

## HYDRA VULGARIS AS A MODEL TO STUDY NOCICEPTIVE-LIKE PATHWAYS

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*Hydra vulgaris* is an elementary organism, belonging to the phylum of Cnidarian, class Hydrozoa, characterized by a saccular structure, composed by an apical end (head) and a basal one (foot). The head has a hypostome with the mouth opening, from which several tentacles (from 5 to 12) depart for defence and feeding. *Hydra* has a simple nervous system consisting of a nerve net that extends through the body.

Here, we show that one of the most important nociceptive-like pathways is conserved in *Hydra vulgaris*. In particular, we found that *H. vulgaris* expresses TRPM3, a nociceptor calcium channel involved in the detection of noxious heat in mammals. Furthermore, we detected that both heat shock and TRPM3 specific agonist (i.e., pregnenolone sulfate) induce the modulation of the heat shock protein 70 (HSP70) and the nitric oxide synthase (NOS), two genes activated by TRP-mediated heat painful stimuli in mammals. As expected, these effects are inhibited by a TRPM3 antagonist (i.e., mefenamic acid). Interestingly, the TRPM3 agonist and heat shock also induce the expression of nuclear transcription erythroid 2-related factor (Nrf2) and superoxide dismutase (SOD), known markers of oxidative stress; noteworthy gene expression was also inhibited by the TRPM3 antagonist. Polyps were cultured at 17°C in a specific medium, maintained in a 16h/8h light/dark cycle and fed once a week. For the heat test, animals were moved from 17°C to 34°C hydra medium for 1 min and then placed at 17°C. Heated specimens were collected at specific time points, ranging from 0 to 24h. Animals were continuously monitored using an optical microscope, in order to analyze behavioural changes. In the meantime, animals were processed for quantitative Real-time PCRs, Western Blotting experiments and immunofluorescence. Biochemical experiments with TRPM3 agonist and antagonist were performed at the same time points. As a whole, our results demonstrate the presence of conserved molecular oxidative/nociceptive-like pathways at the primordial level of the animal kingdom.

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