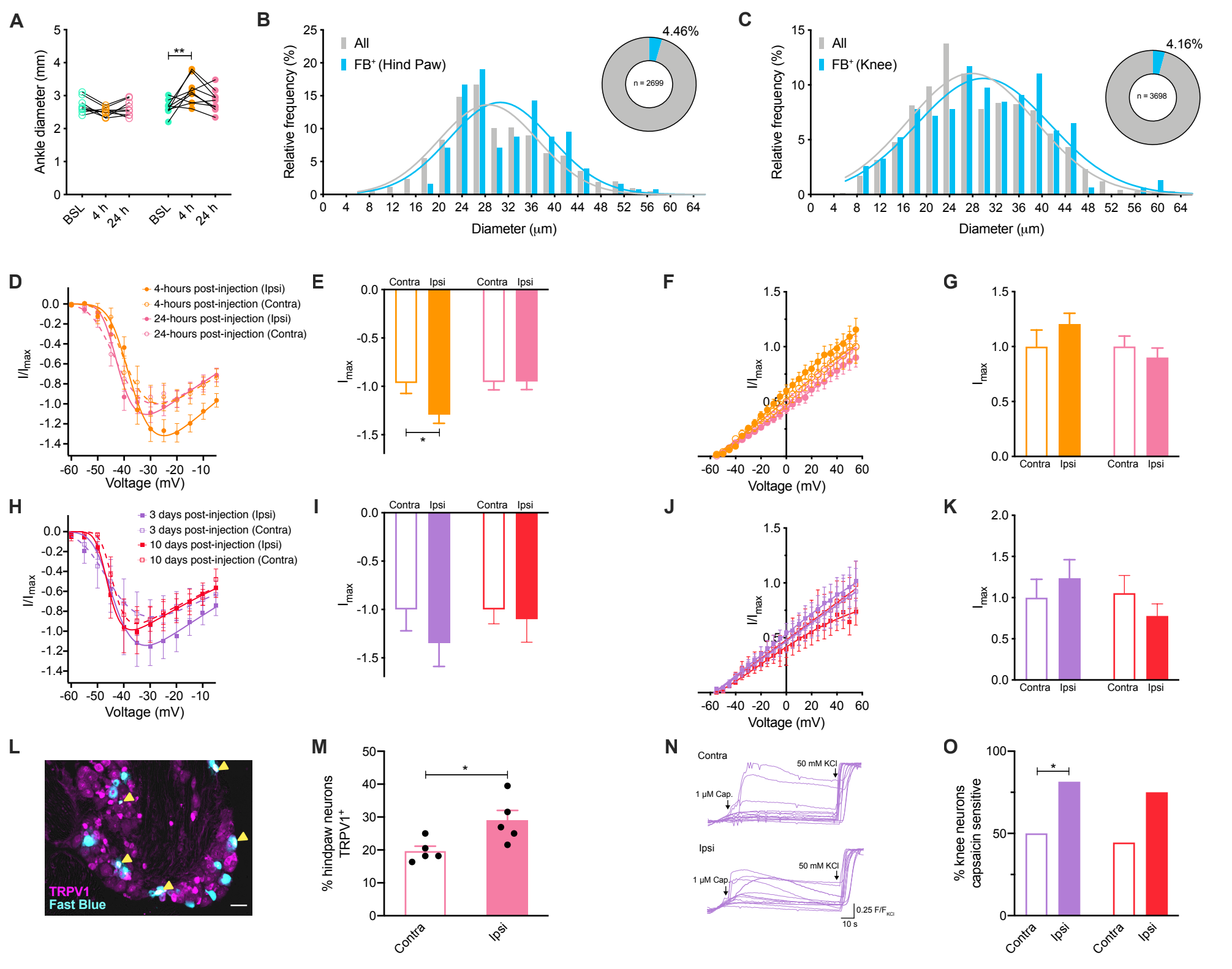

A
Ankle diameter (mm)
BSL
4 h
24 h
**
B
Relative frequency (%)
All
FB+ (Hind Paw)
4.46%
n = 2699
Diameter (μm)
C
Relative frequency (%)
All
FB+ (Knee)
4.16%
n = 3698
Diameter (μm)
D
4-hours post-injection (Ipsi)
4-hours post-injection (Contra)
24-hours post-injection (Ipsi)
24-hours post-injection (Contra)
I/Imax
Voltage (mV)
E
Contra
Ipsi
Imax
*
F
I/Imax
Voltage (mV)
G
Imax
Contra
Ipsi
H
3 days post-injection (Ipsi)
3 days post-injection (Contra)
10 days post-injection (Ipsi)
10 days post-injection (Contra)
I/Imax
Voltage (mV)
I
Contra
Ipsi
Imax
J
I/Imax
Voltage (mV)
K
Imax
Contra
Ipsi
L
TRPV1
Fast Blue
M
% hindpaw neurons TRPV1+
*
Contra
Ipsi
N
Contra
50 mM KCl
1 μM Cap.
Ipsi
50 mM KCl
1 μM Cap.
0.25 F/FKCl
10 s
O
% knee neurons capsaicin sensitive
*
Contra
Ipsi

Figure S1. Effects of Carrageenan-induced inflammation and MIA-induced knee damage on sensory neurons innervating the site of injury, related to Figure 1.
(A) Swelling of the ankle joint was quantified with digital calipers following injection of carrageenan (open circles, Contra, closed circles, Ipsi)**.** Relative frequency distributions of dissociated sensory neuron soma diameter from **(B)** hind paw- or **(C)** knee-innervating and total lumbar populations, Inserts: Proportion of Fast Blue positive cells from acutely dissociated cultures of lumbar DRG. **(D)** Voltage-evoked inward macroscopic current densities of Fast Blue positive sensory neurons isolated from Ipsi and Contra sides and **(E)** normalized peak inward current densities following induction of inflammation with carrageenan. **(F)** Voltage-evoked outward macroscopic current densities of Fast Blue positive sensory neurons isolated from Ipsi and Contra sides and **(G)** normalized peak outward current densities following induction of inflammation with carrageenan. **(H)** Voltage-evoked inward macroscopic current densities of Fast Blue positive sensory neurons isolated from Ipsi and Contra sides and **(I)** normalized peak inward current densities following intra-articular injection of MIA. **(J)** Voltage-evoked outward macroscopic current densities of Fast Blue positive sensory neurons isolated from Ipsi and Contra sides and **(K)** normalized peak outward current densities following intra-articular injection of MIA. All current densities are normalized to the average peak current density of Contra cells for each respective time point. **(L)** A subset of hind paw innervating sensory neurons (blue) express the nociceptive ion channel TRPV1 (magenta), neurons positive for both Fast Blue and TRPV1 are identified by yellow arrowheads, scale bar = 50 µm. **(M)** A higher proportion of Ipsi hind paw innervating neurons expressed TRPV1 24-hours after carrageenan-induced inflammation compared to the Contra paw. **(N)** Ca^{2+} traces of Fast Blue positive sensory neurons isolated from Contra (top) or Ipsi (bottom) sides of a single mouse 3-days post-injection of MIA. Cells were stimulated with 1 µM capsaicin to identify those neurons expressing TRPV1 and 50 mM KCl to identify viable neurons, traces are normalized to the maximal change in fluorescence elicited by KCl (F_{KCl}). **(O)** Proportion of capsaicin sensitive neurons from DRG isolated from the Ipsi and Contra sides 3- or 10- days post-injection of MIA, all Fast Blue positive neurons from 3 animals per side and time point are considered together. * $p < 0.05$, ** $p < 0.01$: **(A)** one-way ANOVA with Bonferroni post hoc; **(E)** t-test test between Ipsi and Contra for individual time points; **(M)** Mann-Whitney test ; **(O)** Chi-squared test.

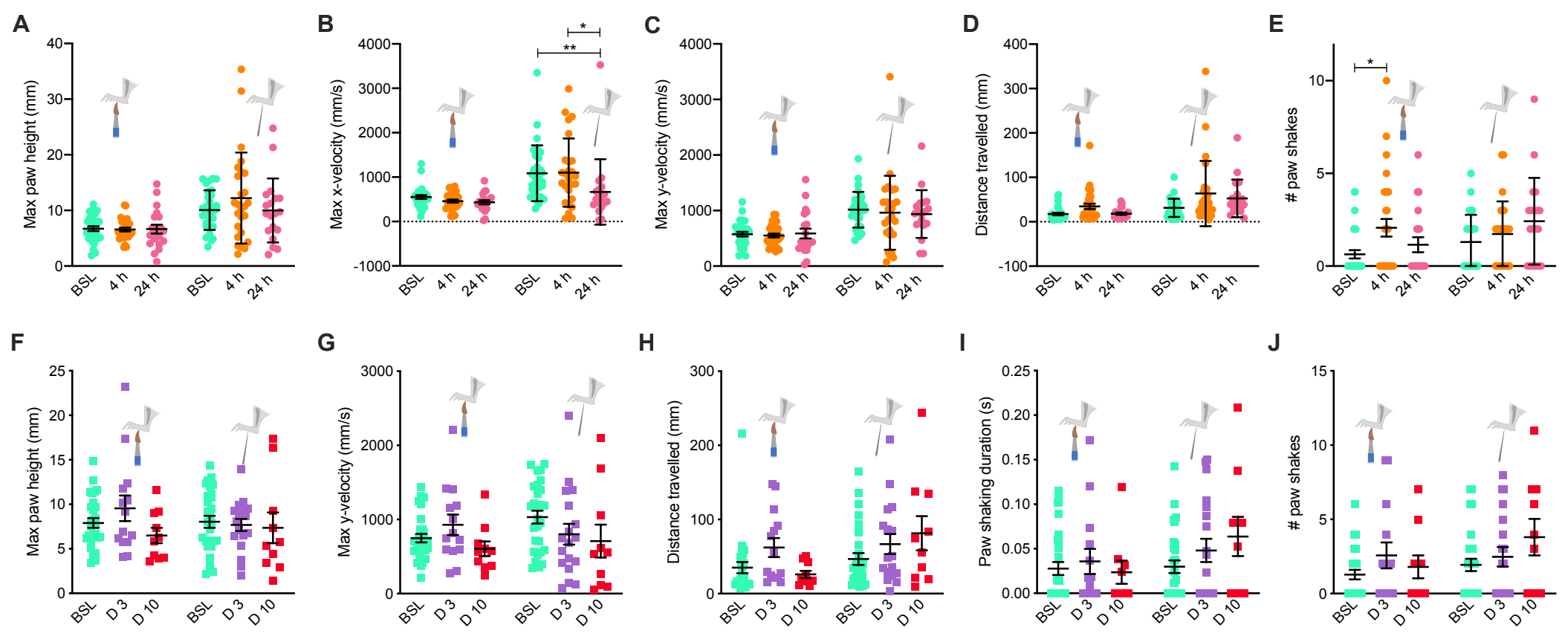
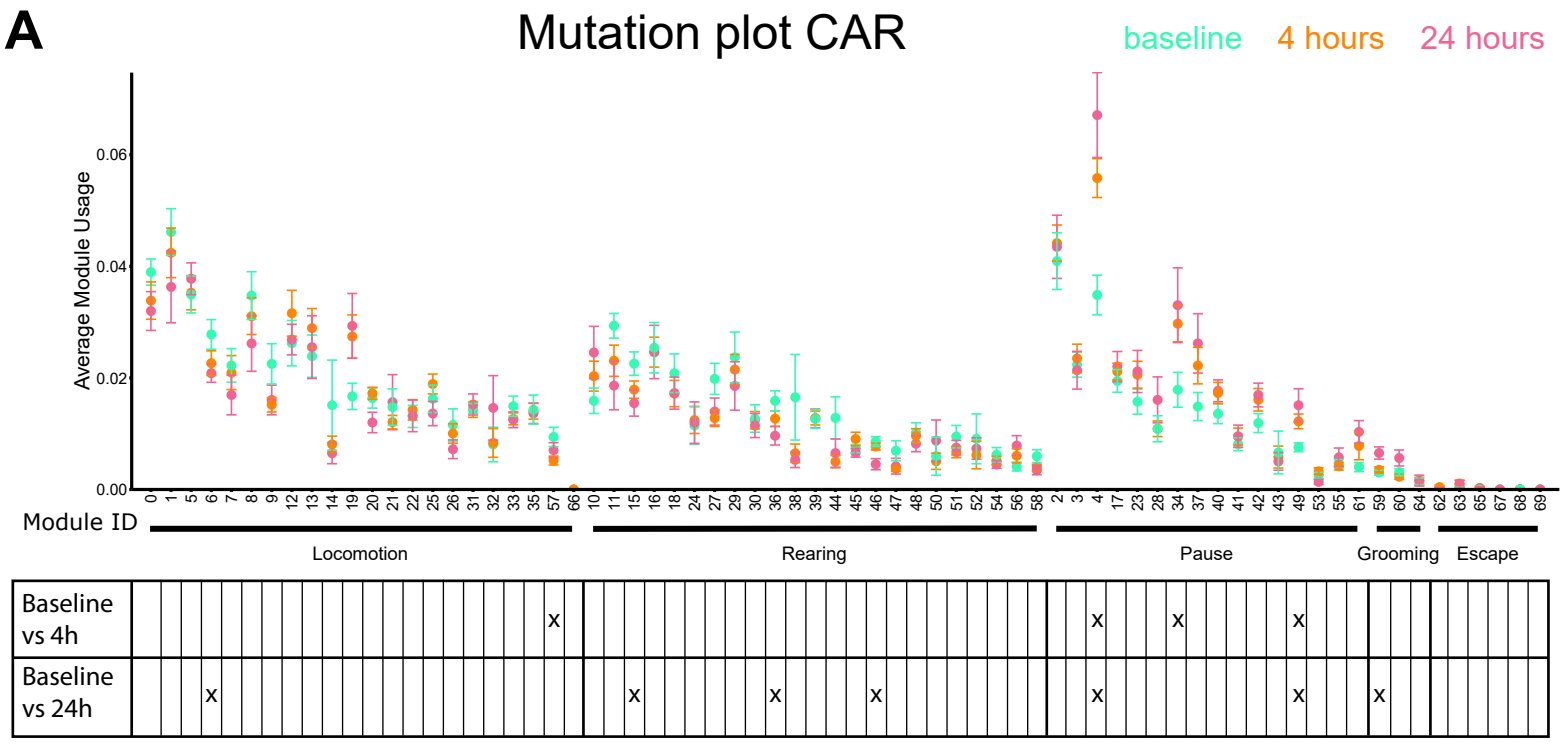

A
Max paw height (mm)
40
30
20
10
0
BSL
4 h
24 h
B
Max x-velocity (mm/s)
4000
3000
2000
1000
0
-1000
*
**
C
Max y-velocity (mm/s)
D
Distance travelled (mm)
400
300
200
100
0
-100
E
paw shakes
10
5
0
F
Max paw height (mm)
25
20
15
10
5
0
D 3
D 10
G
Max y-velocity (mm/s)
H
Distance travelled (mm)
I
Paw shaking duration (s)
0.25
0.20
0.15
0.10
0.05
0.00
J
paw shakes

Figure S2. Reflexive and affective PAWS parameters in response to dynamic brush or pinprick, related to Figure 2. Additional reflexive features of the behavioral response to a stimulation with brush (left) or pinprick (right) at baseline, 4- and 24-hours post-carrageenan injection include **(A)** maximum paw height, **(B)** maximum velocity in x-plane, **(C)** maximum velocity in y-plane and **(D)** distance traveled by paw during withdrawal. **(E)** The number of paw shakes immediately following withdrawal from dynamic brush or pinprick is an additional affective measure. **(F)** Maximum paw height, **(G)** maximum velocity in y-plane and **(H)** distance traveled by paw during withdrawal are reflexive responses to dynamic brush and pinprick stimulation of the hind paw prior and 3- and 10-days post injection of MIA to the knee joint. Affective features following MIA injection include **(I)** paw shaking duration and **(J)** number of paw shakes. * $p < 0.05$, ** $p < 0.01$: Kruskal-Wallis test followed by Dunn's multiple comparisons were performed to determine statistical significance between the responses of mice to each stimuli across time independently.


A
Mutation plot CAR
baseline
4 hours
24 hours
Average Module Usage
0.00
0.02
0.04
0.06
Module ID
Locomotion
Rearing
Pause
Grooming
Escape
Baseline vs 4h
Baseline vs 24h

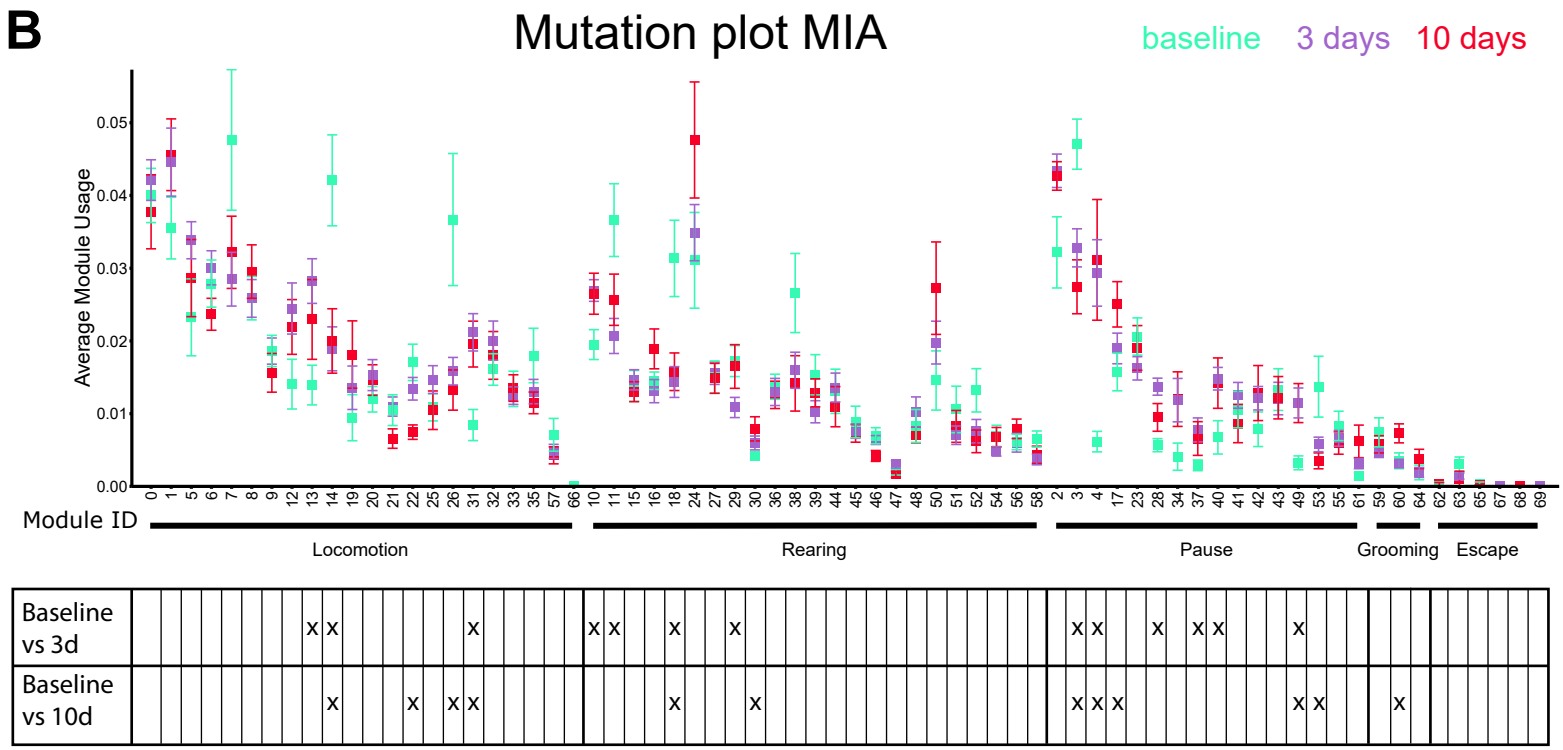
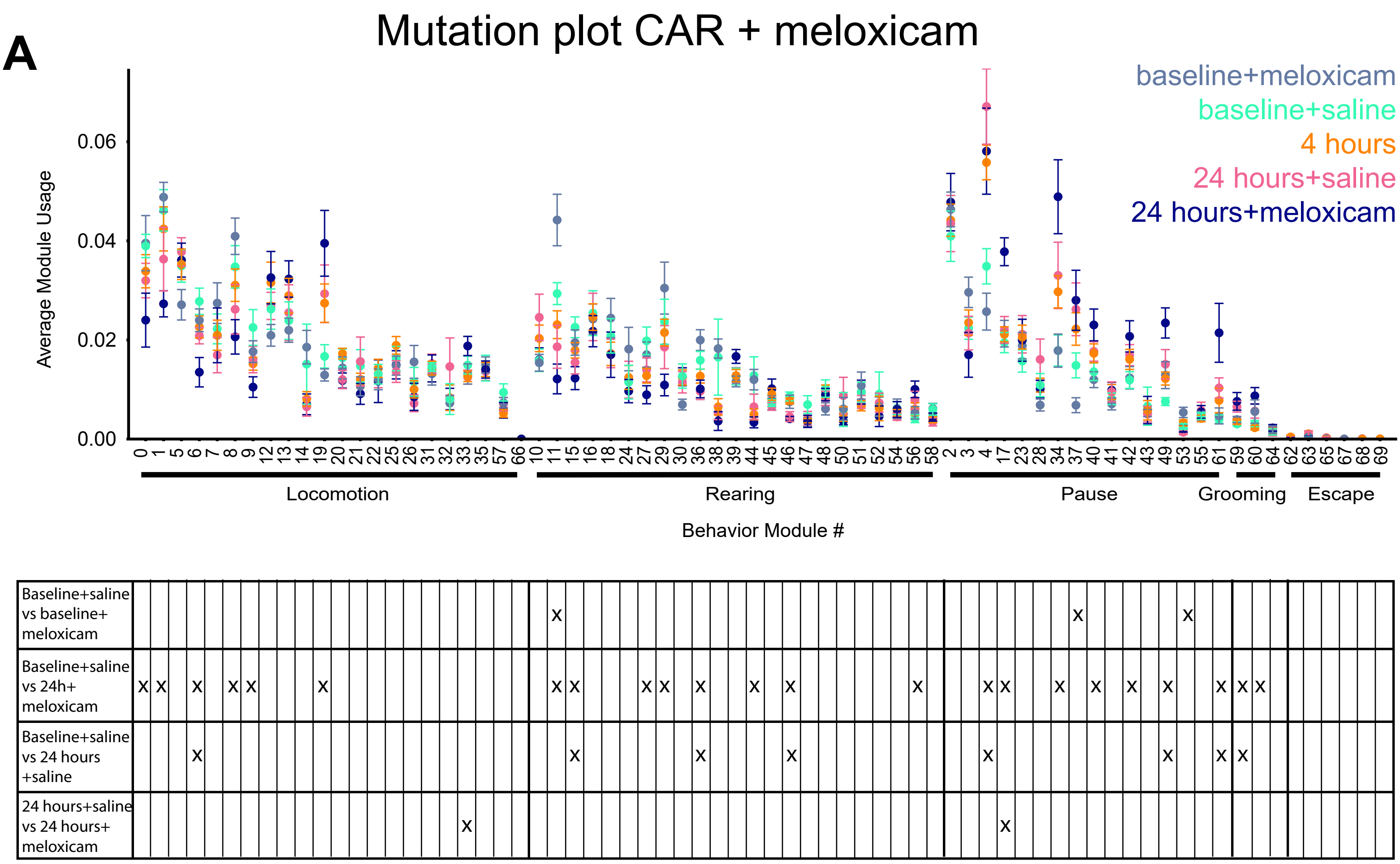

B
Mutation plot MIA
baseline
3 days
10 days
Average Module Usage
0.00
0.01
0.02
0.03
0.04
0.05
Module ID
Locomotion
Rearing
Pause
Grooming
Escape
Baseline vs 3d
Baseline vs 10d

Figure S3. Summary of individual behavioral module usage changes with pain progression, related to Figure 3. Mutation plots summarizing how usage of each behavioral module identified via 3D pose analysis, ordered by behavior type (locomotion, rearing, pause grooming, escape) changes with time following **(A)** intraplantar injection of carrageenan and **(B)** intra-articular injection of MIA. Tables under the mutation plots represent statistically significant differences in module usage between the time points described in the first column. Statistical analysis: corrected bootstrap t-test.


A
Mutation plot CAR + meloxicam
baseline+meloxicam
baseline+saline
4 hours
24 hours+saline
24 hours+meloxicam
Average Module Usage
0.00
0.02
0.04
0.06
0 1 5 6 7 8 9 12 13 14 19 20 21 22 25 26 31 32 33 35 57 66 10 11 15 16 18 24 27 29 30 36 38 39 44 45 46 47 48 50 51 52 54 56 58 2 3 4 17 23 28 34 37 40 41 42 43 49 53 55 61 59 60 64 62 63 65 67 68 69
Locomotion
Rearing
Pause
Grooming
Escape
Behavior Module #
Baseline+saline vs baseline+ meloxicam
Baseline+saline vs 24h+ meloxicam
Baseline+saline vs 24 hours +saline
24 hours+saline vs 24 hours+ meloxicam

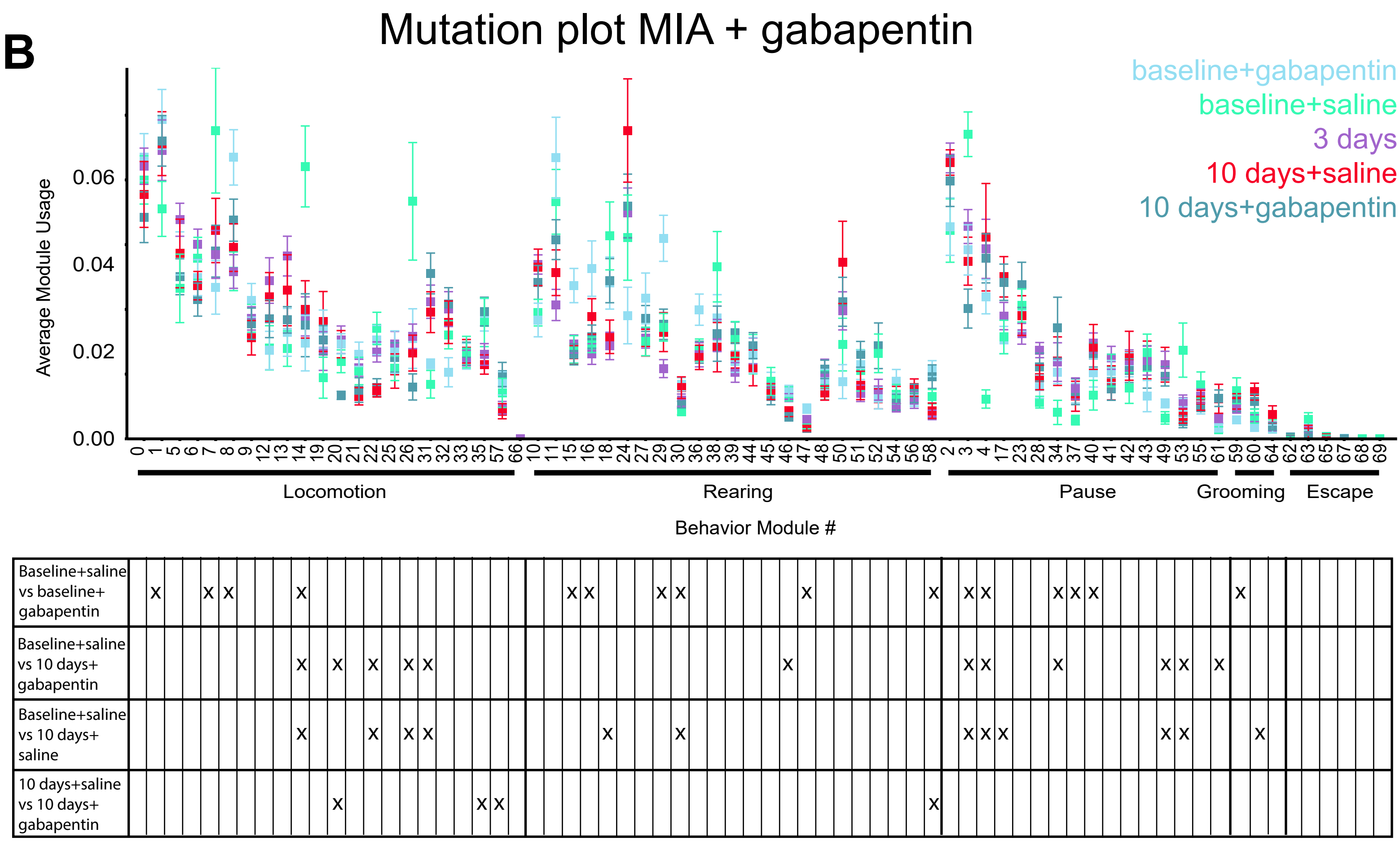

B
Mutation plot MIA + gabapentin
baseline+gabapentin
baseline+saline
3 days
10 days+saline
10 days+gabapentin
Average Module Usage
0.00
0.02
0.04
0.06
0 1 5 6 7 8 9 12 13 14 19 20 21 22 25 26 31 32 33 35 57 66 10 11 15 16 18 24 27 29 30 36 38 39 44 45 46 47 48 50 51 52 54 56 58 2 3 4 17 23 28 34 37 40 41 42 43 49 53 55 61 59 60 64 62 63 65 67 68 69
Locomotion
Rearing
Pause
Grooming
Escape
Behavior Module #
Baseline+saline vs baseline+ gabapentin
Baseline+saline vs 10 days+ gabapentin
Baseline+saline vs 10 days+ saline
10 days+saline vs 10 days+ gabapentin

Figure S4. Summary of individual behavioral module usage changes after administration of meloxicam/gabapentin, related to Figure 4.
Mutation plots summarizing how usage of each behavioral module identified via 3D pose analysis, ordered by behavior type (locomotion, rearing, pause grooming, escape) is changed by administration of meloxicam/gabapentin following **(A)** intraplantar injection of carrageenan and **(B)** intra-articular injection of MIA. Tables under the mutation plots represent statistically significant differences in module usage between the time points described in the first column. Statistical analysis: corrected bootstrap t-test.

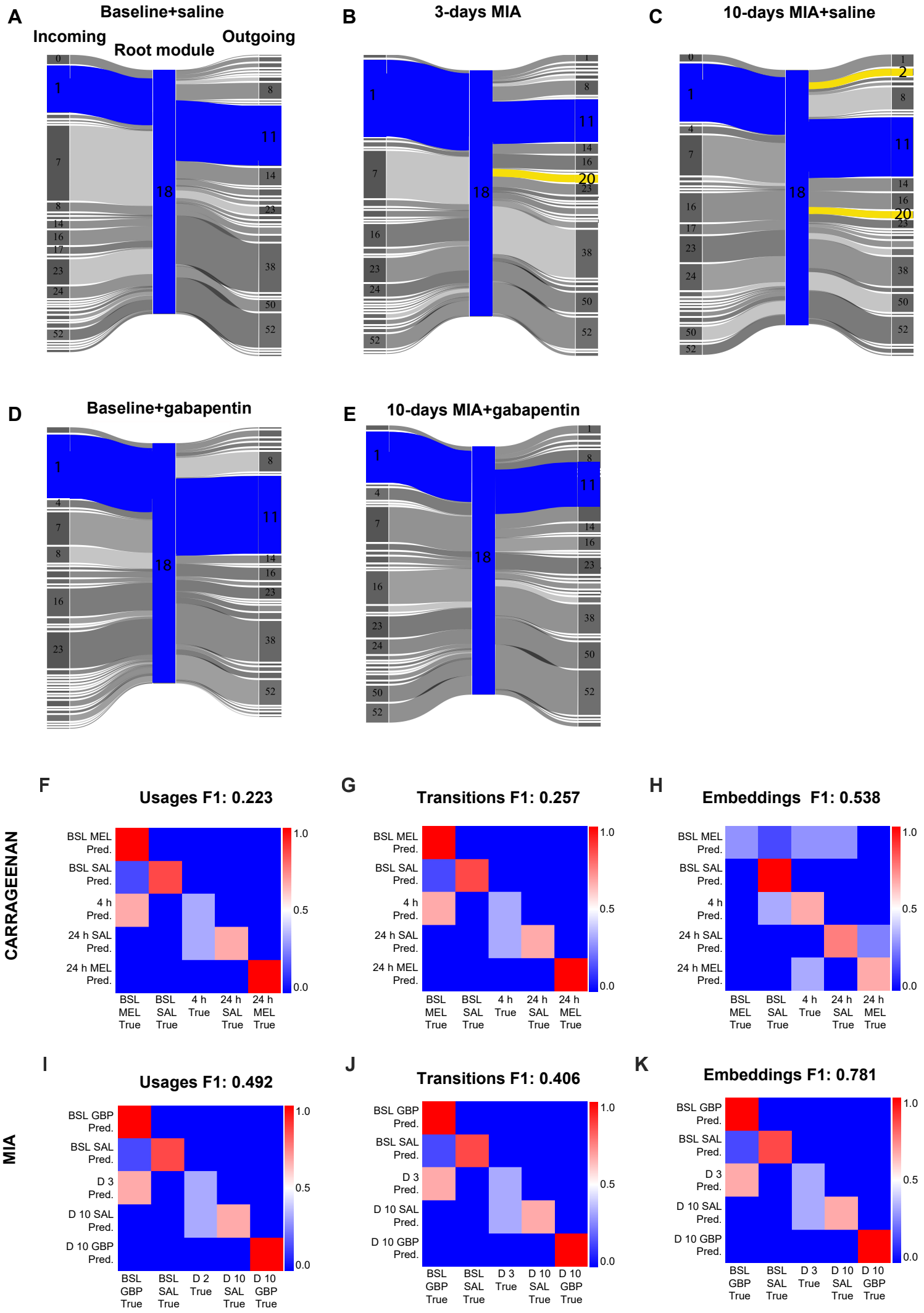

A
Baseline+saline
Incoming
Root module
Outgoing
B
3-days MIA
C
10-days MIA+saline
D
Baseline+gabapentin
E
10-days MIA+gabapentin
CARRAGEENAN
F
Usages F1: 0.223
G
Transitions F1: 0.257
H
Embeddings F1: 0.538
BSL MEL Pred.
BSL SAL Pred.
4 h Pred.
24 h SAL Pred.
24 h MEL Pred.
BSL MEL True
BSL SAL True
4 h True
24 h SAL True
24 h MEL True
1.0
0.5
0.0
MIA
I
Usages F1: 0.492
J
Transitions F1: 0.406
K
Embeddings F1: 0.781
BSL GBP Pred.
BSL SAL Pred.
D 3 Pred.
D 10 SAL Pred.
D 10 GBP Pred.
BSL GBP True
BSL SAL True
D 2 True
D 3 True
D 10 SAL True
D 10 GBP True

Figure S5. Context-dependent learned embeddings are more predictive of experimental groups than raw usage or transition probabilities and show that meloxicam does not promote return to pre-inflammation spontaneous behavior, related to Figure 5. Sankey diagram representation of 3-long module sequences based on root module 18, a rear which shows decreased usage as MIA-induced pain progresses and which is rescued by gabapentin administration (Table 6) and the preceding and following modules at baseline+saline **(A)**, baseline+gabapentin **(D)** and following MIA knee injection at 3-days **(B)**, 10-days+saline intraperitoneal injection **(C)** and 10-days+gabapentin intraperitoneal injection **(E)**. Despite decreased usage, we observe a majority of conserved incoming and outgoing modules (blue). However, we notice the appearance of unique sequences in pain states absent at baseline, such as 18>20 or 18>2 at 3-days and 10-days post-MIA knee injection (yellow).
(F,G,H,I,J,K) Held-out data confusion matrices for an identical classifier trained on raw usage data, transition probabilities and learned embeddings with leave-one-out cross validation. Usages and transition probabilities are less predictive (F1 scores: 0.223 - Carrageenan **(F)**, 0.492 - MIA **(I)**, and 0.257 - Carrageenan **(G)**, 0.406 - MIA **(J)**, respectively) than context-dependent learned embeddings (F1 scores: 0.538 - Carrageenan **(G)**, 0.781 - MIA **(F)**).

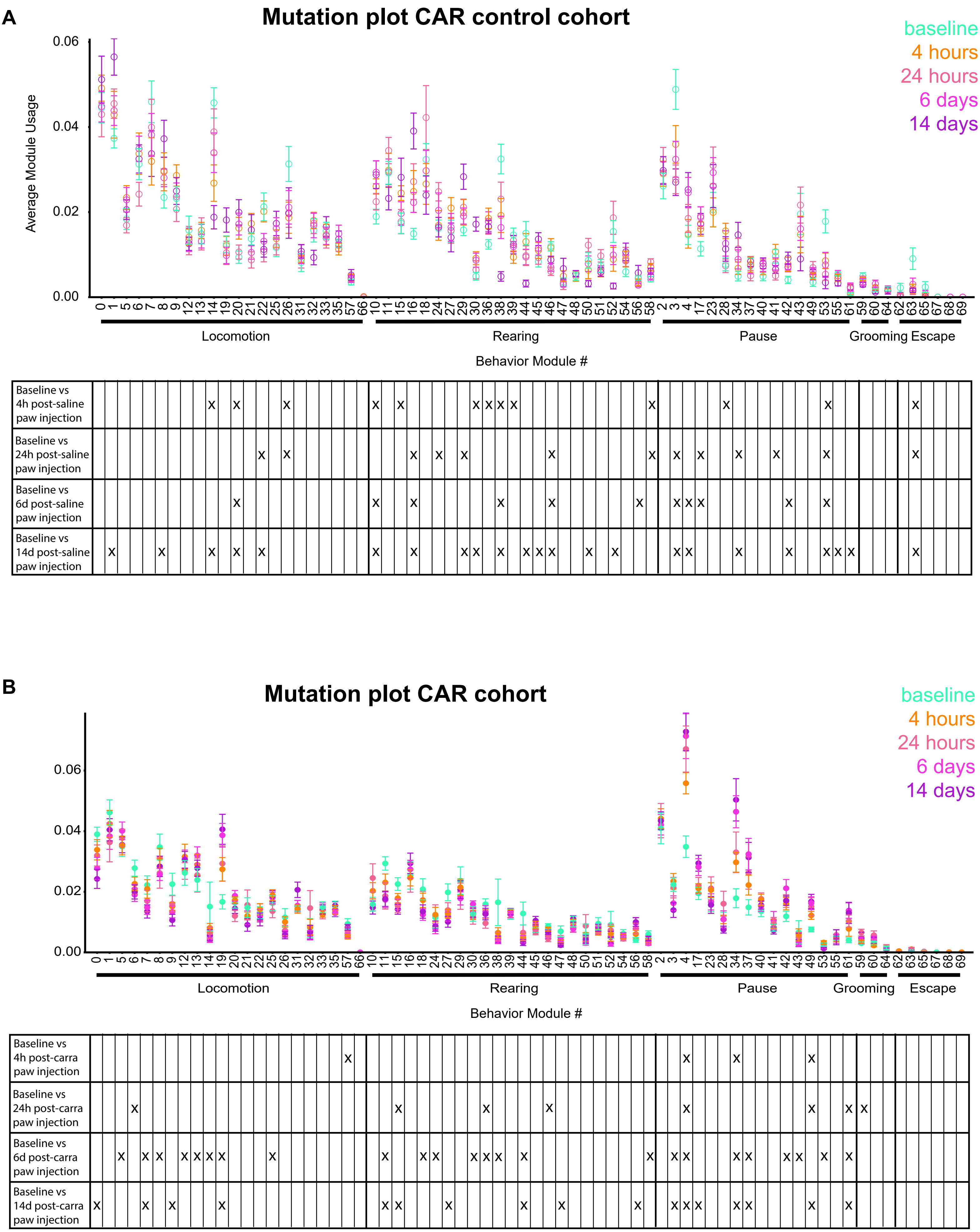

A
Mutation plot CAR control cohort
baseline
4 hours
24 hours
6 days
14 days
Average Module Usage
Locomotion
Rearing
Pause
Grooming
Escape
Behavior Module #
Baseline vs 4h post-saline paw injection
Baseline vs 24h post-saline paw injection
Baseline vs 6d post-saline paw injection
Baseline vs 14d post-saline paw injection
B
Mutation plot CAR cohort
baseline
4 hours
24 hours
6 days
14 days
Locomotion
Rearing
Pause
Grooming
Escape
Behavior Module #
Baseline vs 4h post-carra paw injection
Baseline vs 24h post-carra paw injection
Baseline vs 6d post-carra paw injection
Baseline vs 14d post-carra paw injection

Figure S6. Summary of individual behavioral module usage changes across time from baseline to 14 days post paw injection of saline or carrageenan, related to Figure 6. Mutation plots summarizing how usage of each behavioral module identified via 3D pose analysis, ordered by behavior type (locomotion, rearing, pause grooming, escape) is affected across time by putative habituation to the apparatus **(A)** vs pain (carrageenan paw injection **B**). Tables under the mutation plots represent statistically significant differences in module usage between the time points described in the first column. Statistical analysis: corrected bootstrap t-test.

Ranking	Baseline + meloxicam	Baseline + saline	4-hours	24-hours + saline	24-hours +meloxicam
1.	51>9	18>11>57	65>62	65>62	43>18>14
2.	16>29>11	22>6>21>28	63>65	63>65	29>14>52
3.	29>44	15>27>57	62>63	62>63	51>33>2>13
4.	36>33>13	32>44>9	38>14>23	22>6>21>28	19>30>9
5.	27>14	38>51>8	64>56>41	47>9>21	16>18>16

Table S2 Top ranked module sequence for each behavioral state, CAR condition, related to Figure 5.

Ranking	Baseline + gaba	Baseline + saline	3-days	10-days + saline	10-days + gaba
1.	23>18>11	+	7>26>49	19>30>32	18>8>34
2.	27>9>22	38>14>59	40>4>40>19	29>20>29>11	31>57>51
3.	27>9>0	27>9>3	64>56>41	50>38>50>8	27>44>11>8
4.	28>27>9	26>23>56	27>15	50>38>51	11>38>51
5.	27>9>10	39>45>35	10>27>15	2>49>7>23	18>50>24

Table S3 Top ranked module sequences for each behavioral state, MIA condition, related to Figure 5.