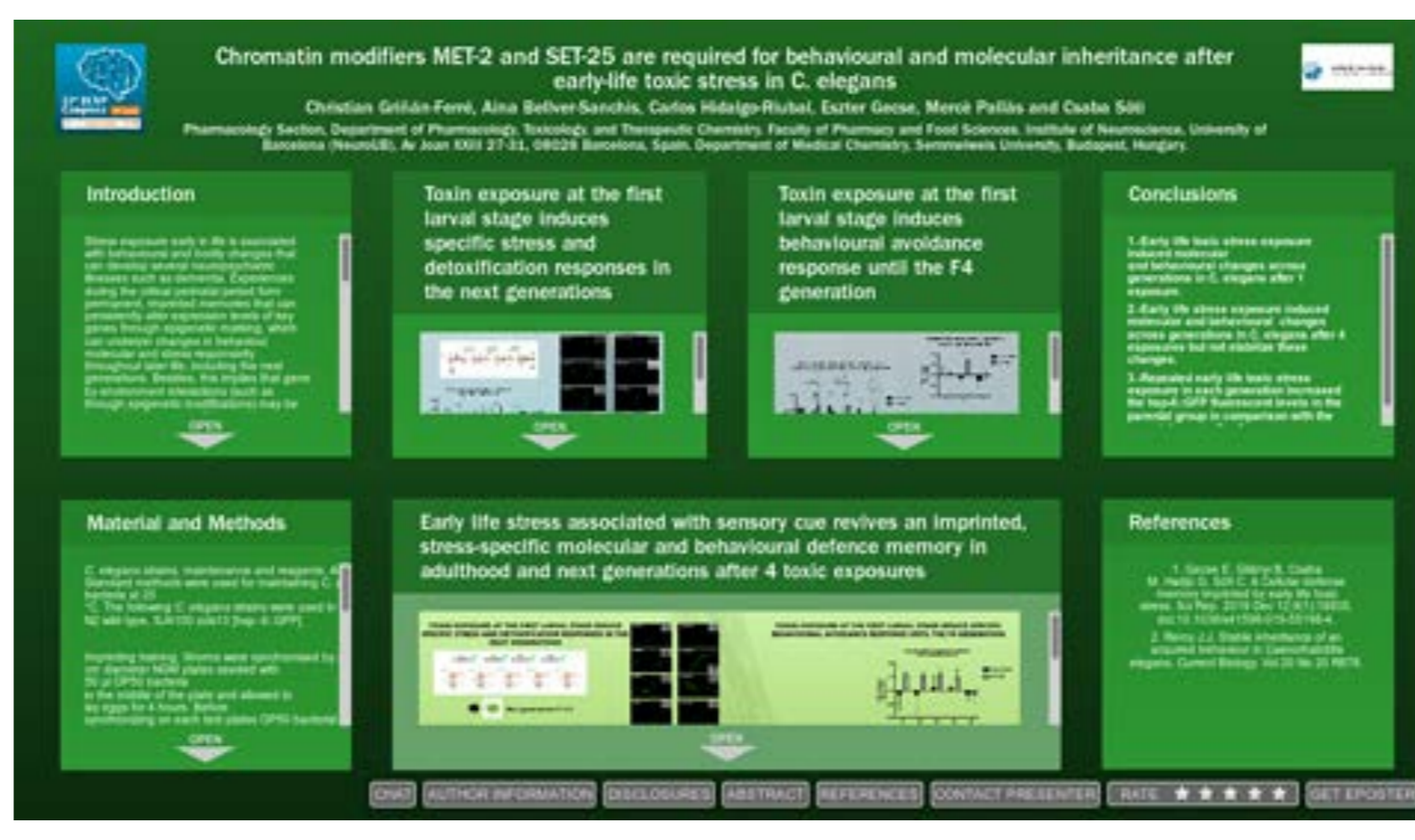
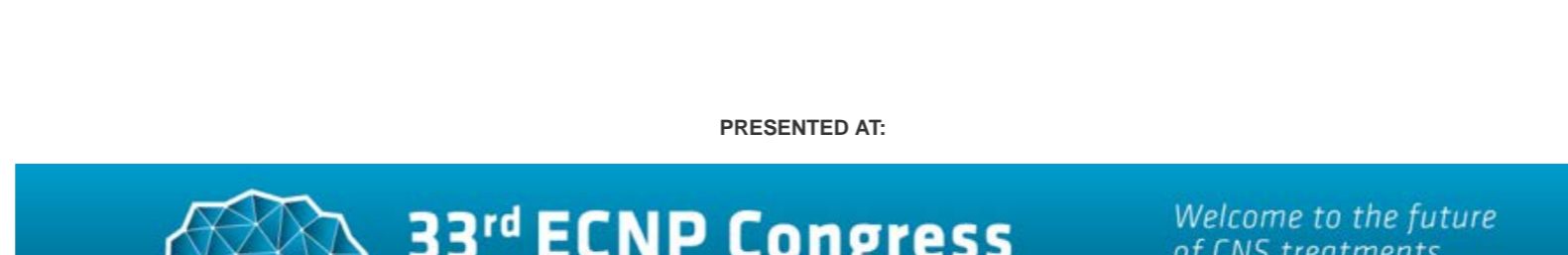


Chromatin modifiers MET-2 and SET-25 are required for behavioural and molecular inheritance after early-life toxic stress in *C. elegans*



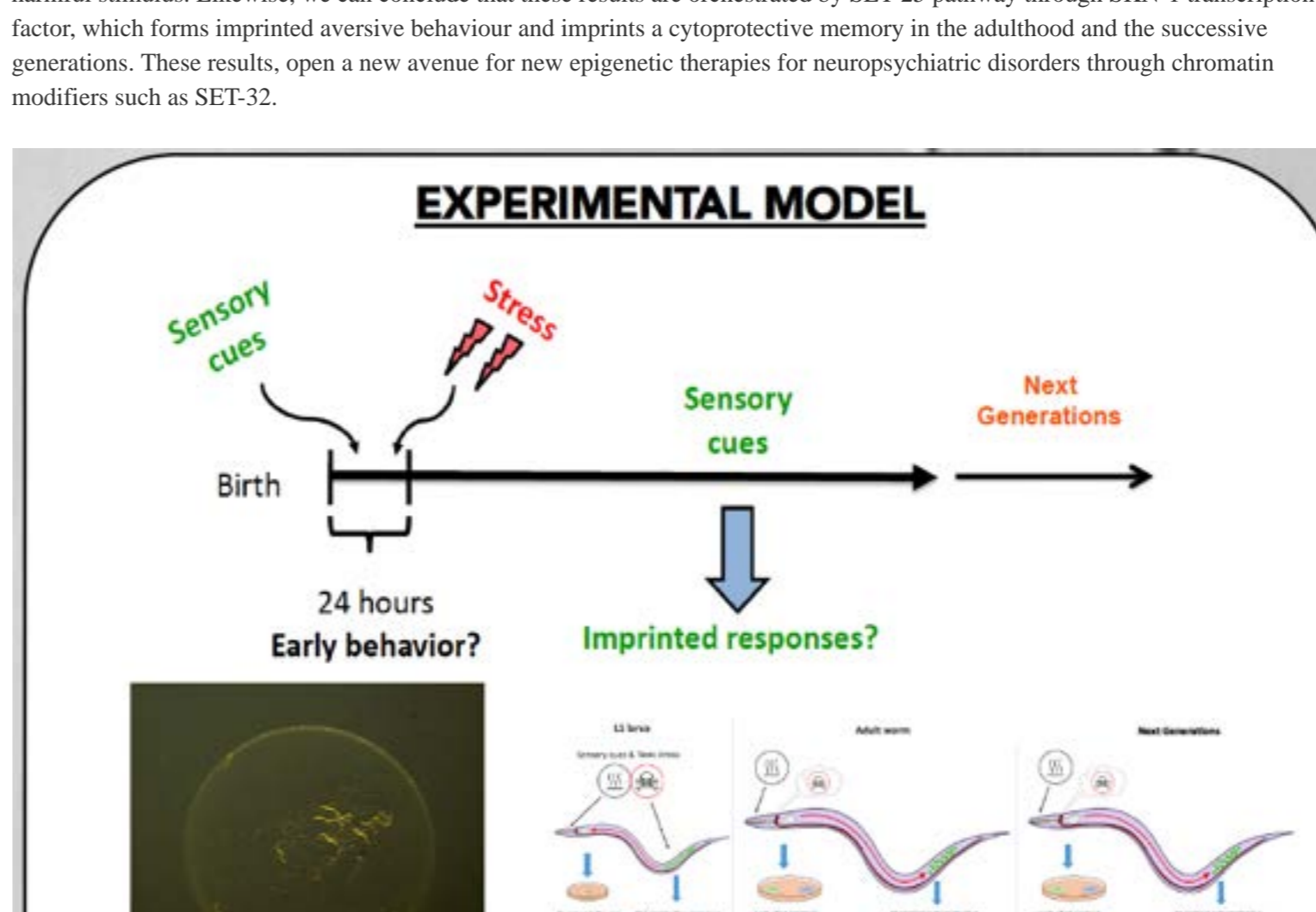
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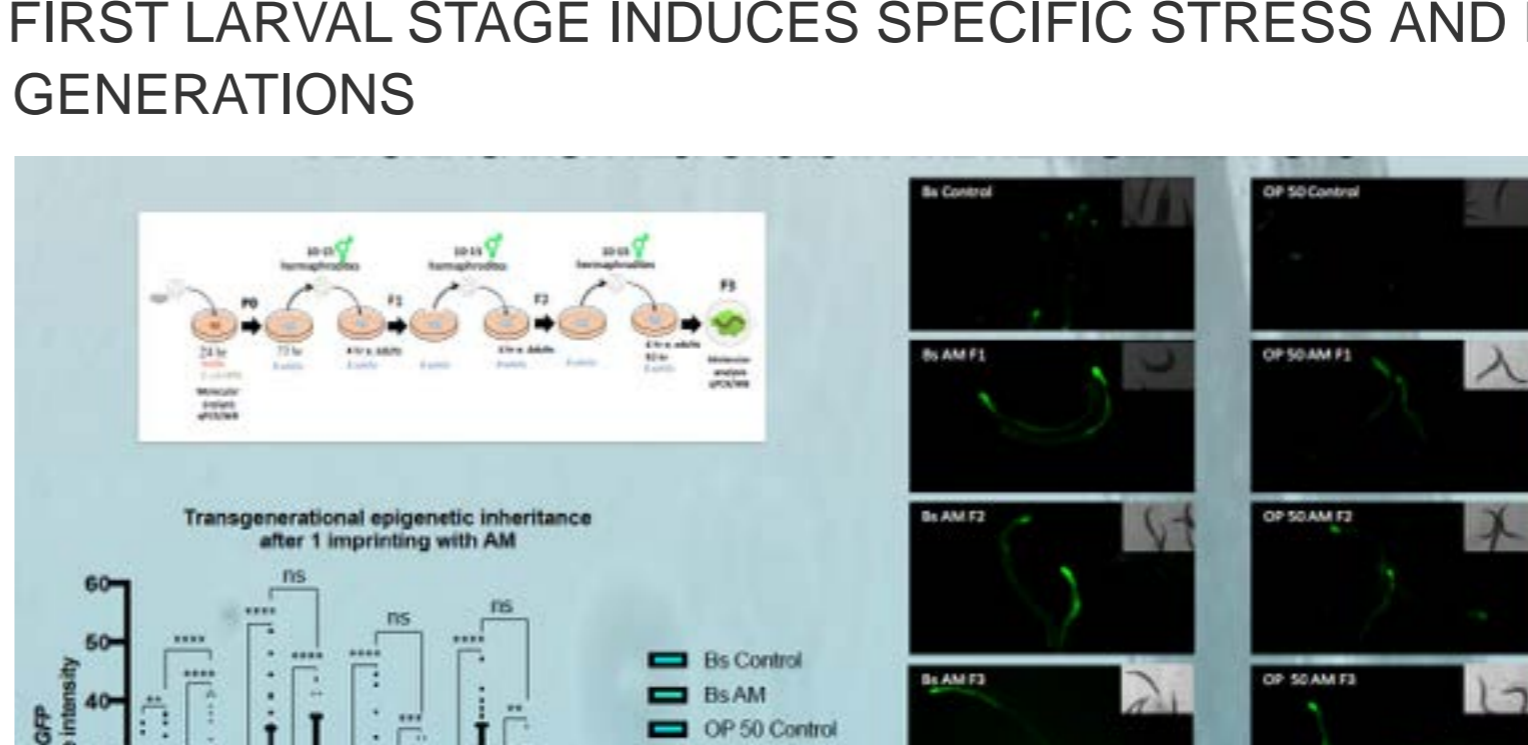


INTRODUCTION

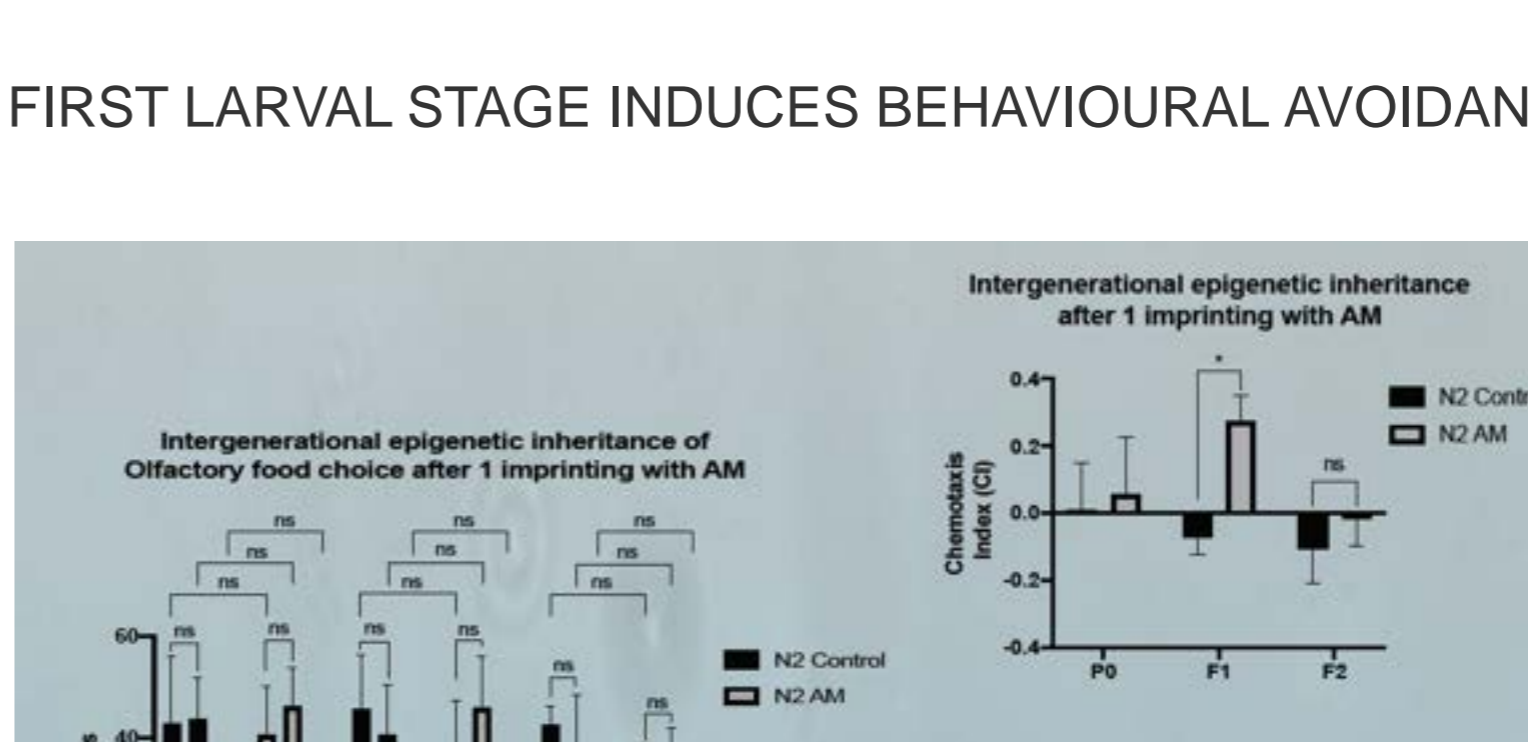
Stress exposure early in life is associated with behavioural and bodily changes that can develop several neuropsychiatric illnesses such as dementia. Experiences during the critical perinatal period form permanent, imprinted memories that can persistently alter expression levels of key genes through epigenetic marking, which can underpin changes in behaviour, molecular and stress reactivity throughout later life, including the next generations. Besides, this implies that gene by environment interactions (such as through epigenetic modifications) may be involved in the onset of these phenotypes. Although there are a number of studies in this field, there are still research gaps. For this reason, understanding the molecular mechanisms underlying the enduring effects of early-life stress is an important research area of the neuroscience. The aim of this project is to determine the behavioural and molecular changes and if there are changes in the epigenetic enzymes that can explain in part this phenomenon. Here using an experimental paradigm, we report that in response to early-life stresses, *Caenorhabditis elegans* nematodes form an imprinted behavioural and cellular defence memory. We show that exposing newly-born worms to toxic antimycin A exposure or repeated exposure, promotes aversive behaviour through chemotaxis assay and stimulates the expression of the *hsp-6* enzyme a toxin-specific cytoprotective. Learned adult defences require memory formation during the L1 larval stage and do not appear to confer increased protection against the toxin. We found that aversive behaviour is inherited only to the F1 generation after 1 exposure to the toxic or can be passed to the F4 generation after 4 exposures to the toxic. At the molecular level, we found changes in the chromatin modifiers MET-2 and SET-25 as well as their target gene SKN-1 until the F3 generation after 1 exposure to the toxic or until the F5 generation after 4 exposures to the toxic stress. Furthermore, we found changes in the lifespan after 1 exposure in the F1 until F3 generations as well as in the F1 until F5 generations after 4 exposures to the toxic stimulus. Regarding the oxidative stress response, we found changes in the same generations after 1 exposure or after 4 exposures to early life toxic stress. Thus, exposure of *Caenorhabditis elegans* to toxic stresses in the critical period elicits adaptive behavioural and cytoprotective responses as well as promote changes in the health outcomes, demonstrating a wide range of alterations that can appear after an early-life harmful stimulus. Likewise, we can conclude that these results are orchestrated by SET-25 pathway through SKN-1 transcription factor, which forms imprinted aversive behaviour and imprints a cytoprotective memory in the adulthood and the successive generations. These results, open a new avenue for new epigenetic therapies for neuropsychiatric disorders through chromatin modifiers such as SET-32.



TOXIN EXPOSURE AT THE FIRST LARVAL STAGE INDUCES SPECIFIC STRESS AND DETOXIFICATION RESPONSES IN THE NEXT GENERATIONS



TOXIN EXPOSURE AT THE FIRST LARVAL STAGE INDUCES BEHAVIOURAL AVOIDANCE RESPONSE UNTIL THE F4 GENERATION



CONCLUSIONS

- 1-Early life toxic stress exposure induced molecular and behavioural changes across generations in *C. elegans* after 1 exposure.
- 2-Early life stress exposure induced molecular and behavioural changes across generations in *C. elegans* after 4 exposures but not stabilize these changes.
- 3-Repeated early life toxic stress exposure in each generation increased the *hsp-6::GFP* fluorescent levels in the parental group in comparison with the parental group after 1 exposure.

MATERIAL AND METHODS

C. elegans strains, maintenance and reagents. All strains were obtained from *Caenorhabditis* Genetics Center. Standard methods were used for maintaining *C. elegans* strains. Worms were grown on Escherichia coli OP50 bacteria at 20 °C. The following *C. elegans* strains were used in this study: N2 wild type, SH4100 *zkl13* [*hsp-6::GFP*].

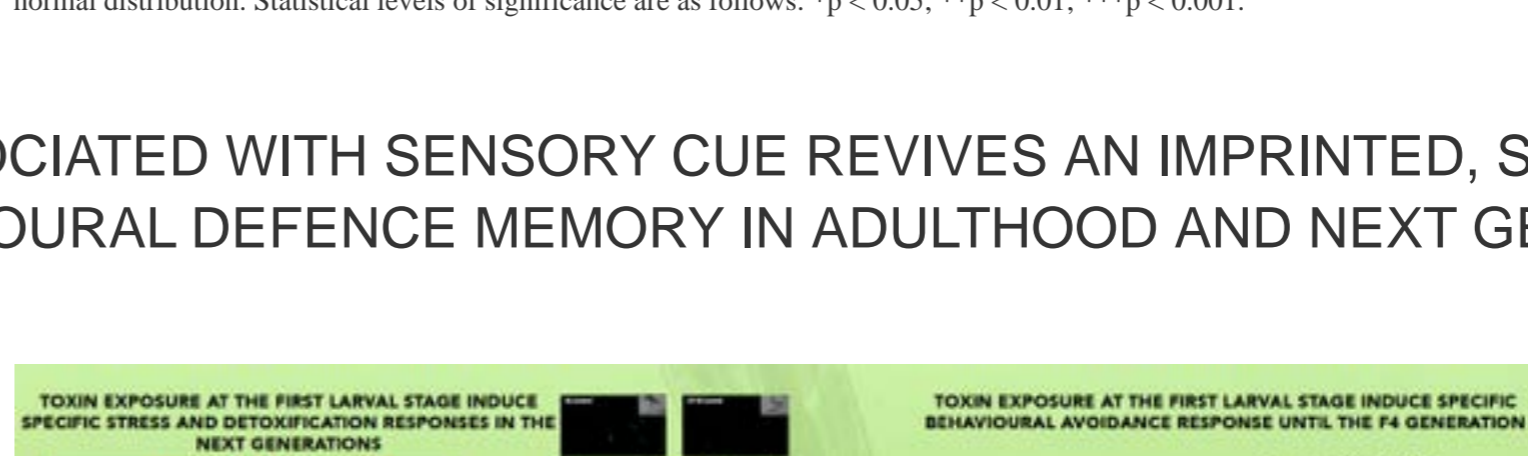
Imprinting training. Worms were synchronised by placing 15–20 hermaphrodites onto 3 cm diameter NGM plates seeded with 50 µl OP50 bacteria in the middle of the plate and allowed to lay eggs for 4 hours. Before synchronizing on each test plates OP50 bacterial lawn was dropped with 20 µl toxin or the appropriate solvent control (enhanced or distilled water, respectively). If not otherwise indicated, antimycin (AM) was used at the concentration of 50 µg/ml. Hermaphrodites were removed and plates were incubated at 20 °C for 24 hours during the L1 larval stage.

Olfactory food choice assay. 9 cm round NGM assay plates were seeded with 30 µl of each bacterial suspension (OD600 = 1) and incubated at room temperature for 1 hour. 80–100 naive and trained 4-day old worms were washed from their growth plates with M9 buffer, rinsed three times, and placed in the middle of the assay plates, containing OP50 and either *Bacillus subtilis* or *Pseudomonas* fluorescence on the opposite sides. Distribution of worms was scored after 1 hour. Using a short assay plate preparation time and the experimental incubation time allowed the assay to be determined by olfactory cues.

GFP reporter expression by fluorescence microscopy. After treatments at least 30 L1 or adult worms per condition were placed on 2% agarose pad and immobilized with 25 mM NaN3 dissolved in M9 buffer. Images were taken by a Nikon Eclipse E400 microscope with Diagnostic Instruments SPOT model 1.5.0 camera using a GFP fluorescent filter. Images were captured at 10x and 20x magnification. GFP expression levels were evaluated by ImageJ software.

Statistical analysis. L1 behavioural assays were analysed by one-way ANOVA with Tukey's HSD post-hoc test. Reporter expressions were analyzed by non-parametric tests. Pairwise comparisons in larvae were made by Kolmogorov-Smirnov test, multiple comparisons in adults were made by Kruskal-Wallis test. Data were expressed as mean ± standard deviation (SD) in behavioural test and reporter expression experiments, which displayed non-normal distribution. Statistical levels of significance are as follows: *p < 0.05; **p < 0.01; ***p < 0.001.

EARLY LIFE STRESS ASSOCIATED WITH SENSORY CUE REVIVES AN IMPRINTED, STRESS-SPECIFIC MOLECULAR AND BEHAVIOURAL DEFENCE MEMORY IN ADULTHOOD AND NEXT GENERATIONS AFTER 4 TOXIC EXPOSURES



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1. Gecse E, Griñán-Ferré C, Sanchis AB, Riubal CH, Söti C. Cellular defence memory imprinted by early life toxic stress. *Sci Rep*. 2019 Dec; 12(9):18935. doi:10.1038/s41598-019-55198-4.
2. Remy JJ. Stable inheritance of an acquired behaviour in *Caenorhabditis elegans*. *Current Biology*. Vol 20 No 20 R878.

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DISCLOSURES

AUTHOR INFORMATION

ABSTRACT

Stress exposure early in life is associated with behavioural and bodily changes that can develop several neuropsychiatric illnesses such as dementia. Experiences during the critical perinatal period form permanent, imprinted memories that can persistently alter expression levels of key genes through epigenetic marking, which can underpin changes in behaviour, molecular and stress reactivity throughout later life, including the next generations. Besides, this implies that gene by environment interactions (such as through epigenetic modifications) may be involved in the onset of these phenotypes. Although there are a number of studies in this field, there are still research gaps. For this reason, understanding the molecular mechanisms underlying the enduring effects of early-life stress is an important research area of the neuroscience. The aim of this project is to determine the behavioural and molecular changes and if there are changes in the epigenetic enzymes that can explain in part this phenomenon. Here using an experimental paradigm, we report that in response to early-life stresses, *Caenorhabditis elegans* nematodes form an imprinted behavioural and cellular defence memory. We show that exposing newly-born worms to toxic antimycin A exposure or repeated exposure, promotes aversive behaviour through chemotaxis assay and stimulates the expression of the *hsp-6* enzyme a toxin-specific cytoprotective. Learned adult defences require memory formation during the L1 larval stage and do not appear to confer increased protection against the toxin. We found that aversive behaviour is inherited only to the F1 generation after 1 exposure to the toxic or can be passed to the F4 generation after 4 exposures to the toxic. At the molecular level, we found changes in the chromatin modifiers MET-2 and SET-25 as well as their target gene SKN-1 until the F3 generation after 1 exposure to the toxic or until the F5 generation after 4 exposures to the toxic stress. Furthermore, we found changes in the lifespan after 1 exposure in the F1 until F3 generations as well as in the F1 until F5 generations after 4 exposures to the toxic stimulus. Regarding the oxidative stress response, we found changes in the same generations after 1 exposure or after 4 exposures to early life toxic stress. Thus, exposure of *Caenorhabditis elegans* to toxic stresses in the critical period elicits adaptive behavioural and cytoprotective responses as well as promote changes in the health outcomes, demonstrating a wide range of alterations that can appear after an early-life harmful stimulus. Likewise, we can conclude that these results are orchestrated by SET-25 pathway through SKN-1 transcription factor, which forms imprinted aversive behaviour and imprints a cytoprotective memory in the adulthood and the successive generations. These results, open a new avenue for new epigenetic therapies for neuropsychiatric disorders through chromatin modifiers such as SET-32. References

REFERENCES

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