

## Behavioral Methods for Assessing OA and Pain in Mouse Models

William C. Wetsel

*Duke University Medical Center*

### Behavioral Tests

*Motor Performance*

*Pain Sensitivities*

*Anxiety-like Behaviors*

*Depressive-like Responses*

*Drug Abuse*

*Learning and Memory*

### *Collagen IX Knockout Mouse*

*Inactivated Col9a1 gene, deleting both the long and short forms of  $\alpha 1(IX)$  molecules*

*Deletion of  $\alpha 1(IX)$  leads to a functional loss; where the  $\alpha 2(IX)$  and  $\alpha 3(IX)$  proteins are not detected in null mice*

### Experimental Design

- 9-month old *Col9a1* mice (C57BL/6 background)
- Mutants show evidence of knee osteoarthritis
- Some intervertebral disc degeneration

*C57BL/6 Mice Fed 10% or 45% Fat*

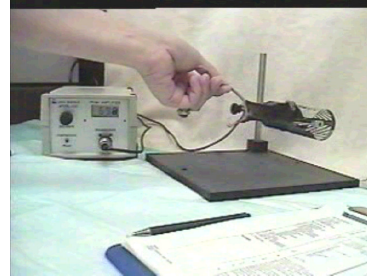
### Experimental Design

- C57BL/6 mice followed longitudinally
- Mice fed 10% or 45% purified fat diet
- Aged obese mice show evidence of knee osteoarthritis

## Motor Performance

1. Overall body position and posture
2. Tail and pelvic elevation
3. Postural/righting reflex
4. Coordination and balance
5. Rear-paw reflex
6. Grip strength
7. Gait analysis
8. Incapacitance
9. Spontaneous activity, running wheel
10. Treadmill performance
11. Rotorod

## Grip Strength



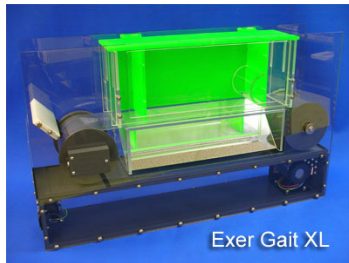
Ugo Basile

## Neuromuscular Screen for *Col9a1* Mice

	Male		Female	
	WT	<i>Col9a1</i> <sup>-/-</sup>	WT	<i>Col9a1</i> <sup>-/-</sup>
Body weight (g) <sup>1</sup>	33.4 ±2.2	35.5 ±4.6	28.4 ±3.0	26.3 ±1.3
Righting reflex	Normal	Impaired*	Normal	Long Delay*
Wire hang (sec)	42.8 ±4.9	11.4 ±3.5*	37.9 ±6.1	11.1 ±3.7*
Forelimb grip (g-force)	51.2 ±3.4	38.7 ±2.6*	50.5 ±4.9	44.3 ±3.4*
Hindlimb grip (g-force)	23.9 ±2.6	15.6 ±1.6*	25.8 ±3.2	21.1 ±0.9*
Pole climb down (sec)	11.4 ±2.3	11.8 ±1.5	9.3 ±0.9	13.4 ±3.7
Pole climb up (sec)	12.8 ±2.7	26.1 ±1.9*	9.7 ±0.9	18.9 ±5.3*
Pole walking (sec)	17.7 ±3.9	19.6 ±1.7	14.8 ±3.6	16.0 ±1.9

<sup>1</sup>Data are presented as means and standard deviations.

## Gait Analyses

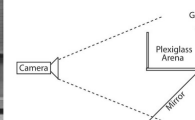
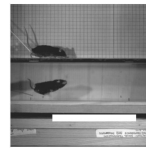


Exer Gait XL

Clever Sys  
Noldus -- CatWalk

## Gait

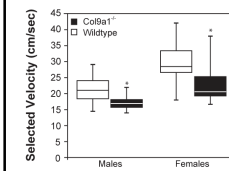
- Unprompted
  - Allowed to freely explore
- Prompted
  - Started via a brush from a cotton swab



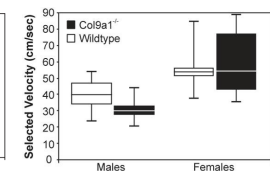
- Recorded at 200 frames per second
- Digitized to know grid coordinates (DLTdataviewer2)

## Gait – Velocity for *Col9a1* Mice

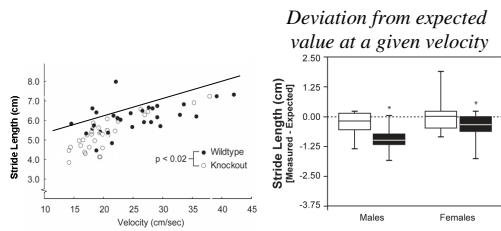
### Unprompted Trials



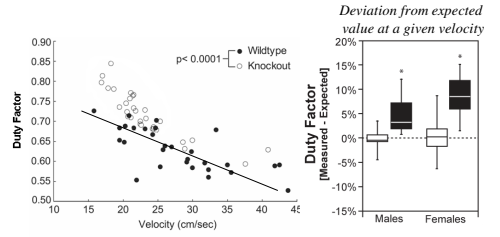
### Prompted Trials



### Gait – Unprompted Stride Length (hind limbs) of *Col9a1* Mice



### Gait – Unprompted Duty Factor (hind limbs) of *Col9a1* Mice



$$DF = \frac{\text{Stance Time}}{\text{Stride Time}} \times 100$$

Percentage time a given limb is in ground contact, and thus, can transmit force

### Incapacitance Test



Columbus Instruments

### Spontaneous Activity in the Open Field



Accuscan Instruments

### Treadmill



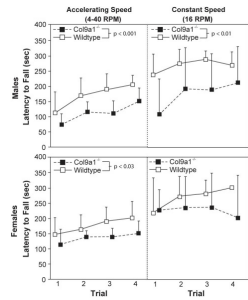
Columbus Instruments

### Rotorod

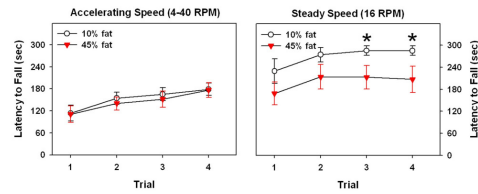


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## Col9a1 Mice are Deficient on the Rotorod



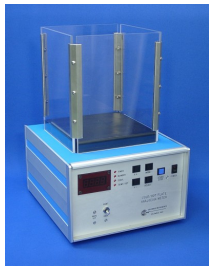
## Mice Fed 45% Fat are Deficient on Rotorod



## Pain Sensitivity

1. Hot plate
2. Tail flick
3. Tactile responses (von Frey)
4. Plethysmometer
5. Threshold withdrawal reflex to knee compression
6. Threshold to struggle to knee compression
7. Vocalization to knee compression

## Hot Plate Analgesia



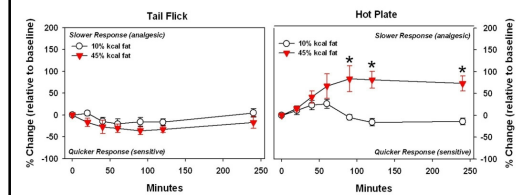
Columbus Instruments

## Tail Flick Analgesia



Columbus Instruments

## Mice Fed 45% Fat are Analgesic on Hot Plate



## Tactile Allodynia



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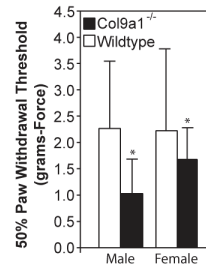
## Allodynia in *Col9a1* Mice

### von Frey Test

- Filaments bend at known force
- Applied to hind plantar surface
- Withdrawal percentage
- Force where the likelihood of withdrawal is 50%



Stocking

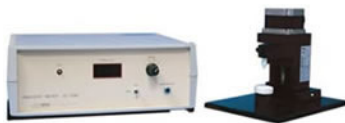


## Plethysmometer



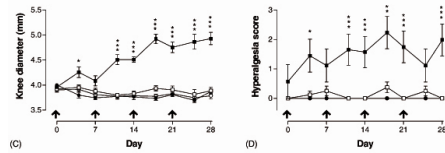
Ugo Basile

## Mechanical Pressure



San Diego Instruments

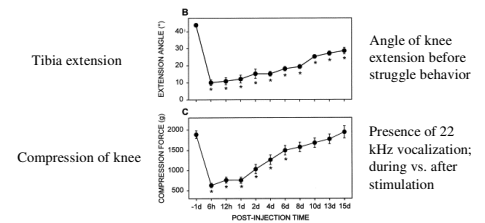
## Rat Withdrawal Reflex to Compression



Rats given FCA once/wk for 4 wks

Gauldie et al., 2004; *J Neurosci Methods*

## Rat Injected With Koalin/Carrageenan



Yu et al., 2002, *J Neurosci Methods*

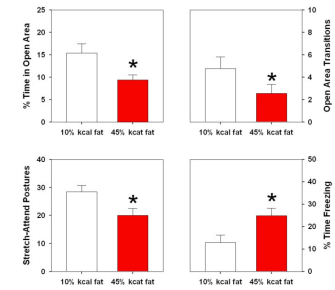
## Anxiety-like Behaviors

1. Zero maze
2. Light-dark emergence test
3. Novelty-suppressed feeding
4. Open field

## Zero Maze



## Mice Fed 45% Fat Show Anxiety-Like Behaviors

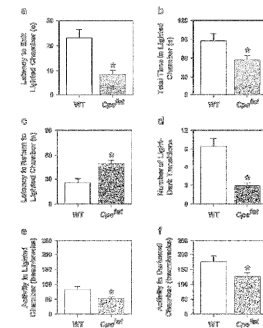


## Light-Dark Box



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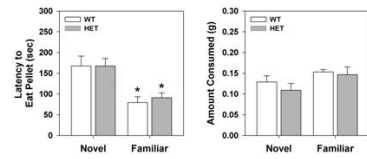
## *Cpe<sup>fat/fat</sup>* Mice Are Anxious in the Light-Dark Box



## Novelty-Suppressed Feeding



## Vmat 2 Mice Are Not Anxious in Novelty-Suppressed Feeding



Fukui et al., 2007

## COLLABORATORS

### Duke University Medical Center

Kyle D. Allen  
Virginia D. Kraus  
Janet L. Hueber  
Lawrence M. Boyd  
Lori A. Setton

Timothy M. Griffin  
Farshid Guilak

Ramona M. Rodriguez  
William C. Wetsel

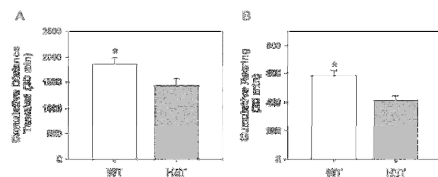
### Support

NIH R01AR47442  
NIH P01AR50245  
NIH T32EB001630

## Depressive-Like Behaviors

1. Locomotor agitation/retardation
2. Anhedonia
3. Forced swim
4. Tail suspension
5. Learned helplessness
6. Serum corticosterone

## VMAT2 HETs Show Locomotor Retardation



Fukui et al., 2007

## Anhedonia Experimental Design

House mice in individual cages for 7 days

Water-deprive mice for 4 h

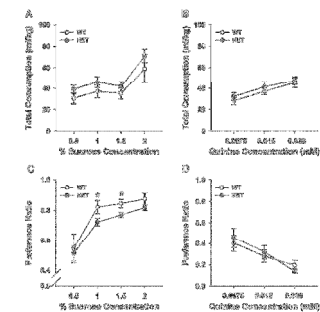
Water/Water pairings for 1 h (4-5 days)

0.5% Sucrose/0.5% Sucrose (3 days)

Water/Sucrose (0.5, 1, 1.5, 2%) pairings

Water/Quinine (0.0075, 0.0150, 0.030 mM) pairings

## VMAT2 HETs Show Anhedonia



Fukui et al., 2007

### Forced Swim

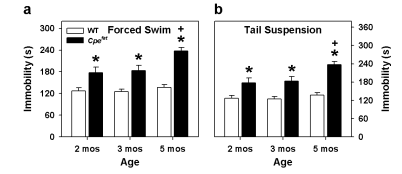


### Tail Suspension

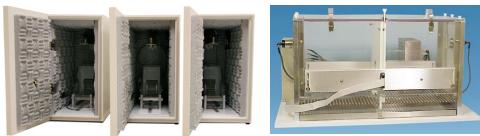


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### *Cpe<sup>fat/fat</sup>* Mice Show Depressive-like Behaviors

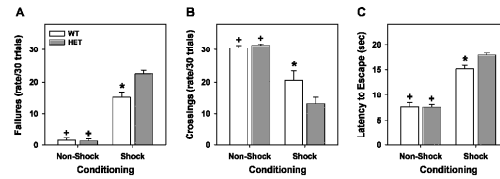


### Learned Helplessness



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### VMAT2 HETs Display “Depressive-like Behaviors” in the Learned Helplessness Test



Fukui et al., 2007