Methods in Neuroscience

PLEASE COMPOSE THE 3 THEMES ON SEPARATED SHEETS

Answers can be provided in either English or French

-1st subject: Marc Pananceau - Visual Evoked Potential - 40 minutes

(Responses should be as short, complete and accurate as possible.)

The non-invasive recording technique of the evoked potentials (EP) is broadly used on human, both in research, to study the organization of the nervous system, as well as in clinic, to detect pathologies that could affect it.

Question 1 (3pts. Out of 10): From your knowledge, how would you define what is an EP? Give a definition.

Question 2 (3pts. Out of 10): What would the basic equipment and the methods necessary to collect EPs in an experiment of your choice? Give a diagram of the experimental set-up if necessary.

In their 2005 paper "The clinical role of evoked potentials", Walsh and colleagues used the recording of visual evoked potentials (VEP) to study the organization of the visual system of a human subject. Figure 1 below shows the VEP obtained in a healthy subject in response to the presentation of stimuli either on the entire visual field or on each of the left or right visual hemi-fields.

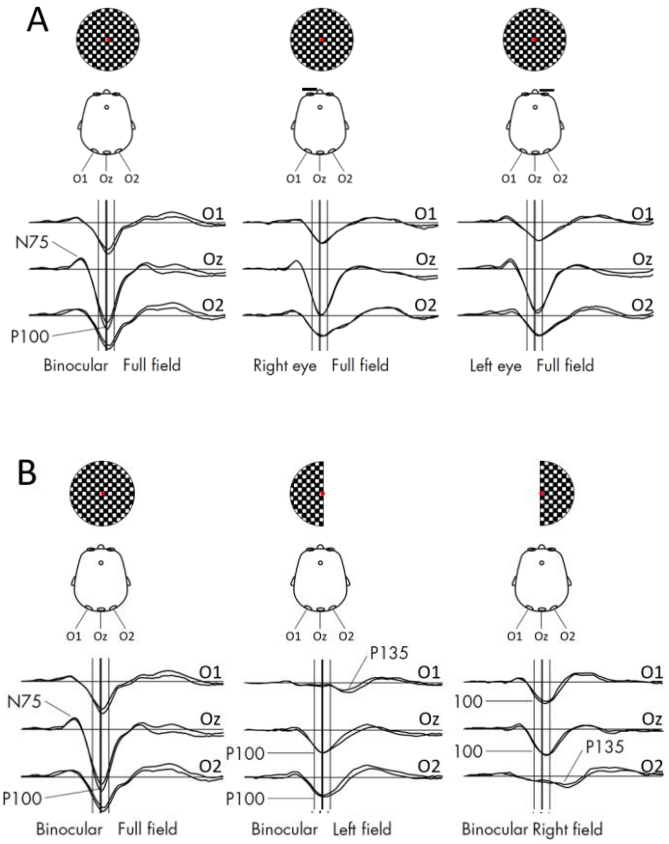


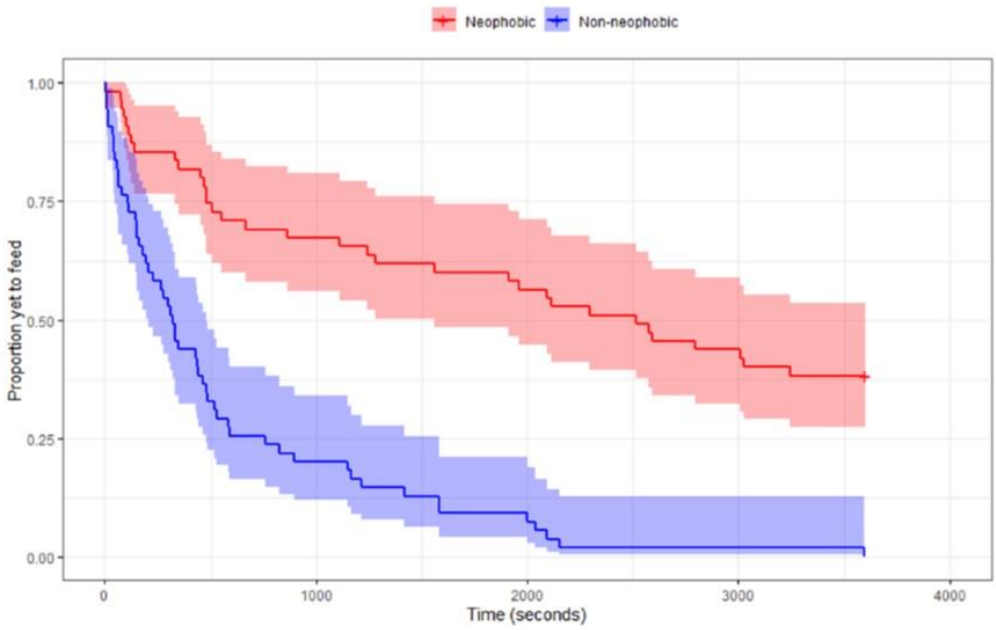
Figure 1: (A) VEP recorded during full field binocular and monocular stimulation of a healthy subject. (B) VEP recorded during full or half field binocular stimulation. In each panel, top depicts the experimental set-up with the visual stimulation screen in front of the head of the subject. At the bottom are shown VEP recorded from O1, Oz and O2 recording sites. During the experiment, the subject had to stare at the fixation point at the center of the screen (red dot).

Question 3 (4pts. Out of 10): What kind of conclusions can you drawn about the organization of the visual system from the results shown on panel A and B of figure 1? Justify your answer.

-2nd subject: Sylvie Granon - Behavioral Neuroscience - 40 minutes

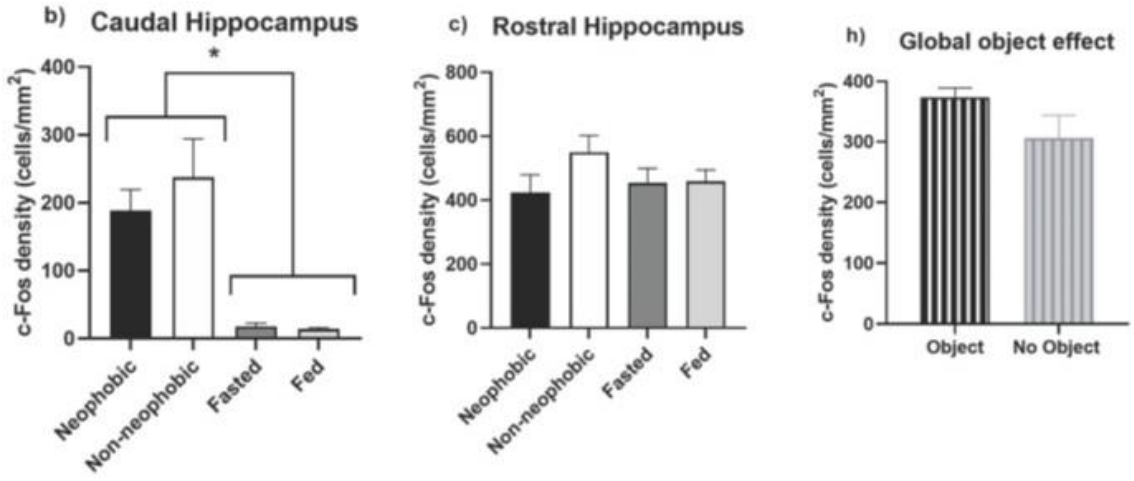
In a research study, neophobia phenotype was assessed using novel object and novel food trials. Then, expression of one immediate early gene (IEG), c-Fos, reflecting neuronal activity in response to novel objects, was measured in house sparrows (*Passer domesticus*), a species exhibiting wide and repeatable individual variation in neophobia. IEG expression in two regions of the dorsomedial hippocampus (one more rostral and one more caudal) was measured. Adult house sparrows (n = 22, 15 males and 7 females) were captured between 28 June and 16 July 2019. Sparrows were housed individually in cages in a vivarium at Louisiana State University with unlimited access to mixed seeds, grit, a vitamin-rich food supplement and water. Animals were classified as neophobic and non-neophobic after an exposure to a novel object and the time to feed when in presence of this object. Neophobic sparrows significantly differed from non-neophobic sparrows in the time to feed in the presence of a novel object (Fig. 1 $p < 0.0001$).

Fig. 1



Question 1. What type of statistical test could be done to estimate whether the difference between neophobic and non-neophobic animals evolve similarly with time (**3 points**).

Fig. 2. Quantification of c-fos expression in the caudal (b) and rostral (c) hippocampus in fasted and fed house sparrows. (h) Global c-fos expression in the entire brain after exposure to an arena containing a novel object or not in all animals taken together. * = $p<0.05$



Question 2. Describe and interpret the results shown in figure 2. Explain what are the role of the fasted and fed conditions (**7 points**).

-3rd subject: Emmanuel Culetto - olfaction, learning, memory and forgetting - 40 minutes

In an effort to identify potential genes involved in associative learning, memory and active forgetting, Hadzi et al. performed a candidate-gene-based test using learning and memory assays in the nematode *C. elegans*.

They investigated the potential role of the Musashi (msi) gene. This gene encodes a protein with an RNA-recognition motif (RRM) which interacts with single-stranded RNAs. There is only one msi gene in *C. elegans*, named *msi-1* and the researcher worked with a loss-of-function deletion allele *msi-1(lf)* of the sole *C. elegans* Musashi gene.

As a first step, they tested the chemotaxis of the loss of function mutant strain *msi-1(lf)* toward different odorants. Results from this analysis are shown on **Figure 1**.

Question 1. Can you interpret these experimental results and indicate the main message shown on **Figure 1** (use the tool phrases: "I observe that ...."; "I conclude that ....").

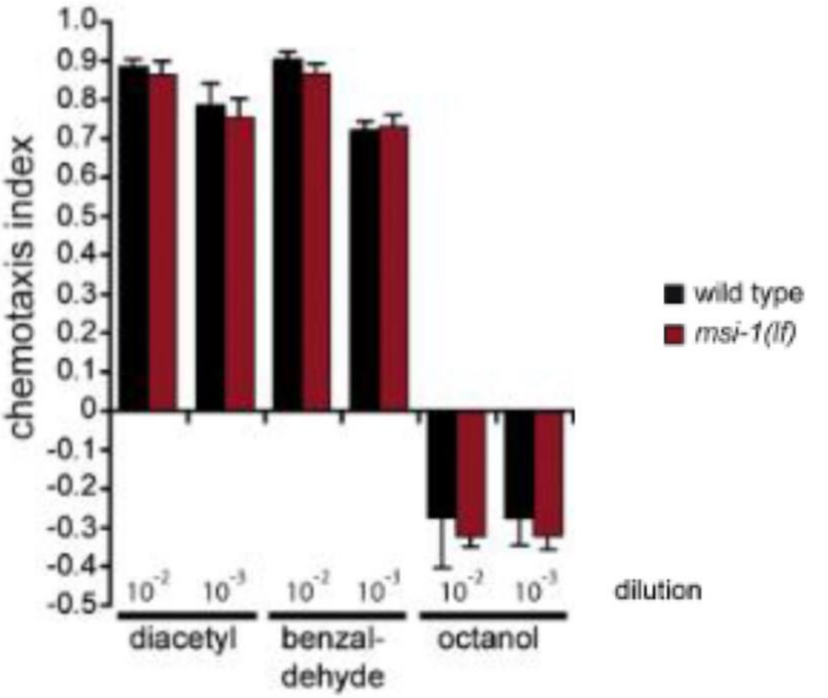


Figure 1. Chemotaxis toward the indicated chemicals was tested in wild-type/control and *msi-1(lf)* worms using the Bargmann test. All experiments were done in triplicate and repeated at least three times. Bars represent mean ± SEM.

Then, the authors tested the role of *msi-1* in the ability of the animals to retain a conditioned behavior over time (short-term associative memory [STAM]). For this experiment, *C. elegans* animals (Wild Type/control and *msi-1(lf)*) were reared in the presence of bacteria (abundant food) from the L1 stage to the young adult stage. Then, conditioning was performed for one hour: worms from each strain are transferred to two plates containing no food. One of these plates contains the olfactory molecule, diacetyl, (0.1% DA) spotted on the lid of the plate (conditioned worms) and the other one contains no odor (naive worms). This protocol gives two populations of animals for each strain, those who experienced no food in the presence of diacetyl (conditioned) and those who experienced no food without diacetyl (naive). Then naive and conditioned worms from both strains were tested with a Bargmann test either right away after conditioning or after a one-hour delay. In both cases, worms have been given a choice between a spot of 0.1% DA in ethanol with 20 mM sodium-azide and a counter spot with ethanol and sodium-azide and after a period of 1 hr, animals were counted and the chemotaxis index was calculated. Results are shown on **Figure 2**.
Question 2: what are the main findings one can draw from this experiment?

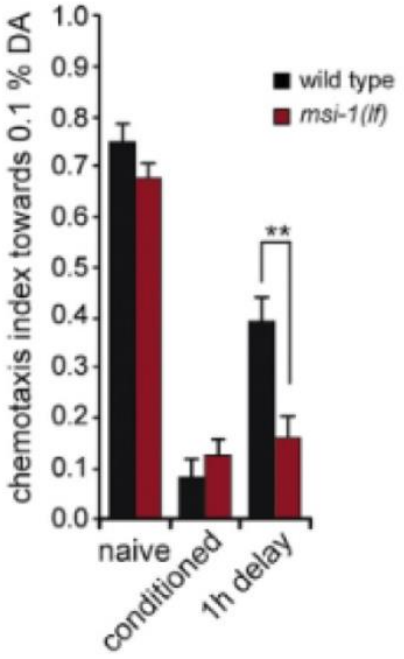


Figure 2. STAM conditioning of WT/control and *msi-1(lf)* worms. Worms were assayed toward DA without (naive) or with (conditioned) preincubation with DA or after 1 hr (1h delay). All experiments were done in triplicates and repeated at least three times. Bars represent mean ± SEM. **p < 0.01, ***p < 0.001.

Subsequently the authors performed a series of complementation assay by expressing, in the *msi-1(lf)* mutant, the *msi-1* minigene/cDNA under the control of different promoters. The wild *type msi-1* minigene/cDNA is therefore expressed in different subgroups of neurons in which *msi-1* is normally expressed. Results are shown on **Figure 3**.

Question 3: What is the purpose/objective of this experiment? Can you interpret these results and present the main finding?

A

promoter	expression in the nervous system
P*msi-1*	RME, RMED, RMEV, RMD, **AVA**,AVE,AFD,SMDV,ADL
P*rig-3*	I1,I4, M4, NSM, **AVA**,PVT
P*nmr-1*	AVE,AVD,RIM, AVG, **AVA**,DVA,PVC

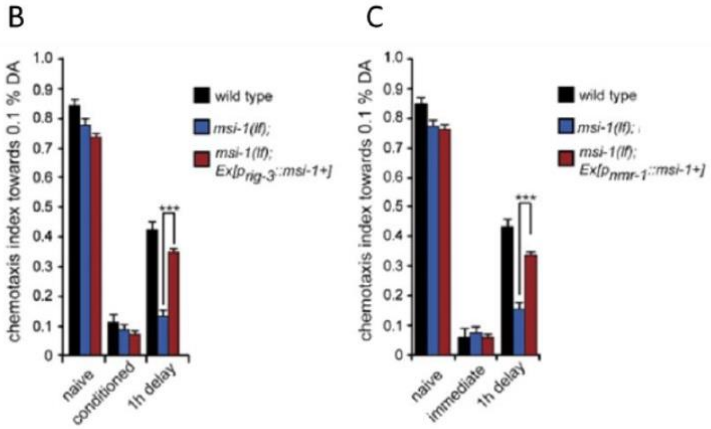


Figure 3. (A) Expression pattern of the *msi-1* promoter (P*msi-1*) and of the two different neural promoters used in B and C. Overlap between nmr-1 and rig-3 promoters with the known msi-1-expressing neurons is highlighted in bold. (B-C) Tissue-specific rescue of the memory loss defect of *msi-1(lf)* mutant worms carrying

the *msi-1* minigene/cDNA under the control of different promoters as indicated. Worms of each transgenic line were conditioned and their preference toward DA was tested immediately (conditioned) or following 1 hr recovery (1h delay). All experiments were done in triplicate and repeated in three independent experiments. Bars represent the average of three independent transgenic lines with (as indicated) or without array (no array) for each construct. Bars represent mean ± SEM. NS, nonsignificant, ***$p < 0.001$.