# Extracellular Recordings of Antennal Lobe MGC neurons in *Agrotis ipsilon:* *olfactory coding of sex pheromone*

*A. Chaffiol, H. Belmabrouk, S. Anton, D. Martinez and J.-P. Rospars*

Spike timing precision in MGC neurons sensitive to pheromone

- Antoine CHAFFIOL
- Post-doc / experiments

PISC INRA UMR 1272
Versailles France

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**Aims:**
Understanding how pheromone information is encoded in a "simple" brain

- *Check the variability across neurons* sensible to the pheromone during responses -> *Pheromone response patterns*
- *Check neurons selectivity (pheromonal compounds)* and pheromone concentration effect -> *Quality & Quantitative Coding*
- *Effect of stimulation's temporal characteristics* on responses -> *Temporal Coding*

- *Quantification of the discharge timing precision during pheromone responses*
- *Check neurons interactions, local field potential dynamics and oscillations*

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→ Get datas to build accurate Projection Neurons models

# Data acquisition

- Insect model: **noctuid moth** *Agrotis ipsilon* males (≈5 days old)

- MGC (or near the MGC) **extracellular recordings of neurons sensible to the pheromone** with two glass pipettes (tip diameter 4µm)

- **long-lasting recordings** of small neuron populations (from 1 to maybe 5 neurons)


*Agrotis ipsilon* male *(from W. Cook)*

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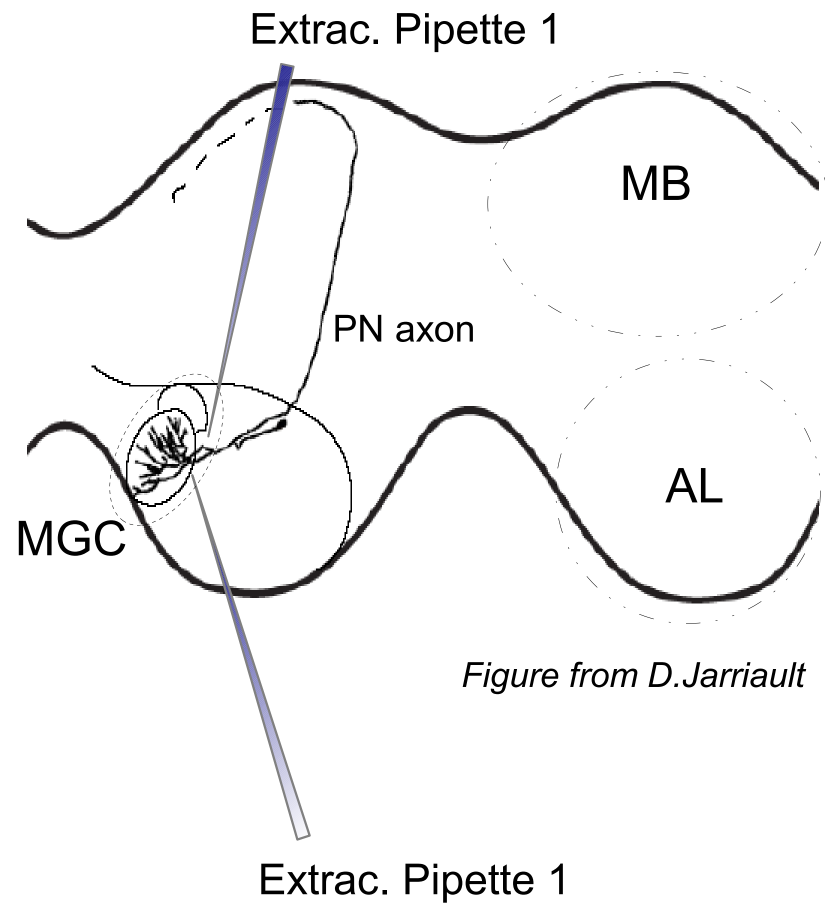
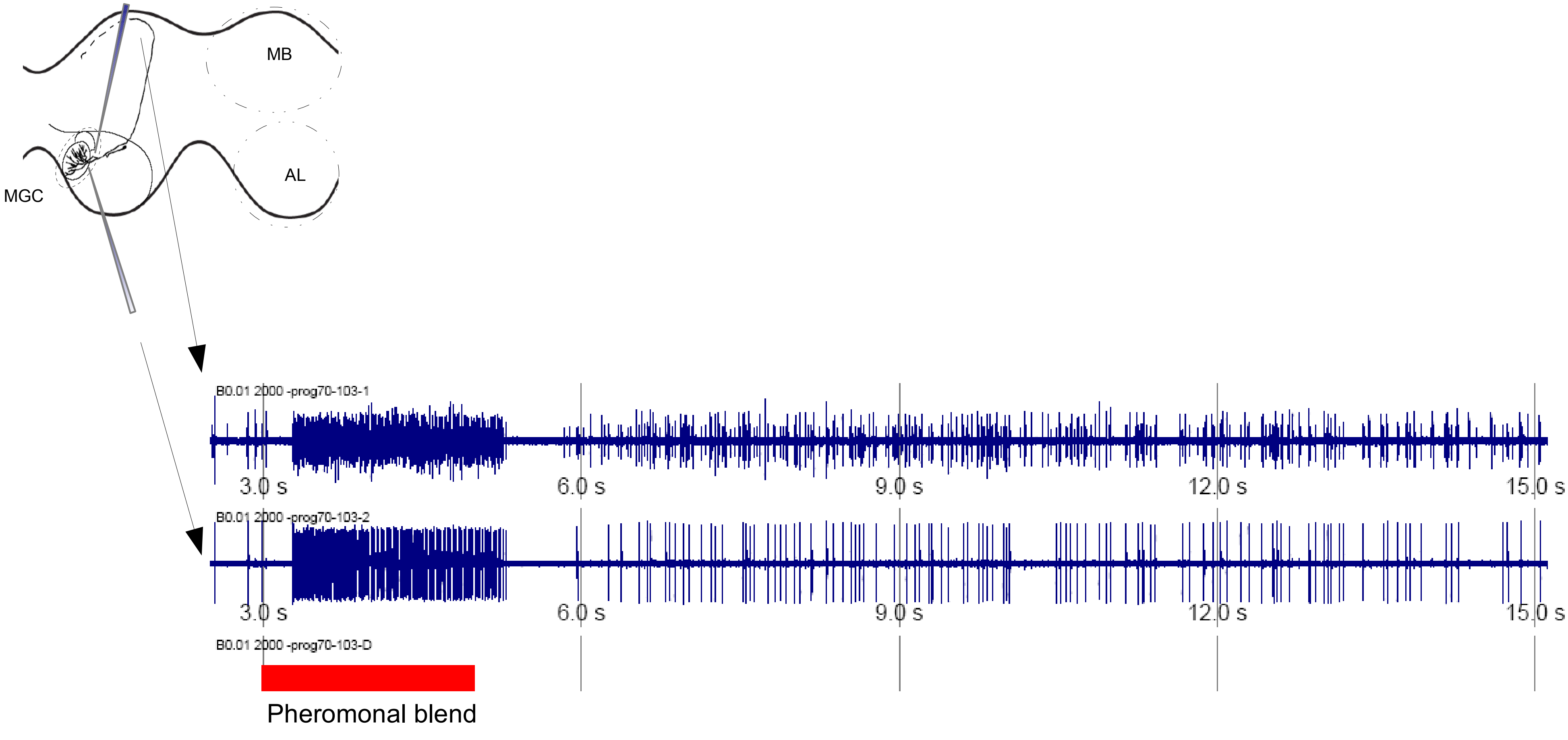



**Insect brain and recording technique**

# Data acquisition




Raw data trace, 2 pipettes, 3 responding neurons

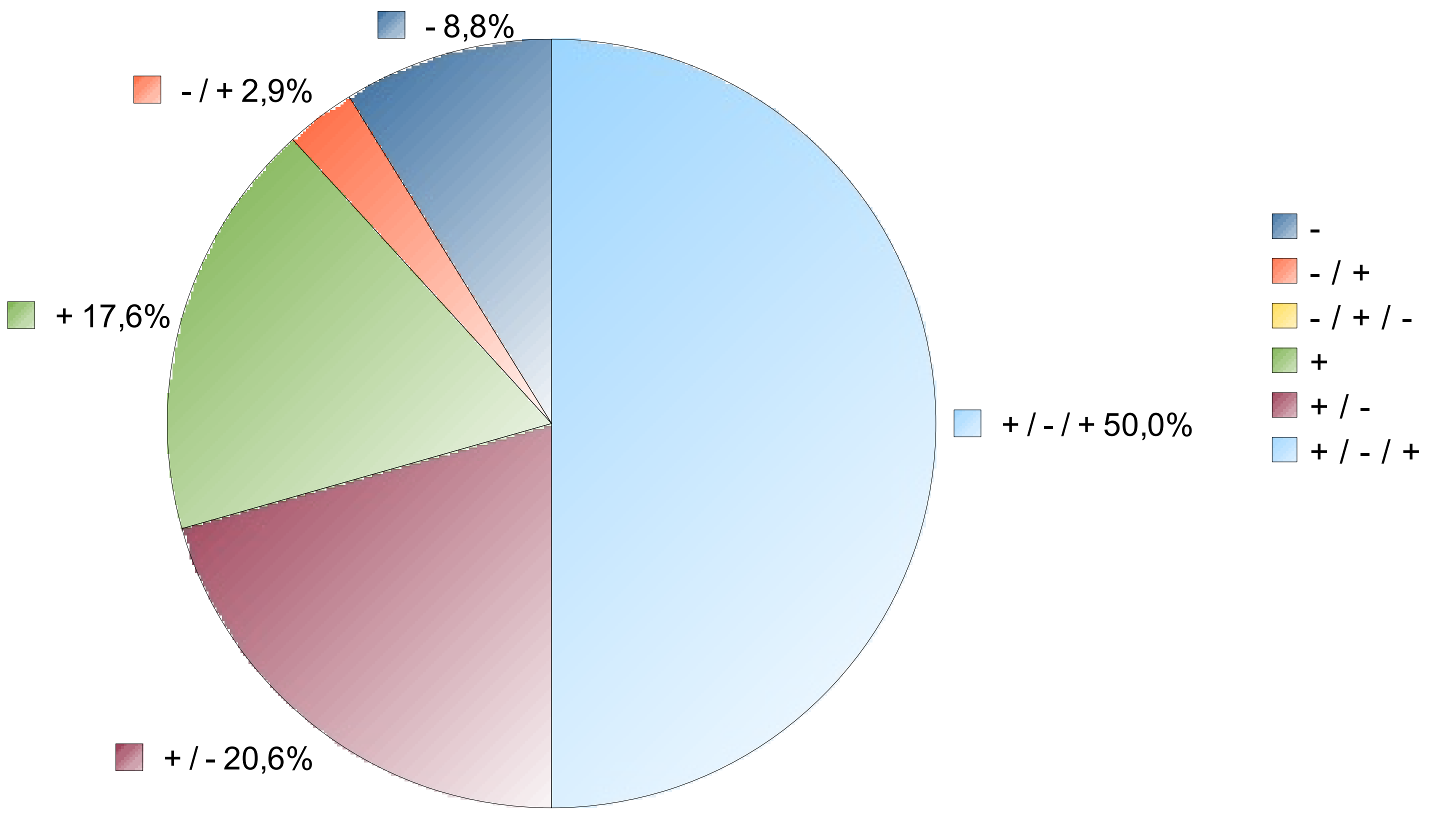
# Pheromone response patterns observed

| | Responses patterns summary |
|---|---|
| + | Excitatory response (tonic) |
| +/- | **Excitation (burst) / Inhibition ('classic PN response')** |
| +/- - | Excitation (burst) / long inhibition |
| + +/- | Excitation (long burst, tonic) / Inhibition |
| -/+ | Inhibition / Excitation |
| -/+/- | Inhibition / Excitation / Inhibition |
| +/-/+ | **Excitation (burst) / Inhibition / Excitation (tonic)** |
| - | Inhibition |

**Table**: Variability observed across recorded neurons sensible to the pheromonal blend

# Pheromone response patterns observed

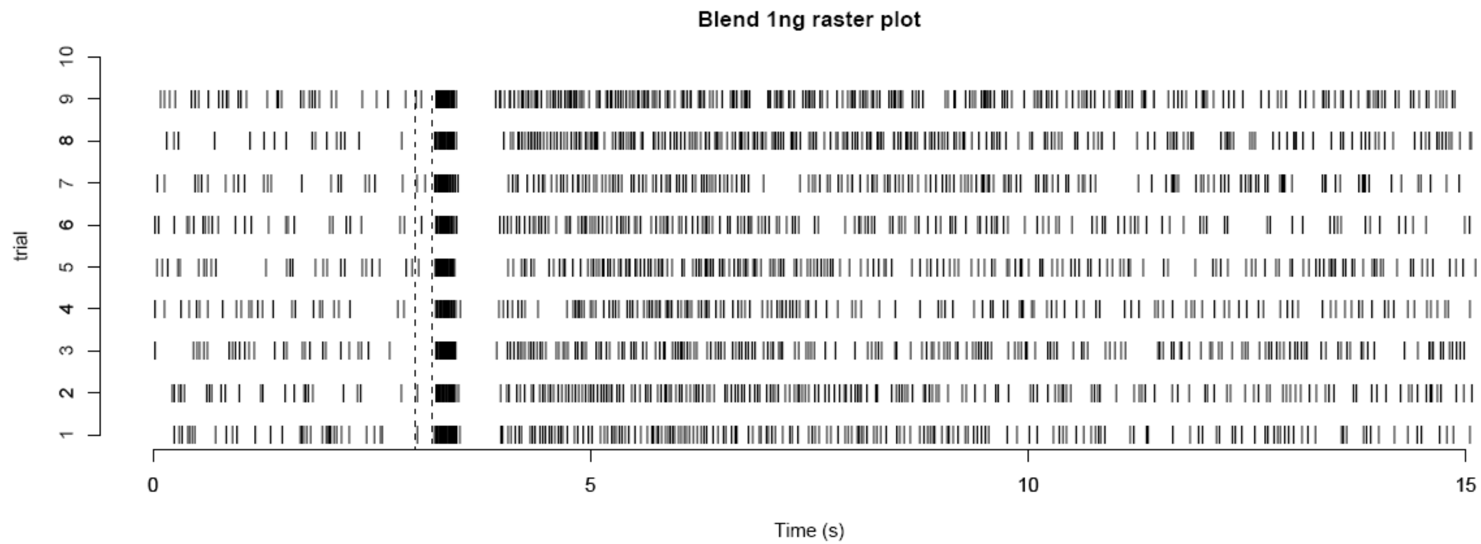
## Pheromone Response PATTERNS



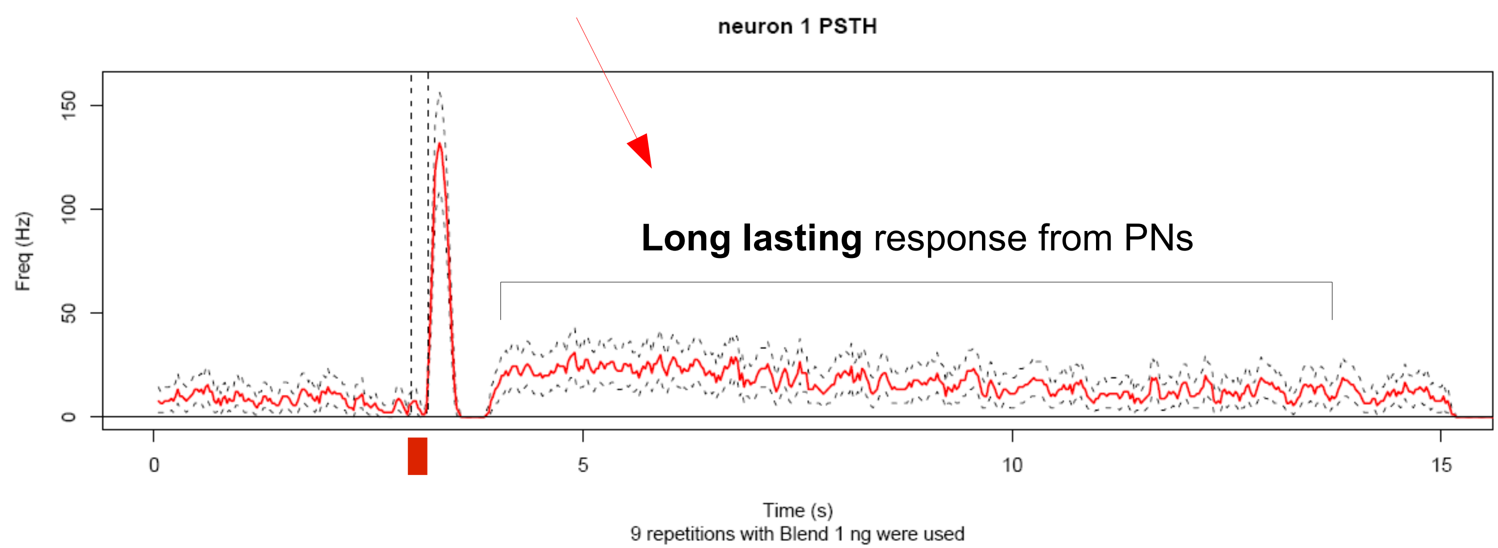

N=34 neurons sensible to the pheromone blend and tested at c=1ng

Pheromone response patterns

# Most frequently observed pattern: + / - / +

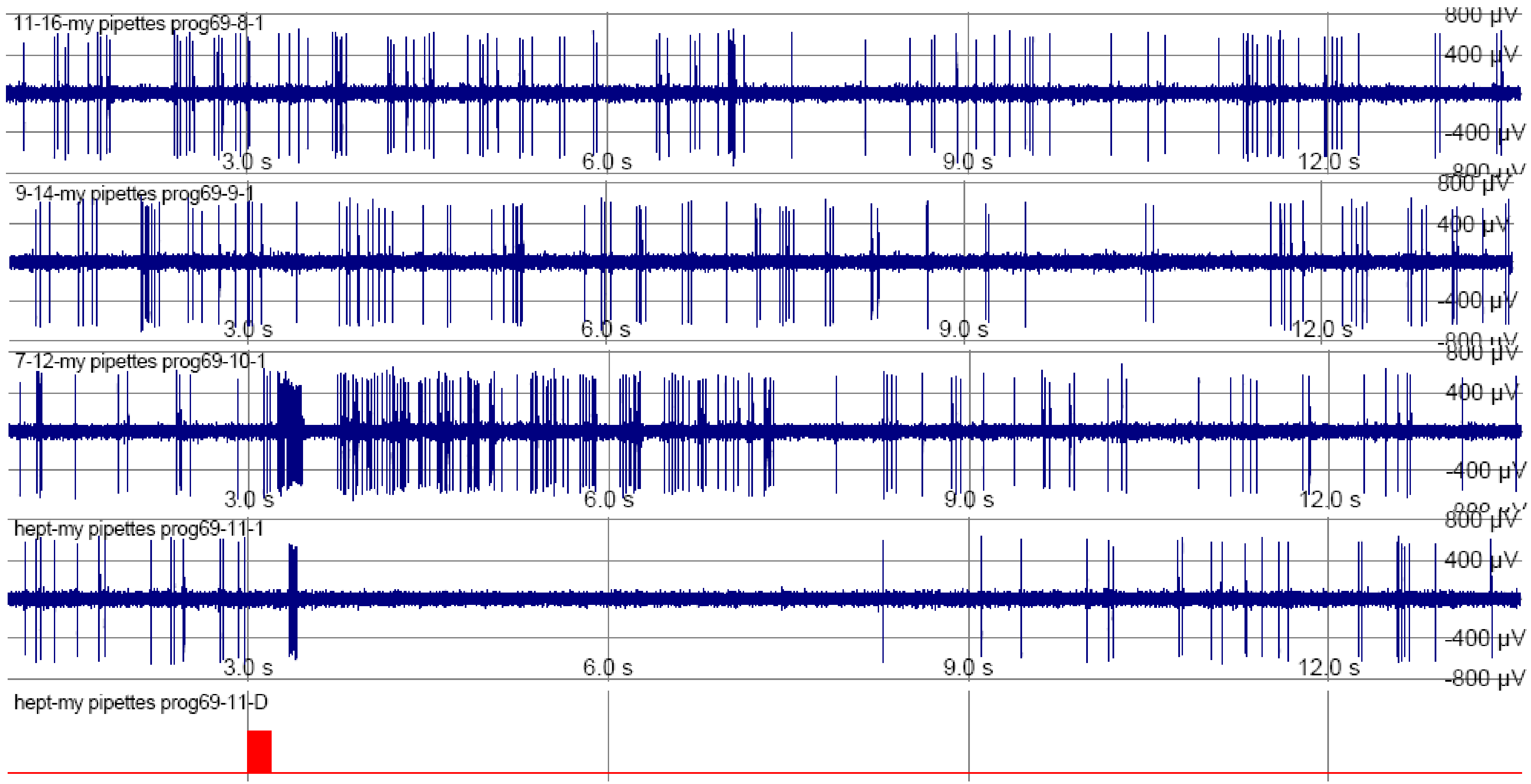



+ / - / +




PSTH: 1 neuron with Blend 1ng & 9 repetitions

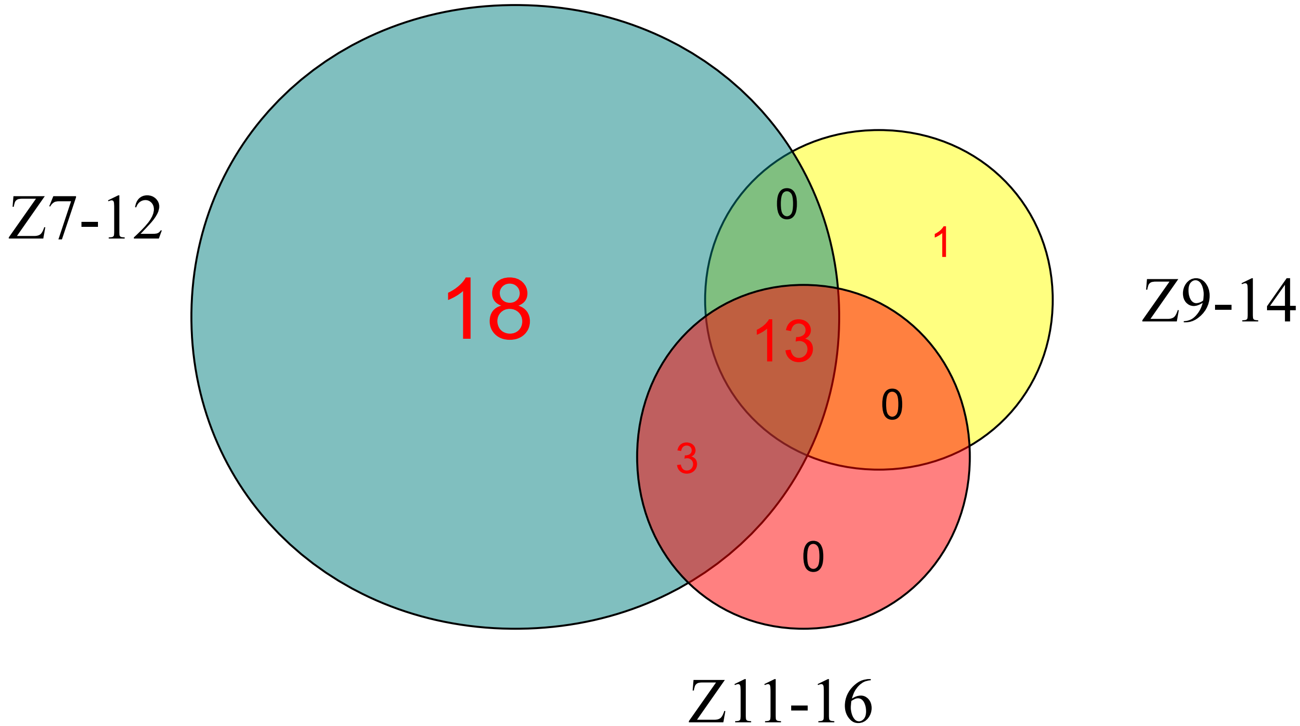
# Quality coding

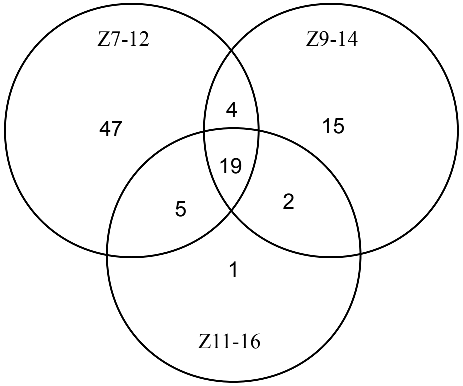



**Neurons selectivity**. Responses of a neuron to the 3 major pheromonal components of the pheromonal blend:

Z11-16:OAc, Z9-14OAc, Z7-12OAc and a host plant odor (heptanal)

# Quality coding



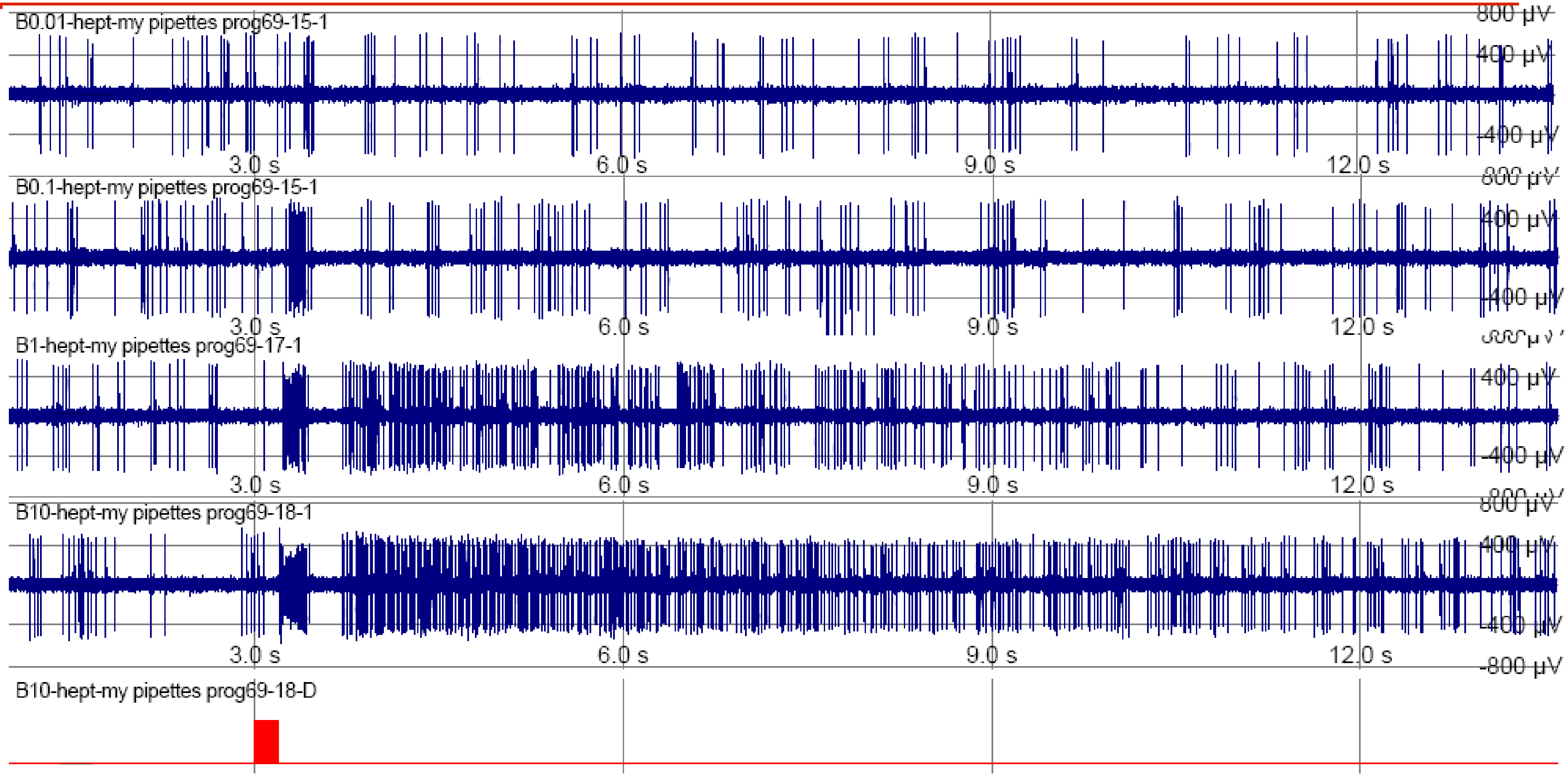

From D. Jarriault
Tests at c=0,01ng

And 100% responses for the pheromonal blend

**Response specificity of 35 neurons** according to their response to three components of the pheromonal blend (at concentration=0,1ng)

# Quantitative coding

pattern switch



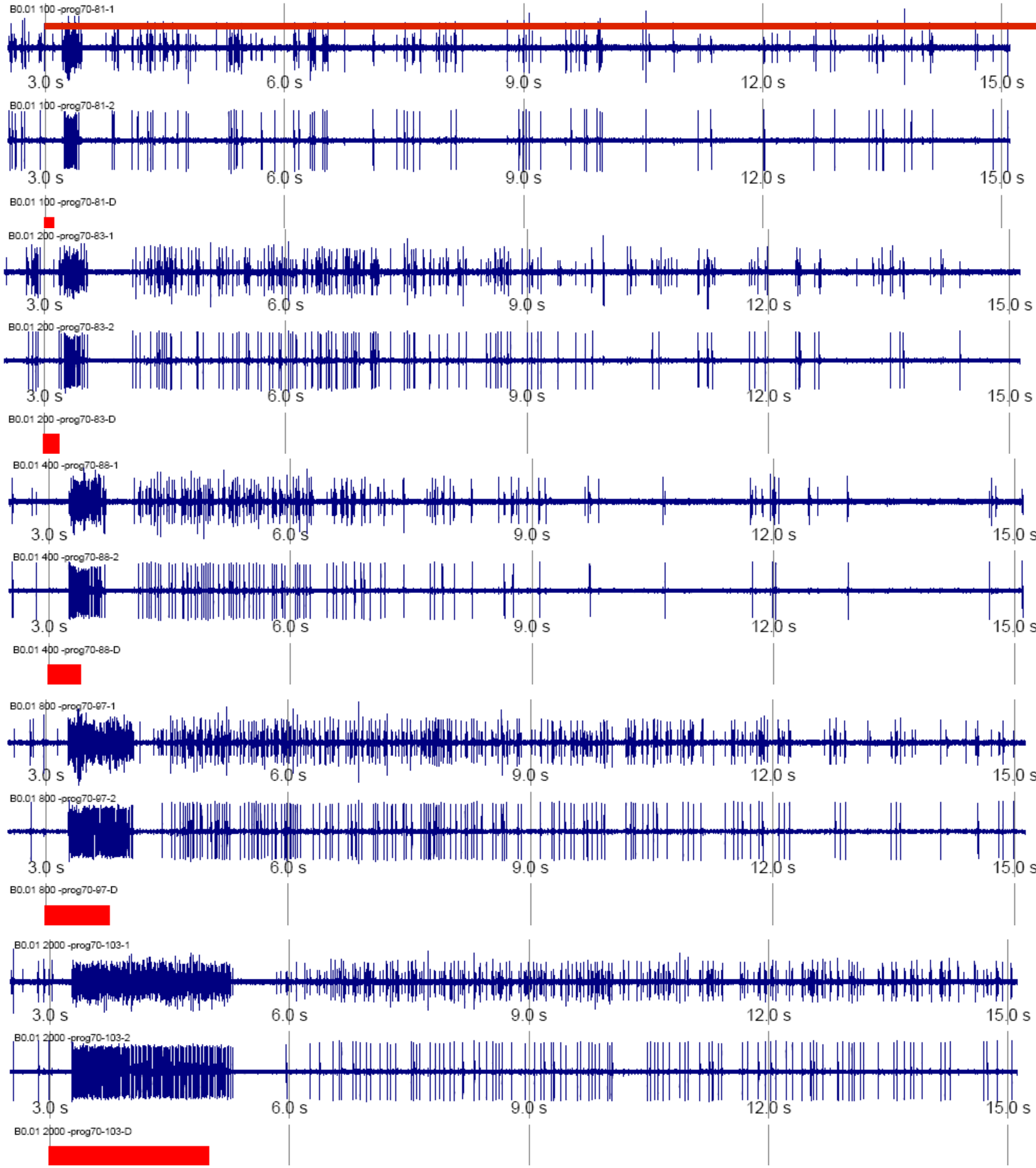

**Dose-response** with the pheromonal blend: from 0,001 ng to 10 ng (only 4 concentrations shown in the example)

We know that increasing the dose leads to a shorter response latency, and a higher max frequency. But no effect on response and inhibition durations have been reported (Jarriault et al., 2009)

→ **We found a Response pattern switch** from a biphasic response (+ / -) to a triphasic response (+ / - / +) at concentrations beetween 0,1 – 1ng

in 70% of neurons displaying a +/- pattern at low dose (17 out of 24 neurons)

# Temporal coding: stimulation duration

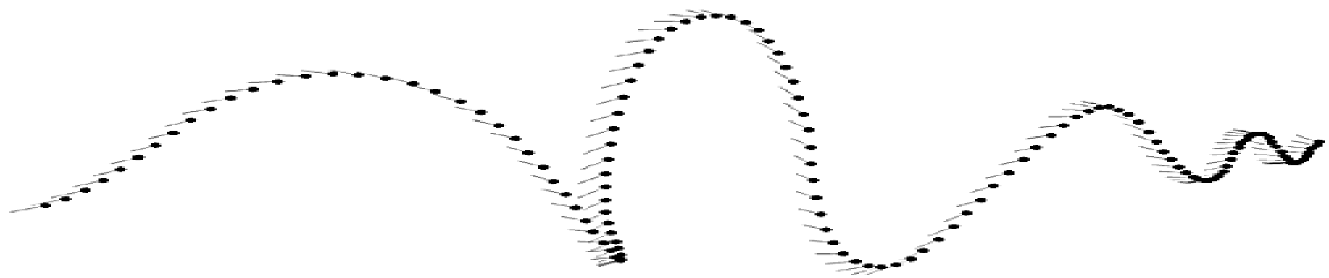



Responses to **increasing stimulation Durations**
(ex: 100, 200, 400, 800 and 2000ms odor puffs)

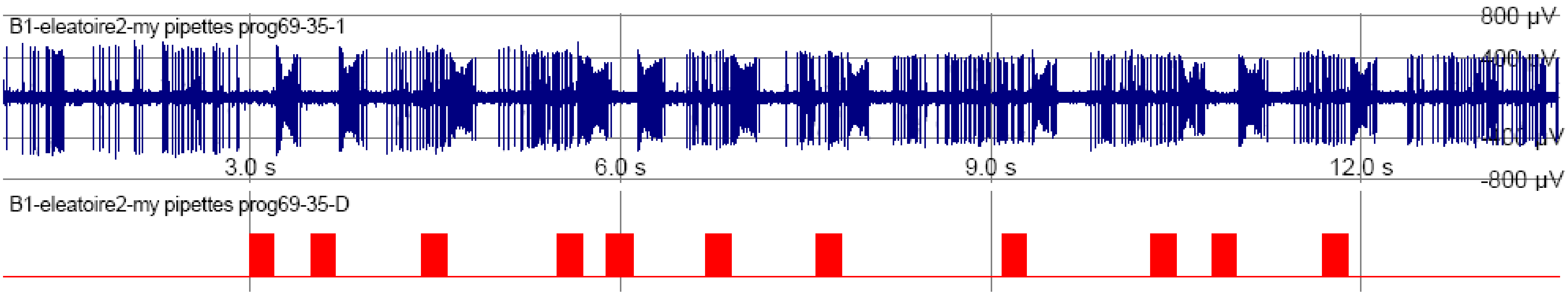
The duration of the excitatory phase is strictly correlated with the stimulus duration (n<10 neurons),

the inhibition phase duration is constant

# Temporal coding: pulsatile stimulations

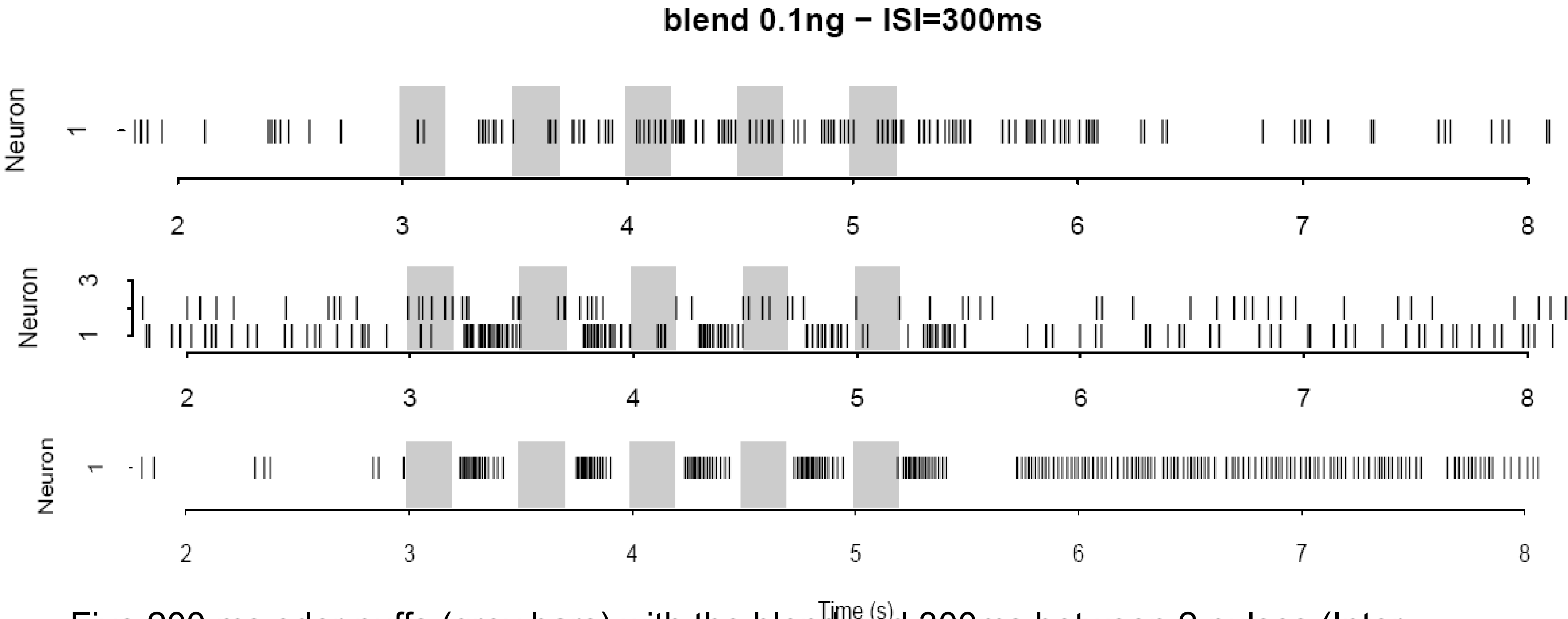



Ex: male moth behavior in odor plume (*Manduca sexta*)
*http://flightpath.neurobio.arizona.edu/*




Responses to blend pulsatile stimulations
raw data trace from 1 “follower” neuron with random pulsatile stimulations

# Temporal coding: pulsatile stimulations




Five 200 ms odor puffs (grey bars) with the blend and 300ms between 2 pulses (Inter Stimulus Interval):

-> responses variability: 3 experiments with neurons responding to the blend but with different degrees of timing precision with the pulses

# Temporal coding: pulsatile stimulations

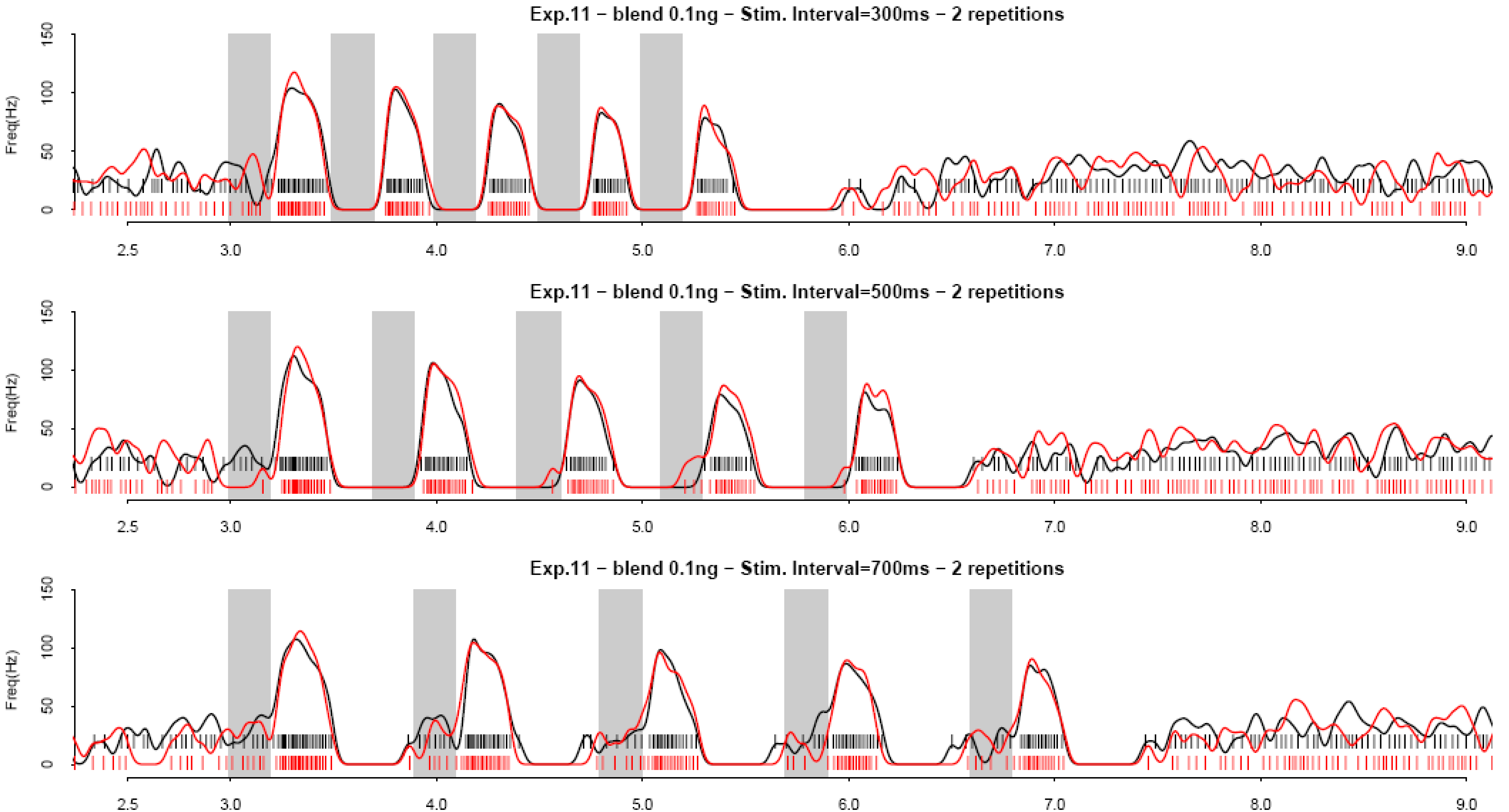
**30 out for 43** neurons tested with pulsatile stimulations can resolve 2 Hz pulses:

-> 69 % of the population
- **26 « +/- » & « +/-/+ » neurons**
- 1 « + » neuron
- 3 « - » neurons

**13 neurons** out of the 26 « + / - / (+) » neurons are kept for a more detailed analysis:
(-> *experiments with best signal to noise ratios*)

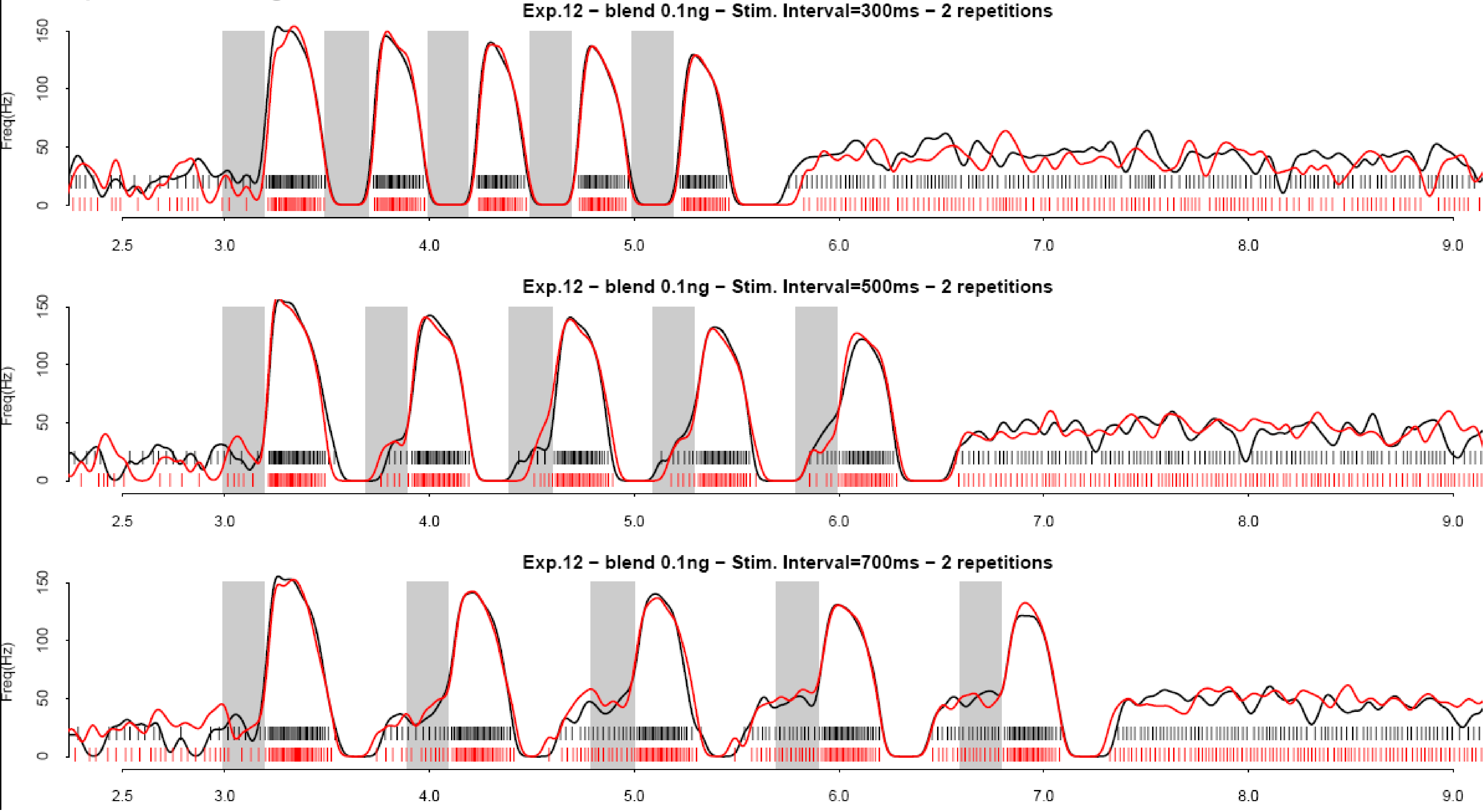
- Automatic segmentation of responses using D.Martinez's detection algorithm (latency, burst duration, mean frequency, spike count, ...)
- Effect of inter stimulus duration between pulses on the observed response patterns ?
- Effect of the pulses repetitions on the observed patterns ?
- Precision and robustness of the responses (timing precision algorithm) ?

## Temporal coding

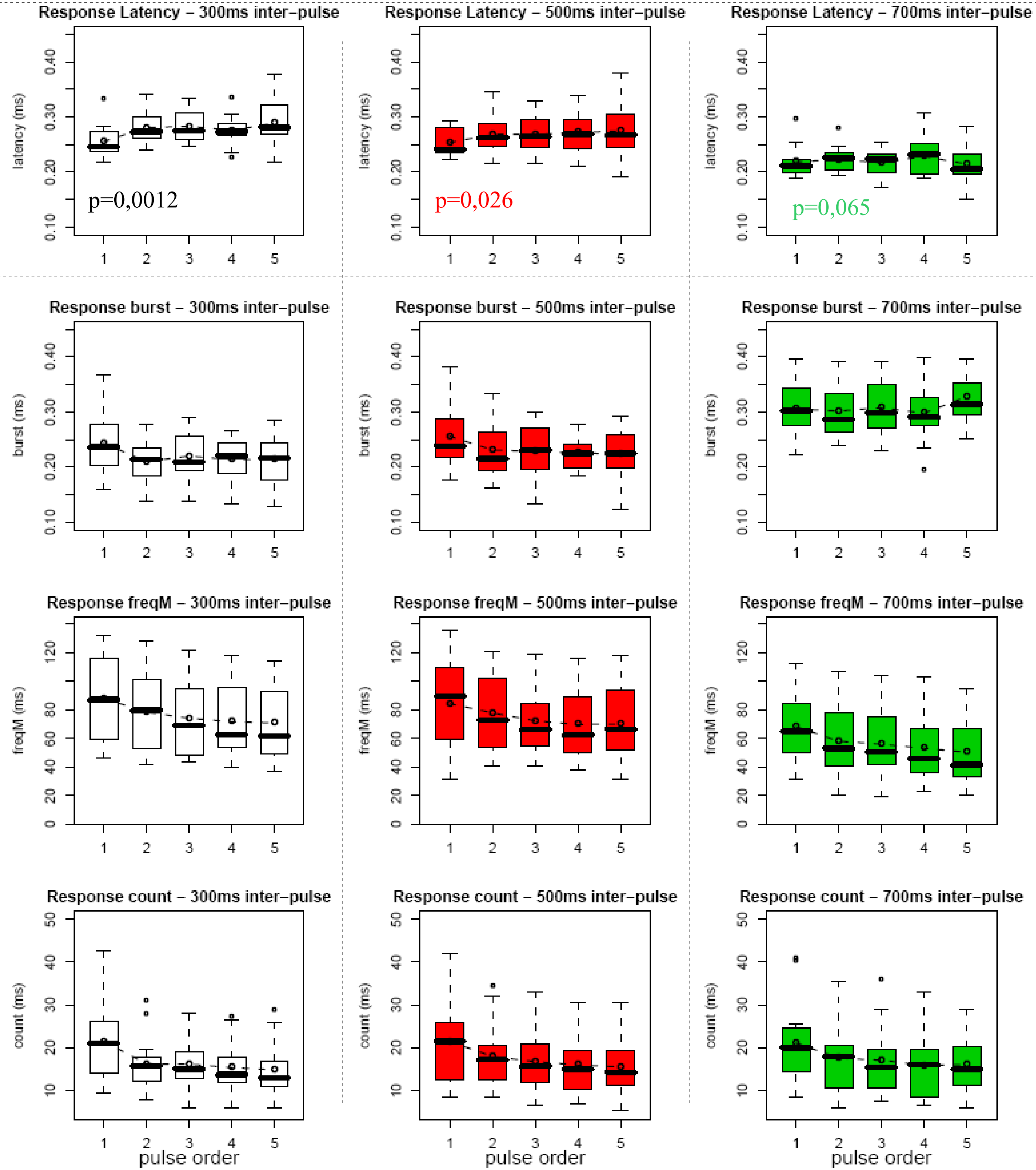



Ex: neuron from exp.11, 5 pulses with an inter stimulus duration of 300, 500 or 700 ms
(2 repetitions: black & red rasters and curves)
-> Raster plots and mean firing frequencies

# Temporal coding




Another example: neuron from exp.12




Latencies:

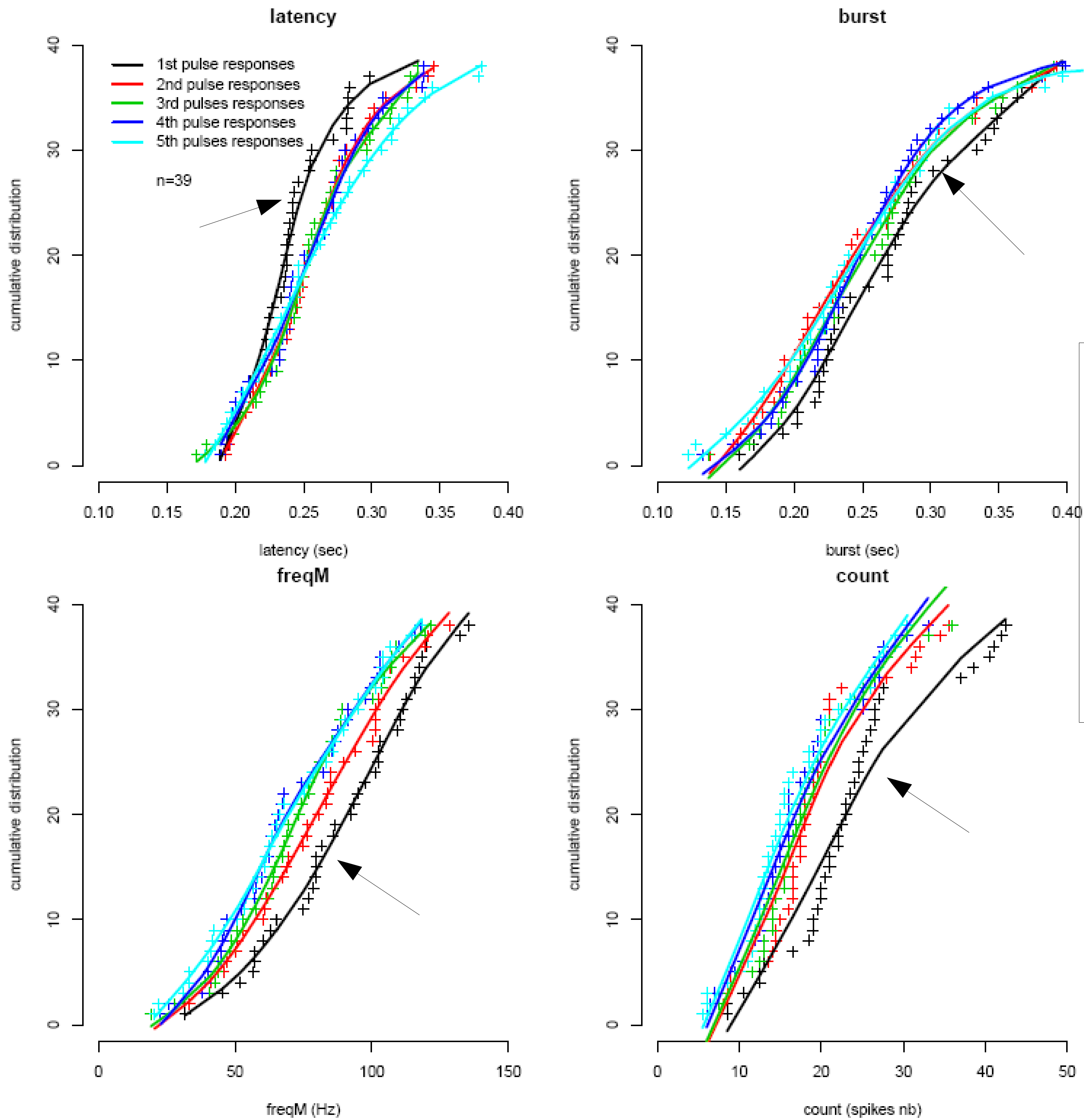
p=0,0012 à 300ms (kruskal wallis).
p=0,026 à 500 et
p=0,065 à 700

13 neurons

4 parameters for each of the 5 pulses

3 different inter-stimulus durations:

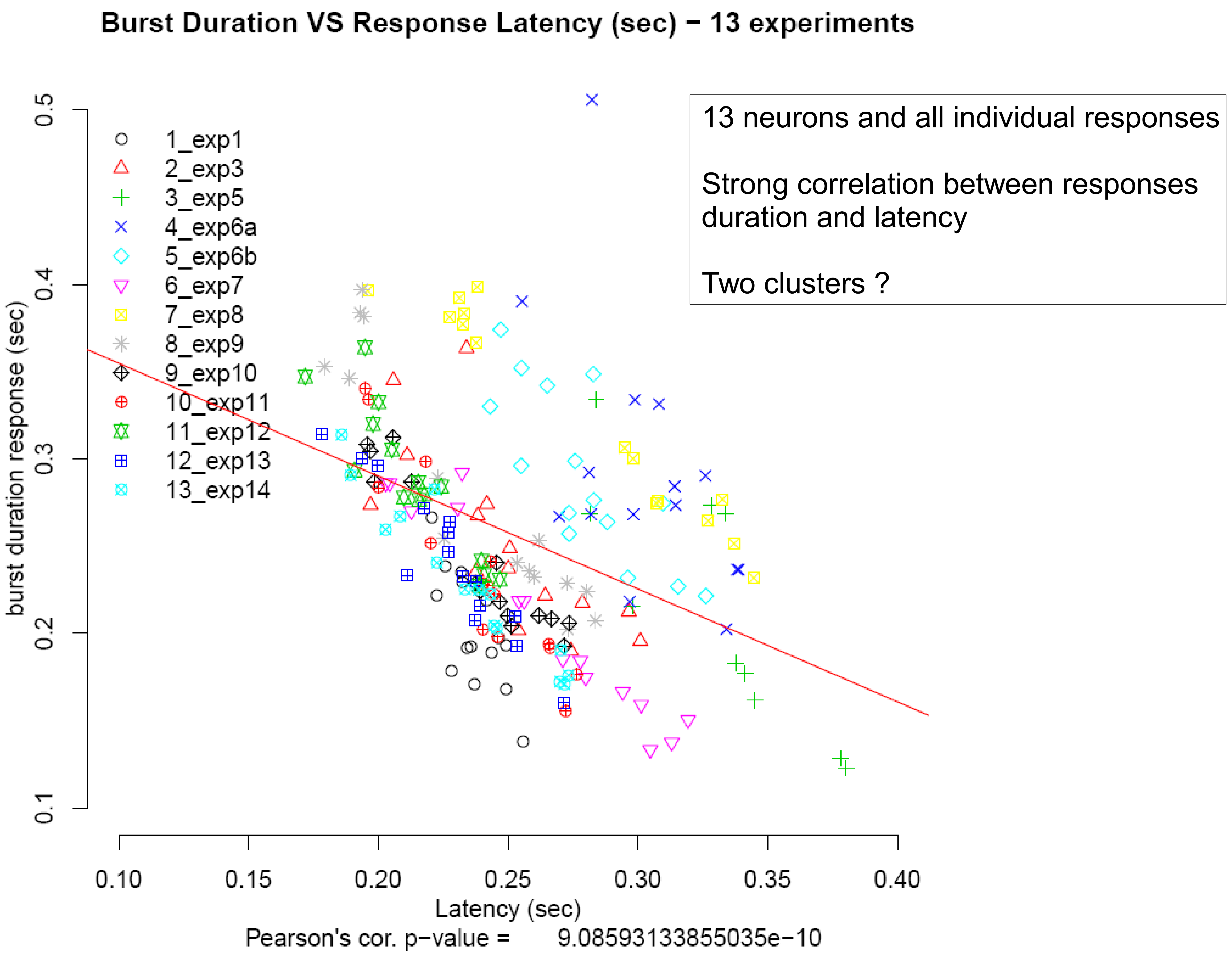
300ms (left column),
500ms (center)
700ms (rigth)



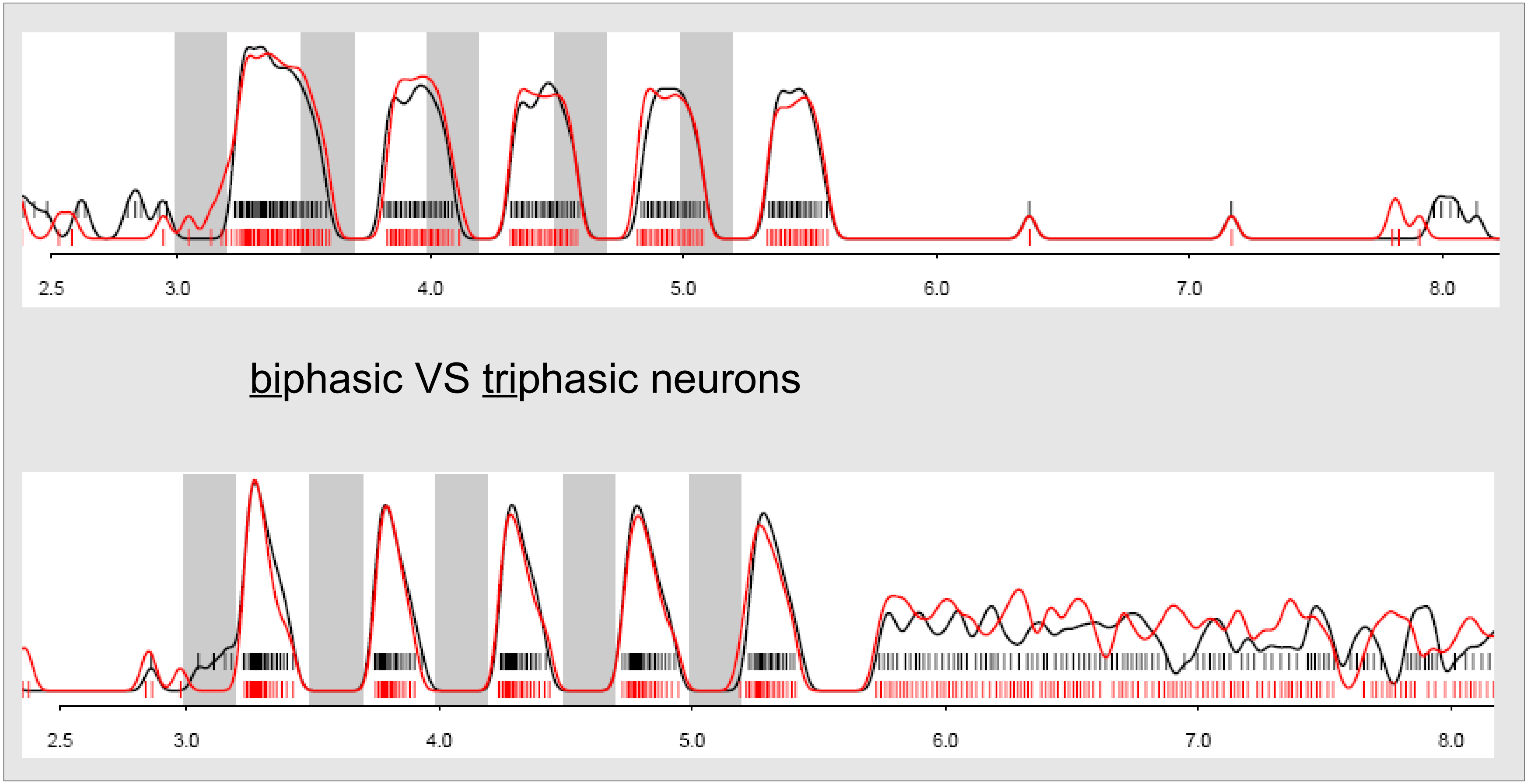

13 neurons

Pulse order effect on the 4 parameters distribution:

-> Different response characteristics from pulse number1


Burst Duration VS Response Latency (sec) − 13 experiments
burst duration response (sec)
0.1
0.2
0.3
0.4
0.5
1_exp1
2_exp3
3_exp5
4_exp6a
5_exp6b
6_exp7
7_exp8
8_exp9
9_exp10
10_exp11
11_exp12
12_exp13
13_exp14
13 neurons and all individual responses
Strong correlation between responses duration and latency
Two clusters ?
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Latency (sec)
Pearson's cor. p−value = 9.08593133855035e−10

# Temporal coding



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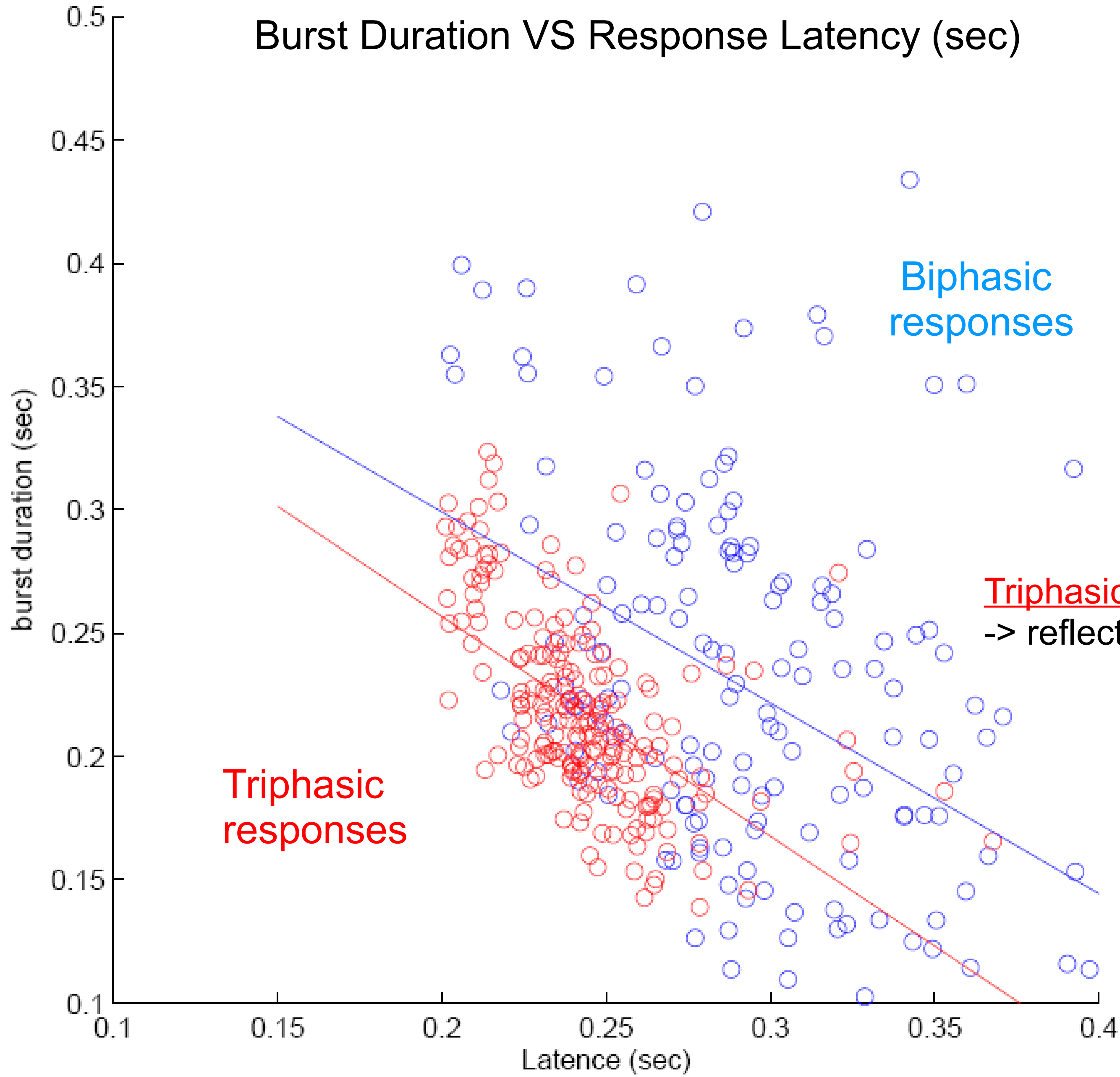



**Mean latencies:**
Neurons +/-: 299 +/- 48ms
Neurons +/-/+: 243 +/- 26ms
**p<0,01**

**Mean response durations:**
Neurons +/-: 222 +/- 81ms
Neurons +/-/+: 218 +/- 39ms
**ns**

Triphasic responses +/-/+ 50ms faster
-> reflect different network configurations ?

# discharge timing precision during pheromone responses

In order to quantify the timing precision of MGC neurons, D.Martinez has adapted for our data a « spike train comparison » algorithm:

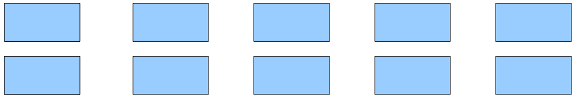
3 parameters can be obtained in the end of the analysis:

- Delta: drifting time of the whole response necessary to optimize the likelihood between 2 spike trains (in ms)
- **Sigma**: « timing precision » / synchronization (in ms)
- **Rhô**: « robustness » or the probability to 'loose' a spike in the response sequence

# discharge timing precision during pheromone responses

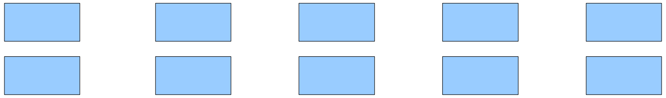
**(in progress)**

300ms interval pulses:



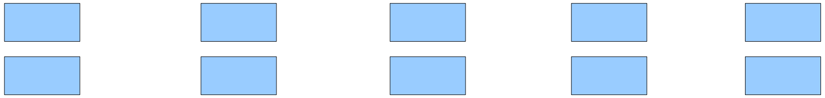
| | | | | | | |
|---|---|---|---|---|---|---|
| Sigma(ms): | 3.29 | 3.61 | 3.67 | 3.79 | 4.13 | ns |
| Rhô(p): | 0.08 | 0.12 | 0.10 | 0.12 | 0.10 | ns |

500ms:



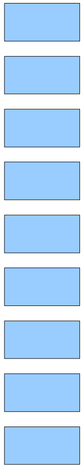
| | | | | | | |
|---|---|---|---|---|---|---|
| Sigma(ms): | 3.04 | 3.37 | 3.35 | 3.65 | 3.73 | ns |
| Rhô(p): | 0.10 | 0.13 | 0.12 | 0.10 | 0.17 | ns |

700ms:



| | | | | | | |
|---|---|---|---|---|---|---|
| Sigma(ms): | 2.88 | 3.11 | 4.32 | 4.16 | 2.90 | ns |
| Rhô(p): | 0.10 | 0.13 | 0.11 | 0.13 | 0.15 | ns |

**Population level values**
N=13 neurons, 2 repetitions/neuron



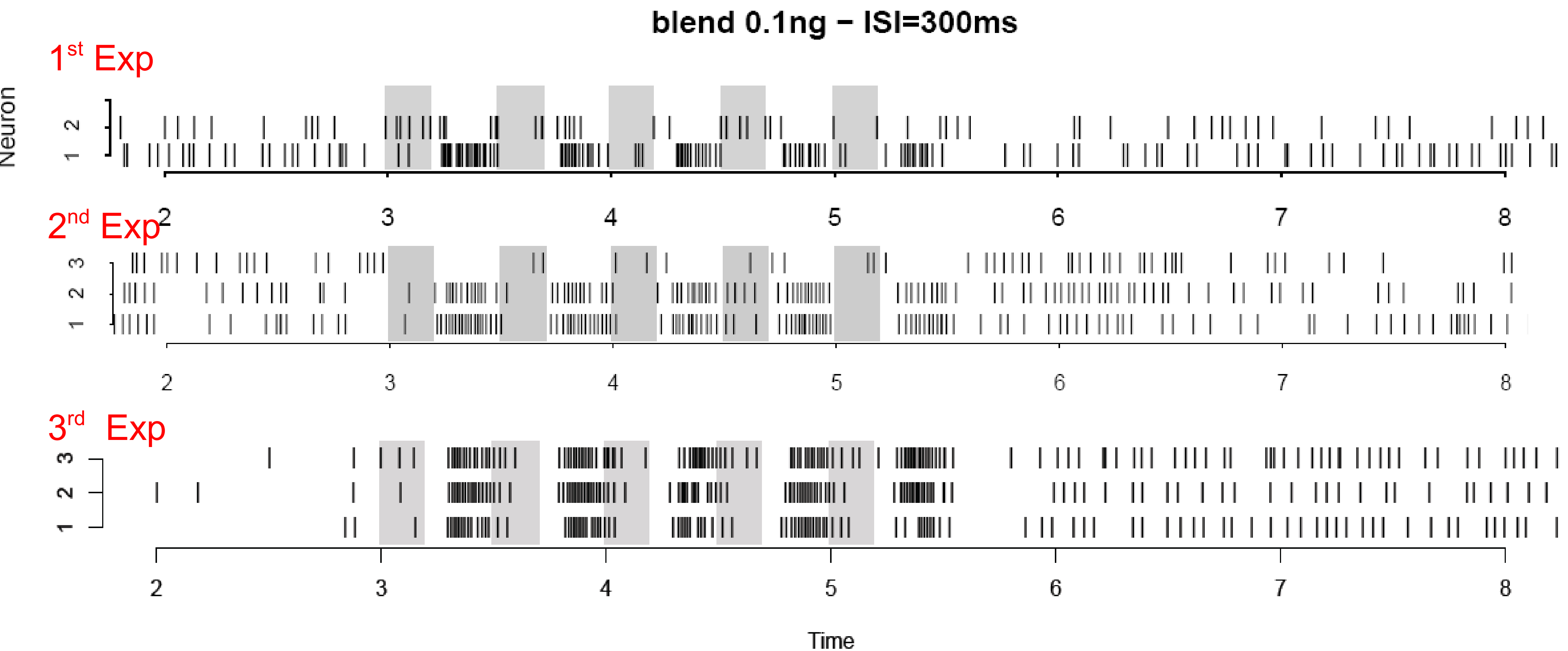
Sigma(ms) = 1,88ms
Rhô = 0,09

From neuron 1 PSTH datas

**Individual level values** (example with 1 neuron and 9 repetitions of a unique odor puff)

# Interactions between simultaneously recorded neurons ?

3 experiments: Puff duration: 200ms, Inter Stimulus Interval: 300ms, 5 pheromonal blend pulses

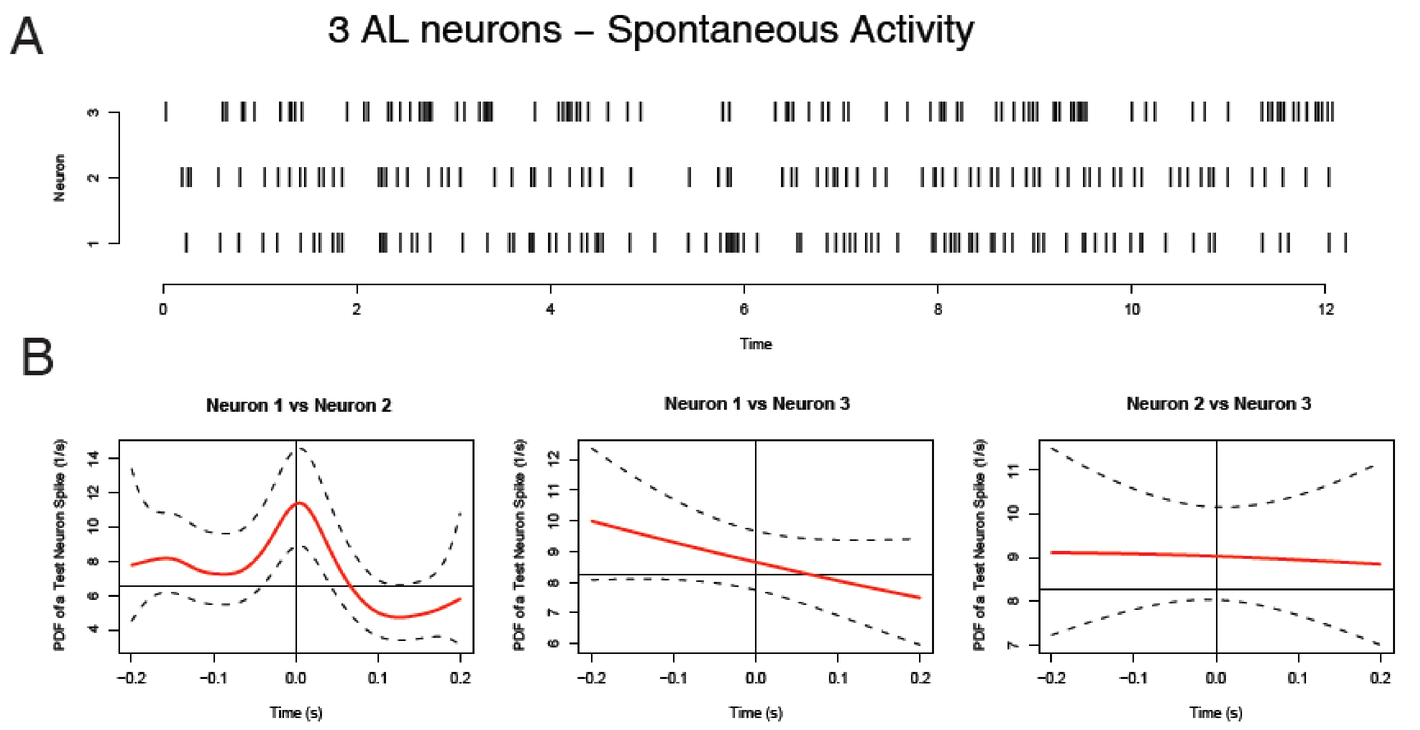



**1st experiment.** 2 simultaneously recorded neurons (after spike-sorting) responding to 5 pulses of the pheromonal blend. : 1 excited (+/- pattern) and 1 inhibited (-) by the pheromonal blend.

**2nd experiment.** 3 simultaneously recorded neurons: 2 synchronized neurons (+/-) and 1 inhibited (-) by the blend.

**3rd experiment.** 3 simultaneously recorded neurons: 3 synchronized neurons (+/-/+).

# Interactions between simultaneously recorded neurons ?



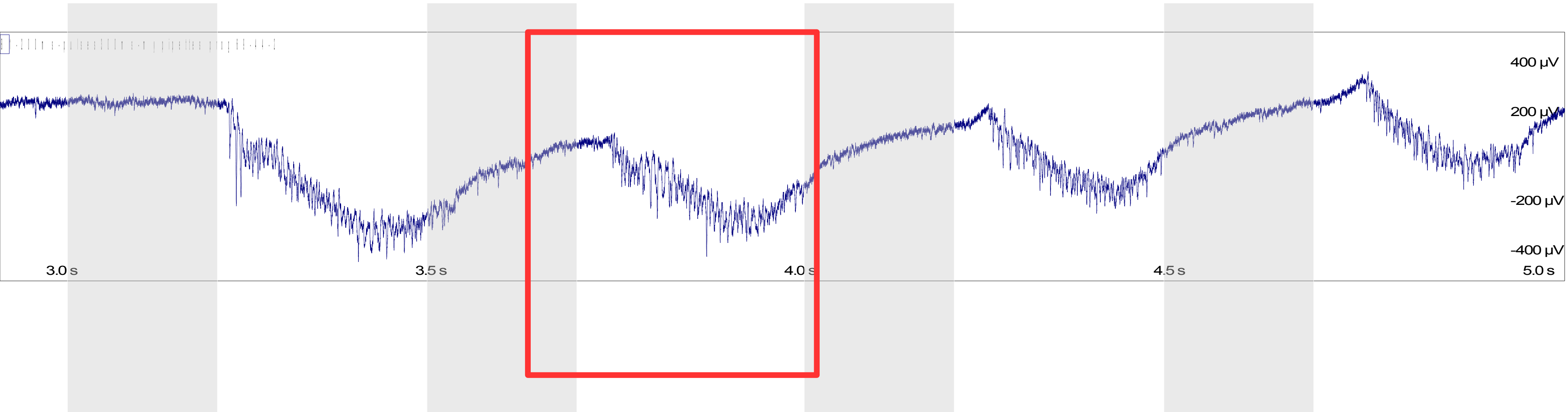

A: rasterplot of 3 neurons
B: cross correlograms

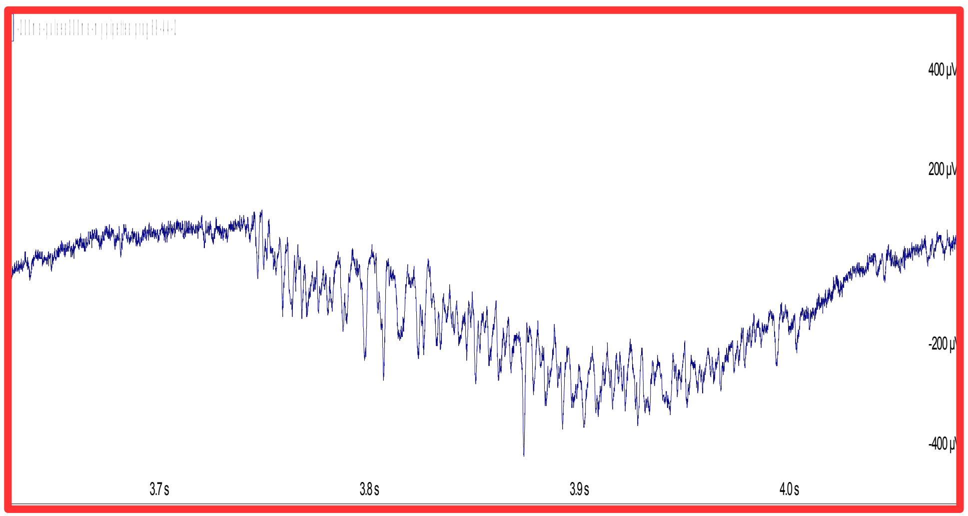
## 2nd Exp details: spontaneous activity

**-> Analysis of correlations between simultaneously recorded neurons revealed correlations** even sometimes during spontaneous activity (neurons 1 and 2):

2 PNs with a common input, interconnected neurons ?

# LFP dynamics and oscillations

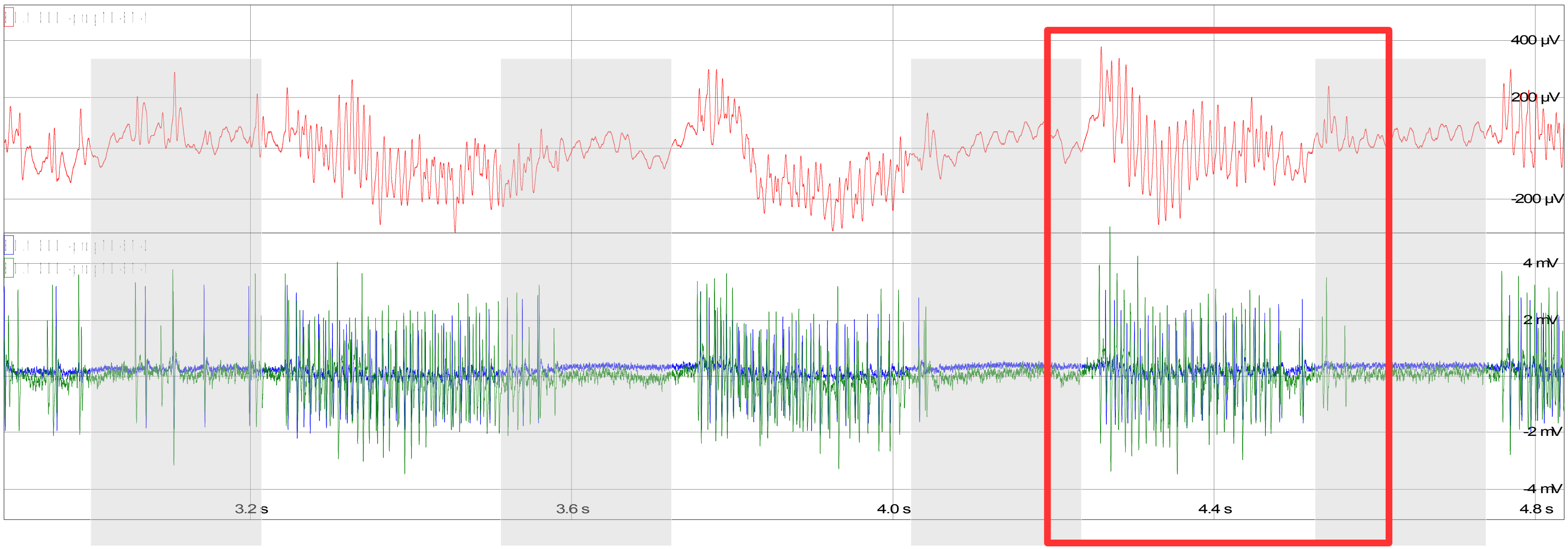


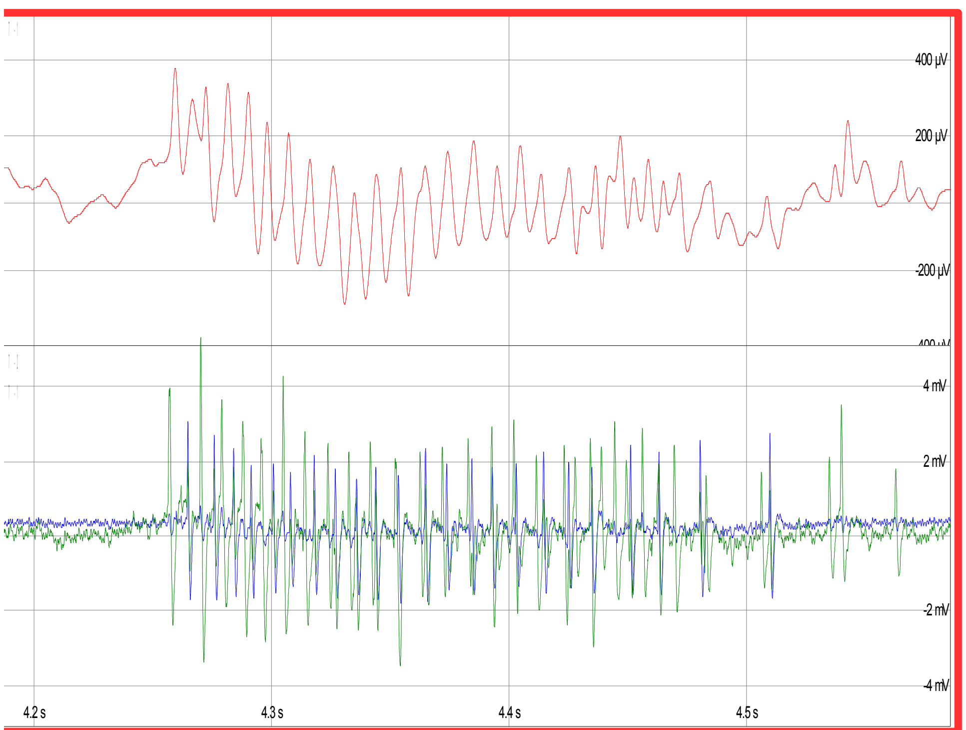




Local Field Potential

Local Field tential :classic response dynamics for a pheromonal pulsatile stimulation in the MGC

# LFP dynamics and oscillations




Local Field Potential :
100Hz oscillation

3 neurons responding to
pheromone pulsatile stimulations

# Conclusions

• Response patterns: Variability across recorded neurons sensible to the pheromonal blend (n=34): different response patterns are observed (70% ' + / - / (+) ': most frequent pattern)

• Quality coding: 51% of the tested neurons (n=35) are 7-12 specific and 37% are generalists

• Quantitative coding: We found a Response pattern switch from a biphasic response (+ / -) to a triphasic response (+ / - / +) at concentrations beetween 0,1 / 1ng in 70% of neurons displaying a +/- pattern at low dose (17 out of 24 neurons)

• Temporal coding:
-69 % of the tested neurons (n=43) can resolve 2Hz pulses (mainly +/-/(+) neurons)
When applying pulsatile stimulations (5 pulses):
-The response for the $1^{st}$ pulse is significatively different from the 4 others: shorter latency and longer bursting duration.
-Burst duration and latency are correlated.
-Triphasic neurons seems to react 50msec faster than biphasic ones (different network?).

• Timing precision/robustness:
- Pairwaise comparisons of pulsed spike-trains reveal very high precision (sigma 3ms, rhô p=0,11) !
-The timing precision or the robustness of the pheromone responses are not increasing over successive pulses repetitions (n=13). The first pulse response is already very precise (compared with the $1^{st}$ pulse of the $2^{nd}$ trial).

• Neurons interactions / LFP:
- Small population recordings reveal interactions between neurons both during spontaneous activity and olfactory responses. Fast oscillations may be observed around 100Hz (n=2).

# To do

• *Analyse what we already have: spike-sorting of all rawdata traces, quantitative analysis, synchronized neurons*

• *Get more datas !*

• *Apply pharmacological blockers*

-> role of inhibition in shaping PN s discharge patterns and synchrony

GABA blockers, Apamin (SK channel)

**Thank You.**

# BONUS TRACKS

## Temporal dynamics and characteristics of sexual pheromone and plant odor mixture olfactory representations displayed by AL neurons

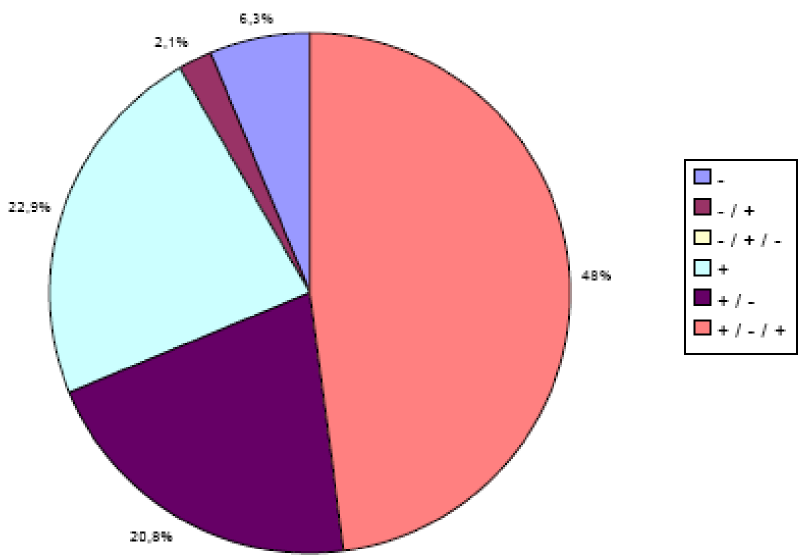
## PART 1: ONLY NEURONS SENSIBLE TO THE PHEROMONAL BLEND & Fixed concentrations

| | PHEROMONAL BLEND 1ng - Observed Response PATTERNS | | | | |
|---|---|---|---|---|---|
| | | A | R | Total | % |
| - | Inhibition | 3 | 0 | 3 | **6,3%** |
| - / + | Inhibition / Excitation | 1 | 0 | 1 | **2,1%** |
| - / + / - | Inhibition / Excitation / Inhibition | 0 | 0 | 0 | **0,0%** |
| + | Tonic Excitation | 6 | 5 | 11 | **22,9%** |
| + / - | Phasic Excitation / Inhibition | 7 | 3 | 10 | **20,8%** |
| + / - / + | Phasic Excitation / Inhibition / Tonic Excitation | 17 | 6 | 23 | **47,9%** |
| | | | | **48** | |

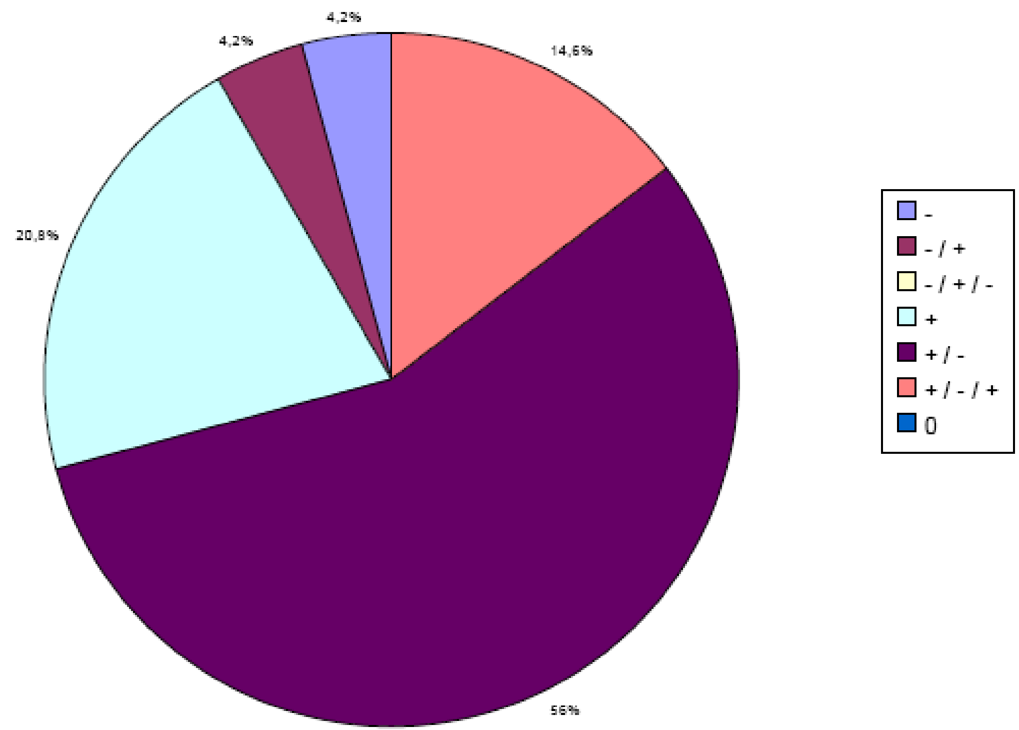
| | Plant Odor: HEPTANAL 100 µg- Observed Response PATTERNS | | | | |
|---|---|---|---|---|---|
| | | A | R | Total | % |
| - | Inhibition | 11 | 2 | 13 | **27,1%** |
| - / + | Inhibition / Excitation | 1 | 0 | 1 | **2,1%** |
| - / + / - | Inhibition / Excitation / Inhibition | 0 | 0 | 0 | **0,0%** |
| + | Tonic Excitation | 3 | 0 | 3 | **6,3%** |
| + / - | Phasic Excitation / Inhibition | 12 | 6 | 18 | **37,5%** |
| + / - / + | Phasic Excitation / Inhibition / Tonic Excitation | 0 | 0 | 0 | **0,0%** |
| **0** | No response | 7 | 6 | 13 | **27,1%** |
| | | | | **48** | |

| | MIXTURE B1ng / HEPTANAL100µg - Observed Response PATTERNS | | | | |
|---|---|---|---|---|---|
| | | A | R | Total | % |
| - | Inhibition | 2 | 0 | 2 | **4,2%** |
| - / + | Inhibition / Excitation | 2 | 0 | 2 | **4,2%** |
| - / + / - | Inhibition / Excitation / Inhibition | 0 | 0 | 0 | **0,0%** |
| + | Tonic Excitation | 5 | 5 | 10 | **20,8%** |
| + / - | Phasic Excitation / Inhibition | 18 | 9 | 27 | **56,3%** |
| + / - / + | Phasic Excitation / Inhibition / Tonic Excitation | 7 | 0 | 7 | **14,6%** |
| **0** | No response | 0 | 0 | 0 | **0,0%** |
| | | | | **48** | |

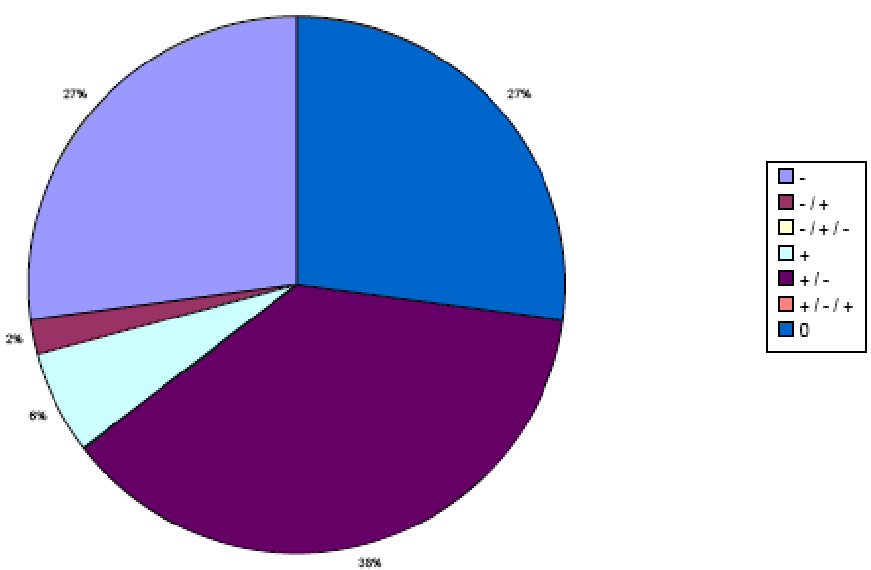
## Pheromone Response PATTERNS

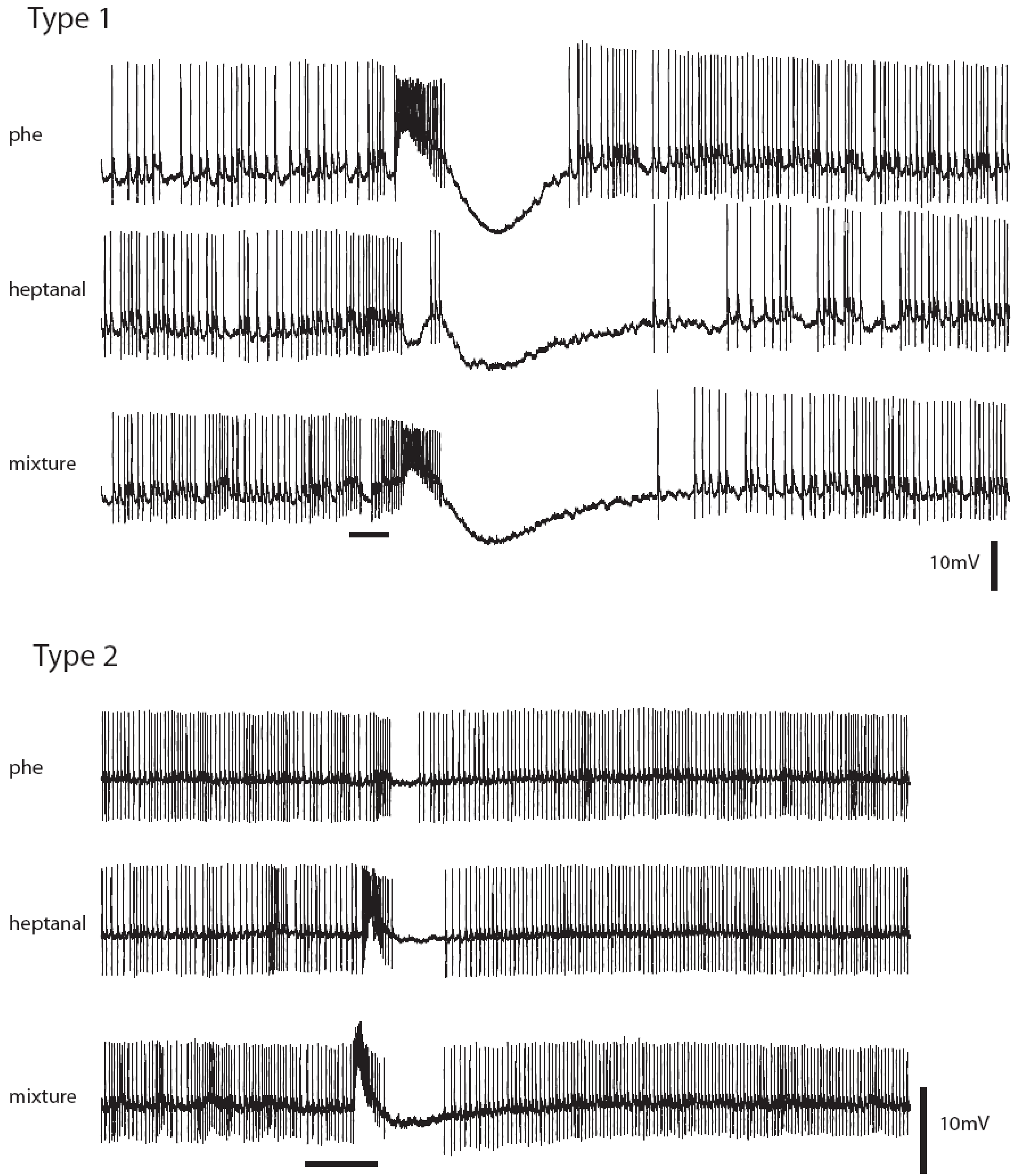


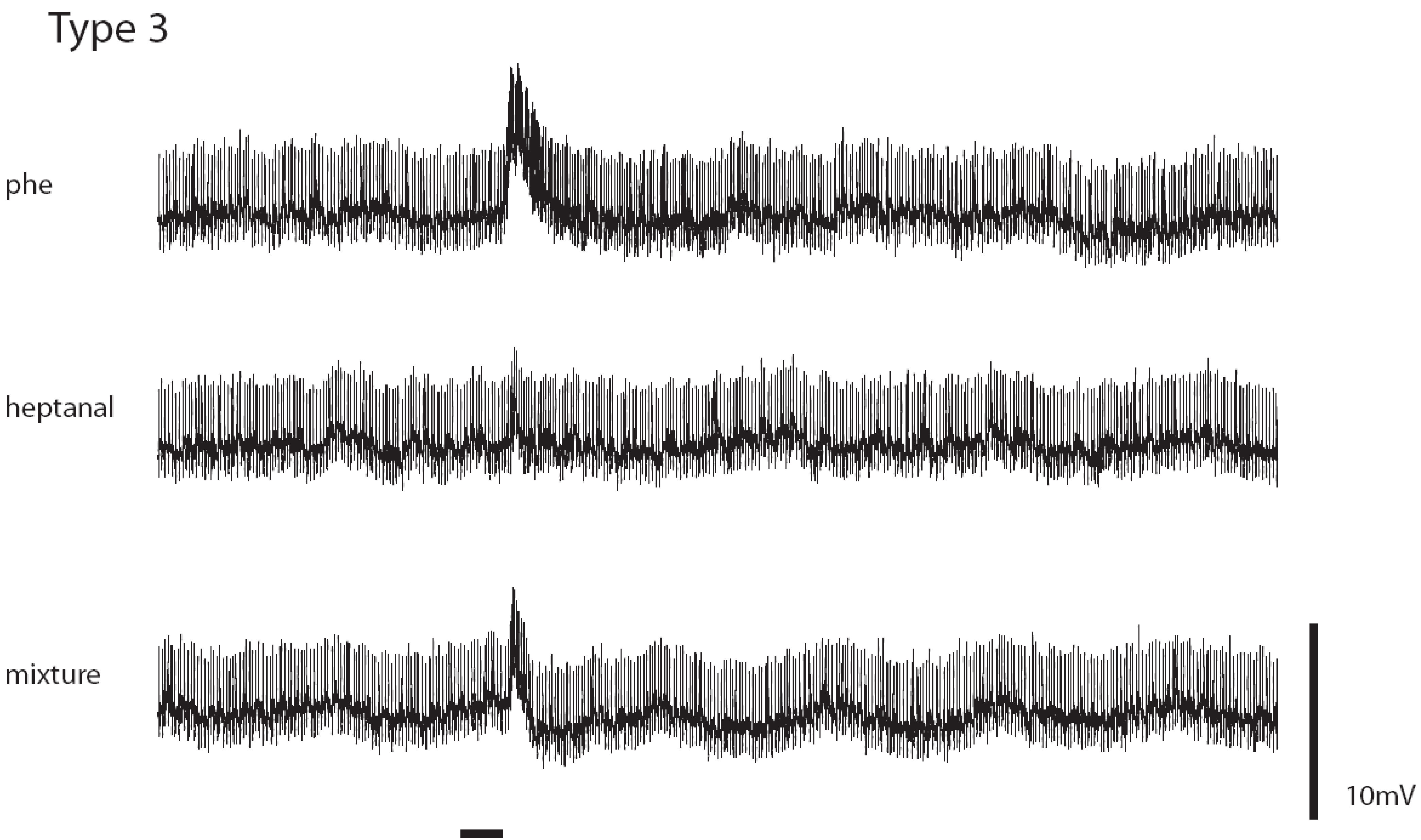

## MIXTURE response PATTERNS

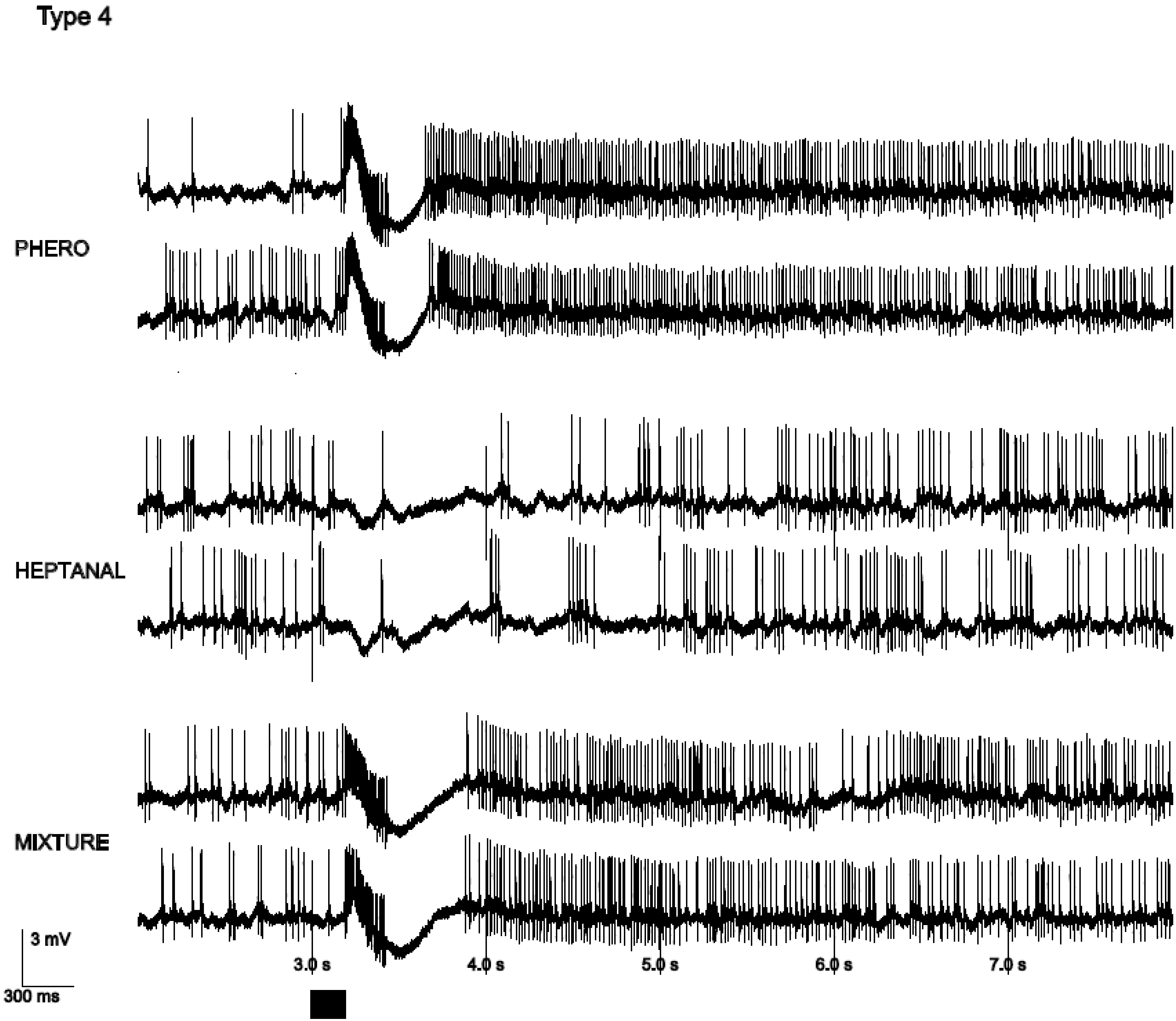



## Heptanal Response PATTERNS




Type 1
phe
heptanal
mixture
10mV
Type 2
phe
heptanal
mixture
10mV


Type 3
phe
heptanal
mixture
10mV



Type 4
PHERO
HEPTANAL
MIXTURE
3 mV
300 ms
3.0 s
4.0 s
5.0 s
6.0 s
7.0 s

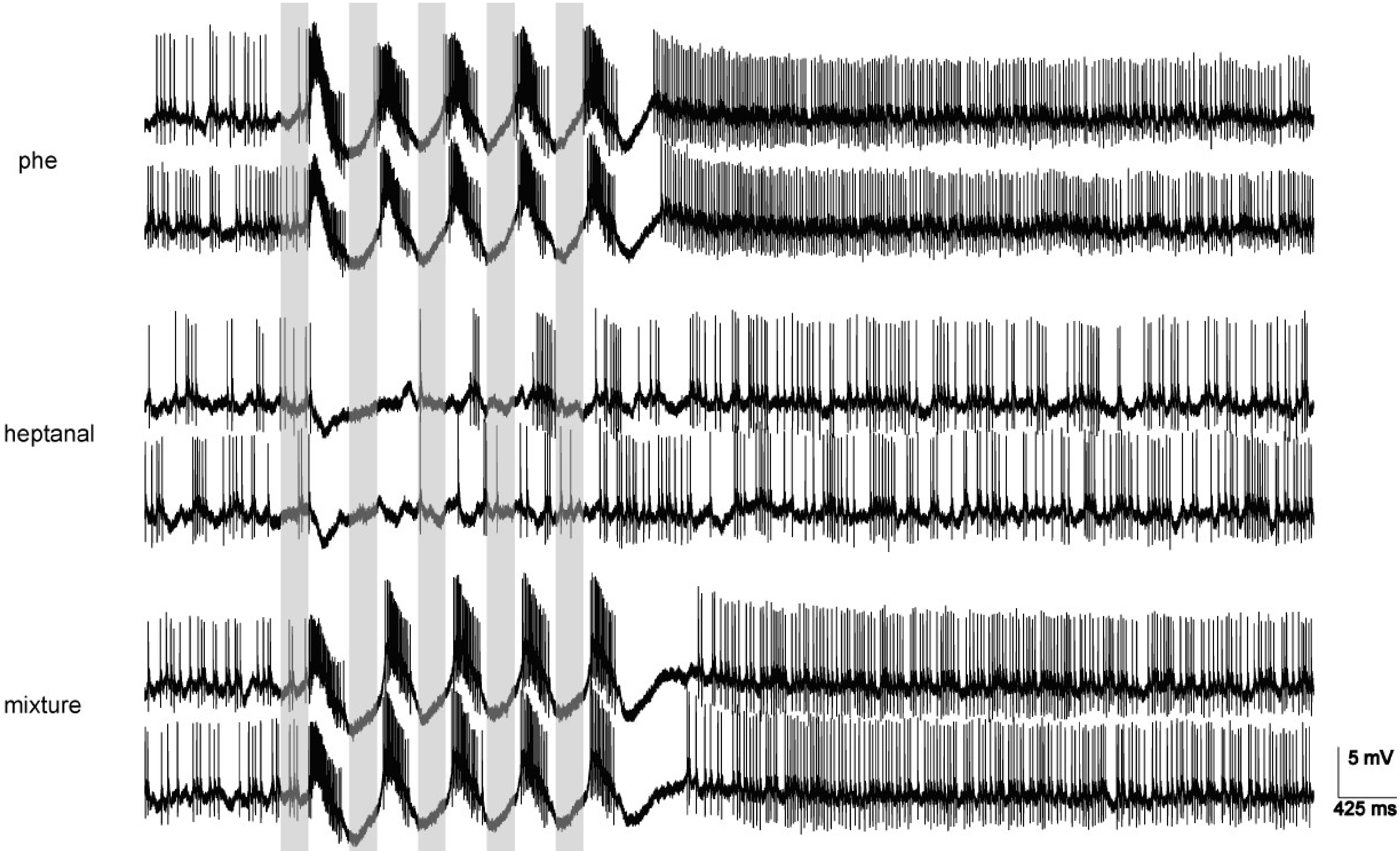



Fig. Aptitude to follow odor pulses. Example from a **Type 4** neuron: **pulsatile stimulations**
with pheromone, heptanal or mixture

**Nb Neurons = 15**

| | **Pheromonal BLEND** | **HEPTANAL** | **MIXTURE** |
|---|---|---|---|
| **Mean** | 4,67 | 1,93 | 4,4 |
| **SD** | 1,05 | 1,91 | 1,4 |

Fig. **Pulse 'score'** from 15 neurons stimulated with 5 pulses of the pheromonal blend, heptanal or the mixture.