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# Effect of Acute and Repeated Nicotine Administration on the Electrical Activity of the Lateral Habenular Neurons in Rats

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## INTRODUCTION

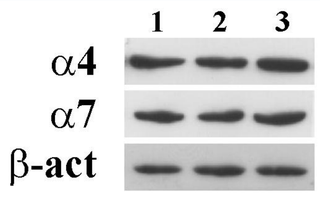
Compelling evidence has shown a pivotal role for dopaminergic function in the addiction to drugs of abuse like nicotine. Nicotinic acetylcholine receptors (nAChRs) are expressed throughout the CNS and can influence different brain areas and functions. Nicotine, acting through these receptors, can affect multiple neurotransmitter systems in the brain inducing neuroadaptations that may account for the observed alterations in brain reward systems involved in the addiction process and withdrawal symptoms. Recently, the Lateral Habenula (LHb) has attracted a great deal of attention as another target for nicotine in the brain because of its role in regulating dopamine (DA) system neuronal activity. In particular, it has been suggested that the symptoms associated with nicotine withdrawal might be associated with an altered output from the LHb induced by continuous exposure to nicotine. Although the presence of nAChRs in the LHb has been shown, the extent to which the LHb may contribute to the neurobiological action of nicotine still needs to be fully addressed. Here, we investigated the effects of both acute and chronic exposure to nicotine on LHb neuronal activity and nAChRs expression at the level of this area.

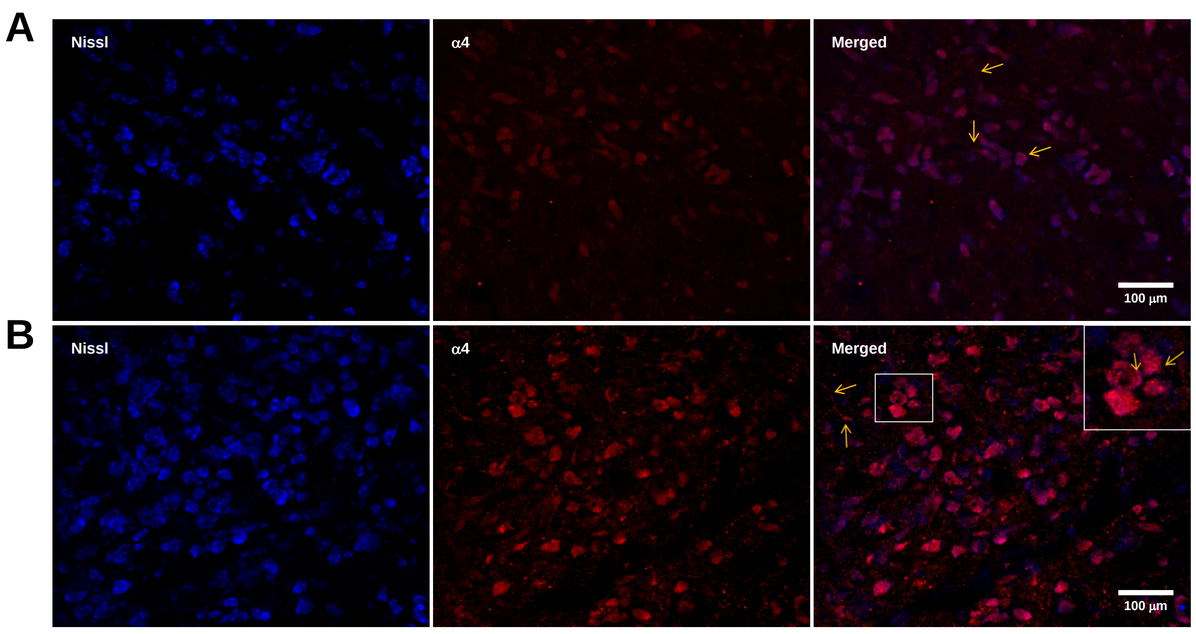
## METHODS

Nicotine (1 mg/kg, *i.p.*, twice a day; n=6). or saline (1 ml/kg, *i.p.*, twice a day; n=6).) were administered for 14 consecutive days to two groups of Sprague-Dawley rats. The day after the nicotine treatment, a group of rats was sacrificed and the LHbs were freshly dissected and homogenized for western blot (WB) analysis of proteins content. The remaining rats were sacrificed, transcardially perfused with formaldehyde and their brains were extracted. Coronary brain sections were successively processed for immunofluorescence and confocal microscopy to assess nAChRs levels of expression. *In vivo* extracellular single unit recordings of LHb neurons were performed in cloral hydrate (400 μg/kg, *i.p.*) anaesthetized male Sprague Dawley rats, (c.300 g). The lateral tail vein was cannulated for endovenous injection of single boluses of nicotine (100 or 200 μg/kg, *i.v.*). Stereotaxic coordinates were used for the placement of the recording electrode. Nicotine was tested on both drug-naïve (n=19) and nicotine chronically treated rats (1 mg/kg, *i.p.*, twice a day for 14 days; n=13). Only one neuron was tested for its response to nicotine for each animal. At the end of each experiment, the recording site was histologically verified.

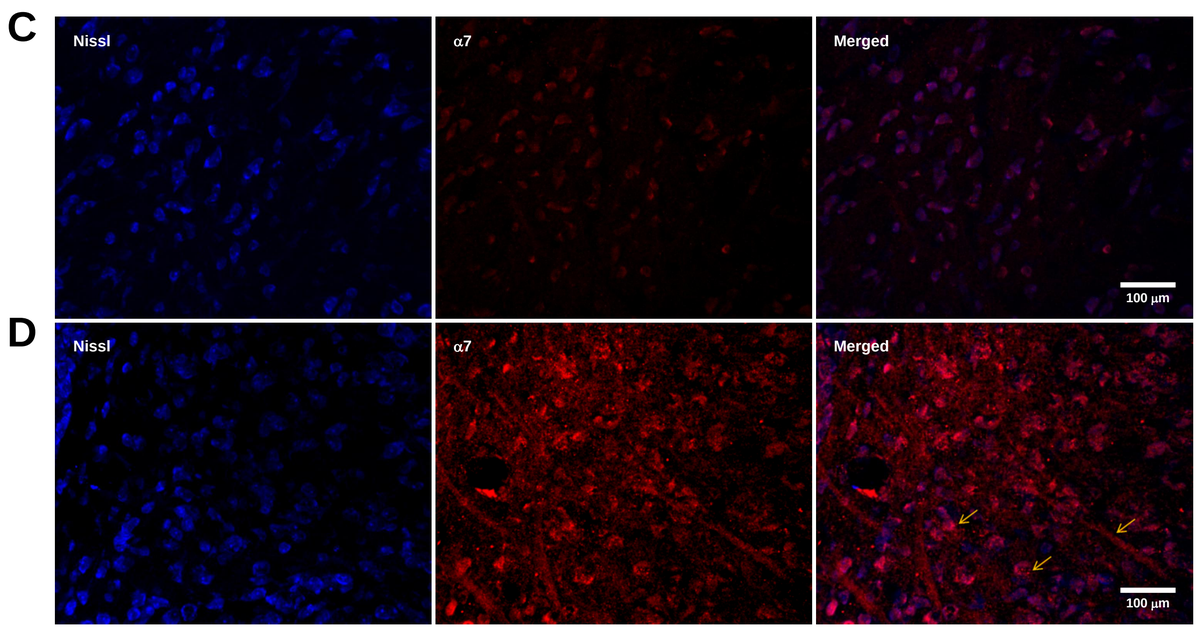
## Figure 1: up-regulation of α4/α7-containing nAChRs expression in the LHb following nicotine chronic treatment.

Western Blot (WB) gel plots showing the total protein content in the LHb of drug-naïve **(1)**, saline **(2)** and nicotine **(3)** treated rats. The WB analysis (density levels normalized to β-actin) showed higher expression of α4-containing nAChRs compared to those containing α7 in the LHb in all groups of rat. No differences in the level of expression nAChRs level of expression were observed between chronically nicotine and saline treated rats or the drug naïve group.

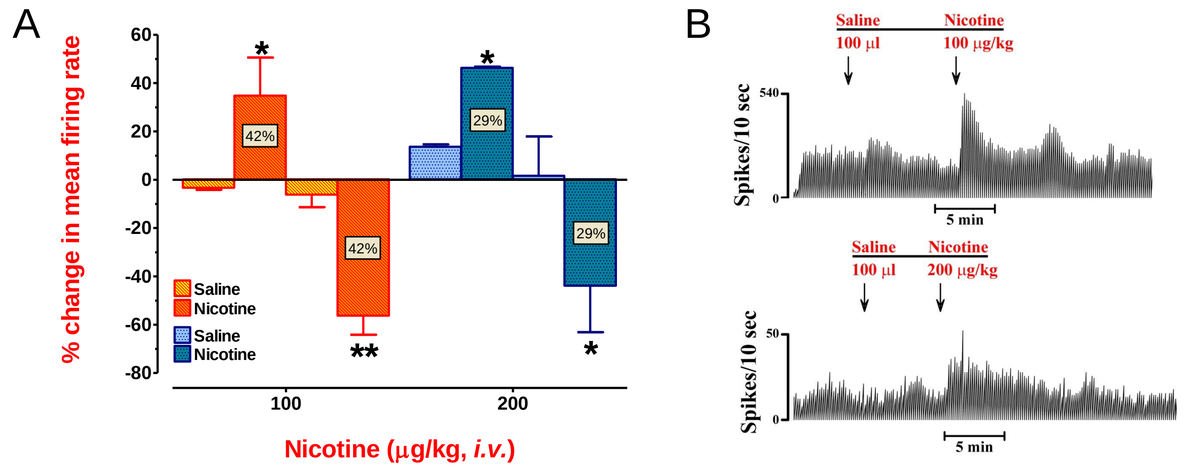



Confocal microscope images showing the expression of α4/α7-containing nAChRs (red) in the LHb of the rat brain. The Nissl staining (blu) shows the localization of neuron cell bodies. **(A)** Expression of α4-containing nAChRs in the LHb of drug-naïve rats. The α4 expression spread throughout the cytoplasm of the cell bodies as well as on cell membranes and fibres **(arrows)**. Following nicotine chronic treatment **(B)**, α4 expression shows an increase, especially at the level of cell membranes **(inset)** and fibres **(arrows)**. The expression of α7-containing nAChRs has been found in the LHb **(C)**, mainly at a cytoplasmatic level as shown by the merged picture. Following nicotine chronic treatment **(D)**, there is an increased expression of this sub-unit not just at a cytoplasmatic level but also on cell membranes and fibres **(arrows)**.




## Figure 2: *i.v.* administration of nicotine either increases or decreases the neuronal activity of single LHb neurons in drug-naïve rats.

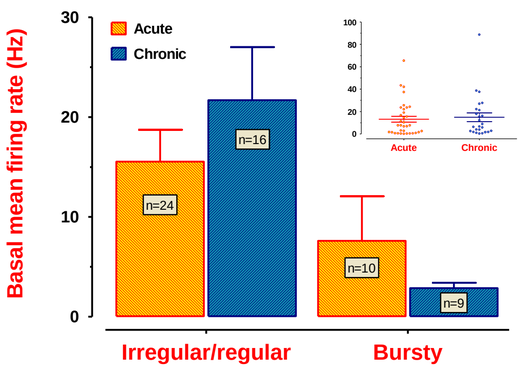



The effect of the systemic administration of nicotine on the neuronal activity of LHb neurons was determined by recording their firing rates extracellularly. For each neuron, the basal firing rate was recorded for a period of 5 min before the injection of saline (control group). Nicotine (100 or 200 μg/kg, i.v.) was administered at least 5 min after the saline injection. Mean firing rates were calculated for the basal activity and the periods following saline and nicotine injections. The last two were normalized to the basal mean firing rate and the nicotine induced changes were expressed as a percentage of the basal rate.

**(A)** Nicotine induced either an increase or decrease of the firing activity of single LHb neurons that were more responsive to the lowest dose, although both doses were able to induce a significant increase or decrease of the basal firing rates (mean±S.E.M.; Paired t-Test; nicotine *vs* saline, * $p < 0.05$, ** $p < 0.01$).

**(B)** Representative rate meters showing the different effects elicited by nicotine on the basal firing rate of LHb neurons. Time of injections is indicated by the arrows.

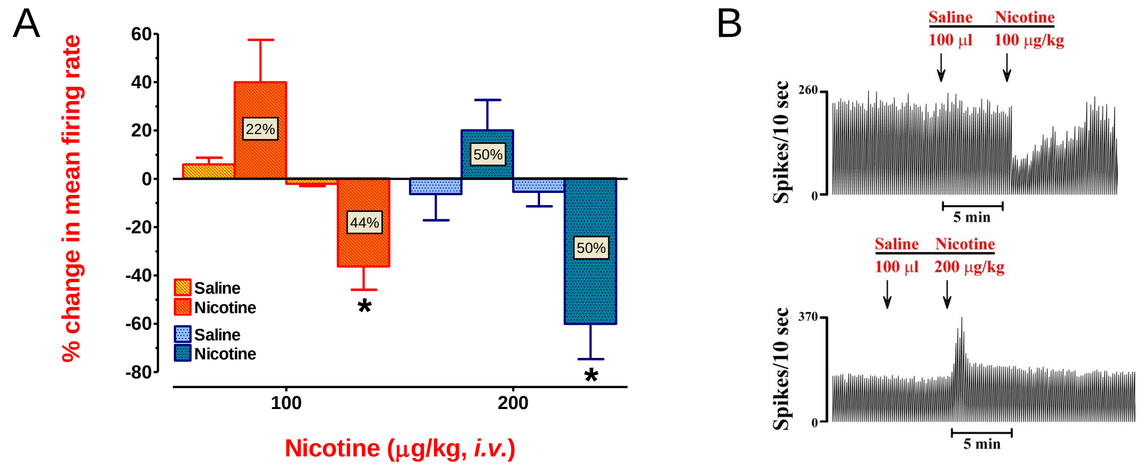
## Figure 3: nicotine chronic treatment did not significantly affect the basal firing activity of single LHb neurons recorded extracellularly *in vivo*.




The inset shows the mean basal firing rates of all neurons recorded from drug-naïve and nicotine chronically treated rats. Lines and bars indicate, respectively, the mean±S.E.M. for each group of rats. No significant difference was found between the two (Unpaired t-Test, *n.s.*).

The histogram shows the mean basal firing rates of drug-naïve *vs* nicotine chronically treated rats for the two main types of basal firing patterns. Although no significant difference was found (Unpaired t-Test, *n.s.*), the data seem to indicate the tendency for irregula/regular neurons to fire at higher rates after nicotine chronic treatment while the bursty neurons show a reduction in their mean basal firing activity.

## Figure 4: *i.v.* nicotine administration induces either an increase or decrease of single LHb neurons neuronal activity following nicotine chronic treatment.




Following the nicotine chronic treatment for 14 days, the effect of the systemic administration of nicotine on the neuronal activity of single LHb neurons was determined. For each neuron, nicotine (100 or 200 μg/kg, *i.v.*) was administered according to the protocol used for the drug-naïve group and the data collected were analysed as previously described.

**(A)** Nicotine induced either an increase or decrease of the firing activity of single LHb neurons but only the latter effect, at both doses, showed a significant effect when compared to the control group (mean±S.E.M.; Paired t-Test; nicotine *vs* saline, * $p < 0.05$).

**(B)** Representative rate meters showing the different effects elicited by nicotine on the basal firing rate of LHb neurons. Time of injections is indicated by the arrows.

## SUMMARY

- Immunofluorescence data show the presence of α4/α7-containing nAChRs in the LHb that undergo up-regulation following the nicotine chronic treatment.
- Single unit extracellular recordings *in vivo* demonstrate a modulatory action of nicotine on LHb neuronal activity that was modified following continuous exposure to this drug, possibly involving plastic changes at the level of this area.
- These results suggest that the LHb, because of its modulatory influence on DA systems, might play an important role in the mechanism of action of nicotine in the brain.