PRE-EXPODOUR

Brief pre-exposure – high expectation: a question of salience or a kind of long-term sensitization?

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Context

In a male moth (*Spodoptera littoralis*), a unique effect of brief pre-exposure on the subsequent behaviour in response to olfactory stimuli has been unveiled. In this moth, pre-exposure to different sensory stimuli enhances the response of male moths to the female-produced sex pheromone. A brief exposure with either sex pheromone (Anderson et al. 2003), or a predator signal (bat sound: pulsed high-frequency sound) were leading to an increased response of males to the sex pheromone (unpublished data). In parallel with the increased behavioural responses, increased sensitivity of neurons in the primary olfactory centre, the antennal lobe, were found (Anderson et al. 2007). Global recordings of the peripheral olfactory system, the antenna, did not show any differences before and after pre-exposure.

Pre-exposure might thus either raise attention and make a stimulus more salient, or initiate a maturation process via long-term sensitization. We propose to use this moth as a model to study the nature of the pre-exposure effect and the neural mechanisms of plasticity occurring after pre-exposure to sensory stimuli. The project fits thus well with point 3 of the project call, "Perception and action". Understanding how experience can modify behaviour and the mechanisms governing plasticity of sensory systems, will allow in the future to interfere specifically at well-defined levels within the sensory pathway. Such manipulations are of wide importance not only from a medical point of view, but equally in the context of animal welfare and agricultural pest management.

Objectives

The objective of the present project was to determine if brief pre-exposure to sensory stimuli in our model insect elicits salience or if the effects of pre-exposure we observe are a new form of learning/ longterm sensitization and involve maturation processes. To answer this question, we studied 1) the specificity of the pre-exposure effect, and 2) the neurobiological and molecular mechanisms behind the behavioural and neuronal changes known so far.

1) Is the increase in responsiveness to sex pheromone an increase in salience/attention, due to a "choice" for the most important stimuli, because of high costs for high sensitivity and therefore linked with a decrease in attention/sensitivity to other stimuli? We have tested this hypothesis, by looking at the response to food stimuli. If the pre-exposure effect implies a "choice", pheromone pre-exposed males should respond less than naïve males to floral odours and nectar. If the effect we observe is due to a more general priming effect (maturation effect), where an important signal increases sensitivity to any kind of stimulus, we should see an increase in the responsiveness to any kind of behaviourally relevant stimulus.

2) What is the time-course of pre-exposure effects and what are the peripheral and central nervous mechanisms, which allow the changes in behavioural and neuronal sensitivity after pre-exposure? Are molecules involved in the reception and transduction of pheromones (OBPs, receptors, channels,…) expressed differentially before and after pre-exposure? Are neuromodulators such as biogenic amines involved in peripheral and/or central modulation? Is pre-exposure changing the branching patterns and synaptic interactions between the different neurons in the olfactory pathway? Will we see cross-modal effects of pre-exposure and in that case on which level?

Results and conclusions

Our results show that the increase in responsiveness after brief pre-exposure to a sensory stimulus is indeed a form of general sensitization rather than a case of specific attention. Behavioural experiments using different behaviourally relevant auditory, gustatory, and olfactory stimuli during pre-exposure and subsequent behavioural tests show that sensitization occurs not only within, but also across modalities. We were able to identify both physiological and structural modifications at different levels of the olfactory pathway. Depending on the sensory signals used for pre-exposure, peripheral and/or central nervous changes were detected. So far no effects were found at the level of selected gene expression, at least in the peripheral olfactory system.

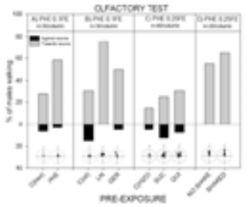
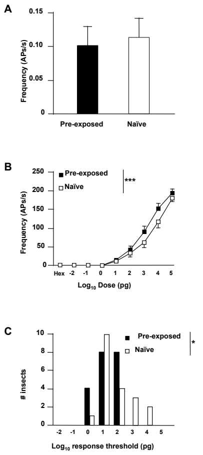


Figure 1. Behavioral responses to the main sex pheromone compound (PHE) of males pre-exposed to olfactory (**A** PHE, **B** plant compounds linalool LIN and geraniol GER) and gustatory stimuli (**C** sucrose SUC and quinine QUI) on a locomotion compensator. **D** Responses of males to PHE after pre-exposure to non-specific shaking. Grey and black columns show the percentage of males walking towards and against the source, respectively. The length of the whole column (grey+black) shows their activity level. Within each frame (A, B, C, D), different letters and numbers denote significant differences in the activity and orientation levels, respectively, of naïve and pre-exposed males (Chi-Square, p<0.05). Circular diagrams show the mean angle of individual males (the stimulus is situated at 0°). Asterisks in circular diagrams show groups of insects that did not walk randomly (Raleigh test, p<0.05).



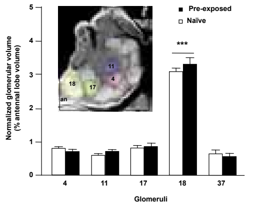
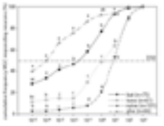


Figure 3. The cumulus of the MGC is enlarged in pre-exposed males. Volumes (mean ± s.e.m.) of reconstructed glomeruli in naïve (n = 20) *vs.* pre-exposed (n = 18) males, normalised to the whole AL volume. Macroglomerulus 18 (cumulus) was significantly larger in pre-exposed than in naïve males. No significant volume difference was observed in any other reconstructed glomerulus. **Inset:** confocal optical section of a left antennal lobe showing the superimposed reconstruction of four of the analyzed glomeruli (glomerulus 37 was in another plane). *an*: antennal nerve. * *P*<0.05 (Mann-Whitney test).

Figure 2. ORNs are more sensitive to pheromone after pheromone pre-exposure. **(A)** Average spontaneous firing activity (mean ± s.e.m.) in pre-exposed and naïve males. No significant differences were observed between the two treatments. **(B)** Mean (± s.e.m.) firing activity during the 200-ms stimulation period with 10^{-2} to 10^{5} pg of *Z*9,*E*11-14:Ac or Hexane (*Hex*) as a control in pre-exposed and naïve males. The two dose-response curves differ significantly (two-way ANOVA for repeated measures, P < 0.001). **(C)** Distribution of response thresholds of ORNs in naïve and pre-exposed males. Response thresholds were different between the two groups (c^2, *P*<0.05) (n=20 pre-exposed and 20 naïve in all three panels). *APs:* action potentials. *: *P*<0.05 (Chi-square test); ***: *P*<0.001 (ANOVA).

Figure. 4. Cumulative response threshold distribution of antennal lobe neurons to the main pheromone component, *EZ-9,*11-14:OAc. Mainly neurons with a high threshold were found in naïve and tone-exposed males, while lower threshold neurons were found in males that had been pre-exposed to either pheromone or bat sound. The D50, dose at which the 50% of neurons responded, is indicated as a dashed line. Neurons in pheromone- and bat-exposed males reached the D50 at lower doses than in tone-exposed and naïves males. bat: neurons in bat sound-exposed males, tone: neurons in tone-exposed males, phe: neurons in pheromone-exposed males.



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In preparation:

Minoli S, Kauer I ,Colson V, Party V, Renou M, Anderson P, Marion-Poll F, Anton S. Intra- and cross-modal sensitization of olfactory and gustatory responses induced by brief pre-exposure in a moth

Schmidt-Büsser D, Chabaud MA, Iqbal J, Gaertner C, Devaud JM, Anton S. Anatomical plasticity within the olfactory pathway after cross-modal pre-exposure.

Schmidt-Büsser D, Monsempes C, Anton S, Jacquin-Joly E, Frérot B. Effects of long-term pheromone exposure with high doses on behavioural responses and expression of olfaction-related molecules in the antenna of *S. littoralis*.

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