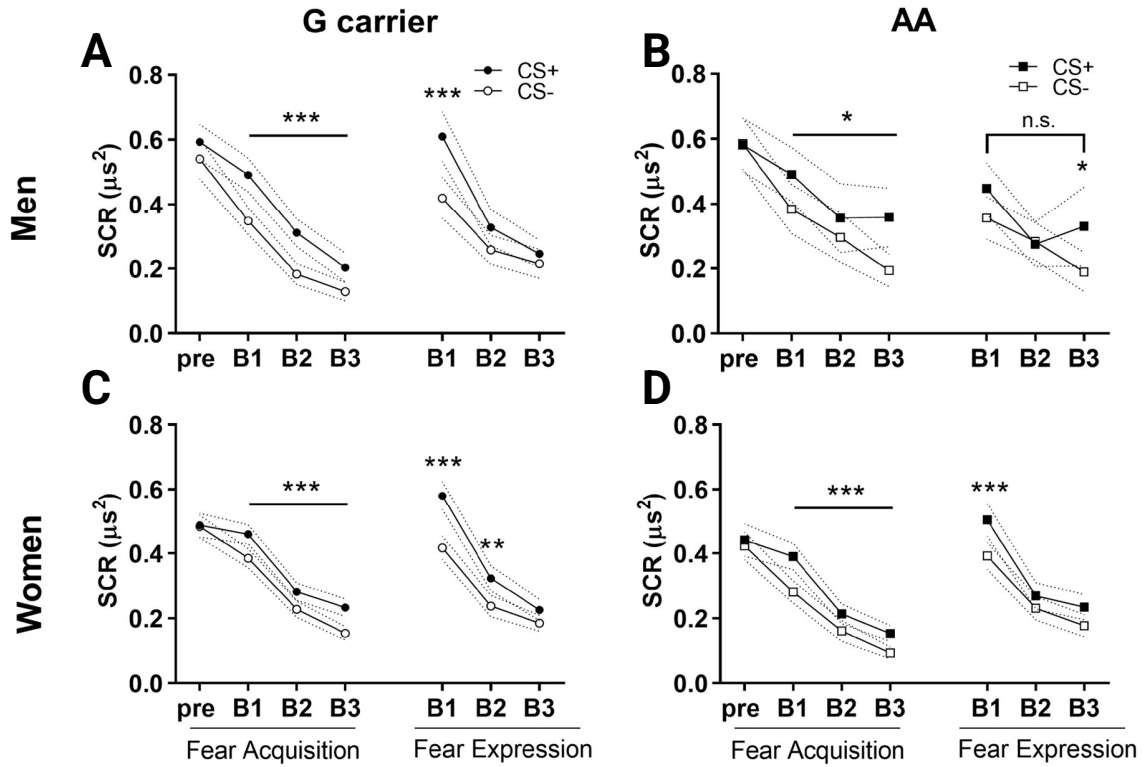


Fig. S9. Skin conductance response: fear acquisition and expression according to genotype and sex.

Men are shown in the upper panels G carriers (A) and AA genotype (B). Women are shown in the lower panels G carriers (C) and AA genotype (D). B1, B2, B3: block, CS+: reinforced CS, CS-: non-reinforced CS, pre: pre-acquisition trials. Asterisks above a bar indicate main effect stimulus. * Indicates CS discrimination (i.e., higher responses to CS+>CS-). Data were analyzed using Repeated measures ANOVA. Data are shown as Mean \pm SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

RR

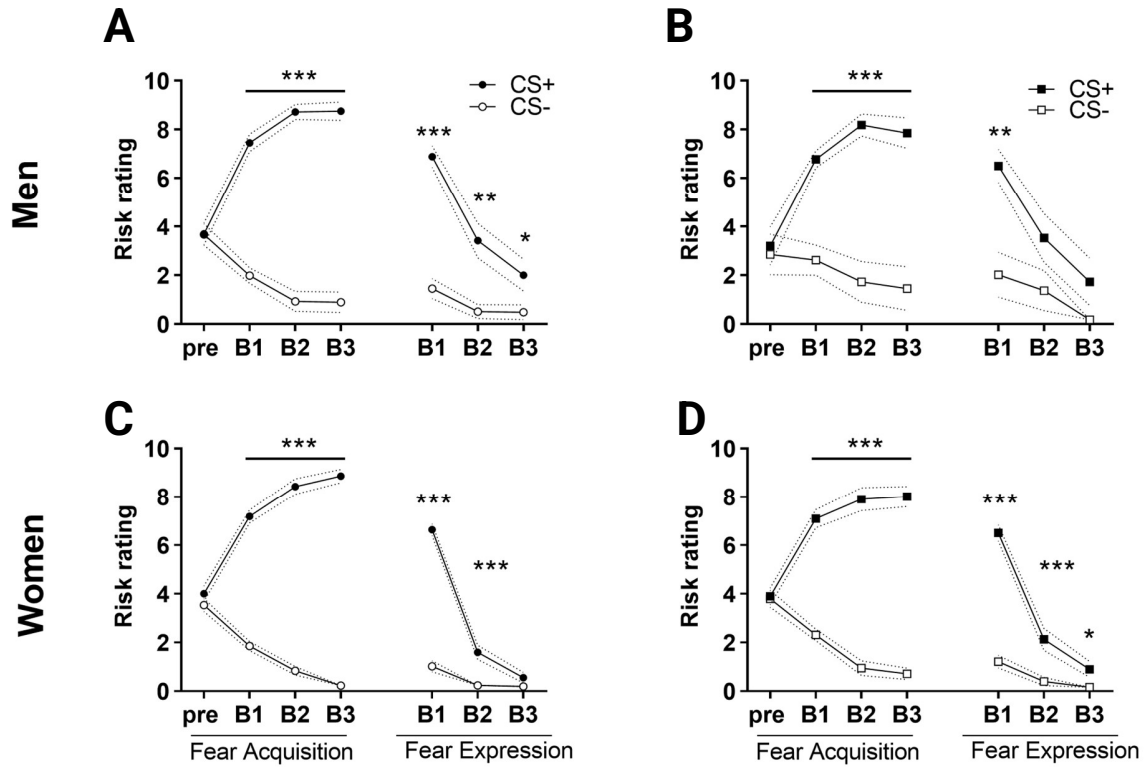


Fig. S10. Subjective risk ratings (RR): fear acquisition and expression according to genotype and sex.

Men are shown in the upper panels G carriers (**A**) and AA genotype (**B**). Women are shown in the lower panels G carriers (**C**) and AA genotype (**D**). B1,B2,B3: block, CS+: reinforced CS, CS-: non-reinforced CS, pre: pre-acquisition trials. Asterisks above a bar indicate main effect stimulus. * Indicates CS discrimination (i.e., higher responses to CS+>CS-). Data were analyzed using Repeated measures ANOVA. Data are shown as Mean \pm SEM. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Data S1. Statistics file.

Movie S1. Fluorescence of a calcium imaging recording.