nature | **methods**

Coding of facial expressions of pain in the laboratory mouse

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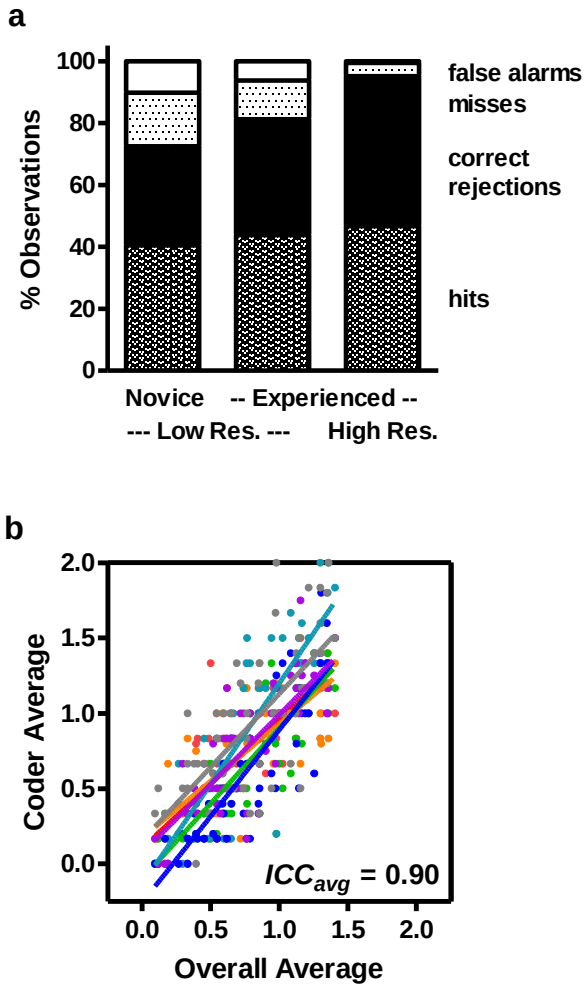
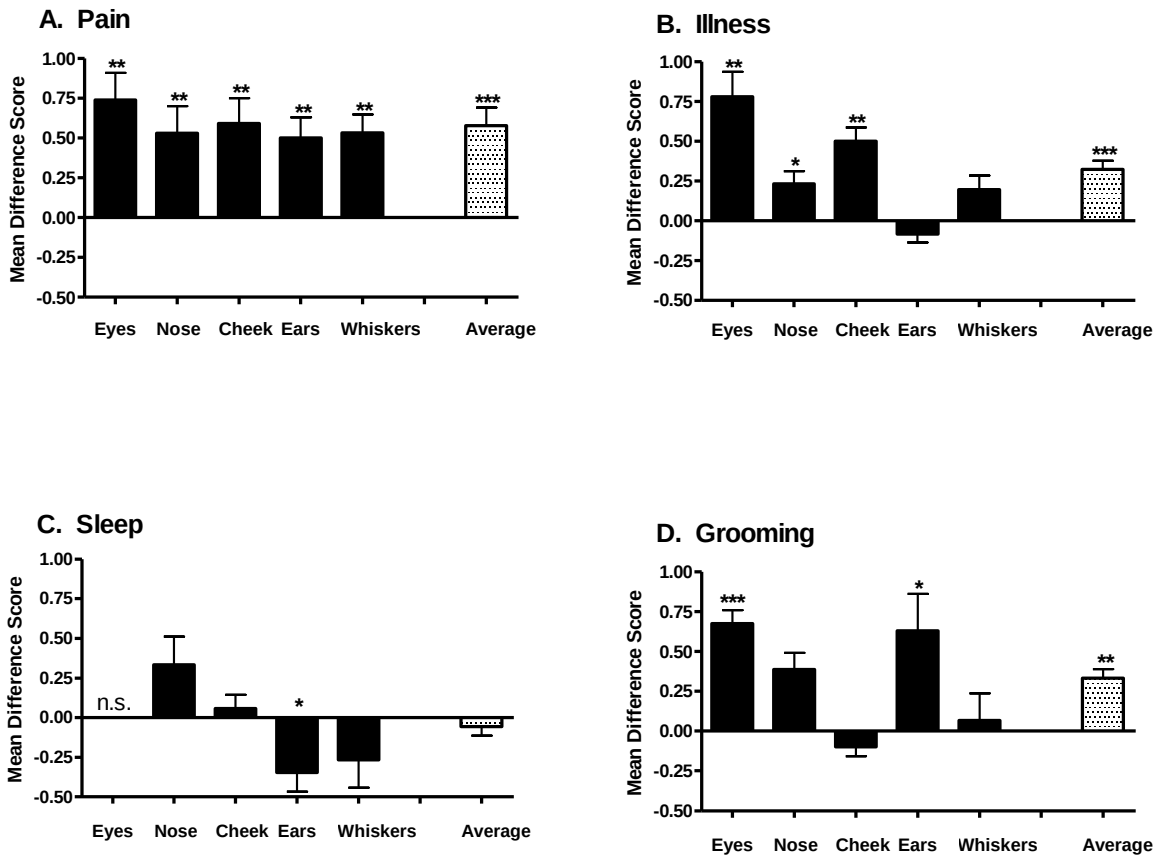
Supplementary figures and text:

Supplementary Figure 1



Supplementary Fig. 1. Accuracy and reliability of the Mouse Grimace Scale. (**a**) Signal detection of novice and experienced coders on the abdominal constriction test, and the improvement in accuracy obtained using a high-definition (1920 x 1080) video camera (High Res.), using a selection of 64 randomized (*pain* and *no pain*) photographs. (**b**) Interrater reliability of the MGS scale. The mean MGS scores of each of six novice coders were compared to the average of all coders. ICC_{avg} = average intraclass correlation coefficient (see **Online Methods**).

Supplementary Figure 2

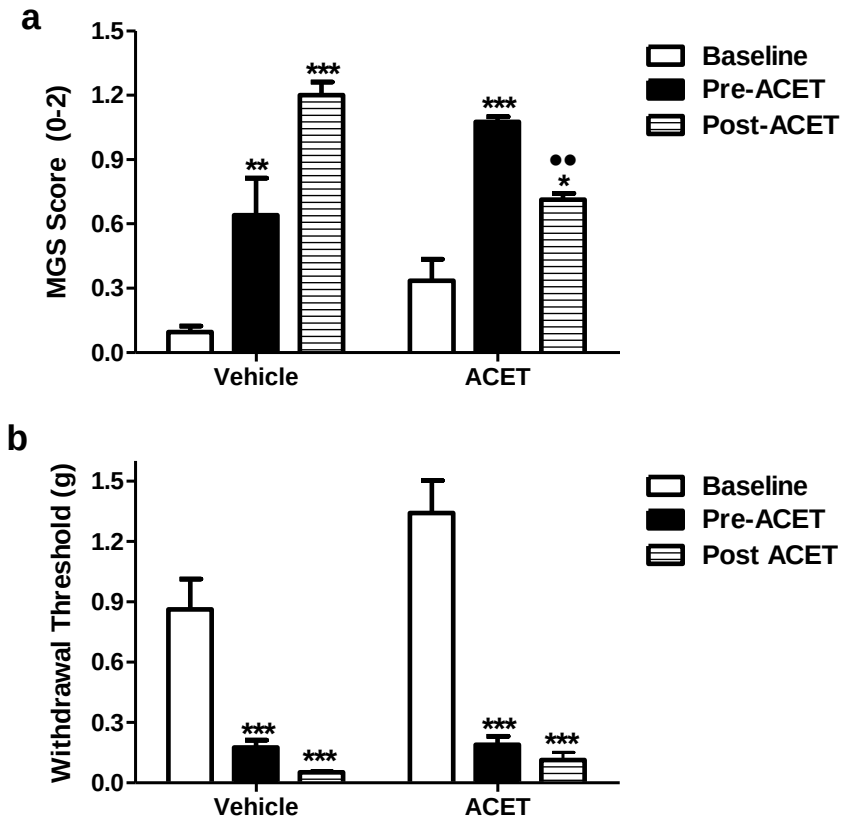


Supplementary Figure 2. Mean MGS difference scores by action unit (Eyes: orbital tightening; Nose: nose bulge; Cheek: cheek bulge; Ears: ear position; Whiskers: whisker change; see main text for details) and overall average score for mice **(A)** injected with 0.9% acetic acid, **(B)** injected with 100 mg/kg lithium chloride (a dose effectively producing conditioned place aversion[1]), **(C)** sleeping, and **(D)** actively exhibiting grooming behavior. Bars represent mean ± s.e.m. difference scores. *Significantly different from zero by one-sample Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). n.s., not scored.

Reference

1. Tenk, C.M., Kavaliers, M., & Ossenkopp, K.-P. *Eur. J. Pharmacol.* **515**, 117-127 (2005).

Supplementary Figure 3



Supplementary Figure 3. Detection of the analgesic efficacy of 300 mg kg^{-1} acetaminophen (ACET) against zymosan inflammatory nociception by the MGS (**a**) but not von Frey withdrawal testing for mechanical allodynia (**b**). Separate groups of mice (n = 4-8/group) were tested at baseline (for details see **Online Methods**), then injected into the plantar hindpaw (20 μl) with 5 mg ml^{-1} zymosan (100 μg). From 4-4.5 h post-zymosan, mice were retested, and then injected with 300 mg kg^{-1} ACET or vehicle (polyethylene glycol; 10 ml kg^{-1}). From 30–60 min post-ACET, mice were tested yet again. Bars represent mean ± s.e.m. MGS scores (**a**) and withdrawal thresholds in g (**b**). As can be seen, ACET reduced MGS scores by approximately 50% compared to baseline, but was wholly ineffective against mechanical allodynia, which continued to increase in both vehicle and ACET-treated groups. Note that the 50% reversal of MGS scores is likely an underestimate, because vehicle group data in both experiments reveal the increasing intensity of the noxious stimulus from the pre-ACET to post-ACET time points. One-between (drug) repeated measures ANOVAs revealed a significant drug x repeated measure interaction for MGS ($F_{2,16}$ = 17.2, P < 0.001), but only a significant effect of repeated measure on von Frey withdrawal thresholds ($F_{2,20}$ = 28.0, P < 0.001). * P < 0.05, ** P < 0.01, *** P <

0.001 compared to within-drug baseline, •• $P < 0.01$ compared to within-drug Pre-ACET (post-hoc test for repeated measures; Systat v. 11).