

## Supplementary information

### **Neuromodulatory effect of interleukin 1 $\beta$ in the dorsal raphe nucleus on individual differences in aggression**

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Supplementary Table 1, 2, 3, 4, 5, 6

Supplemental Figure 1, 2, 3, 4, 5, 6, 7

Supplementary Table 1. IL1R1 shRNA sequences

<b>IL1RI-1a</b>	gatcccc <u>gtgcctctgctgctgcctggacttctgtcatccagcgacagcagaggcactttttggaat</u>
<b>IL1RI-1b</b>	ctagattccaaaaaag <u>tgctctgctgctgcctggatgacaggaagtccagcgacagcagaggcacggg</u>
<b>IL1RI-2a</b>	gatcccc <u>gtcttcaaggttgacatagtgcttctgtcacactatgtcaacctgaagactttttggaat</u>
<b>IL1RI-2b</b>	ctagattccaaaaaag <u>tcttcaaggttgacatagtgacaggaagcactatgtcaacctgaagacggg</u>

**Supplementary Table 2. Statistics for supplementary figures.**

<b>Supplementary Figure 1: Behavior characterization of AGGs, NONs, and variables (n=123)</b>			
Repeated 2-way ANOVA			
	Main effect of Groups	Main effect of Days	Groups x Days Interaction
Attack bites (frequency)	F(2,246)=62.58, p<.0001	F(2,246)=18.97, p<.0001	F(4,246)=3.985, p<.0001
Sideways threats (duration)	F(2,246)=68.15, p<.0001	F(2,246)=17.54, p<.0001	F(4,246)=34.932, p=.0008
Tail rattles (duration)	F(2,246)=40.73, p<.0001	F(2,246)=12.13, p<.0001	F(4,246)=7.026, p<.0001
Pursuit (duration)	F(2,246)=9.917, p=.0002	F(2,246)=3.464, p=.0328	-
Locomotion (duration)	F(2,246)=6.752, p=.0017	F(2,246)=7.933, p=.0005	F(4,246)=2.216, p=.0678
Rearing (duration)	F(2,246)=3.292, p=.0405	F(2,246)=21.96, p<.0001	F(4,246)=3.194, p=.0140
Grooming (duration)	F(2,246)=5.659, p=.0045	-	-
Contact (duration)	F(2,246)=19.13, p<.0001	F(2,246)=74.11, p<.0001	F(4,246)=3.469, p<.0001
Total aggression (duration)	F(2,246)=56.52, p<.0001	F(2,246)=8.169, p=.0004	F(4,246)=3.215, p=.0136
Attack latency	F(2,246)=189.8, p<.0001	F(2,246)=47.39, p<.0001	F(4,246)=15.18, p<.0001
<b>Supplementary Figure 2: i.c.v. injection of IL-1RA (n=17)</b>			
Repeated 2-way ANOVA			
	Main effect of Drug	Main effect of Days	Drug x Days Interaction
Attack latency	F(1,45)=9.179, p=0.0084	F(3,45)=6.897, p=0.0006	-
Attack bites (frequency)	F(1,45)=7.027, p=0.0197	F(3,45)=7.575, p=0.0003	F(3,45)=3.388, p=0.0260
Sideways threats (duration)	F(1,45)=4.574, p=0.0493	F(3,45)=6.992, p=0.0006	-
Tail rattles (duration)	F(1,45)=3.362, p=0.0866	F(3,45)=4.895, p=0.0050	-
Pursuit (duration)	-	-	-
Locomotion (duration)	-	-	-
Rearing (duration)	-	F(3,45)=2.304, p=0.0897	-
Grooming (duration)	F(1,45)=8.251, p=0.0116	-	-
Contact (duration)	-	F(3,45)=6.069, p=0.0015	-
<b>Supplementary Figure 3: Intra-DRN injection of IL-1RA (n=24)</b>			
Repeated 2-way ANOVA			
	Main effect of Drug	Main effect of Days	Drug x Days Interaction
Attack bites (frequency)	F(1,22)=9.786, p=0.0049	F(1,22)=19.25, p=0.0002	-
Sideways threats (duration)	F(1,22)=2.689, p=0.0715	-	-
Tail rattles (duration)	-	-	-
Pursuit (duration)	F(1,22)=6.468, p=0.0185	F(1,22)=4.467, p=0.0461	-
Locomotion (duration)	-	-	-
Rearing (duration)	-	-	-
Grooming (duration)	F(1,22)=5.780, p=0.0251	F(1,22)=6.362, p=0.0194	F(1,22)=3.630, p=0.0699
Contact (duration)	F(1,22)=2.079, p=0.0989	F(1,22)=17.69, p=0.0004	-

<b>Supplementary Figure 4: Intra-DRN injection of IL-1RA in NON animals (n=12)</b>			
Repeated 2-way ANOVA			
	Main effect of Drug	Main effect of Days	Drug x Days Interaction
Attack bites (frequency)	-	-	-
Sideways threats (duration)	-	-	-
Tail rattles (duration)	-	-	-
Pursuit (duration)	-	-	-
Locomotion (duration)	-	$F(1,10)=3.582, p=0.0877$	-
Rearing (duration)	-	-	-
Grooming (duration)	-	$F(1,10)=3.663, p=0.0846$	-
Contact (duration)	-	$F(1,10)=7.849, p=0.0187$	-
<b>Supplementary Figure 5: DRN IL-1R1 knockdown (n=36)</b>			
Repeated 2-way ANOVA			
	Main effect of Virus	Main effect of Days	Virus x Days Interaction
Attack bites (frequency)	$F(1,68)=7.515, p=0.0097$	$F(2,68)=6.780, p=0.0021$	-
Sideways threats (duration)	$F(1,68)=4.539, p=0.0404$	$F(2,68)=9.721, p=0.0002$	-
Tail rattles (duration)	$F(1,68)=3.497, p=0.0701$	$F(2,68)=5.097, p=0.0087$	-
Pursuit (duration)	$F(1,68)=6.913, p=0.0128$	$F(2,68)=3.733, p=0.0290$	-
Locomotion (duration)	-	$F(2,68)=11.47, p<0.0001$	-
Rearing (duration)	$F(1,68)=5.912, p=0.0205$	-	-
Grooming (duration)	$F(1,68)=6.389, p=0.0163$	$F(2,68)=4.205, p=0.0190$	-
Contact (duration)	-	$F(2,68)=4.939, p=0.0099$	-
<b>Supplementary Figure 6: Intra-DRN injection of IL-1B (n=16)</b>			
Repeated 2-way ANOVA			
	Main effect of Drug	Main effect of Days	Drug x Days Interaction
Attack latency	$F(1,14)=3.814, p=0.0711$	-	-
Total aggression (duration)	$F(1,14)=7.311, p=0.0171$	-	-
Unpaired t test			
	Day1	Day2&3	
Attack bites (frequency)	-	$t(14)=3.041, p=0.0088$	
Sideways threats (duration)	-	$t(14)=2.076, p=0.0568$	
Tail rattles (duration)	-	$t(14)=2.808, p=0.0139$	
Pursuit (duration)	-	-	

**Supplementary Table 3. Blood cytokine and chemokine responses to aggressive encounter in aggressor (AGG) and non-aggressor (NON).** No significant interaction was detected in any cytokines or chemokines.

	20 min		24 hrs		Repeated 2-way ANOVA	
	AGG	NON	AGG	NON	AGG vs NON	20 min vs 24 hrs
	n = 12	n = 9	n = 9	n = 9		
IL-1 $\alpha$	449.2 $\pm$ 40.1	355.5 $\pm$ 56.4	489.4 $\pm$ 63.5	685.9 $\pm$ 139.7	<i>ns</i>	F(1,34)=4.276, p=0.0463
IL-2	1.9 $\pm$ 0.2	2.2 $\pm$ 0.6	2.2 $\pm$ 0.4	1.7 $\pm$ 0.4	<i>ns</i>	<i>ns</i>
IL-4	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	<i>ns</i>	F(1,33)=6.954, p=0.0125
IL-6	3.2 $\pm$ 1.1	4.2 $\pm$ 2.3	2.2 $\pm$ 1.7	0.6 $\pm$ 0.3	<i>ns</i>	F(1,33)=5.315, p=0.0276
IL-7	5.3 $\pm$ 2.3	7.9 $\pm$ 5.0	0.6 $\pm$ 0.6	0.3 $\pm$ 0.2	<i>ns</i>	F(1,34)=5.384, p=0.0265
IL-9	93.8 $\pm$ 20.6	82.2 $\pm$ 23.7	43.8 $\pm$ 7.5	56.5 $\pm$ 15.0	<i>ns</i>	F(1,34)=3.410, p=0.0735
IL-15	52.1 $\pm$ 31.1	58.9 $\pm$ 31.4	14.1 $\pm$ 2.0	10.9 $\pm$ 3.1	<i>ns</i>	F(1,34)=3.302, p=0.0780
IL-17	8.3 $\pm$ 1.7	8.7 $\pm$ 3.4	3.8 $\pm$ 0.9	2.6 $\pm$ 0.5	<i>ns</i>	F(1,34)=7.865, p=0.0083
IP-10	59.3 $\pm$ 5.0	58.3 $\pm$ 3.0	64.5 $\pm$ 5.4	57.0 $\pm$ 3.6	<i>ns</i>	<i>ns</i>
KC	42.3 $\pm$ 3.2	38.6 $\pm$ 1.8	29.6 $\pm$ 2.7	25.1 $\pm$ 3.8	<i>ns</i>	F(1,35)=18.42, p=0.0001
LIX	9012 $\pm$ 661	10215 $\pm$ 345	8816 $\pm$ 414	9264 $\pm$ 416	<i>ns</i>	<i>ns</i>
MCP-1	15.4 $\pm$ 2.8	15.7 $\pm$ 1.8	10.0 $\pm$ 2.4	7.9 $\pm$ 2.0	<i>ns</i>	F(1,34)=8.105, p=0.0074
M-CSF	7.5 $\pm$ 0.9	8.6 $\pm$ 1.7	9.4 $\pm$ 2.2	6.1 $\pm$ 0.4	<i>ns</i>	<i>ns</i>
MIG	97.1 $\pm$ 17.5	139.4 $\pm$ 40.8	82.1 $\pm$ 4.6	70.1 $\pm$ 6.1	<i>ns</i>	F(1,34)=2.958, p=0.0945
MIP-1a	33.4 $\pm$ 4.7	27.2 $\pm$ 6.5	34.5 $\pm$ 6.4	25.1 $\pm$ 6.3	<i>ns</i>	<i>ns</i>
MIP-2	92.3 $\pm$ 4.4	84.5 $\pm$ 5.1	91.3 $\pm$ 11.3	88.7 $\pm$ 7.9	<i>ns</i>	<i>ns</i>
RANTES	11.8 $\pm$ 1.4	12.5 $\pm$ 1.3	15.7 $\pm$ 1.6	16.1 $\pm$ 1.3	<i>ns</i>	F(1,35)=6.487, p=0.0154
TNF $\alpha$	3.6 $\pm$ 1.0	2.5 $\pm$ 0.6	2.5 $\pm$ 0.4	1.5 $\pm$ 0.3	<i>ns</i>	<i>ns</i>
VEGF	0.7 $\pm$ 0.3	1.0 $\pm$ 0.4	1.1 $\pm$ 0.4	1.0 $\pm$ 0.3	<i>ns</i>	<i>ns</i>
Eotaxin	347.0 $\pm$ 42.8	378.2 $\pm$ 29.1	337.8 $\pm$ 34.6	365.1 $\pm$ 45.4	<i>ns</i>	<i>ns</i>
G-CSF	134.7 $\pm$ 31.9	113.6 $\pm$ 34.9	92.6 $\pm$ 25.9	50.0 $\pm$ 7.7	F(1,33)=3.315, p=0.0777	<i>ns</i>

**Supplementary Table 4. DRN cytokine responses to aggressive encounter in aggressor (AGG) and non-aggressor (NON). Multiplex ELISA analysis showed no significant difference was detected in any cytokines other than IL-1 $\beta$  (see Figure 1).**

	AGG	NON	t-test
	n = 8	n = 6	
IL-4	3.9 $\pm$ 0.9	3.6 $\pm$ 0.5	<i>ns</i>
IL-6	2.7 $\pm$ 0.1	2.7 $\pm$ 0.2	<i>ns</i>
IL-10	8.6 $\pm$ 0.4	9.2 $\pm$ 0.7	<i>ns</i>
TNF- $\alpha$	3.7 $\pm$ 0.1	3.9 $\pm$ 0.3	<i>ns</i>
IFN- $\gamma$	3.0 $\pm$ 0.3	4.5 $\pm$ 1.3	<i>ns</i>

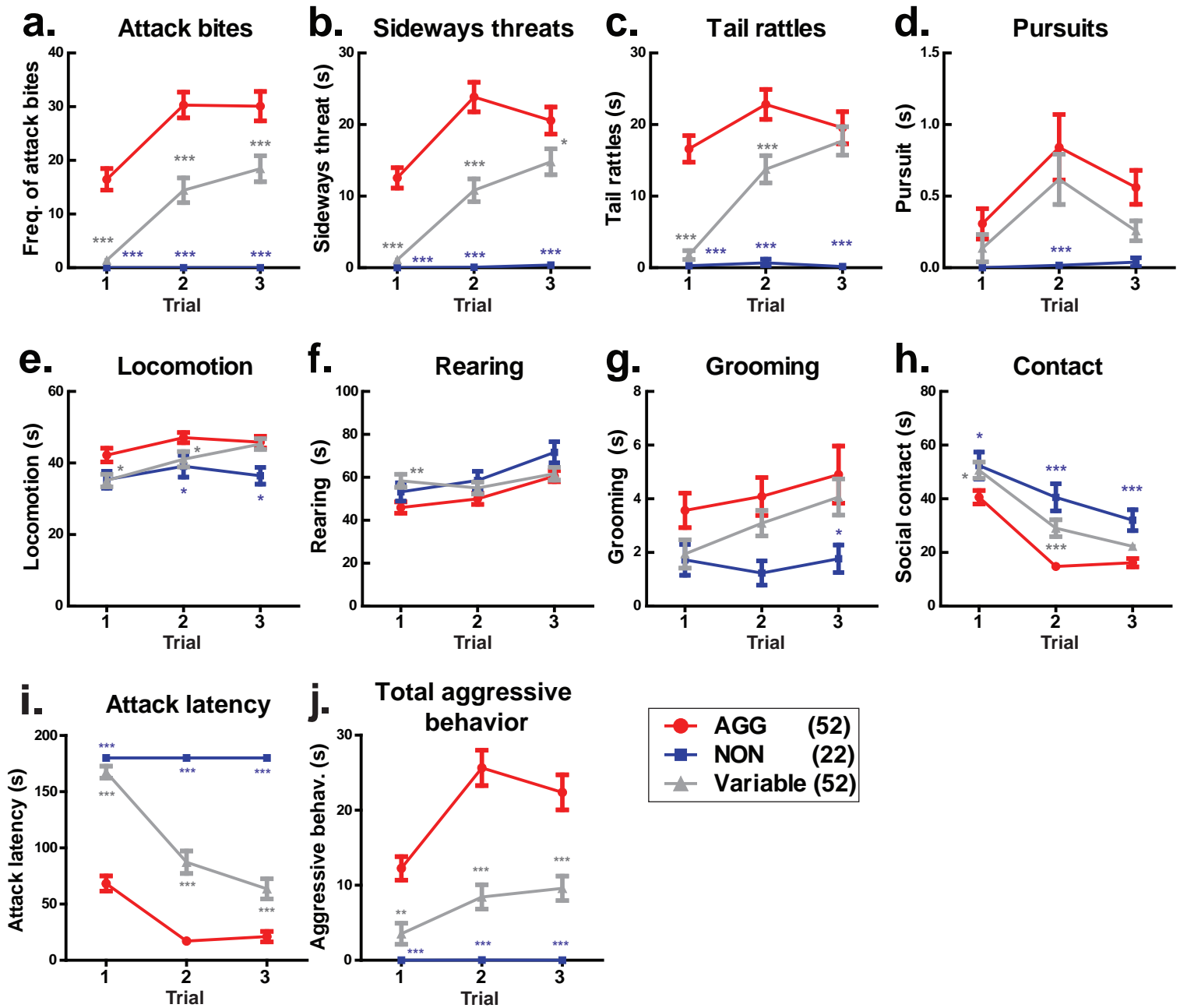
Supplementary Table 5. mRNA expression of IL-1 $\beta$ , IL-1 receptors (IL-1R1 and IL-1R2), IL-1 receptor antagonist (IL-1RA), and adaptor proteins of IL-1 receptor (IL-1RaP and IL-1RaPb) in the DRN of AGG and NON animals. Values indicate normalized expression of each mRNA relative to Gapdh mRNA expression.

	AGG	NON	t-test
	n = 11	n = 11	
IL-1 $\beta$	0.83 $\pm$ 0.12	1.31 $\pm$ 0.18	$t(20)=2.239, p=0.0367$
IL-1R1	0.89 $\pm$ 0.08	1.12 $\pm$ 0.20	<i>ns</i>
IL-1R2	0.87 $\pm$ 0.12	1.05 $\pm$ 0.15	<i>ns</i>
IL-1RA	0.96 $\pm$ 0.13	1.06 $\pm$ 0.06	<i>ns</i>
IL-1RaP	0.94 $\pm$ 0.08	0.94 $\pm$ 0.09	<i>ns</i>
IL-1RaPb	0.95 $\pm$ 0.05	0.96 $\pm$ 0.08	<i>ns</i>

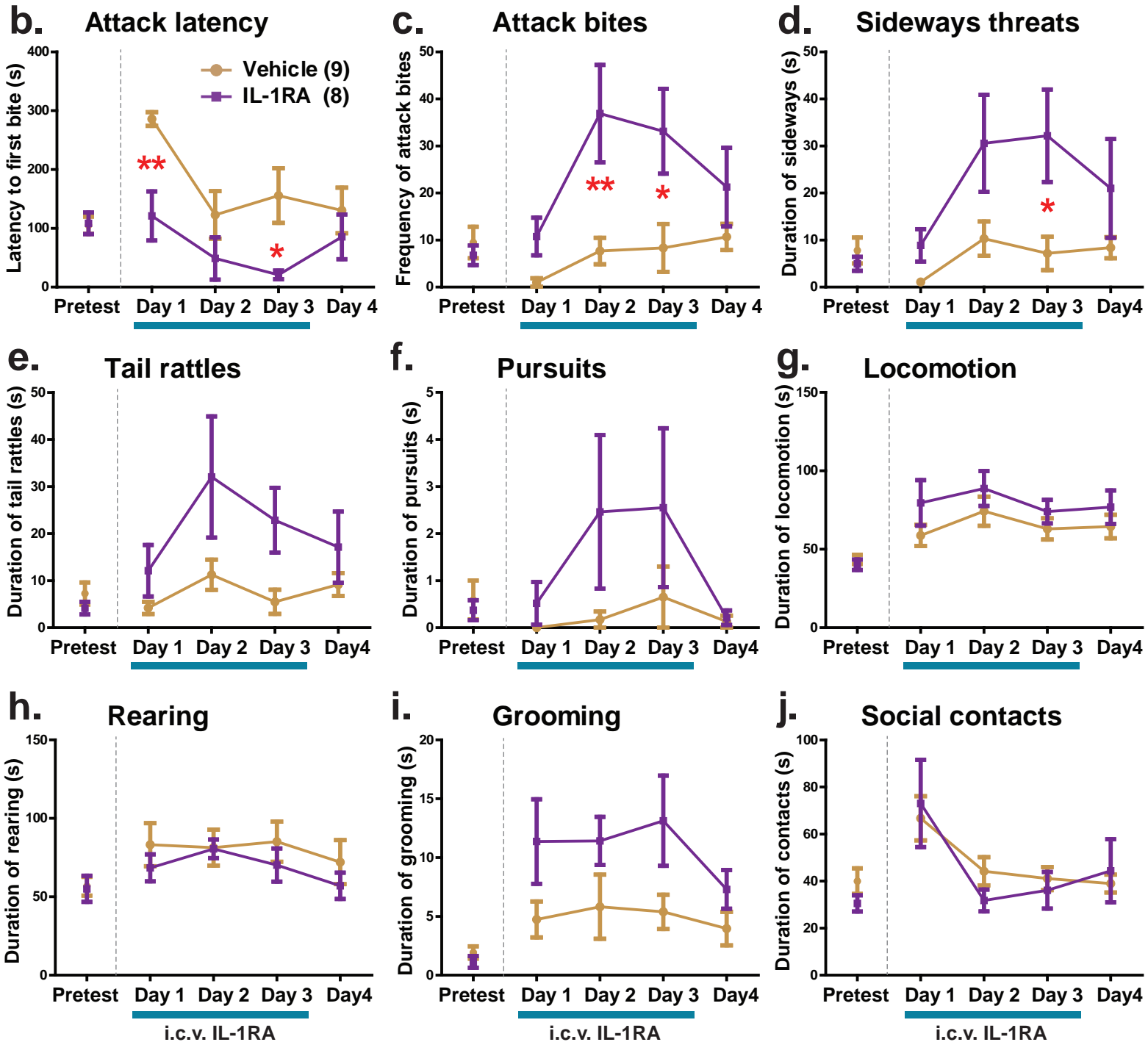
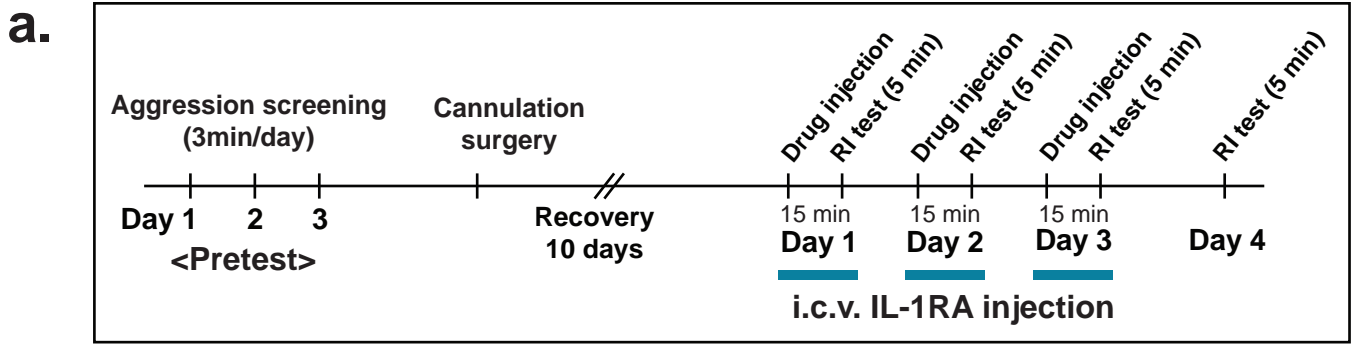
**Supplementary Table 6. mRNA expression of IL-1RaP, IL-1RA, IL-1 $\beta$ , and TNF $\alpha$  genes after IL-1R1 knockdown in the DRN. Significant reduction of IL-1RaP mRNA expression was observed by IL-1R1 KD compared to Luc control. IL-1R2 expression was below the detection in any group (ND). Values indicate normalized expression of each mRNA relative to Gapdh mRNA expression.**

	KD	Luc	t-test
	n = 6	n = 5	
IL-1RaP	0.84 $\pm$ 0.04	1.19 $\pm$ 0.12	<i>t</i> (9)=3.075, <i>p</i> =0.0132
IL1R2	ND	ND	-
IL-1RA	0.87 $\pm$ 0.08	1.16 $\pm$ 0.34	<i>ns</i>
L-1 $\beta$	1.00 $\pm$ 0.31	1.01 $\pm$ 0.58	<i>ns</i>
TNF- $\alpha$	0.93 $\pm$ 0.33	1.08 $\pm$ 0.35	<i>ns</i>

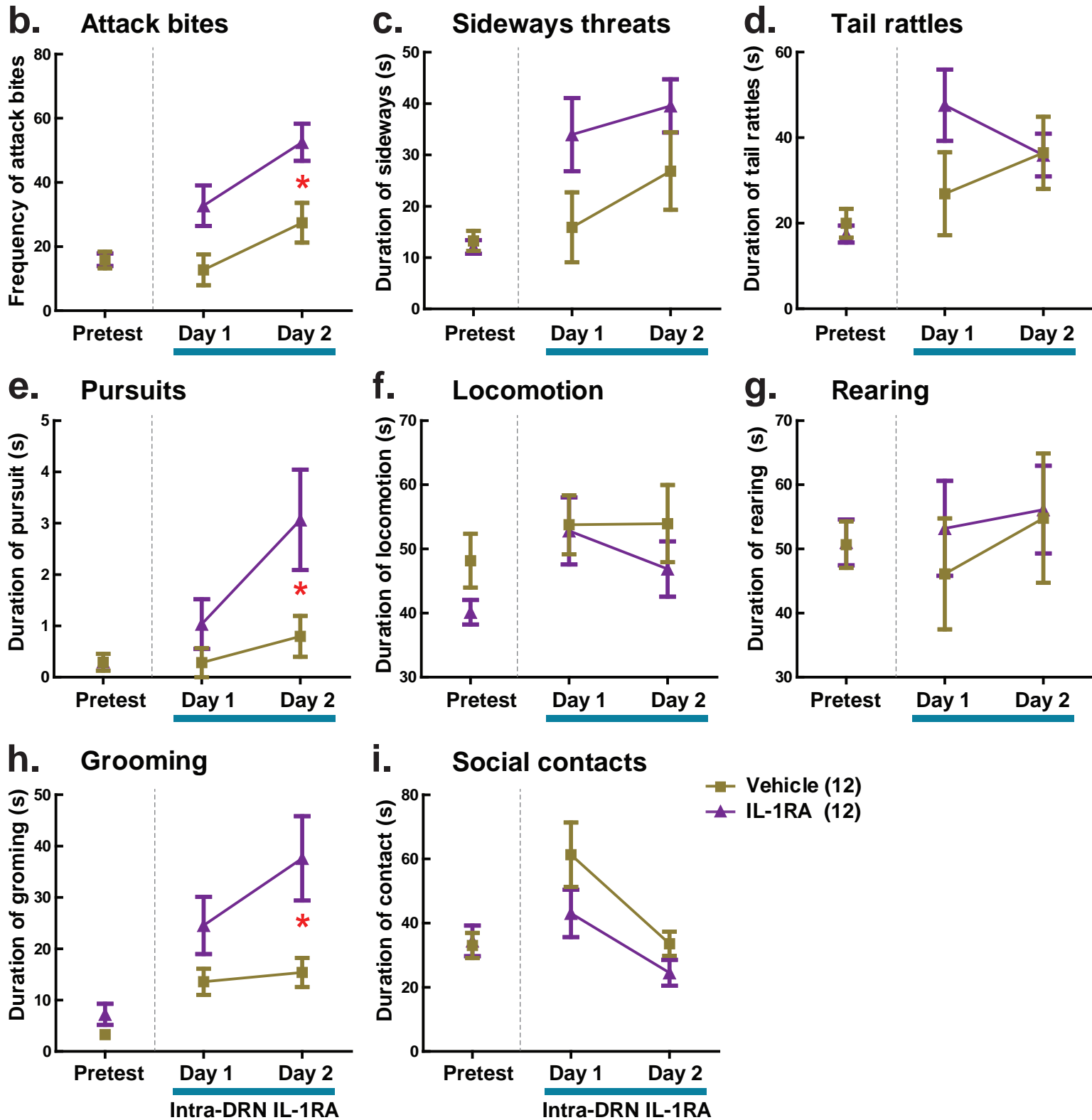
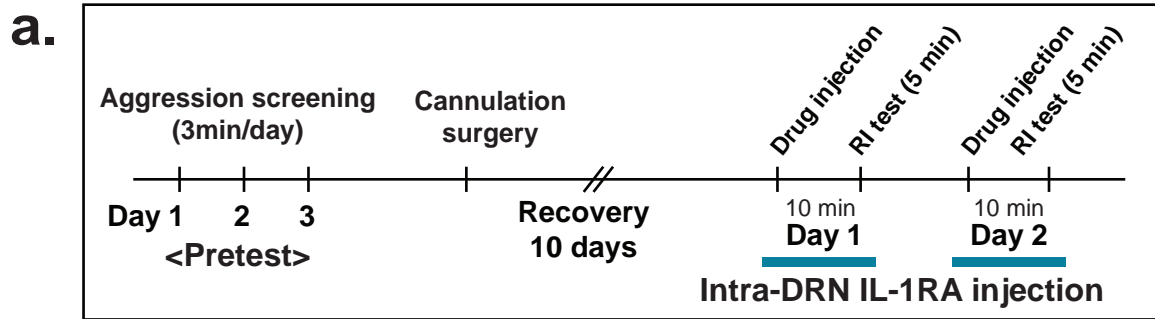




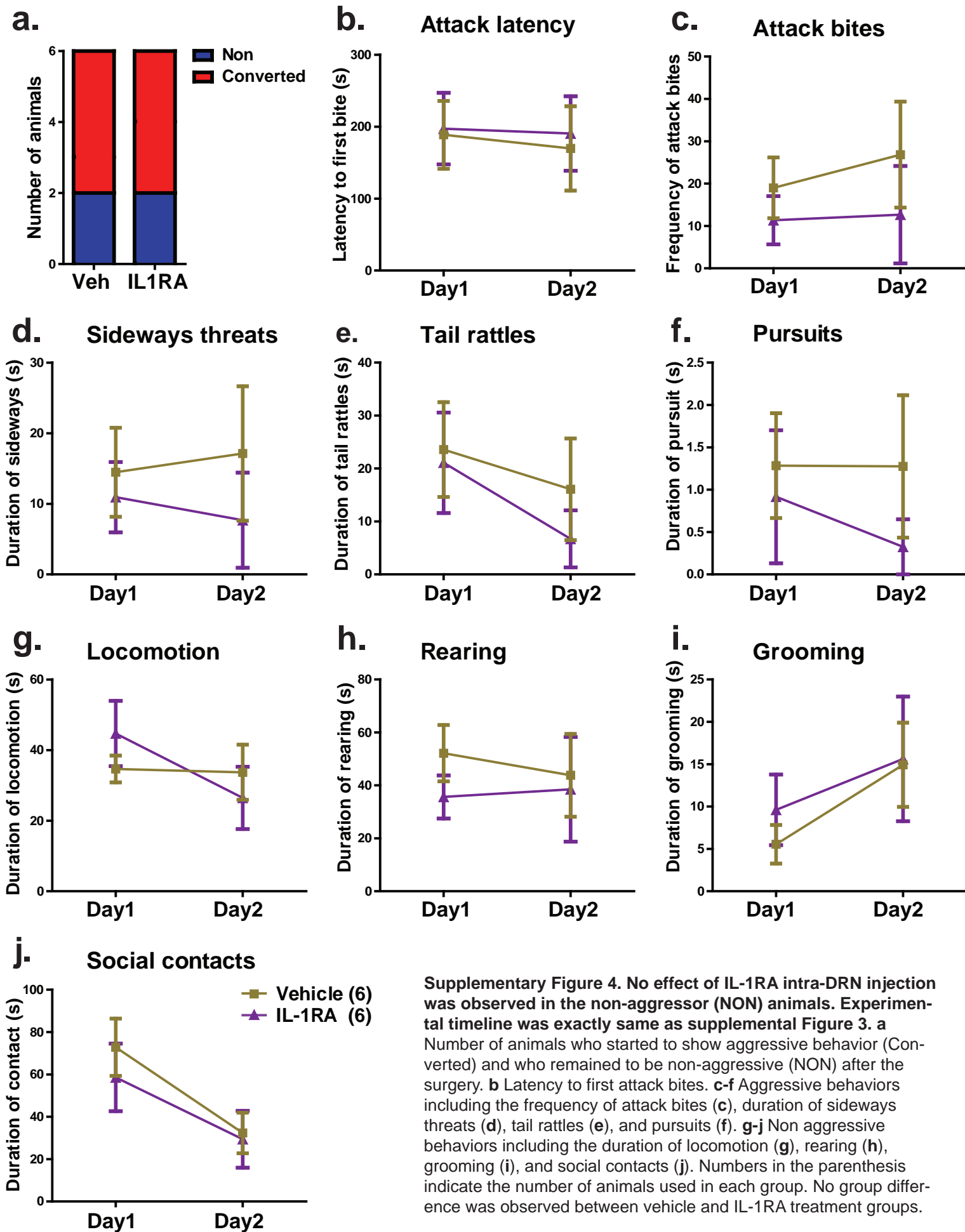
**Supplementary Figure 1. Detailed behavioral profile of Aggressor (AGG), Non-aggressor (NON) and variable animals in resident-intruder test.** a-d Aggressive behaviors including the frequency of attack bites (a), duration of sideways threats (b), tail rattles (c), and pursuits (d). e-h Non aggressive behaviors including the duration of locomotion (e), rearing (f), grooming (g), and social contacts (h). i Latency to the first bite. j Total duration of aggressive behavior. Asterisks indicates significant difference between AGG vs NON (blue asterisks), and AGG vs variables (gray asterisks). \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

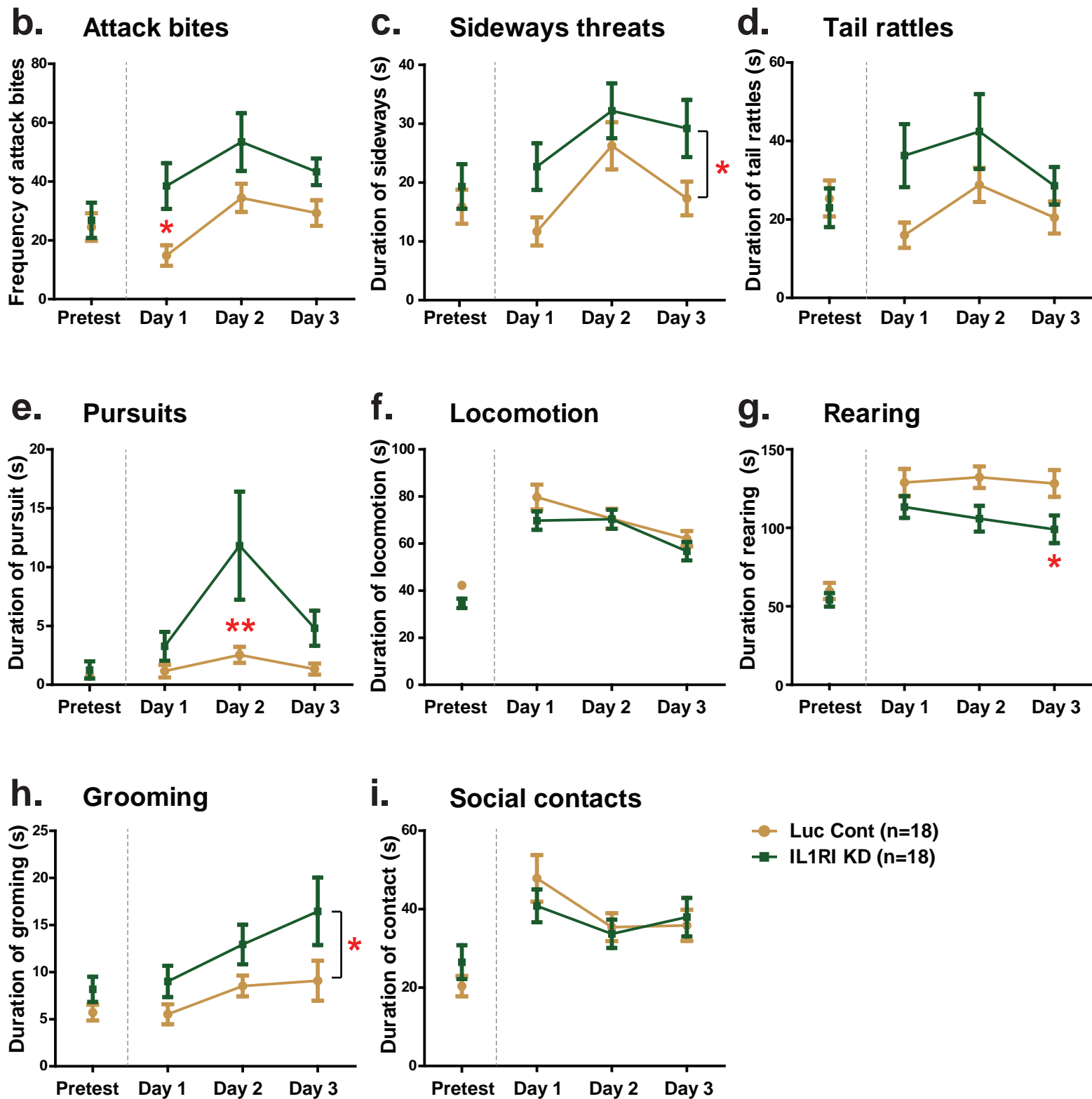
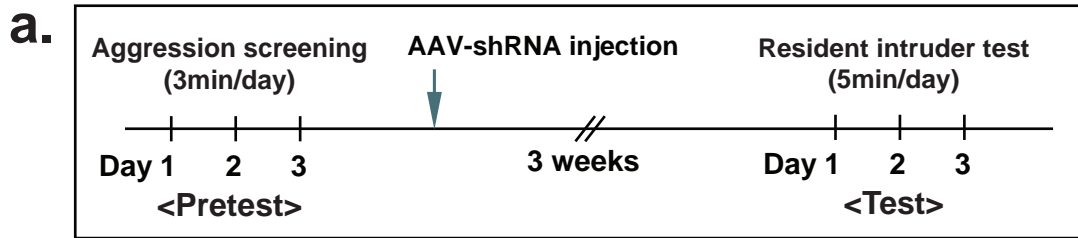


**Supplementary Figure 2. Detailed behavioral profile of male mice after i.c.v. injection of IL-1 receptor antagonist (IL-1RA) in the resident-intruder test.** **a** Schematics of IL-1RA i.c.v. injection experiment. RI test: resident-intruder test. Pretest data is an average of 3-days RI tests before the surgery. In the test, IL-1RA was injected into the lateral ventricle 15 min before the RI test, and this encounter was repeated for 3 days. On the day 4, RI test was conducted without any injection. **b-f** Aggressive behaviors including the attack latency (**b**), frequency of attack bites (**c**), duration of sideways threats (**d**), tail rattles (**e**), and pursuits (**f**). **g-j** Non aggressive behaviors including the duration of locomotion (**g**), rearing (**h**), grooming (**i**), and social contact (**j**). Numbers in the parenthesis indicate the number of animals used in each group. \*\* $p < 0.01$ , \* $p < 0.05$  between vehicle and IL-1RA in each test day.

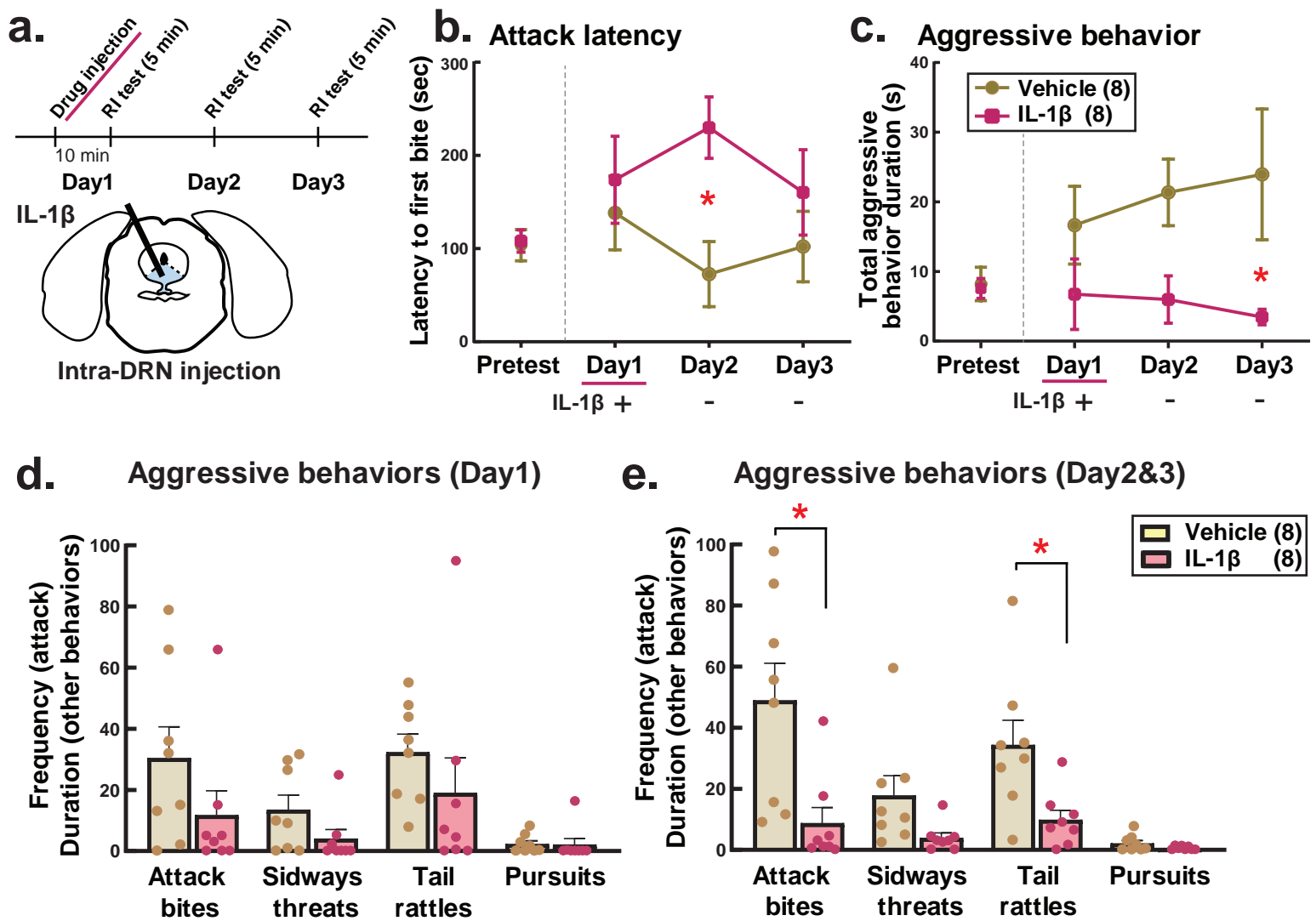


**Supplementary Figure 3. Detailed behavioral profile of male mice after intra-DRN injection of IL-1RA in the resident-intruder test.** **a** Schematics of IL-1RA intra-DRN injection experiment. RI test: resident-intruder test. Pretest data is an average of 3-days RI tests before the surgery. In the test, IL-1RA was injected into the DRN 10 min before the RI test, and this encounter was repeated for 2 days. **b-e** Aggressive behaviors including the frequency of attack bites (**b**), duration of sideways threats (**c**), tail rattles (**d**), and pursuits (**e**). **f-j** Non aggressive behaviors including the duration of locomotion (**f**), rearing (**g**), grooming (**h**), and social contacts (**i**). Numbers in the parenthesis indicate the number of animals used in each group. \* $p < 0.05$  between vehicle and IL-1RA in each test day (post-hoc test).

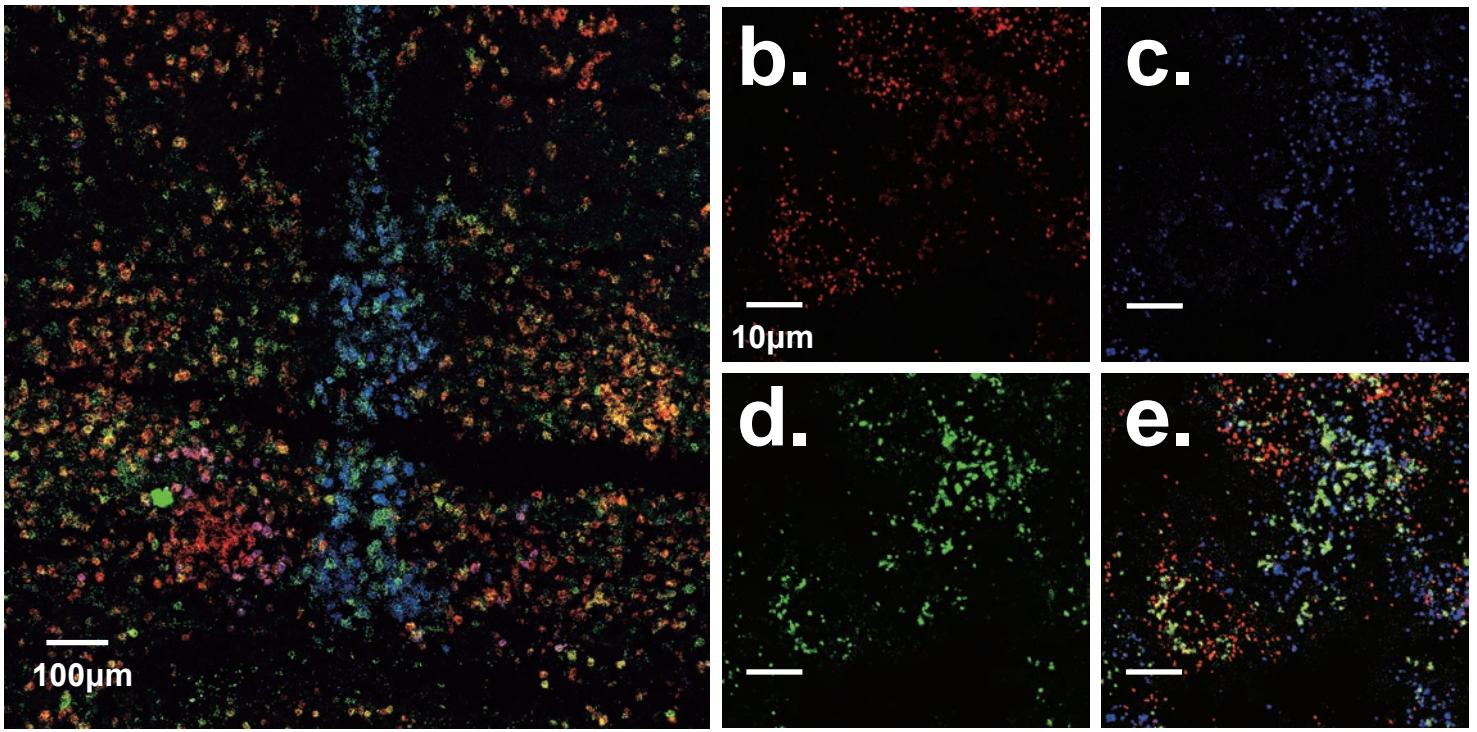




**Supplementary Figure 5. Detailed behavioral profile of male mice after IL-1 receptor type 1 (IL-1R1) knockdown in the resident-intruder test.** **a** Schematic timeline of IL-1R1 KD using shRNA expressing AAV injection. Either control luciferase (Luc) shRNA or IL-1R1 shRNA expressing AAV was injected into the DRN 2 weeks before the test. RI test: resident-intruder test. Pretest data is an average of 3-days RI tests before the surgery. **b-e** Aggressive behaviors including the frequency of attack bites (**b**), duration of sideways threats (**c**), tail rattles (**d**), and pursuits (**e**). **f-j** Non aggressive behaviors including the duration of locomotion (**f**), rearing (**g**), grooming (**h**), and social contacts (**i**). Numbers in the parenthesis indicate the number of animals used in each group. \*\* $p < 0.01$ , \* $p < 0.05$  between vehicle and IL-1RA in each test day (post-hoc test) or significant main effect of drug treatment in the 2-way ANOVA.



**Supplementary figure 6. Long-term effect of IL-1 $\beta$  injection into the DRN on aggressive behavior of male mice.**  
**a** Schematic timeline of intra-DRN IL-1 $\beta$  injection experiment. RI test: resident-intruder test. IL-1 $\beta$  (200 pg) was injected into the DRN only Day1 of the experiment. **b-c** Effect of i.v.c. IL-1RA injection on the attack latency (**b**) and duration of aggressive behaviors (**c**). **d-e** Detailed behavioral analysis for aggressive behaviors on Day1 (**d**) and Day2&3 (**e**). For Day2&3, average data of Day2 and Day3 of resident intruder test was presented. Numbers in the parenthesis indicate the number of animals used in each group. \* $p < 0.05$ .



**Supplementary Figure 7. Expression of c-Fos mRNA on GABA neurons and vGlut3-positive glutamatergic neurons in the DRN.** **a** Representative picture of the expression of c-Fos (green), GAD67 (red) and vGlut3 (blue) in the DRN by RNAscope analysis. **b-e** Enlarged pictures of the DRN showing GAD67 (**b**), vGlut3 (**c**), c-Fos (**d**), and their co-localization (**e**).