**Designing Thermal Stimuli for Ca2+ Imaging**

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Goal: design thermal stimuli that can be generated by the thermal imaging stimulator. Include both non-physiological stimuli and stimuli that recapitulate the thermosensory experience of a *S. stercoralis* iL3 engaging in temperature-driven behaviors.

For all stimuli, worms should be held at starting temperature for 5 minutes prior to the start of imaging.

**Non-Physiological Stimuli**

Based on stimulus protocols from the lab of Daniel Colon-Ramos.

Positive Thermotaxis Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 23 | 10 | 60 | 0.2°C/s |
| 17 | 60 | 120 | 0.1°C/s |
| 40 | 230 | 120 | 0.1°C/s |
| 23 | 66 | 60 | 0.25°C/s |

Total time = 726 seconds or 12.1 minutes; use a 13 minute recording period

Negative Thermotaxis Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 23 | 10 | 60 | 0.2°C/s |
| 22 | 10 | 120 | 0.1°C/s |
| 13 | 90 | 120 | 0.1°C/s |
| 23 | 100 | 120 | 0.1°C/s |

Total time = 630 seconds or 10.5 minutes; use a 13 minute recording period

**Near-Fictive Stimuli**

Positive Thermotaxis

Calculate change in temperature of each worm per second (divide temp per frame times 2), take the median to generate an average °C/second for each worm. Take the median again to get the value across worms.

* Using Switch Point Tracking worms (5 PT and 5 NT from worms placed at 23°C). Selecting only worms that do PT, ramp is **0.029°C/second**.
* Using Steep Gradient worm tracks from Bryant *et al* 2018 – these are the ones that we used to quantify worm speed from 25-27°C, plus they’re plotted in Figure 1a. As calculated when worms are migrating from 25-34°C, the ramp is **0.0268°C/second**.
* Use Tax-4 No Cas 9 worms from Bryant *et al* 2018 – these worms are placed near 30°C. The ramp is **0.0137°C/second**.
* Using 29-41°C worm tracks from Bryant *et al* 2018, the average ramp is 0.0089°C/second for the whole range. From 29-37°C range, average ramp is **0.0127°C/second**

Pseudofictive Positive Thermotaxis Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 23 | 10 | 60 | 0.2°C/s |
| 20 | 30 | 120 | 0.1°C/s |
| 34 | 560 | 120 | 0.025°C/s |
| 23 | 44 | 120 | 0.25°C/s |

Total time = 1064 seconds or 17.73 minutes; use an 18 minute recording period

Pseudofictive Positive Thermotaxis Extended Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 23 | 10 | 60 | 0.2°C/s |
| 20 | 30 | 120 | 0.1°C/s |
| 40 | 800 | 120 | 0.025°C/s |
| 23 | 66 | 120 | 0.25°C/s |

Total time = 1304 seconds or 21.73 minutes; use a 23 minute recording period

Negative Thermotaxis – NOT USED

Calculate change in temperature of each worm per second (divide temp per frame times 2), take the median to generate an average °C/second for each worm. Take the median again to get the value across worms.

* Using Switch Point Tracking worms (5 PT and 5 NT from worms placed at 23°C). Selecting only worms that do NT, ramp is **-0.0055°C/second.**

Fictive Negative Thermotaxis Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 22 | 5 | 120 | 0.2°C/s |
| 20 | 400 | 120 | 0.005°C/s |
| 23 | 60 | 120 | 0.05°C/s |

Total time = 830 seconds or 13.833 minutes

Reversal Behavior – NOT USED

Use the reversal behaviors observed in a 15-25°C gradient, Tc = 23°C. Defining PT as start of track to first time worms reach maxtemp-0.5°C. Defining NT zone as last time worms are at maxtemp-0.5°C to end of track. PT ramp = **0.0095°C/second**. NT ramp = **-0.0043°C/second.**

* Time in PT = 158 sec
* Time @ maxtemp +/- 0.5°C = 262 sec
* Time in NT = 650 sec

Fictive Reversal Behavior Stimulus:

|  |  |  |  |
| --- | --- | --- | --- |
| Temperature | Rise Time (s) | Hold Time (s) | °C/sec |
| 23 | 10 | 60 | n/a |
| 25 | 200 | 270 | 0.01°C/s |
| 23 | 400 | 60 | 0.005°C/s |

Total time = 1,000 seconds or 16 minutes and 40 seconds.