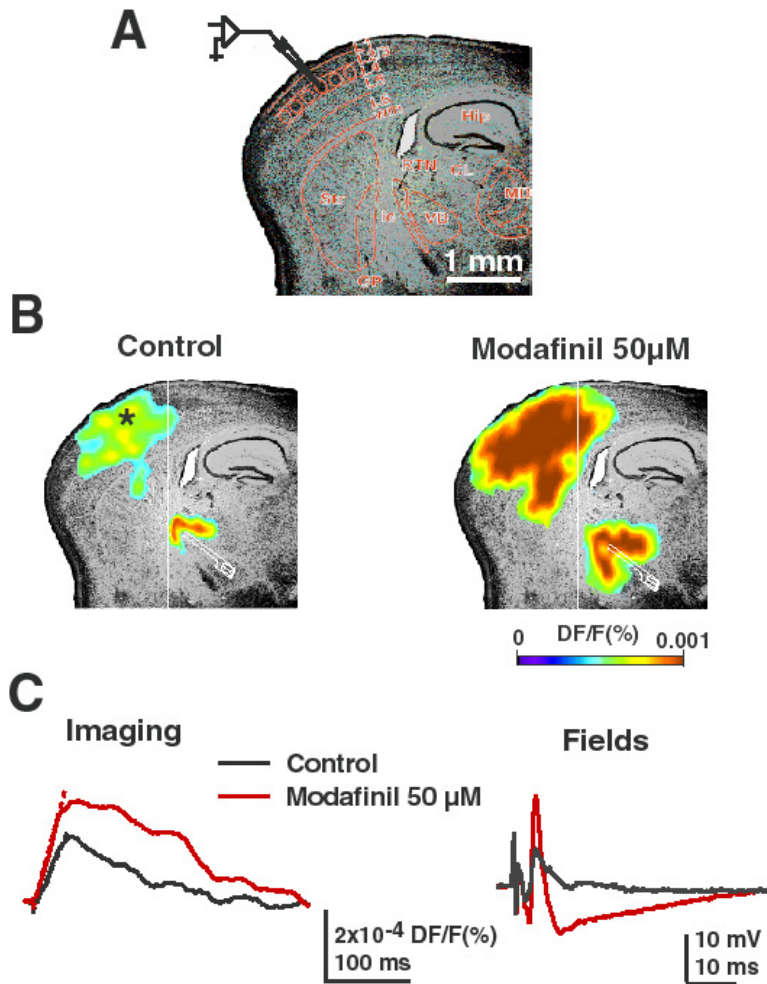


# MODAFINIL ENHANCED THALAMOCORTICAL ACTIVATION: AN IN-VITRO VOLTAGE DEPENDENT IMAGING STUDY

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Cortical and thalamocortical *in vitro* slice preparations were used to study the effect of Modafinil on brain tissue from rats and mice, using voltage dependent dye imaging and extracellular field potentials. The paradigm allowed specific activation of the somatosensory "barrel" cortex via direct electrical stimulation of white matter or of the thalamic ventrobasal nucleus, respectively. Initially the effects of Modafinil were addressed by combining voltage-sensitive dye imaging (dye RH-795, Molecular Probes) and extracellular field potentials.

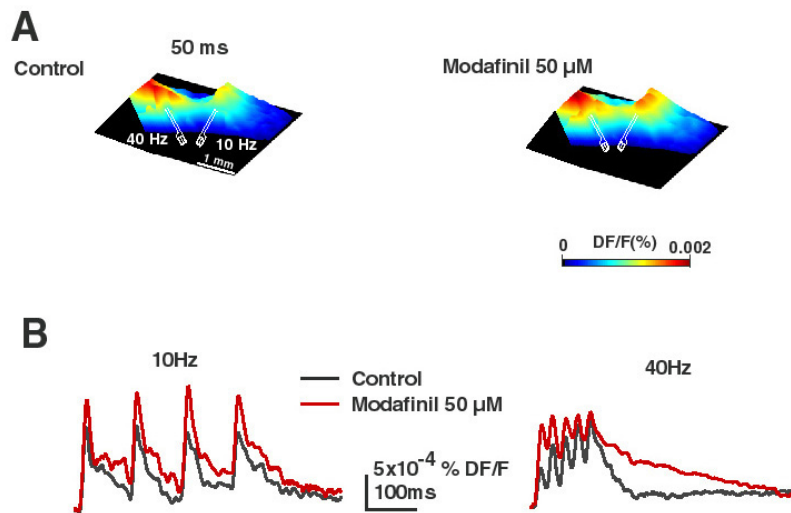


A. Digital image of a Nissl-stained mouse thalamocortical slice. Nuclei and layer subdivisions are demarcated with red lines. Hip, hippocampus; ic, internal capsule; Str, striatum; GP, globus pallidus; VB, ventrobasal nucleus; CL, centrolateral; MD, mediodorsal; wm, white matter; L1, 2/3, 4, 5, 6, different cortical layers. Borders of the cortical "barrels" are outlined for illustration. Position of pipette for extracellular field recordings on the cortex is indicated. B, Spread of voltage-sensitive dye responses after a

VB single shock stimulation before (Control) and after application of Modafinil 50 $\mu$ M are shown on top of a Nissl-stained thalamocortical slice. Note that after VB stimulation, Reticular nucleus, Striatum and cortex were much more activated in the presence of Modafinil compare to Control. Color scale for fluorescence DF/F intensity is from 0 (blue) to 0.001% (red). C, Comparison of pixel profiles (left) and fields recordings (right) taken from layer 4 (see asterisk in panel B). Both pixel profiles and fields were facilitated following the Modafinil application (in red). Furthermore, in the presence of Modafinil the rising slope of the pixels profiles clearly increased (0.7 and 1.3 in Control and Modafinil, respectively). Thalamocortical slices from mice were obtained as previously described (Agmon & Connors, 1991).

Modafinil (50  $\mu$ M) enhanced thalamocortical voltage-sensitive dye responses as shown in Figure 1B. Indeed, at 50  $\mu$ M Modafinil a greater spread of activity was observed on the thalamus, striatum/putamen, and cortex after a single shock stimulation. Figure 1C illustrates Modafinil's increase of both amplitude and rising slope (dotted lines; 0.7 and 1.3 for control and Modafinil, respectively) of the response. This facilitation continued after 30 minutes washout (n=3 slices) as did the amplitude of the extracellular field potentials recorded in layer 4 (Figure 1C, fields). Similar results were obtained from 19 other slices.

The effects of Modafinil were also studied directly on the cortex using coronal somatosensory slices. In this case, two bipolar electrodes were placed on the white-matter (~1 mm of separation) delivering both high (40 Hz) and low frequencies (10 Hz), allowing the study of Modafinil following repetitive stimulation at different frequencies. Figure 2A show typical cortical voltage-sensitive dye imaging control responses and the field potentials at (50, 150 and 250 ms).



**Figure 2: Spatiotemporal patterns of cortical activity in the presence of Modafinil.**

A. 3-D snapshots illustrate the spread of cortical activity for the same slice at t=50, when 40Hz + 10Hz white matter stimulation was used in absence (Control) and presence of Modafinil. Stimulation electrodes were separated by ~1.0 mm.

Both area and amplitude of both low and high frequency cortical responses were larger after Modafinil. Color scale for fluorescence DF/F intensity is from 0 (blue) to 0.002% (red). **B.** pixel profiles taken from layer 5 during 10 Hz (left) and 40 Hz (right) stimulation for both control and Modafinil. Note that during 40 Hz stimulation was also able to increase duration of the steady state activity. After Modafinil (50  $\mu$ M), both frequency-dependent components were facilitated in both amplitude and area. The 40 Hz increase was larger at the initial events but the steady-state change was only slightly enhanced. Figure 2B compares the pixel profiles obtained from the L5 area “on beam” for both 10 and 40 Hz electrodes, in both control and Modafinil conditions. Similar results were observed on 12 different slices. Moreover, there was also an increment on the duration of the voltage-sensitive responses at steady state (i.e., after the peak of the responses was reached), being bigger after a 40 Hz train of stimulation (Figure 2B, 10 Hz and 40 Hz). Cortical slices were obtained from rats.

Since this increment of the steady state by Modafinil may be viewed as an action through some of the metabotropic receptors present on the cortex, our first pharmacological step was to manipulate muscarinic ACh receptors. Thus, we studied the effects of Modafinil (10  $\mu$ M) in the presence of a “cocktail” of well-known non-competitive muscarinic antagonists (atropine and scopolamine). As shown Figure 3, the presence of AChR-antagonists (Figure 3B) didn’t reduce the Modafinil-mediated facilitation of the thalamocortical responses (Figure 3A).

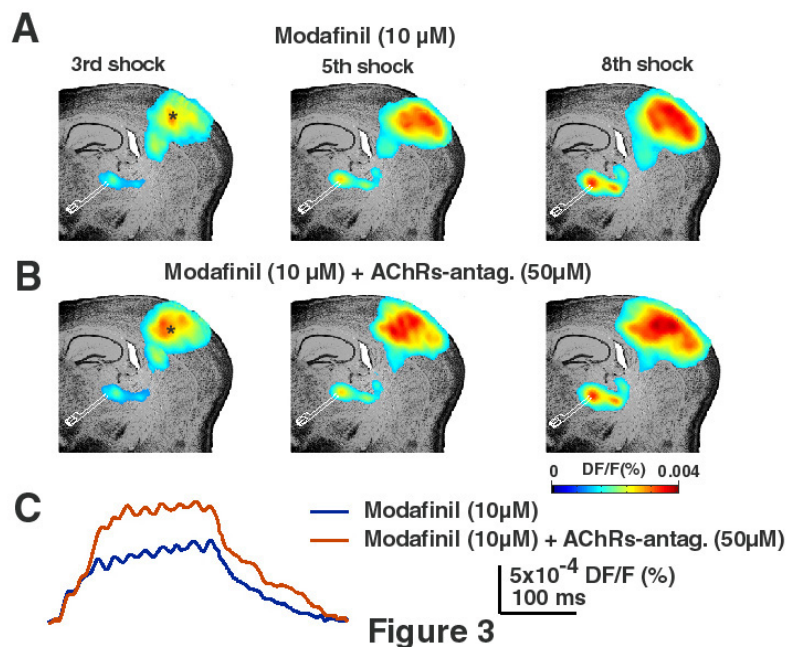


Figure 3: Effects of the muscarinic AChR antagonists atropine and scopolamine on the Modafinil-mediated increment of thalamocortical spatiotemporal voltage-sensitive dye responses.

Spatiotemporal profiles of the voltage-sensitive dye responses during the 3rd, 5th and 8th shock of a train at 40 Hz stimulation of VB nucleus in the presence of Modafinil (10  $\mu$ M) are shown in A, and those after application of 50  $\mu$ M of both scopolamine and atropine are shown in B. Note that in the presence of AChR antagonists there was no reduction of the Modafinil-facilitated responses. Color scale for fluorescence DF/F intensity is from 0 (blue) to 0.001% (red). C, pixel profiles taken from layer 4 (see asterisk in panels A and B) are shown for both conditions, showing that the presence of AChR antagonists further increased Modafinil responses. This facilitation is related to the removal of a muscarinic-mediated inhibition as previously described in this preparation.

A clear increment of the imaging responses was observed in the presence of AchRs-antagonists (Figure 3C), probably related to a dis-inhibition of the postsynaptic muscarinic receptors present on the cortex. The same results were obtained from 6 different slices. In addition, the presence of Carbachol (10 $\mu$ M), muscarinic and nicotinic agonist did not prevent Modafinil's facilitation of the thalamocortical voltage sensitive dye responses (n=3 slices; data not shown). Thalamocortical slices from mice were obtained as previously described (Agmon & Connors, 1991).

In order to determine the minimum effective dose of Modafinil that could enhance the voltage-sensitive dye responses, concentrations as low as 0.2  $\mu$ M (200 nM) were able to enhance the rising slope of voltage-sensitive dye responses. Figure 4A shows pixel profiles taken from layer 5 during white-matter 40 Hz stimulation for both control (black) and Modafinil (0.2 and 20  $\mu$ M; green and red, respectively) conditions from a cortical rat slice. In figure 4B, a more detailed plot of the first shock corresponding to the three conditions is shown together with the fitting to a first order line of their rising phase (dotted lines). In this case, Modafinil increased the slope of the voltage-sensitive dye responses from 2.1 in Control condition to 2.9 and 3.7 in 0.2 and 20  $\mu$ M, respectively. In average, the slope increment during 40 Hz stimulation was 39 $\pm$ 30% (n=5 slices; Mean $\pm$ Std. dev.). Nevertheless, additional experiments will be needed to characterize the dose-response of Modafinil.

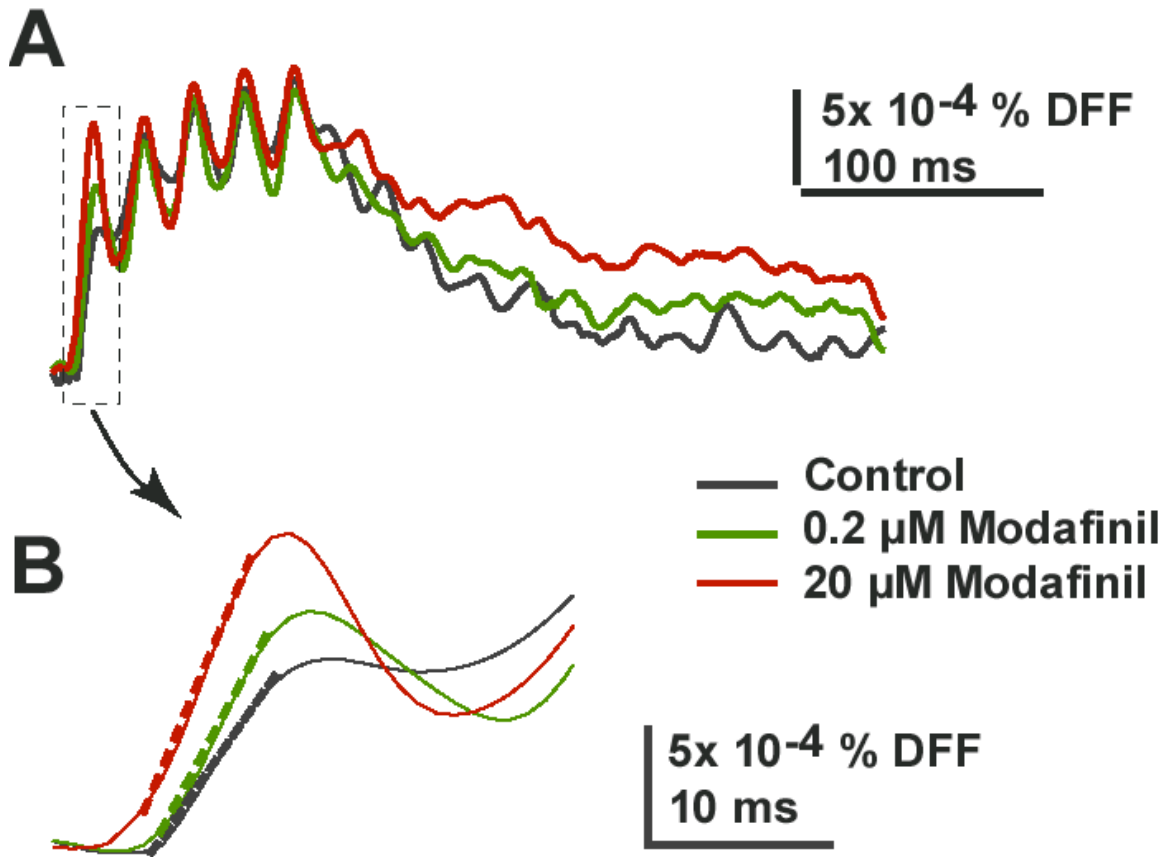


Figure 4: Effects of two concentrations of Modafinil on the cortical voltage-sensitive dye imaging responses. **A.** Pixel profiles taken from layer 5 during 40 Hz stimulation for control (black lines) and Modafinil 0.2 μM (green lines) and 20 μM (red lines). **B.** Comparison of the rising phases of pixels profiles corresponding to the first shock in A. The best fits to a first order line are shown in dotted lines. Slopes were 2.1, 2.9 and 3.7 to Control, 0.2 μM Modafinil and 20 μM Modafinil, respectively. Cortical slices were obtained from rats.

#### References

- Agmon A. & Connors BW (1991) Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience* **41**: 365-379.
- Llinás R, Leznik E and Urbano FJ (2002). Temporal Binding via Cortical Coincidence Detection of Specific and Non-specific Thalamocortical Inputs: A Voltage Dependent Dye Imaging Study in Mouse Brain Slices. *Proceedings of the National Academy of Sciences of the USA*. **99(1)**: 449-454.