# MOUSE GRIMACE SCALE (MGS): THE MANUAL

I. VIDEO & FRAME CAPTURE PROCEDURES:

Place mice individually in cubicles (9 x 5 x 5 cm high), with two walls of transparent Plexiglas® and two opaque side walls (to encourage mice to face either forward or backward). Place two digital video cameras immediately outside both Plexiglas walls to maximize opportunity for clear head shots. Higher-resolution color video is highly recommended (e.g., 1920 x 1080, Sony High Definition Handycam® Camcorder)
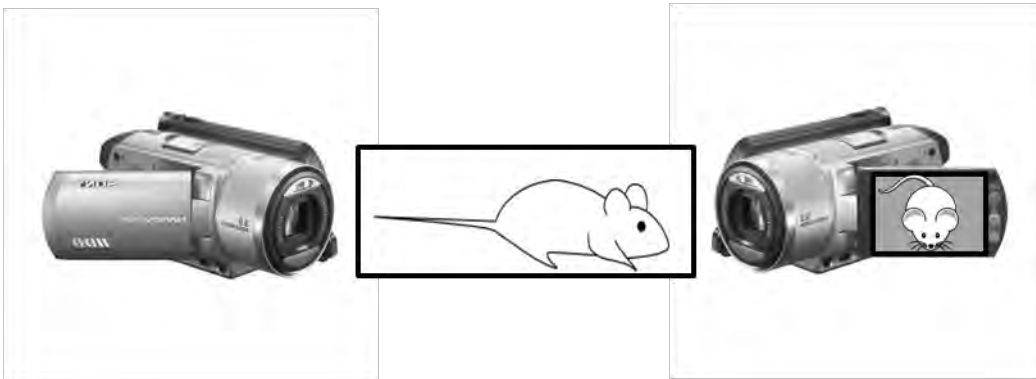
Digitally videotape mice during habituation phase, prior to administration of noxious stimulus (i.e., baseline/"no pain" video). This habituation period should not exceed 45 min, as animals generally fall asleep by this time, and sleeping photos should not be coded. At the appropriate time point after noxious stimulation (i.e., "pain" video), place mice back into cubicle and film for appropriate time; this time will depend on the assay being used.

Using Windows Media Player® or its equivalent, review "no pain" video and capture frames at 2-3 minute intervals, when a clear, unobstructed head shot is observed. On a 30 minute video, this method would yield 10 baseline photos per animal. (*Note: In Windows Media Player Version 9.0 or less, "Ctrl-1" can be used to grab a frame and save it in JPG [or equivalent, e.g., TIF] format). For "pain" videos, if pain is associated with an overt behavioral response, frames should be grabbed during the exhibition of such behavior, unless it involves purposeful head movement (e.g., licking of the hind paw). For assays which are not associated with any behavioral response, use the same frame capture method as for "no pain" videos, grabbing frames at regular intervals.

The resultant JPG files can then be cropped (so that body position is no longer visible), and auto-adjusted for contrast and brightness using Adobe PhotoShop®. JPGs can be copied into PowerPoint, one photo per slide. A macro can be obtained from the web (www.tushar-mehta.com/powerpoint/randomslideshow/index.htm) in order to randomize slide order.

II. CODING PROCEDURES:

Hide photo identifications (for example by placing them in the Notes panel within PowerPoint) during coding to ensure that coding is performed blind. Coders should always use the computer when coding, as photographs may lose resolution with printing. Before beginning the coding process the coder should view and familiarize oneself with baseline photos provided to determine the specific mouse features present. All faces are to be coded for the presence and intensity of the following specific facial Action Units (AU), relative to the status of the region of the baseline prototype.

A. INTENSITY RATINGS:

Intensity ratings are coded for each AU.

AU is not present =   0
AU moderately visible = 1
AU severe =   2

An MGS score for each photograph is calculated by averaging intensity ratings for each AU. An MGS difference score (relative to baseline/"no pain") can then be calculated for each subject, and averaged across a group. These calculations are described in detail at the end of the document.

B. ACTION UNITS:

1. Orbital Tightening
Mouse must display a narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. An eye squeeze is defined as the orbital muscles around the eyes being contracted. A wrinkle may be visible around the eye. As a guideline, any eye closure that reduces the eye size by more than half should be coded as a "2". *Note that sleeping mice display closed eyes, and this may be mistaken for a tightly closed eyelid. Photographs of sleeping mice should therefore not be taken and/or coded.
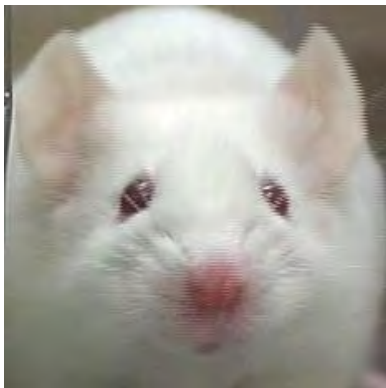

"0"   "1"   "2"

2. Nose Bulge

Mouse must display a bulge on top of the nose. The skin and muscles around the nose will be contracted creating a rounded extension of skin visible on the bridge of the nose. A nose bulge may also be coded if a coder sees vertical wrinkles extending down the side of the nose from the bridge. In frontal headshots, a bulge may be seen as a widening of the nose area (i.e., V-shape connecting eyes to nose appears broader). *Note that a nose bulge may also appear when mice are actively exploring (i.e., sniffing). Ideally, these photographs should not be taken and/or coded, as they may inflate baseline MGS scores.
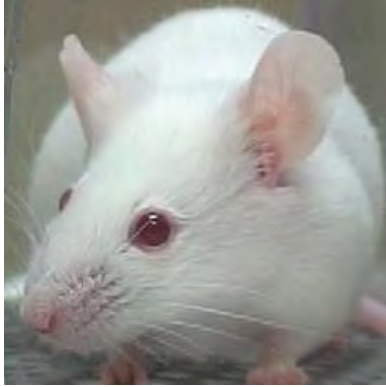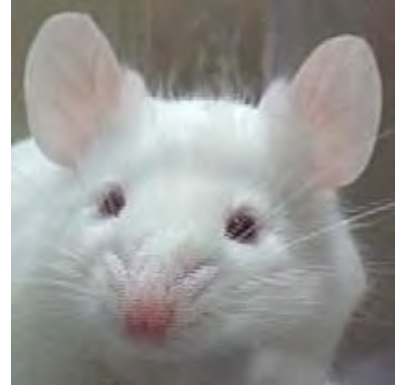
"0"   "1"   "2"

3. Cheek Bulge

The cheek muscle is contracted and extended relative to the baseline condition; it will appear to be convex from its neutral position. Note: The cheek is considered to be the area directly below the eye and extending to the beginning of the whiskers on the nose (in humans, the infraorbital triangle). The distance from eye to whisker pad may appear shortened relative to baseline.
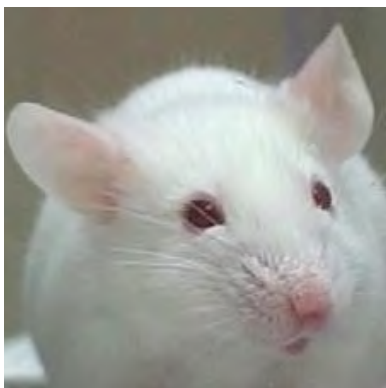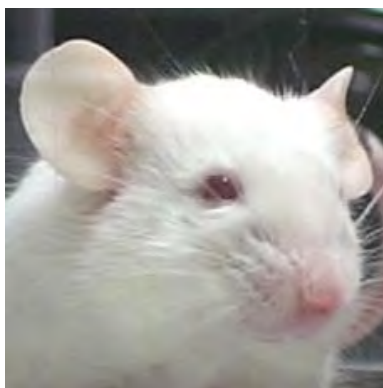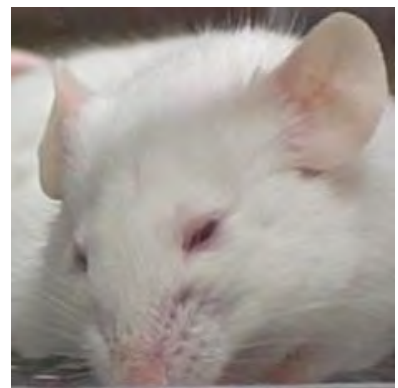
"0"   "1"   "2"

4. Ear Position

Ears may be pulled back from their baseline position, or may be seen as laid flat against the head. In a typical baseline position ears are roughly perpendicular to the head and are directed forward. In pain, the ears tend to rotate outwards and/or back, away from the face. As a result, the space between the ears may appear wider relative to baseline.*Note that mice engaged in exploration or grooming may also pull ears back, but distance between ears tends to narrow rather than widen. In any case, these may cause confusion, and it is advised that photographs of mice actively exploring or grooming not be taken and/or coded.
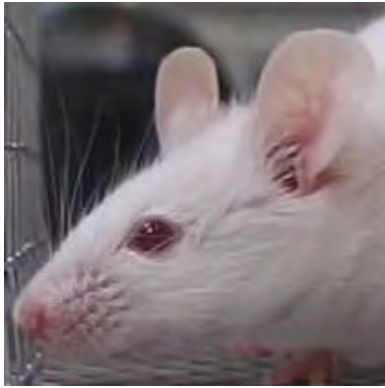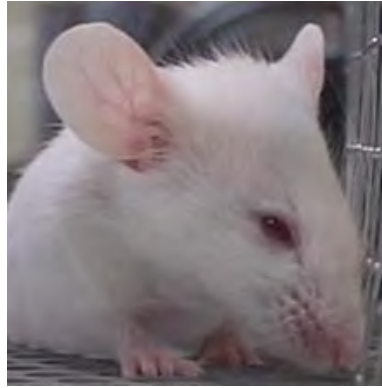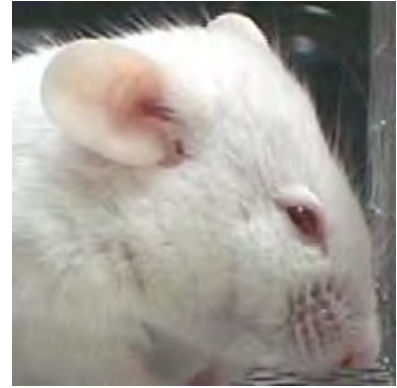
"0" "1" "2"

5. Whisker Change

Whiskers must have moved from the baseline position. They could either be pulled back to lay flat against the cheek or pulled forward as if to be "standing on end". Whiskers may also clump together compared to baseline whiskers, which tend to be fairly evenly spaced.

"0" "1" "2"

III. CALCULATING MGS SCORES:

1. Average all AUs for each photograph to give MGS score. Most of these preliminary calculations are easily carried out using Excel (see example below):

	A	B	C	D	E	F	G
1	Photo ID#	Orbital	Nose	Cheek	Ears	Whiskers	MGS
2	1	2	1	1	2	1	1.4
3	2	0	0	1	0	1	0.4
4	3	1	2	2	2	1	=AVERAGE(B4:F4)

2. After unblinding, sort data by subject (e.g., "Mouse") then by condition. Note that "no pain" photographs are almost always baseline photographs taken prior to noxious stimulation:

	A	B	C	D	E	F	G	H	I
1	Photo ID#	Mouse	Condition	Orbital	Nose	Cheek	Ears	Whiskers	MGS
	27	A1	no pain	0	0	1	0	1	0.4
3	58	A1	no pain	0	0	0	0	0	0
4	1	A1	pain	2	1	1	2	1	1.4
5	206	A1	pain	1	2	2	2	1	1.6

3. Calculate mean MGS score for all "no pain" and all "pain" photographs for each subject and subtract the mean for the "no pain" photographs from the mean for the "pain" photographs to give an MGS difference score for each subject:

	A	C	D	E
1	Mouse	MGS_no pain	MGS_pain	MGS_diff
2	A1	0.2	1.5	1.3
3	A2	0.4	1	0.6
4	A3	=AVERAGE(no pain)	=AVERAGE(pain)	=MGS_pain-MGS-no pain

4. Difference scores can then be averaged across subjects to give a mean difference score, which can be analyzed for significance using a one-sample Student's t-test compared to 0.

IV. ATTRIBUTION:

For all uses of the MGS, please attribute our companion publication as follows:

Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., LaCroix-Fralish, M.L., Matsumiya, L., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wong, D., van den Maagdenberg, A.M.J.M., Ferrari, M.D., Craig, K.D., and Mogil, J.S. Coding of facial expressions of pain in the laboratory mouse. *Nature Methods*, in press.

Please address all questions to Dr. Jeffrey S. Mogil at jeffrey.mogil@mcgill.ca.

Good luck!