

## *fMRI in anxiety*

Anxiety disorders are highly prevalent. They induce individual distress and impairment, and are responsible for significant social costs. Owing to this, the quest for more efficient and safer treatments with lesser addictive potential is a major challenge for the pharmaceutical industry. In order to improve the development of anxiolytic drugs, the development of anxiety models in healthy volunteers is useful. The available models for induction in healthy individuals of anxious states comparable to states observed in anxiety disorders basically fall into the two categories of behavioral and pharmacological. Both categories are investigated in this study; the pharmacological model was used to study panic attack underlying neuronal mechanisms, and behavioral models to study anticipatory anxiety.

### Methods

The pharmacological model uses the attenuated panic-like symptoms induced by cholecystokinin-4 (CCK-4) administration and is composed of three functional magnetic resonance imaging (fMRI) scans. During scan 1, the healthy male subjects are informed that they are going to be injected with placebo. This scan is a control to assess the effects of a simple injection on the brain activity. During scan 2, the subjects are injected with CCK-4. A 0.9% saline solution for placebo or 50 µg of CCK-4 is injected in a bolus fashion in less than 10 s via an intravenous (IV) catheter placed into the vein of the forearm (the side of injection depends on subject vein quality and this was determined by the medical staff; however, the right side has prevailed). Scan 3 is a behavioral classical conditioning model, in which the panic attack experienced on CCK-4 acts as the unconditional stimulus and a red square coupled with a clock acts as the conditional stimulus.

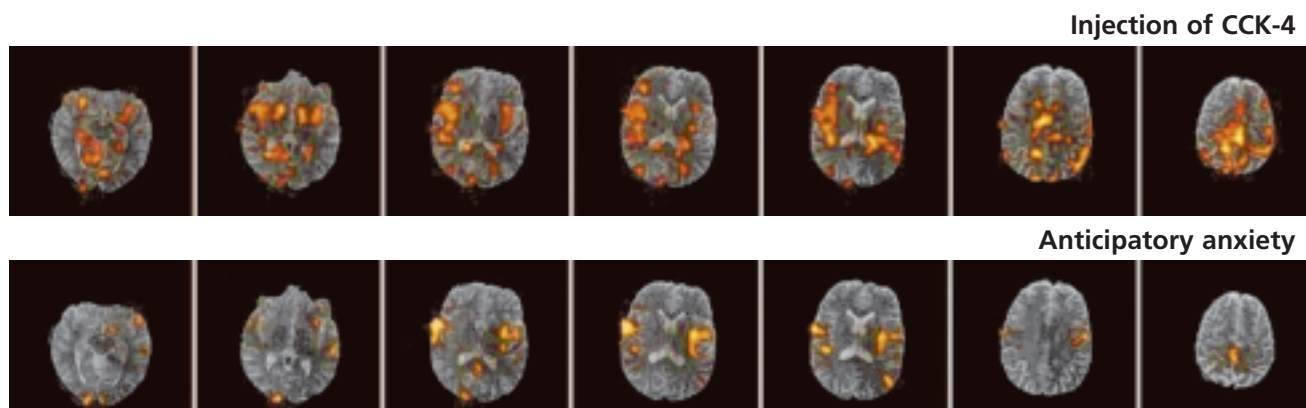
- The first and the second scans last 10 min: 3 min of baseline, before placebo (first) or CCK-4 (second) injection, and 7 min after the IV injection.
- The third scan lasts 13 min: 7 blue and 6 red squares are alternately presented, blue squares are presented for 68 s (17 images) and the red for 52 s (13 images).

The blue square presentation is the rest period and the red one is the threat condition period. A timer is used during the threat condition period, the subject is instructed that he could be administered CCK-4 within the last 10 s.

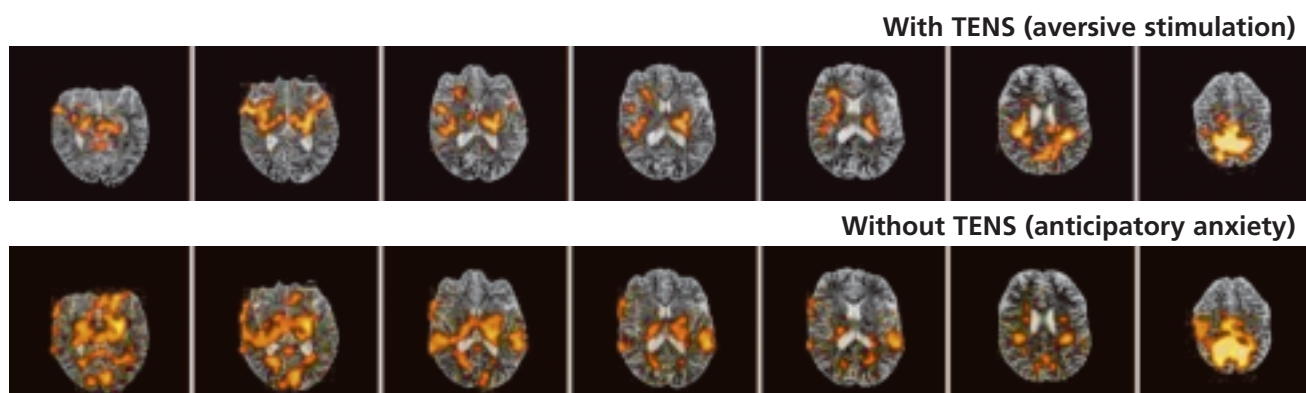
The behavioral model is based on classical aversive conditioning: the conditioned stimulus is a visual presentation and the unconditioned stimulus is a somatosensory stimulation. The task is composed of one fMRI session. The acquisition lasts 11 min: 12 blue and 12 red circles are alternately presented for about 27 s (8 images); the blue circle presentation is the rest period and the red the threat condition period. The subject is instructed that he could receive none, one, or two transcutaneous electrical nerve stimulations (TENS) of the sural nerve within the threat condition period.

### Image processing

Image processing and statistical analysis were performed with freeware software Medimax (Institut de Physique Biologique, GITIM, Louis Pasteur University, Strasbourg, France). All functional images were registered to the first functional image in the series using an automated registration algorithm (rigid registration). A Gaussian filtering (FWHM [full width at half maximum] = 8 mm) and a temporal filtering were applied on each EPI (echo-planar imaging) image. A correlation coefficient between the observed response function and a waveform representing the expected response was computed for each voxel. For each subject, the activation map was obtained from the correlation image using a cluster pixels analysis procedure. Pixels with a correlation coefficient >0.6 were considered as seed points of the clusters identified with a high-connectivity algorithm and a correlation coefficient >0.4. For anatomic registration, a mean image was created with the realigned functional images and was coregistered to the anatomical image with an affine transformation. So the statistical maps could be superimposed on the anatomical image using the transform maps obtained after the affine registration.



**Figure 1.** Axial slices in the pharmacological model. CCK-4, cholecystokinin-4.



**Figure 2.** Axial slices in the behavioral model. TENS, transcutaneous electrical nerve stimulations.

## Results

### *Pharmacological model*

Axial slices showing the patterns of brain activity for one subject in the postinjection period contrasted with preinjection period and the anticipatory anxiety challenge opposed to rest are presented in *Figure 1*. (The pixels represented have a correlation coefficient comprised between 0.4 and 0.6 for CCK-4 injection and between 0.2 and 0.3 for anticipatory anxiety challenge.) CCK-4 injection has produced an intense functional response in insular cortex, temporal poles, amygdala, and middle cingulate gyri. In the anticipatory anxiety condition, cerebral activation is observed in same regions.

### *Behavioral model*

Axial slices showing the patterns of brain activity for one subject in the stimulation presentation contrasted with

rest and in the threat condition contrasted with rest are presented in *Figure 2*. (The pixels represented have a correlation coefficient comprised between 0.4 and 0.6.) In the two conditions, with or without TENS, cerebral activation is observed in loci known to be implicated in the modulation of nociceptive message and its affective and cognitive components: middle cingulate gyri and ipsilateral primary somatosensory cortex, insular cortex, contralateral thalamic nucleus, superior temporal gyri, temporal poles, and internal face of temporal lobes.

### Conclusion

fMRI examinations performed on the healthy volunteers (n=11) were reproducible in terms of the cortical areas involved and support the role of paralimbic structures as neural substrates of anticipatory anxiety.

This work is supported by Johnson & Johnson Pharmaceutical Research and Development. We wish to thank R. M. Vasseur for her assistance in acquisition of the fMRI data.