***Supplementary statistical modelling***

In the main document, we used an aversion index as it allows an easiest comparison of the various experiments. In addition, using indexes is very common in the field to illustrate habituation performance. Nonetheless, here we present the analysis performed on raw data to demonstrate that the exact same conclusions can be drawn from them.

Models:

We used cox mixed models (function coxme; package coxme) when the dependent variables were durations until an event occur: *i.e.* the time to contact the bridge, the time to cross the bridge and the time to deploy the first pseudopod of 10mm². We used linear mixed models for the exponential growth rate (functions: lmer, ranova; packages: lme4, lmerTest).

To compare CA: controls tested without NaCl; CS: controls tested with NaCl; HA: habituated tested without NaCl and HS: habituated tested with NaCl we used two fixed factors:

* The training: controls (C) or habituated (H) slime moulds.
* The substrate used for the test (bridge or arena): agar gel (A) or agar gel with NaCl (S).

For the “mass habituation” experiment the plasmodia identity was incorporated to the model as random factor.

For the long-term habituation, we incorporated the sclerotia identity nested in the plasmodia identity as random factors as each plasmodium gave 12 different sclerotia, that were each cut into several samples. The full model also contains another fixed factor: the weight of the sclerotia sample and its area.

For each models, we calculated the 95%CI of each estimate using the function “dredge” in the package MuMIn.

Interpretations of the models :

If the substrate has a significant effect, it means that NaCl impacts slime mold movement.

If the training has a significant effect it means that training impacts slime mold movement.

If the interaction is significant between substrate and training and is positive, it means that NaCl is less aversive for habituated slime molds (H) than for control ones (C) and we have habituation.

Supplementary table 1: **Short term habituation**: Results of a Cox mixed model to test for the effect of training and substrate on the time to cross the bridge.

|  |  |  |  |
| --- | --- | --- | --- |
| Full model with interaction | df = 14 | AIC = 3618.6 | BIC = 3677 |
| Model without interaction | df = 10 | AIC = 3790.3 | BIC = 3833 |
| Names of fixed factors | Substrate | Training | Interaction |
| Estimates of fixed factors | -3.85 | -0.32 | 3.57 |
| Lower 2.5% CI | -4.33 | -0.62 | 2.99 |
| Higher 97.5% CI | -3.36 | -0.02 | 4.15 |
| P value | **<0.001** | **0.035** | **<0.001** |
| Name of random factor | Plasmodia |  |  |
| Variance | 8.19\*10^-2 |  |  |
| Integrated LR | p-value = | 0.001 |  |
| Penalized LR | p-value = | <0.001 |  |

LR: likelihood-ratio tests

Supplementary table 2: **Long-term habituation.** Results of a Cox mixed model to test for the effect of training and substrate on the time to contact the bridge.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model with interaction | df = 22 | AIC = 3845.4 | BIC = 3935 |  |
| Model without interaction | df = 21 | AIC = 3858.6 | BIC = 3943 |  |
| Names of fixed factors | Substrate | Training | Interaction | Weight |
| Estimates of fixed factors | -1.19 | -0.54 | 0.80 | 0.037 |
| Lower 2.5% CI | -1.50 | -1.08 | 0.38 | 0.021 |
| Higher 97.5% CI | -0.88 | 0.0012 | 1.23 | 0.054 |
| P value | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Names of random factors | Plasmodia | Plasm/Scler |  |  |
| Variance | 1.01\*10^-1 | 2.64\*10^-1 |  |  |
| Integrated LR | p-value = | <0.001 |  |  |
| Penalized LR | p-value = | <0.001 |  |  |

LR: likelihood-ratio tests

Supplementary table 3: **Long-term habituation.** Results of a Cox mixed model to test for the effect of training and substrate on the time to cross the bridge.

|  |  |  |  |
| --- | --- | --- | --- |
| Model with interaction | df = 19 | AIC = 3420.3 | BIC = 3497 |
| Full model (+weight) | df = 20 | AIC = 3421.7 | BIC = 3503 |
| Model without interaction | df = 16 | AIC = 3436.4 | BIC = 3503 |
| Names of fixed factors | Substrate | Training | Interaction |
| Estimates of fixed factors | -1.45 | 0.22 | 0.99 |
| Lower 2.5% CI | -1.82 | -1.08 | 0.51 |
| Higher 97.5% CI | -1.07 | 1.53 | 1.46 |
| P value | **<0.001** | **0.007** | **<0.001** |
| Names of random factors | Plasmodia | Plasm/Scler |  |
| Variance | 4.1\*10^-1 | 1.63\*10^-1 |  |
| Integrated LR | p-value = | <0.001 |  |
| Penalized LR | p-value = | <0.001 |  |

LR: likelihood-ratio tests

Supplementary table 4: **Long-term habituation.** Results of a Cox mixed model to test for the effect of training and substrate on the time to deploy the first pseudopod within the arena.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Full model with interaction | df = 19 | AIC = 4392.5 |  |  |
| Model without interaction | df = 18 | AIC = 4416.9 |  |  |
| Names of fixed factors | Substrate | Training | Interaction | Weight |
| Estimates of fixed factors | -1.71 | -1.17 | 1.02 | 0.031 |
| Lower 2.5% CI | -2.01 | -1.60 | 0.62 | 0.0085 |
| Higher 97.5% CI | -1.41 | -0.73 | 1.43 | 0.054 |
| P value | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| Names of random factors | Plasmodia | Plasm/Scler |  |  |
| Variance | 2.71\*10^-4 | 1.48\*10^-1 |  |  |
| Integrated LR | p-value = | <0.001 |  |  |
| Penalized LR | p-value = | <0.001 |  |  |

LR: likelihood-ratio tests

Supplementary table 5: **Long-term habituation.** Results of a linear mixed model to test for the effect of training and substrate on the exponential growth rate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Full model with interaction | df = 8 | AIC = -4119.8 | R²(fixed effects) = 0.36 | R²(all effects) = 0.44 |
| Model without interaction | df = 7 | AIC = -4094.6 | R²(fixed effects) = 0.31 | R²(all effects) = 0.38 |
| Names of fixed factors | Substrate | Training | Interaction | Weight |
| Estimates of fixed factors | -4.1\*10^-3 | -9.6\*10^-4 | 2.7\*10^-3 | 1.7\*10^-4 |
| Lower 2.5% CI | -4.7\*10^-3 | -1.8\*10^-3 | 1.9\*10^-3 | 1.2\*10^-4 |
| Higher 97.5% CI | -3.5\*10^-3 | -1.4\*10^-4 | 3.5\*10^-3 | 2.18\*10^-4 |
| P value | **<0.001** | **0.003** | **<0.001** | **<0.001** |
| Names of random factors | Plasmodia | Plasm/Scler |  |  |
| Variance | 1.37\*10^-21 | 5.93\*10^-7 |  |  |
| Lower 2.5% CI | 0 | 1.89\*10^-7 |  |  |
| Higher 97.5% CI | 2.24\*10^-7 | 1.21\*10^-6 |  |  |
| P value | 1 | <0.001 |  |  |

Supplementary table 6: **Long-term habituation and Recovery.** Results of a Cox mixed model to test for the effect of training and substrate on the time to deploy the first pseudopod within the arena.

|  |  |  |  |
| --- | --- | --- | --- |
| Model with interaction | df = 21 | AIC = 3661 |  |
| Full model (+weight) | df = 22 | AIC = 3662.4 |  |
| Model without interaction | df = 21 | AIC = 3674.8 |  |
| Names of fixed factors | Substrate | Training | Interaction |
| Estimates of fixed factors | -1.77 | -0.65 | 0.93 |
| Lower 2.5% CI | -2.10 | -1.30 | 0.48 |
| Higher 97.5% CI | -1.45 | -1.68\*10^-3 | 1.37 |
| P value | **<0.001** | **0.005** | **<0.001** |
| Names of random factors | Plasmodia | Plasm/Scler |  |
| Variance | 0.23 | 0.068 |  |
| Integrated LR | p-value = | <0.001 |  |
| Penalized LR | p-value = | <0.001 |  |

LR: likelihood-ratio tests

Supplementary table 7: **Long-term habituation and recovery.** Results of a linear mixed model to test for the effect of training and substrate on the exponential growth rate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model with interaction | df = 7 | AIC = -3635.5 | R²(fixed effects) = 0.42 | R²(all effects) = 0.50 |
| Model without interaction | df = 6 | AIC = -3618.4 | R²(fixed effects) = 0.37 | R²(all effects) = 0.46 |
| Full model (+weight) | df = 8 | AIC = -3615.6 | R²(fixed effects) = 0.42 | R²(all effects) = 0.50 |
| Names of fixed factors | Substrate | Training | Interaction |  |
| Estimates of fixed factors | -4.31\*10^-3 | 3.92\*10^-5 | 2.36\*10^-3 |  |
| Lower 2.5% CI | -4.86\*10^-3 | -8.33\*10^-4 | 1.58\*10^-3 |  |
| Higher 97.5% CI | -3.76\*10^-3 | 9.22\*10^-4 | 3.14\*10^-3 |  |
| P value | **<0.001** | **0.859** | **<0.001** |  |
| Names of random factors | Plasmodia | Plasm/Scler |  |  |
| Variance | 6.98\*10^-8 | 5.75\*10^-7 |  |  |
| Lower 2.5% CI | 0 | 2.19\*10^-7 |  |  |
| Higher 97.5% CI | 5.39\*10^-7 | 1.21\*10^-6 |  |  |
| P value | 0.568 | <0.001 |  |  |

LR: likelihood-ratio tests

Supplementary table 8: Cox model of the habituation by constrained absorption of repellent, using the time to deploy a first pseudopod in an arena, the selected model incorporate only the effect of substrate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model of effect of susbtrate | df = 1 | | AIC = 1267.6 | |
| Full model with interaction | df = 3 | | AIC = 1271.3 | |
| Name of fixed factor | | Substrate | |
| P value | | **<0.001** | |

Supplementary table 9: Linear model of the habituation by constrained absorption of repellent, using the exponential growth rate in an arena, the selected model incorporate only the effect of substrate and the effect of training.

|  |  |  |  |
| --- | --- | --- | --- |
| Model without interaction | df = 4 | AIC = -1851 | R² = 0.26 |
| Full model with interaction | df = 5 | AIC = -1850.9 | R² = 0.27 |
| Names of fixed factors | Substrate | Training |  |
| Estimates of fixed factors | -6.79\*10^-4 | 5.38\*10^-4 |  |
| Lower 2.5% CI | -9.07\*10^-4 | 3.09\*10^-4 |  |
| Higher 97.5% CI | -4.50\*10^-4 | 7.67\*10^-4 |  |
| P value | **<0.001** | **<0.001** |  |