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Project Acronym: NovelTune

Project title:

Novelty Tuning: Behavioural, electrophysiological and molecular mechanisms of novelty detection

Instrument: Specific targeted research project

PERIODIC ACTIVITY REPORT

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Project coordinator name: Jacques Mallet
Project coordinator organization name: CNRS

PUBLISHABLE EXECUTIVE SUMMARY

PROJECT REFERENCE

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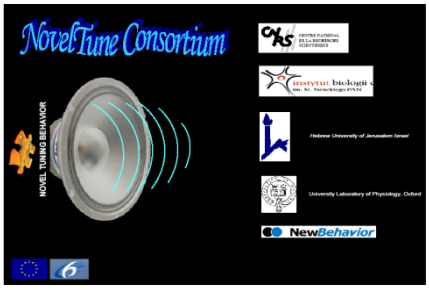
PROJECT LOGO



PROJECT PUBLIC WEBSITE

http://www.noveltune.org

DESCRIPTION OF THE PROJECT OBJECTIVES

The nervous system has constantly to adapt to its changing environment. Modifications in the neuronal circuitry, presumably due to synaptic plasticity, occur in response to unexpected events. It follows that novelty detection is crucial for the successful adaptation of an animal to a new environment. A cogent paradigm is that of the auditory system.

Mechanisms underlying novelty detection in auditory cortex are regulated by and may depend on modulatory neurotransmitter systems such as cholinergic, dopaminergic and serotonergic systems, and additionally may be modulated by stress response, such as glucocorticoid (GC) release following environmental changes. Also, the induction of c-Fos and mismatch negativity (MMN), share the dependence not only on neuromodulatory signalling but also on normal NMDA receptor function.

Overall, the mechanism of processing of novel, as opposed to non-novel, stimuli detection can be considered as a 3-stage process in which: (1) novelty is detected in the cortex by the conjunction of sensory information with novelty signal that is carried by the neuromodulatory systems; (2) this conjunction leads to novelty-related electrophysiological responses such as MMN in humans and to their close correlates such as stimulus-specific adaptation (SSA) on the one hand, and to the associated induction of the expression of IEGs on the other hand; (3) transcription factors initiate a cascade of events, including adaptive changes in mRNAs, microRNAs and alternative splicing, eventually leading to synaptic plasticity.

The specific aim of this project is to study the interactions between the behavioural, molecular and electrophysiological aspects of the processing of novel stimuli. More precisely, our objective is to understand the implication of electrophysiological responses, the induction of IEGs (c-Fos in particular), the modulation by neurotransmitters and the resulting long-term

synaptic changes, which together combine to adapt the sensory responses. Our approach will help in generating high resolution cortical maps and in defining auditory-motor cortex areas that are jointly involved in the control of a specific behaviour. Finally, it will give a complete circle of predictions for the processing of novel vs. non-novel stimuli, including the electrophysiological, behavioural and molecular effects that follow.

The project relies on an operant version of avoidance training, where the conditioned stimulus is a pure tone. Novelty is manipulated by pre-exposing animals to a tone stimulus, and then training the animals either with the same stimulus (the non-novel condition) or with a different stimulus (the novel condition) as warning signals. In the non-novel condition, it is well known that avoidance caused by re-exposing the animals to the training stimulus is reduced (latent inhibition). By varying the tone frequency used during training relative to that used during the pre-exposure, a behavioural frequency tuning curve for novelty can be generated. The model presented above entails a correlation between the behavioural read-out of novelty, as indexed by the amount of latent inhibition, and the electrophysiological (MMN, SSA) and molecular (c-Fos, IEG, splicing and microRNA profiles) read-outs of novelty.

Molecular genetics techniques are used to disrupt the processing of novel stimuli at a number of stages and thus directly test causality. In particular, we expect that (1) disrupting neuromodulatory signalling globally will abolish behavioural novelty responses, together with the associated electrophysiological and molecular responses; (2) disrupting neuromodulatory signalling only in auditory cortex will leave the behavioural novelty responses intact, while abolishing electrophysiological novelty responses and c-Fos induction in auditory cortex; (3) disrupting c-Fos induction in auditory cortex will leave behavioural and electrophysiological novelty responses intact, while abolishing c-Fos staining and the changes in electrophysiological novelty responses associated with conditioning; (4) disrupting the molecular pathways downstream of c-Fos induction will only disrupt the changes in the electrophysiological novelty responses associated with conditioning.

CONTRACTORS INVOLVED

Partner Role	Partner N°	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1a	Centre National de la Recherche Scientifique	CNRSa	France	1	42
PA	1b	Centre National de la Recherche Scientifique	CNRSb	France	1	42
PA	2	Nencki Institute of Experimental Biology	NIEB	Poland	1	42
PA	3a	The Hebrew University	HUJIa	Israel	1	42
PA	3b	The Hebrew University	HUJIb	Israel	1	42
PA	4	Oxford University	UOXF AS	U.K.	1	42
PA	5	New Behavior	NBHVZUR	Switzerland	1	42

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WORK PERFORMED, RESULT ACHIEVED SO FAR AND EXPECTED END RESULTS

Novelty detection is crucial for the successful adaptation of an animal to a new environment. The auditory system is, to date, the most pertinent model system to address such an issue. In that context, NovelTune aims to analyze the interactions between the behavioral, electrophysiological and molecular aspects of the processing of a novel auditory stimulus in an animal model.

The project is based on an operant version of avoidance training, where the conditioned stimulus is a pure tone. Novelty is manipulated by pre-exposing animals to a tone stimulus, and then training the animals either with the same stimulus (the non-novel condition) or with a different stimulus (the novel condition) as warning signals. In the non-novel condition, it is well known that avoidance caused by re-exposing the animals to the training stimulus is reduced (latent inhibition). The model presented above entails a correlation between the behavioural read-out of novelty, as indexed by the amount of latent inhibition, and the electrophysiological (MMN, SSA) and molecular (c-Fos, IEG, splicing and microRNA profiles) read-outs of novelty.

The first challenge was to define a standardized behavioural paradigm and use the behavioural test to measure the behavioural tuning curve for novelty obtained by varying the tone frequency used during training relative to that used during the pre-exposure, as well as establishing electrophysiological tests to be used by all member groups. To achieve this goal, one of the partners, NewBehavior AG, have initially developed a device derived from their well-established IntelliCage system and adapted the system for the auditory avoidance training expanding the conditioning system with an auditory avoidance part (NovelTune-corner). Four functional prototypes of the IntelliCage system were made available to the partners. Recently, an industrialized version of IntelliCage for auditory fear conditioning has been commercialized.

Progress towards the generation of a common behavioural paradigm evolved through a collaboration between the Jerusalem and Warsaw groups. The paradigm that was developed consists of three phases. In a habituation period, the mice learn to drink from the IC-NovelTune corner. During that time, the doors leading to the drinking spots are open, and the mice can drink as much as they need, but must do so at the IC- NovelTune corner. Mice learn this behaviour easily. At the second training phase, a sound (tone busts at 6.67 kHz, duration of 30 ms with 5 ms rise/fall time, every 300 ms) is presented every time the mice enter the corner. Furthermore, the mice learn to push their nose (nose-poke) into the drinking hole in

order to open the door leading to the water. Nose-poking is a natural behaviour for mice. The mice do not seem to be disturbed by the presentation of the tone, so that their rate of visits to the corner and amount of drinking do not decrease. Finally, the conditioning phase consists of presenting, with low probability, a deviant sound (tone bursts at 13.4 kHz, same timing as above). A presentation of this sound serves as a warning: if the mouse tries to nose-poke in order to open the door to the water it is punished with a moderate air puff. The probability of deviant presentations is low enough so that the mice get essentially the same amount of water in the conditioning stage as in the exposure stage. The performance of the mice on an initial version of this paradigm is shown in Figure 1.

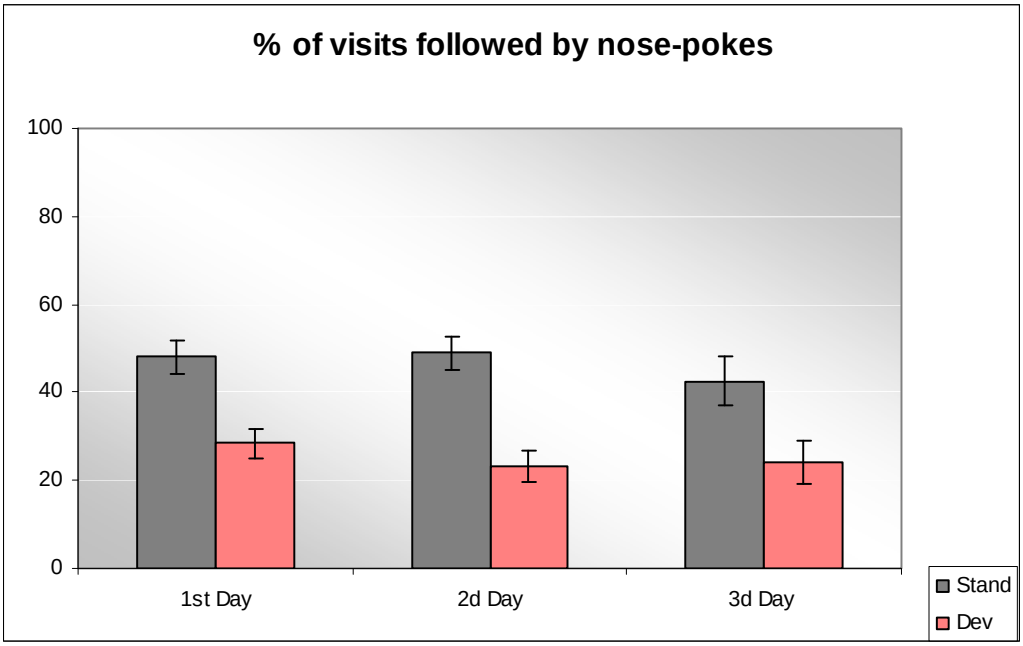

Figure 1: Results of the behavioural paradigm. *Standard and deviant tone bursts (6.67 and 13.4 kHz, duration 30 ms with 5 ms rise/fall time, every 300 ms) were presented during corner visits. Mice continued to make nose pokes during standard visits (gray; 75% of visits; no air puff) but made fewer nose pokes during deviant visits (orange; 25% of visits; nose poke triggering air-puff), indicating they could discriminate between the two sounds and had learned their significance.*

The above discrimination paradigm led to rapid and stable learning. Thus, it has an enormous potential for applications in different aspects of auditory research and was further explored. Firstly, optimizations were performed to determine the 'just noticeable differences' (JND) in mice performing this task.

The standard paradigm was slightly modified. NewBehavior delivered a new corner with modified doors and a fan, with the purpose of preventing the doors from getting stuck in the open position during training. Animals can become stressed when conditioning begins and refrain from nose-poking although to a lesser extent in the neutral (standard) visits. To prevent this, the conditioned tone was introduced more gradually. Rather than introducing the conditioned (deviant) visits at a probability of appearance of 25%, the first conditioning phase included only 5% of conditioned visits, then 10% and finally 17%. After testing different types of sounds, the neutral visits were accompanied by a pulsed 6670Hz tone (30ms pips

every 333ms) and conditioned visits by a pulsed 13340Hz tone (30ms pips every 333ms). These two tones are one octave apart.

In these conditions, mice were capable of learning not to nose-poke in the conditioned visits while maintaining a normal level of nosepoking during the standard visits (Figure 2). Animals learned the discrimination very fast, in a single trial. The training is compressed as much as possible without affecting learning, allowing a manageable experimental protocol that can be used to test many mice types efficiently. This protocol was used successfully in Oxford, Warsaw and Jerusalem.

These developments led us to adopt a protocol with rather short lengths of the phases, as follows:
3 days of habituation (instead of 7 days)
4 days of 100% neutral visits (neutral tone is 6670Hz)
2 days of 5% conditioned visits (instead of 3 days, conditioned tone is 13340Hz)
2 days of 10% conditioned visits (instead of 3 days)
2 days of 17% conditioned visits (instead of 3 days).

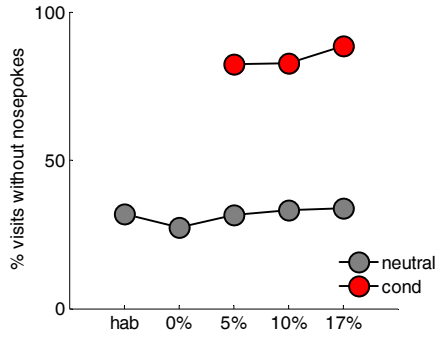

Figure 2. *Discrimination: % visits without nosepokes by phase (average of 3 days) for the neutral visits (gray) and conditioned visits (red). Note the baseline of 30-40% visits without nosepokes in the neutral visits. and the comparatively high avoidance to nosepoke in the conditioned visits. The baseline remains constant from habituation, through the neutral only phase, up to phases containing 5, 10 or 17% of conditioned visits.*

Further, the discrimination capacity of these mice was tested by progressively reducing the frequency difference between the neutral and the conditioned tone. The neutral tone was kept constant, and the conditioned tone was gradually reduced until a difference of 2% was reached. In these conditions, mice could discriminate well a conditioned tone that was 4% above the neutral tone.

These above refinements allowed us to establish the **behavioural tuning curve of novelty detection** which was based on the phenomenon of latent inhibition. Latent inhibition occurs when the animal is pre-exposed to the foreground cues (e.g. either context or tone). By measuring the extent of latent inhibition as a function of the frequency of the adapting tone, it is possible to quantify behaviourally the frequency tuning curve for novelty. Thus, animals were trained as above with the exception that a pre-exposure phase was introduced between the neutral visits only phase and the conditioning phase.

The following experimental protocol was used:

- 3 days of habituation: no tones, doors open.

- 11 days of 100% neutral visits (6670Hz): as before, only neutral visits, nose-poke leads to opening of the doors and access to water.

- 5 days of pre-exposure (17% of visits, no air-puff) to a test tone different from 6670Hz: In 17% of the visits a test tone is presented. During these visits a nose-pokes leads to opening of the doors and access to water as in the neutral visits. The rest of the visits are neutral.

- 5 days of conditioning (17% of visits, air-puff upon nose-poke) to 13340Hz: In 17% of the visits the conditioned tone is presented, and a nose-poke leads to the delivery of an air-puff and no access to water. The rest of the visits are neutral. In all these replications the neutral tone was 6670Hz and the conditioned tone was 13340Hz, two tones that are an octave apart and are easily discriminable (see above).

It is anticipated that pre-exposure to tones which are progressively closer to the conditioned tone will lead to latent-inhibition in the form of a reduction in the % visits without nose-pokes in the conditioned visits, at least initially. As expected, pre-exposure to 3335Hz (an octave below the neutral tone and 2 below the conditioned tone), did not result in latent-inhibition (Figure 3). On the other extreme, pre-exposure to the conditioned tone itself, did result in latent inhibition.

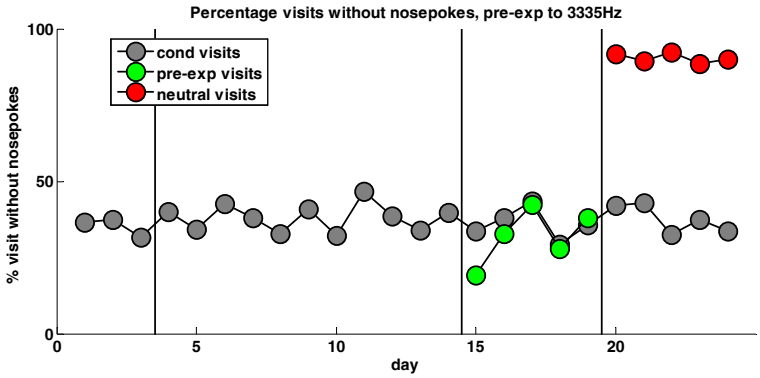

Figure 3. *The baseline % of visits without nosepokes is unchanged by the introduction of the unconditioned pre-exposure frequency (3335Hz, green) in 17% of the visits. When the conditioned visits are introduced (13340Hz, red), animals stop nose-poking in these visits, indicating that they discriminate well between these and the neutral visits. The neutral visits are unaffected by the introduction of the conditioned visits.*

Behavioural tuning curves of novelty detection were obtained as a relation between the pre-exposure frequency and the level of discrimination on the first day of conditioning (Figure 4).

Clearly, the behavioural tuning curve is wide (about half an octave, latent-inhibition is observed already at pre-exposure frequencies of 9433Hz and above).

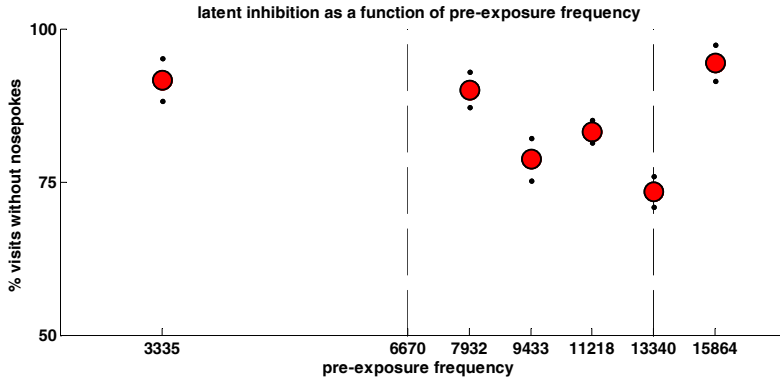

Figure 4. *Performance on the first day of conditioning was clearly dependent on the frequency used during the preceding pre-exposure phase. When the frequency used during pre-exposure was more than 1/2 and octave below or 1/4 of an octave above the conditioning frequency we observed no latent-inhibtion. However, frequencies that were within 1/2 octave below the conditioned frequency were capable of generating latent-inhibtion that was close in level to that caused by pre-exposure to the conditioned frequency itself.*

In conclusion, the behavioural tuning curve of novelty detection is wider, spanning almost 1 octave (more than 1/2 an octave below the conditioning frequency, and less than 1/4 of an octave above), than the discrimination capacity of these mice, who can discriminate up to ΔFs of 4 to 7%.

Are there correlations between the behavioural read-out of novelty, as indexed by the amount of latent inhibition, and the **electrophysiological level** measured in term of MMN and SSA?

During the first part of the project a standardized, rapid electrophysiological screening method using evoked potentials was developed and subsequently used for pre-screening of molecularly modified animal models. Having these electrophysiological measurements in hand, the goal was then to thoroughly characterize the electrophysiological correlates of novelty detection both in naive wild type animals and in wild type animals that have undergone behavioural training, using both local field (MMN) and single neuron (SSA) recording methods. Responses of wild-type C57BL6 animals were characterized using local field potential methods. Strong mismatch effects are obtained in the responses of cortical auditory neurons. The effects of varying the frequencies of the tones, duration and inter-stimulus interval were explored. Results provide strong evidence of stimulus-specific adaptation in which the amplitude of the response to the deviant stimulus is larger than the response to the common or standard stimulus. A manuscript reporting data obtained from wild-type C57BL6 mice will be submitted for publication shortly. Notably, the behavioural training paradigm was recently extended to investigate whether the frequency discrimination performance of the animals decreased after a short period in which the animals were not exposed to the conditioning sounds. These experiments show that performance remained unchanged after such period of time. The above methodology has been used by the Oxford and Jerusalem groups to describe the responses of animals following behavioural training in a two-tone discrimination task.

Following the above developments, several recording sessions took place in one of the partner labs in Paris (CNRS partner 1b). In each of these sessions, electrophysiological responses were recorded from a variety of genetically manipulated animals and animals treated with pharmacological agents. In particular, the responses of animals from the transgenic mouse

line GR^{D1aCre}, characterized by the lack of expression of the glucocorticoid receptor (GR) in neurons that express the dopamine D1a receptor was tested. These animals exhibit an overall reduction in the amplitude of their responses with respect to control animals. Furthermore, differences were observed in the responses between these two genotypes in terms of SSA. Taken together, these results suggest that the GR may play a role in the processing of novel stimuli. Further experiments are in progress to characterize the electrophysiological responses of behaviourally trained GR^{D1aCre} animals.

Finally, what are the correlations between the **behavioural**, **electrophysiological** and **molecular** read-outs of novelty?

The behavioral paradigms described above were used with the goal to identify cortical network components within auditory and motor cortices that subserve sound frequency discrimination in a behavioral context. For that purpose, we studied c-Fos activation, an IEG that is hypothesized to reflect novelty detection in different cortical structures after exposure of an animal to novel sounds associated with a particular behavioural meaning. Clearly, c-Fos induction in the mouse auditory cortex was only observed in response to a novel sound when this sound did not carry any significant behavioural meaning. Surprisingly, the observed level of c-Fos expression following exposure to a sound presented together with a shock was significantly lower compared to the animals presented to the same sound alone. One of the most plausible explanations is that the observed effect is related to the type of the behavioural training that was used. Classical fear conditioning might have activated rather sub-cortical than cortical structures, the latter being even inhibited.

Thus, expression of c-Fos was analyzed after the training in the modified version of the behavioural paradigm within different parts of the auditory cortex, motor cortex and inferior colliculus according to the protocol shown in Figure. 5.

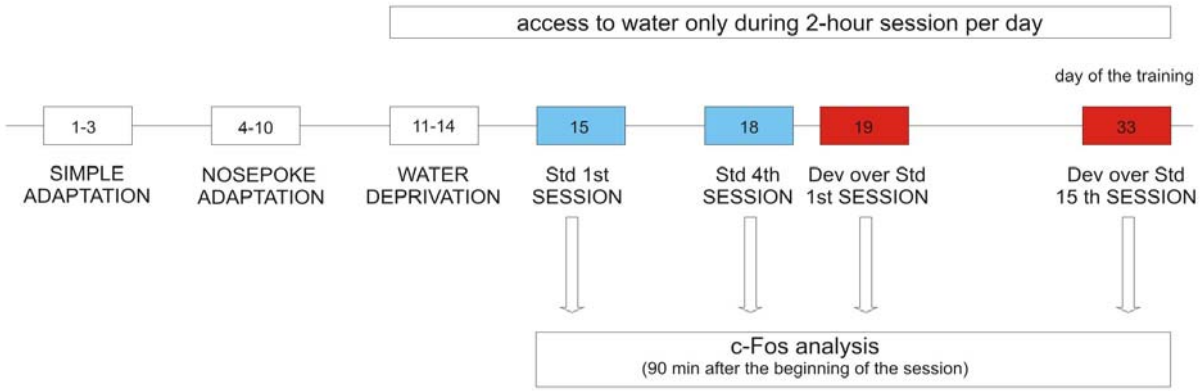

Figure 5. *The scheme of the modified behavioural training in the NovelTune cage.*

The behavioural and c-Fos results were obtained in three repetitions of the experiment. The brain sections for c-Fos analysis were collected every sixth section between -2.06 mm and -3.40 mm from bregma. The expression was assessed bilaterally in six 40µm-sections for every animal. The expression was analyzed separately for the ventral and dorsal part of the

auditory cortex, as well as the anterior (sections 1-3) and posterior part (sections 4-6). The expression was also analyzed in the primary motor cortex and inferior colliculus.

Significant changes in c-Fos expression were observed between groups in the anterior parts of both the ventral and dorsal auditory cortex. The increase in expression was clear in the Deviant over Standard group (after the 1st session). After the long training (15 sessions), c-Fos expression was low in the animals that acquired the avoidance response effectively (good learners) but remained high in the animals that did not avoid the air-puffs (bad learners).

Therefore, the level of c-Fos expression does not correlate with the number of air-puffs received (as measured during the first training session). However, there is a strong correlation (~0.7) between the number of nose-pokes and the level of c-Fos expression in both the ventral and dorsal auditory cortex after the 15th session of the training. Interestingly, the level of c-Fos expression in the primary motor cortex decreased after 4 sessions of the training with Standard sound alone; however, c-Fos level is significantly increased after the 1st as well as the 15th session in the Deviant over Standard group. The disparity between the pattern of c-Fos expression within the auditory and motor cortices may reflect their different involvement in the discrimination training.

The functional role of c-Fos in the auditory cortex was ascertained by RNA interference experiments using lentiviral vectors. *In vivo* testing was performed into the cortex of adult rat in which the c-fos promoter was activated by a single injection of metrazol. Immunostaining experiments clearly revealed knock-down of c-Fos in the transduced cells.

Overall, the molecular analysis showed (i) there was a clear increase in c-Fos expression within the auditory cortex (especially the anterior part) following the discrimination training in the NovelTune cage. The level of c-Fos expression was closely correlated to the level of performance of an animal, being the highest at the beginning of learning and in animals that did not master the response properly; (ii) the pattern of c-Fos activation in the auditory cortex was partially followed by the pattern of c-Fos expression in the inferior colliculus, whereas in the motor cortex we observed a different pattern of activation. The disparity between the patterns of c-Fos expression within the auditory and motor cortices may reflect their different involvement in the discrimination training; (iii) these developments allow analysis of the functional role of c-Fos during the discrimination training in the NovelTune cage.

Neuromodulary systems play a crucial role in cortical plasticity. In that context, the dopaminergic, cholinergic and serotonergic systems are thought to play a pivotal role in the auditory system. Perturbation of these systems is crucial to establish causation. The serotonergic neurons from the raphe are known to project directly into all cortical areas including the primary auditory cortex. Therefore, reducing or abolishing serotonin synthesis in this tissue should clarify the role of this system in auditory processing of novelty detection. The spatial inhibition of 5-HT synthesis in the neurons of the dorsal raphe in female mice of the C57/BL6 strain was carried out by an RNA interference approach targeting the TPH2 mRNA. Appropriate lentiviral vectors and shRNA constructs proved to be quite efficient.

A single stereotactic injection into the dorsal raphe inhibits serotonin production, as revealed by antibodies against TPH2 and serotonin (Figure 6). These findings have been confirmed by HPLC analysis. Moreover, initial electrophysiological experiments show an altered response of the primary auditory cortex neurons to the tone presentation. Further work will decipher the underlying mechanisms which implicate the serotonergic system in the creation of the novelty response.

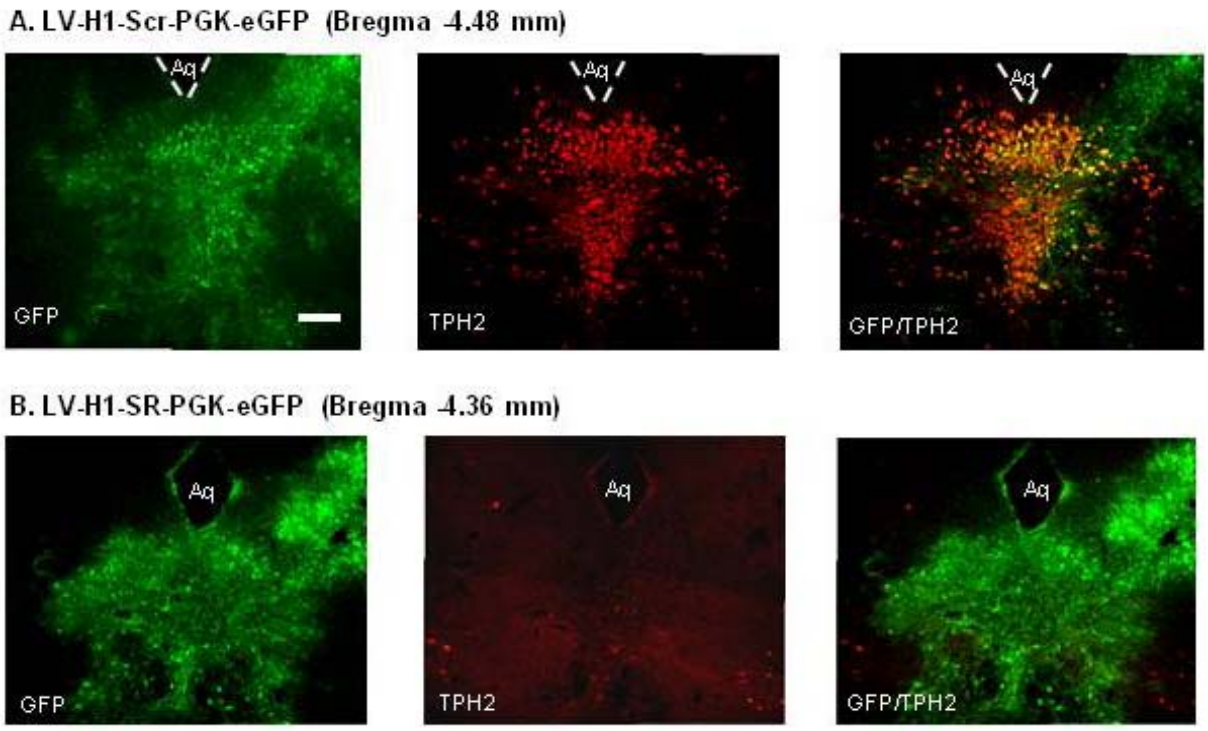

Figure 6. *Down regulation of TPH2 in the DRN under in vivo delivery of recombinant lentiviral vector into the DRN of an adult mouse. Photomontages of the DRN are shown in A and B panels. Fluorescent immunostaining for GFP (green) and TPH2 (red) 2 weeks post-injection of the control, LV-H1-Scr-PGK-eGFP (LV-Sc-shRNA), or the inhibiting, LV-H1-SR-PGK-eGFP (LV-TPH2-shRNA), vectors (5 x 108 TU/mL). GFP labeling shows the transduced cells. TPH2 and GFP were co-localized in LV-H1-Scr-PGK-eGFP (control vector) transduced neurons (A) but not in LV-H1-SR-PGK-eGFP transduced neurons in which TPH2 immunoreactivity is clearly lost. Scale bar = 200 µm.*

Experiments implying **the cholinergic system** and in particular the **cholinergic basal forebrain**, which has been implicated in auditory cortical responses proved to be more tedious. The inhibition of the expression of choline acetyltransferase (ChAT), was achieved either by conditional gene knockout in mouse or by posttranscriptional gene knockdown through RNA interference (RNAi). Using this last strategy, the number of neurons containing ChAT was reduced by 30-60% in the nucleus Basalis of Meynert (nBM). In the first strategy, the Cre lentiviral vector was delivered in the nBM of adult $ChAT^{lox/lox}$ mice. Analysis of transduction efficiency of the vector showed moderate to strong reduction of ChAT expression in those neurons transduced with the Cre recombinase as compared to the contralateral non injected neurons (50-80%). The shape of the nBM, which required injections of the vector in 8 sites instead of 1 in the raphe is likely to account for a lower efficacy in the level neurotransmitter depletion in the nBM than in the raphe. Electrophysiological experiments, now in progress, will assess the role of the cholinergic system underlying novelty detection in the cortex.

Stress responses such as glucocorticoid (GC) release following environmental changes have been thought to modulate mechanisms underlying novelty detection in the auditory cortex. To test this hypothesis the Paris group (CNRS 1b) undertook a series of experiments with mice deprived of **glucocorticoid receptors** (GR) or, conversely, with enhanced expression of GR, taking advantage of the Cre/loxP or the “tetracycline” systems. GR effects were also tested on animals chronically treated for two weeks with GC in the drinking water and in

adrenalectomized animals (depletion). In most cases, MMN was not affected in these animal models. $GR^{5\text{-}HT1Acre}$ animals in which GR expression was shown to be abolished in regions comprising cortex, hypothalamus, brainstem, and with the highest density in the hippocampus (taking advantage of the 5-HT1A receptor promoter) exhibit increased anxiety whereas resignation was significantly reduced. Most notably, the behavioural phenotype observed in $GR^{5\text{-}HT1ACre}$ mutant is reminiscent of the phenotype observed in 5-HT1A-$R^{-/-}$ mutant mice. However these investigations strongly indicate that, in the contrary to what has been suggested from GCs treatment in animals, GR is a positive direct regulator of 5-HT1A receptor gene expression. When absent, 5HT1A-R gene expression is reduced leading to changes in emotional behaviours.

Animals that conditionally over-express the human GR gene locally, mimic a stress induced release of GC by increasing GR local concentration, and furthermore allow distinguishing between GR effects in the adult and developmental effects since hGR is only expressed during precise time windows. Mice over-expressing hGR in hippocampus (in the CamkIIalpha-tTA transgenic line) show increased anxiety and decreased resignation. At the biochemical level, they exhibit reduced levels in the transcripts of BDNF, glutamate receptor transcripts (gria1 and grin2b), and phosphatase dusp1, involved in the modulation of the MAPKinase pathway. These changes in mRNAs were performed in the hippocampus of the transgenic animals since this structure displays the lowest mosaicism in neuronal hGR over-expression.

Another series of investigations relied on GR^{D1Cre} mice (Cre expression under the control of the dopamine receptor D1A promoter) in which only the deepest layers of the cortex were affected, whereas 80% of the neurons were recombined in the striatum. The absence of GR in these dopaminoceptive neurons strongly reduces the spontaneous firing of dopamine neurons showing that GR is required for the feedback control that post-synaptic neurons exert on dopamine neurons. Interestingly, GR deprivation in D1A-expressing neurons confers an impaired response to cocaine but not to morphine. Sensitisations to cocaine, Conditioned Place Preference to cocaine as well as motivation for cocaine self-administration, are deeply reduced, while none of the responses to morphine are affected. At the biochemical level, it was demonstrated that, in the absence of GR in dopaminoceptive neurons, dopamine release in the striatum, as well as the release of norepinephrine in the cortex are impaired. Moreover, the absence of GR affects IEGs (c-fos and zif268) induction in response to abuse drugs in different structures including the striatum and the cortex.

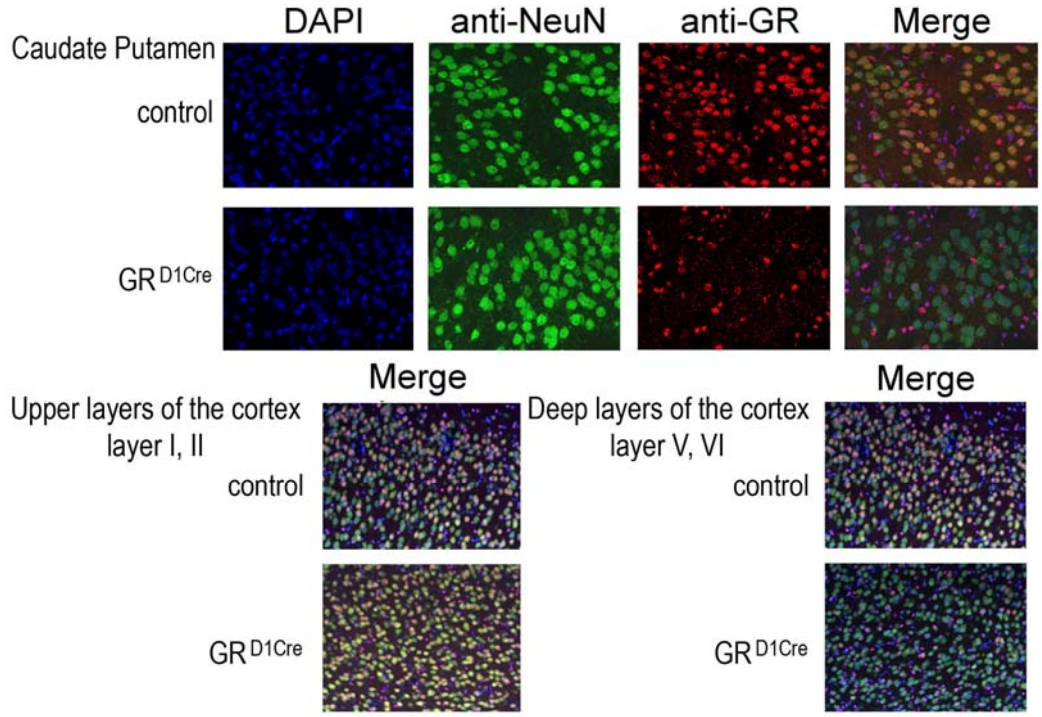

Figure 7. *Immunofluorescence co-labeling of NeuN and GR proteins in 30µm microtome sections of Caudate Putamen and Cortex from GR^{D1Cre} and control littermates. For every section, a DAPI staining of the nuclei (blue), a labeling of the neuronal specific antigen NeuN (green), and a labeling of GR protein (red) are presented. In the upper part, the last column presents the merged pictures. In sections from the GR^{D1Cre} mice, the GR protein disappears from a large majority of striatal neurons. In the lower part, GR protein disappears from some neurons of the deeper cortical layers but not upper ones.*

Most interestingly, GR^{D1Cre} mice display a distorted MMN. This finding is in line with molecular analysis also performed by the CNRS 1b group showing that this mutation affects a group of genes involved in the glutamatergic transmission. In addition, parallel electrophysiological studies showed that the absence of GR in striatal Medium Spiny neurons reduces their sensitivity to glutamate. Thus, the mutation affects the activity of the targeted neurons in a cell-autonomous way. It is also known that the mutation has some indirect "non-cell autonomous" consequences, such as reducing the firing rate of the dopamine neurons that are not affected by the mutation. These results suggest that the dopaminergic tonus may play a key role in the alteration of the MMN.

In view of the cogent MMN findings presented above, a particular attention was paid to the GR^{D1Cre} mouse line. Two main aspects were investigated. Firstly, latent-inhibition experiments were initiated using NovelTune cormer, as a collaboration between the Paris and Oxford groups. Secondly and most importantly, in view of the association of MMN with schizophrenia in humans, a detailed behavioural characterization of this animal model was undertaken to investigate whether defects in MMN, such as the one we observed in GR^{D1Cre} mice, would correlate with other phenotypes reminiscent of schizophrenia (SZ).

Schizophrenia is characterized by a constellation of pre-attention and attention cognitive processes and electrophysiological deficits such as MMN. The latter are specific to schizophrenics and schizotypic individuals, since they were not observed in other mental illnesses such as major depression or bipolar disorders. SZ is characterized by positive, negative and cognitive symptoms. Although certain aspects of the symptomatology of the SZ,

such as positive symptom (delusions or hallucinations), cannot be modelled in animals, others are well established in rodents. For instance, forebrain-specific calcineurin mutant mice have been shown to exhibit a severe deficit in social interaction that may reflect the social withdrawal observed in schizophrenics. Working memory disturbances have served as a heuristic model to mimic SZ in animals and object recognition paradigms have been widely used to evaluate the reaction to a spatial change. Finally, other pre-attention processing events such as pre-pulse inhibition (PPI) have been extensively used in both preclinical and clinical research to investigate the neurobiology of SZ. Indeed, SZ patients exhibit abnormal PPI despite having relatively normal responses to startling stimuli. Interestingly, this paradigm is easily translated across species and antipsychotic medications have been shown to increase PPI. Most of these paradigms appear to be sensitive to non-competitive NDMA antagonist, drugs that have been shown to produce or exacerbate symptoms of SZ.

These observations served as a rationale to explore the effects of the GR^{D1Cre} genetic manipulation in different paradigms relevant to negative and cognitive symptoms of SZ such as pre-pulse inhibition, social interaction and novel object recognition. Initial findings suggest that the biological substrates underlying the behaviour abnormalities observed in SZ patients might diverge. Indeed, GR^{D1Cre} mice appear to exhibit impairment in pre-attention processes such as MMN although they behave normally in social interaction and pre-pulse inhibition paradigm. Further, this genetic manipulation attenuates distinctive behaviour alterations elicited by administration of non-competitive NMDA antagonists. The findings in this part of the project open new venues for discovery of previously non-pervceived targets for therapeutic intervention with SZ symptoms.