Master 2 Neurosciences
UE **Réseaux de neurones** (Neuronal networks) - C0SB9506
Monday, the 10th of December - 9h-12h

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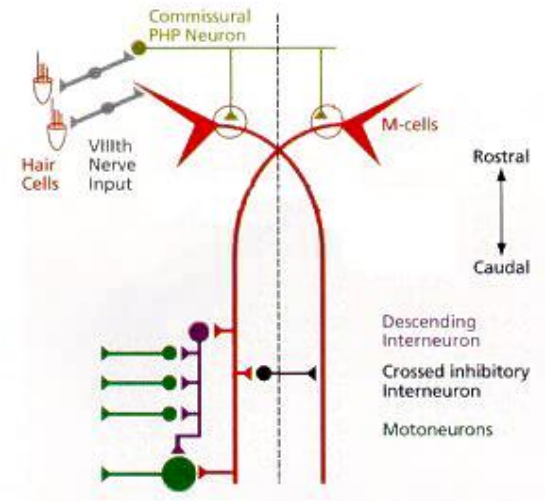
UNIVERSITÉ BORDEAUX SEGALEN

UNIVERSITÉ BORDEAUX 1 Sciences Technologies

Role of reticulospinal interneurons in escape behaviour in fish

Background:
Brainstem reticulospinal neurons (RSNs) serve as the major descending system in vertebrate sensorimotor integration. Escape behaviour in fish is controlled by one of a pair of giant, fast conducting RSNs, called Mauthner (M) cells. Each M cell is activated by strong inputs from hair cells of the ear and lateral line sensory systems via the VIIIth cranial nerve. A single impulse in the M cell on one side is conducted along its contralateral spinal axon to activate axial motorneurons so that the opposite side to the stimulus contracts and shortens. This contraction bends the fish into a C shape before it swims away to safety. An extremely quick mutual feedback inhibitory circuit (via commissural PHP interneurons) ensures that only one M-cell reaches spiking threshold (as the C-start has to be unilateral by definition) and that only one action potential is fired.

Figure 1. Neural circuitry of the M cell which mediates rapid escape behaviour in fish.



Recently the role of the M cell as a command neuron for escape has been studied in zebrafish larvae (Kohashi and Oda (2008); Figures 2,3). In a separate study on the cichlid fish *Astatotilapia burtoni* (Figure 4) changes in the M cell circuit associated with the formation social dominance hierarchies have been investigated (Whittaker et al, 2010 ; Figures 5-8).

Questions:

A. Carefully examine the data presented in the figures 2 to 7 below and provide an account of what can be concluded from them regarding:

1) The role of the M cell as a command neuron in escape behaviour.

2) The M cell circuit as a site for neuromodulation of escape threshold in a social context.

B. Bonus question: what can you conclude from figure 8 with regard to the results of Figure 7 in terms of the possible sites of serotonin modulation of escape behaviour in this fish?

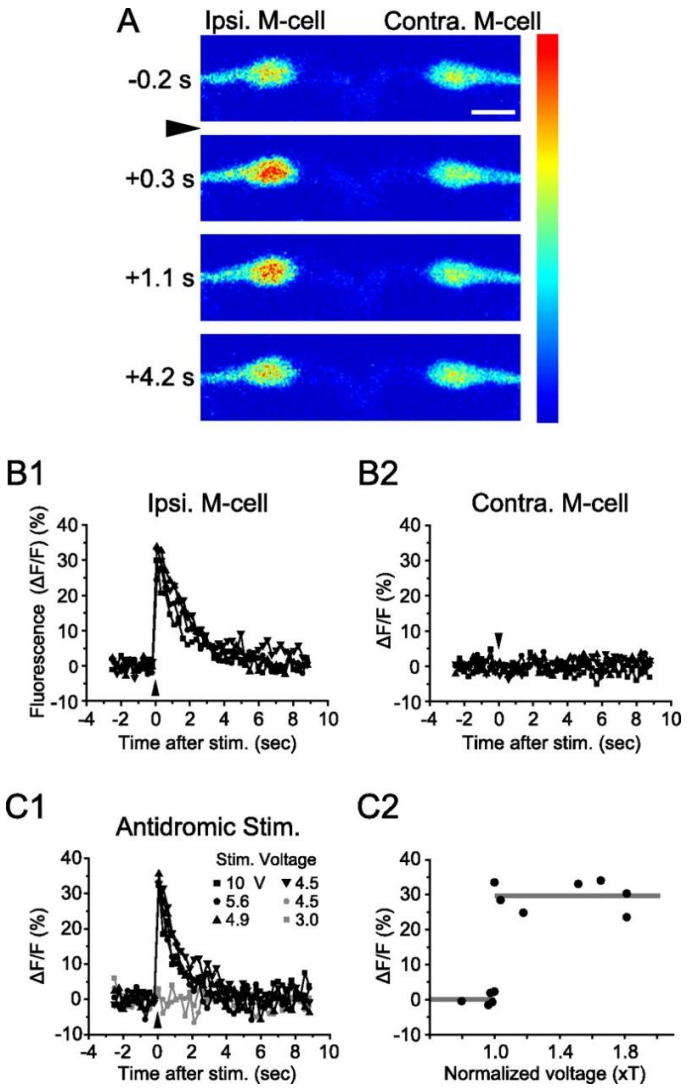


Figure 2. Fluorescence response of the paired M-cells associated with escape.
A , Typical fluorescence responses (pseudocolored images) of the paired M-cells (obtained simultaneously with the tail response: not shown) before (−0.2 s) and after (+0.3, 1.1, and 4.2 s) the water pulse arrival (arrowhead). Scale bar: 20 μm. The color scale on the right represents fluorescence intensity (blue, lowest; red, highest). ***B*** , Fractional changes in fluorescence (Δ*F*/*F*; ordinate) in the somata of the M-cells were plotted against the time after stimulus arrival (abscissa). The responses of four trials obtained from the pair shown in ***A*** are superimposed (data exemplified in ***A*** are shown as triangles). ***C1*** , Fluorescence responses associated with an antidromic spike of the same ipsilateral M-cell in response to electrical stimulation applied to the spinal cord, which appeared in an all-or-nothing manner with different stimulus intensity (volts) as denoted. The amplitude of suprathreshold fluorescence responses is comparable with that of the large sensory-evoked responses in ***B1*** . ***C2*** , The amplitude of the fluorescence response (ordinate) associated with the AD spike of another M-cell is plotted against the intensities of spinal cord stimulation (abscissa; normalized by the threshold voltage, *T*). Note the constant amplitude of fluorescence at the suprathreshold stimulus (average amplitude, 29.6%; right gray line). The left gray line indicates 0%.

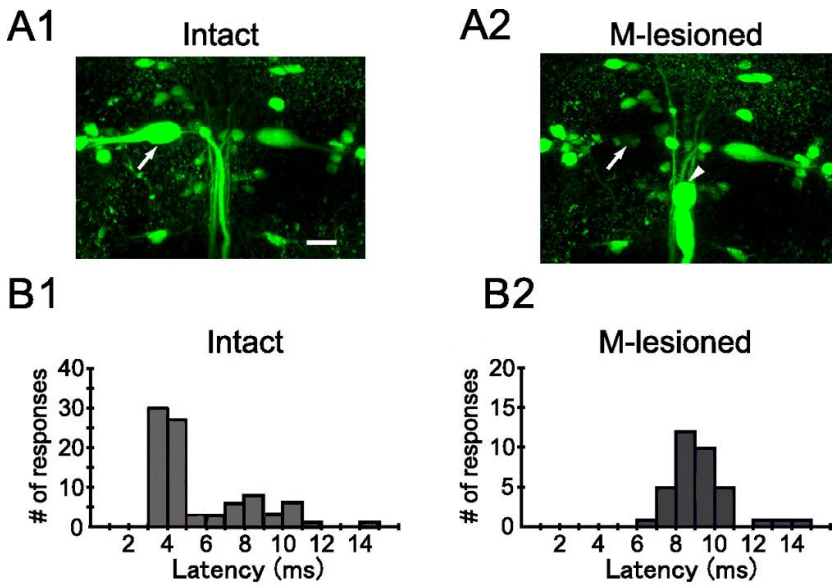


Figure 3. Effects of laser ablation of M cells on escape.
A, Fluorescence images of reticulospinal neurons before (***A1***) and a day after (***A2*** ; M-lesioned) laser irradiation aimed at the left M-cell soma (arrows). The loss of fluorescence from soma and the stumped axon of the irradiated M-cell (arrowhead) without effect on nearby neurons show that the M-cell was successfully and selectively killed. Scale bar, 20 μm. ***B***, Frequency distribution of onset latencies of escapes elicited by the OV stimulation in intact (***B1***) or M-lesioned (***B2***) fish.

Figure 4. **The African cichlid *Astatotilapia burtoni* is a powerful model system in social neuroscience.**
In this species, males alternate between two socially dependent phenotypes throughout their lives. When an individual is dominant (DOM) in a social group, he maintains a territory, bright body coloration, and displays a vigorous behavioural repertoire to defend his territory and attract females. In contrast, when subordinate (SUB), males will shoal with conspecifics and females, display fewer and less conspicuous behavioural patterns, and adopt cryptic coloration that allows them to blend in with the surrounding environment. Based on the differences in coloration and behaviour, DOMs are more vulnerable to predation than SUBs.

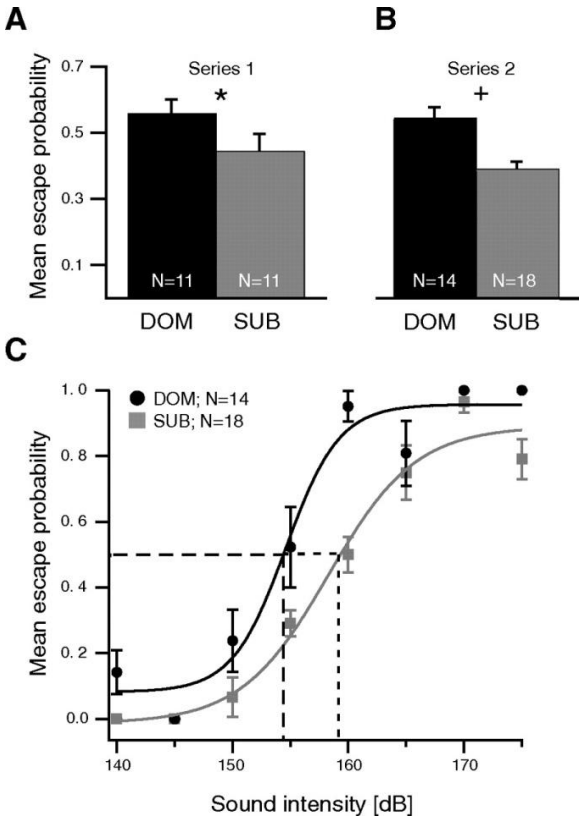


Figure 5. Dominance hierarchies and escape behaviour.
A and ***B***: mean escape probabilities (±SE) in dominants (DOMs) and subordinates (SUBs) for auditory stimulus. Note: these results involved fish populations used for experiments in different laboratories, underlining the reproducibility of the results. *Series 1*: **P* = <0.025; *series 2*: +*P* < 0.01. ***C***: stimulus–response curves for DOMs (black) and SUBs (gray) from the data in *B* (means ± SE). Narrow and wide-gaped dashed lines indicate the stimulus intensity that evokes a response 50% of the time.

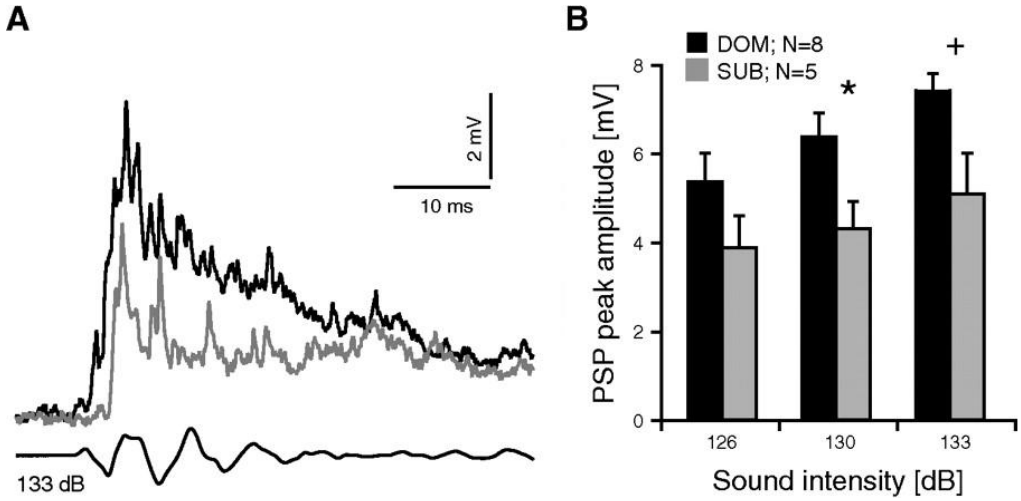


Figure 6. Modulation of M cells in dominance hierarchies.
Differential Mauthner cell (M-cell) responses in DOMs and SUBs. ***A***: somatically recorded postsynaptic potentials (PSPs; (averages of 5 sweeps each) in a DOM (black) and a SUB (gray) in response to a 133 dB sound pip. Bottom trace: microphone recording of the sound stimulus. ***B***: plots of mean PSP peak amplitudes (±SE) for 3 sound intensities. **P* = 0.026; +*P* = 0.018.

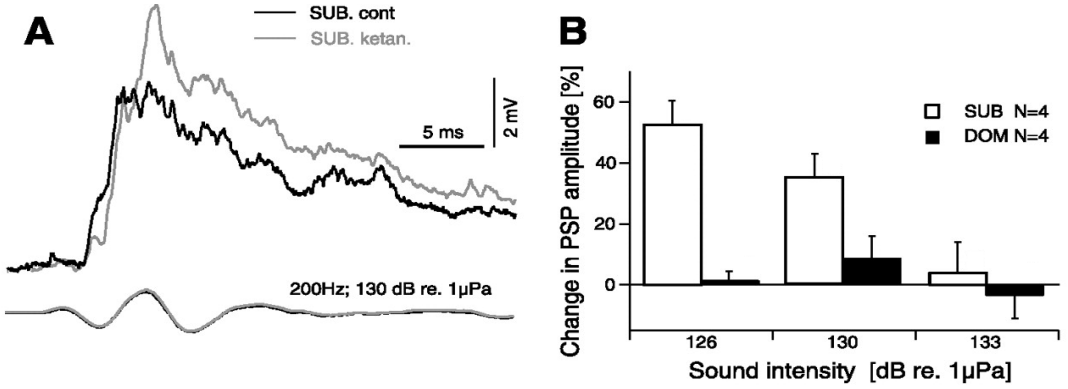


Figure 7. Serotonin receptors and M cell modulation.
Differential effects of ketanserin on M-cell properties in SUBs and DOMs. ***A***: somatically recorded postsynaptic response (PSPs, averages of 5 sweeps) in a SUB in response to a sound pip before (black) and after (gray) the administration of the 5-HTR_{2A} antagonist ketanserin. *Bottom* trace shows a record of the sound stimulations. ***B***: quantification of the effects of ketanserin on the PSP peak amplitude in SUBs and DOMs (means ± SE). The histogram shows the changes in PSP amplitude in response to different stimulus intensities relative to controls after application of ketanserin.

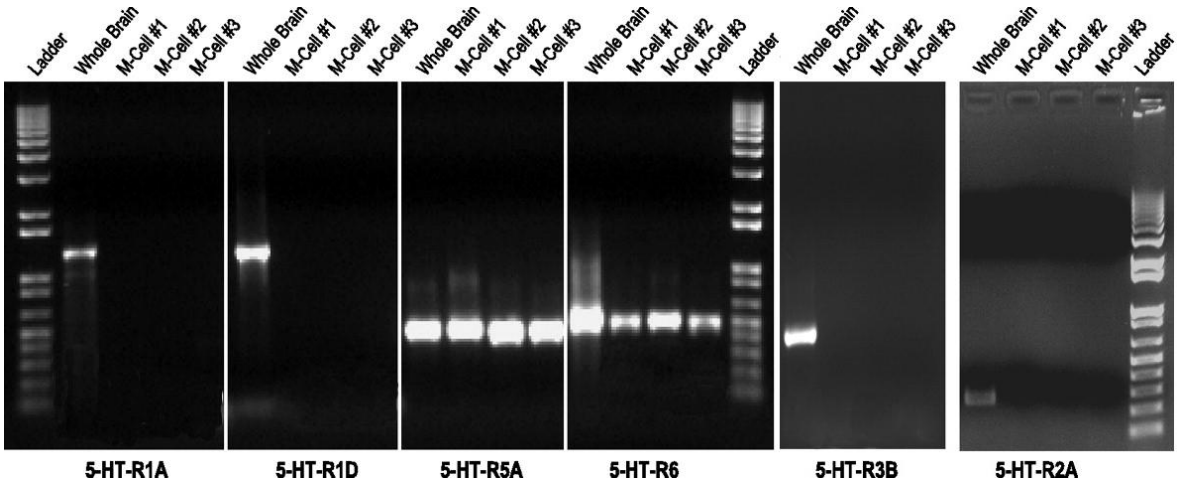


Figure 8. Serotonin receptors expressed by M cells.
Expression of 5-HTR subtypes in single M-cells. mRNAs extracted from a homogenized whole brain and 3 different M-cells were used as the template for a PCR reaction with primers specific to 5-HTR subtypes. All subtypes are present in the brain, as expected (see "whole brain trace), but not in individual M-cells.

RNA extraction protocol (for your information; you don't have to comment this protocol)**.**
RNA was isolated from whole brain of three *A. burtoni* individuals (1 DOM, 1 SUB, 1 female) and from single M-cells of three fish. For the whole brain sample, the tissue was homogenized in 500 µl of TRIzol (Invitrogen) and processed according to the manufacturer's instructions. Pellets were redissolved in RNA Storage Solution (Ambion). For single-cell RNA isolation, we harvested the cytoplasm of one M-cell each from one DOM, one SUB, and one female at the end of the respective electrophysiological recording session. We aspirated the cytoplasm with a low-resistance (2–4 MΩ) glass electrode pulled from 3-mm

glass with a vertical puller (PE-22, Narishige) filled with a 2.5 M KCl solution made up with RNase-free water. After penetration of the M-cell, we applied a small negative pressure for ~15–20 min with a 60-ml syringe and aspirated the cytoplasmic contents into the tip of the electrode. During aspiration we monitored the M-cell RMP and, every 4 s, the amplitude of an antidromically evoked M-cell spike (i.e., input resistance) to verify a good seal. The electrode was then quickly removed, the tip broken off, immediately frozen in powdered dry ice, and stored at −80°C until RNA isolation and amplification. To avoid contamination from extracellular sources, the electrodes were coated before the experiment with a film of Sigmacote (Sigma; neutral, hydrophobic film on the electrode surface), which repels water, retards clotting, and prevents adsorption of macromolecules. We extracted the RNA by previously established protocols.

The figures presented here are from the following articles:
Zottoli and Faber (2000) The neuroscientist 6, 26-38
Kohashi and Oda (2008) J. Neuroscience 28, 10641-10653
Neumeister et al (2010) J. Neurophysiol 104, 3180-3188
Whitaker et al (2011) J. Neurophysiol 106, 127-137

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UNIVERSITÉ BORDEAUX SEGALEN

Master 2 Neurosciences
Neural networks
Monday, the 4th of December - 9h-12h

Jean-Marie CABELGUEN

UNIVERSITÉ BORDEAUX 1 Sciences Technologies

Role of sensory inputs in the control of the transition from stance-to-swing in walking cats. Duration : 1h

Physiological data from a decerebrated cat walking on a treadmill

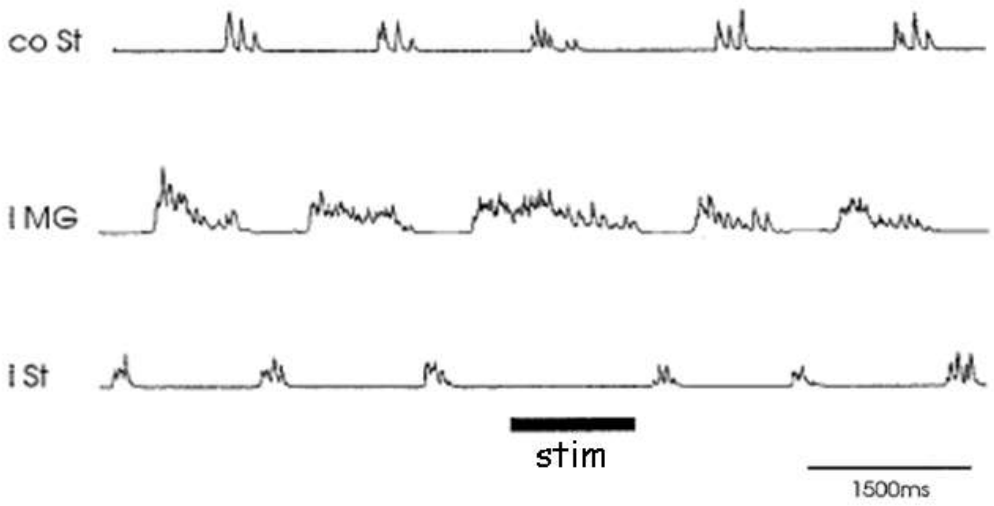

Fig.1. Effects of stimulating the group I afferents in the nerves supplying an ankle extensor muscle group (*gastrocnemius lateralis+soleus*). The black bar (*stim)* indicates the time of stimulation.
From top to bottom: rectified and filtered EMG of hindlimb muscles during spontaneous stepping on a treadmill.
co St : contralateral knee flexor (*semitendinosus*);
i MG : ipsilateral ankle extensor (*gastrocnemius medialis*);
i St: ipsilateral knee flexor (*semitendinosus*).

Physiological data from chronic spinal cats walking on a treadmill.

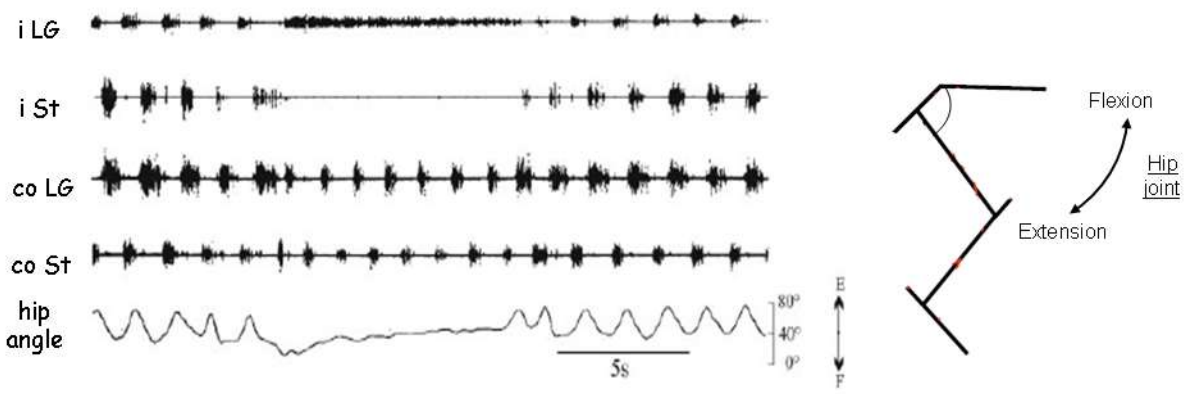

Fig. 2. Effects of manipulation of the hip.
Left panel: EMGs were recorded from flexor and extensor muscles of each hindlimb of a chronic spinal cat walking on a treadmill. The hip angle (lower trace) was continuously monitored (E: hip extension, upwards; F: hip flexion, downwards). The ipsilateral limb was lifted and held during the stance phase, then the hip was progressively extended to the point at which swing was normally initiated in the steps preceding this. i LG: ipsilateral ankle extensor (*gastrocnemius lateralis*); i St: ipsilateral knee flexor (*semitendinosus*). co LG : contralateral ankle extensor (*gastrocnemius lateralis*); co St : contralateral knee flexor (*semitendinosus*).
Right panel: Schematic drawing of the cat hindlimb.

From these physiological studies, it has been concluded that the stance-to-swing transition is influences by at least two sensory signals (Duysens & Pearson, 1980; Grillner & Rossignol, 1980; Whelan et al., 1995).

1. What are these two signals? **Specify the sensory afferents involved.**

However, physiological studies did not provided information on the relative importance of these two sensory signals.

2. Why this information cannot be obtained in physiological experiments?

3. Do you think that the aforementioned findings can confidently be extended to walking in intact cats?

Computer simulations

In an attempt to evaluate the relative roles of the two sensory signals, Ekeberg and Pearson (2005) used computer simulations that incorporate known properties of neural networks, muscles, and body mechanics of the hindlimbs of walking cats.

4. Give the main advantage of computer simulations to address this issue.

5. Do you know another approach? Give its limitations for addressing the present issue.

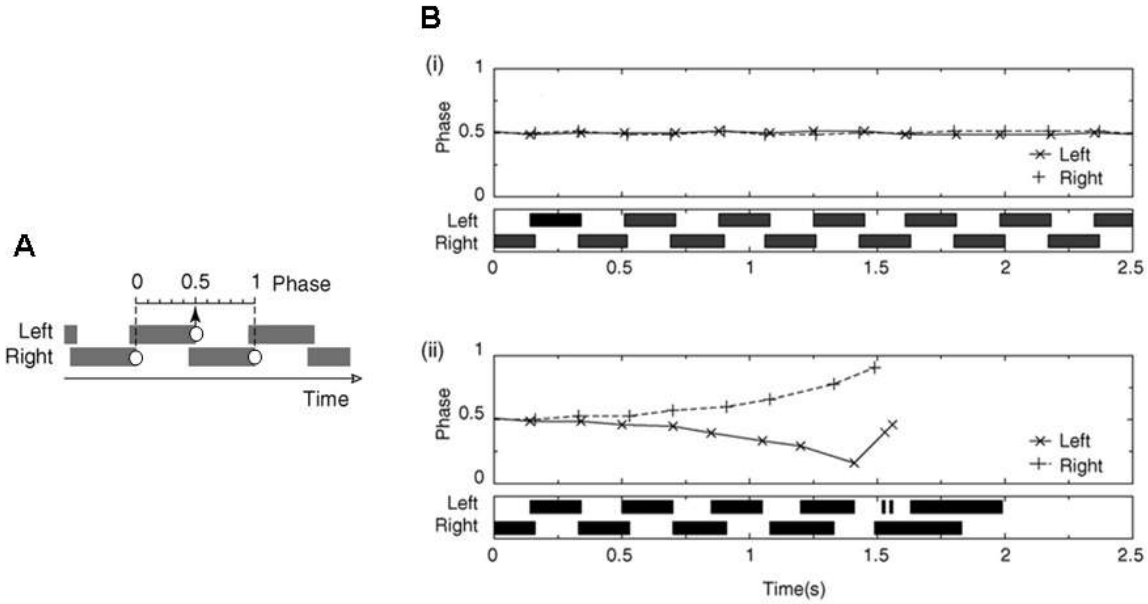

Fig. 3. Computer simulation of the gait patterns in the hindlimbs of the cat.
A. Phase plots were constructed by measuring the time of swing onset in one leg (arrow) relative to the timing of the preceding and following swing onsets in the opposite leg (phase values of 0 and 1). The dark bars represent the time when the foot was in contact with the ground and the three white dots indicate the swing onsets used in this particular phase measurement.
B. Phase plots showing the pattern of coordination when the stance-to-swing transitions were initiated by sensory signal from the ankle extensors (i), and by sensory signals from the hip (ii).

6. What do these computer simulations suggest?

7. Which additional computer simulations do you suggest to assess the whole picture about the role of the sensory feedback in the stance-to-swing transition?

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