GABAERGIC INTERNEURONS ARE INVOLVED IN THE EXCITATORY ACTION OF 8-OH-DPAT ON PREFRONTAL CORTEX PYRAMIDAL NEURONS

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INTRODUCTION

Some marketed atypical antipsychotics and new antipsychotic drugs in development display direct or indirect $5HT_{1A}$ agonism. This mechanism may be important to elevate DA release in medial prefrontal cortex (mPFC), an effect thought to be beneficial for the treatment of cognitive and negative symptoms in schizophrenia (1, 2).

The $5HT_{1A}$ receptor is expressed postsynaptically (mPFC, hippocampus, lateral septum…) and presynaptically (Raphe nuclei). In the mPFC –key area in the symptomatology and treatment of schizophrenia- this receptor is expressed by 50-60% of pyramidal neurons and 20-30% of GABAergic interneurons (3,4).

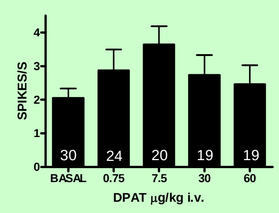
Endogenous 5HT mainly inhibits mPFC pyramidal neurons acting through $5HT_{1A}$ receptor yet the systemic administration of 5-HT_{1A} receptor agonists paradoxically increases pyramidal cell activity (5), which suggests the involvement of 5-HT_{1A} receptors in other cell types (e.g. GABAergic interneurons) or in afferent areas to the PFC, such as hippocampus, raphe, entorhinal cortex, etc.

General aim: To study the neuronal networks involved in the effect of $5HT_{1A}$ agonists and atypical antipsychotic drugs. **Specific objective:** To evaluate the role of prefrontocortical GABAergic interneurons, hippocampal pyramidal neurons and R-(+)-8-OH-DPAT enantiomer on the disinhibitory effect of low doses of 8-OH-DPAT on PFC pyramidal neurons.

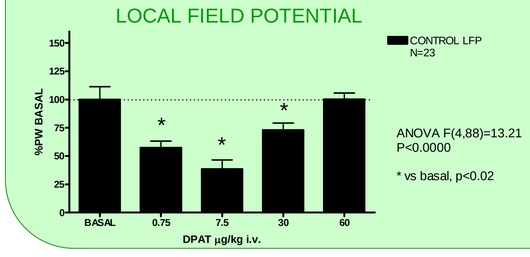
RESULTS

CONTROL SITUATION: LOW DOSES OF 8-OH-DPAT CAUSE AN INCREASE OF ACTIVITY ON mPFC PYRAMIDAL NEURONS

PYRAMIDAL NEURON FIRING



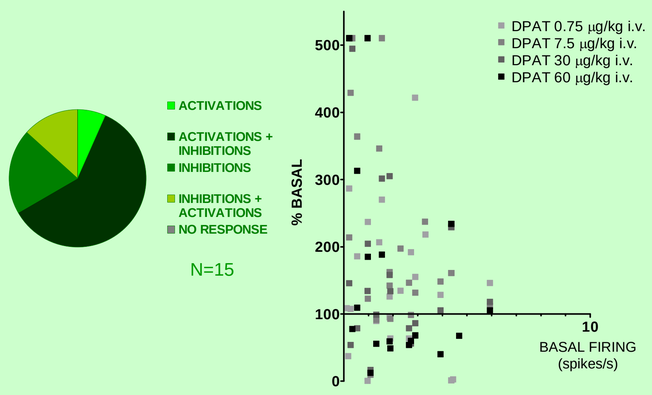
LOCAL FIELD POTENTIAL



ANOVA F(4,88)=13.21
P<0.0000

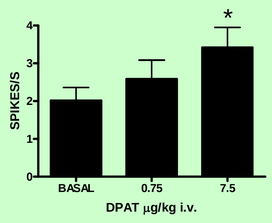
* vs basal, p<0.02

PATTERNS OF RESPONSE



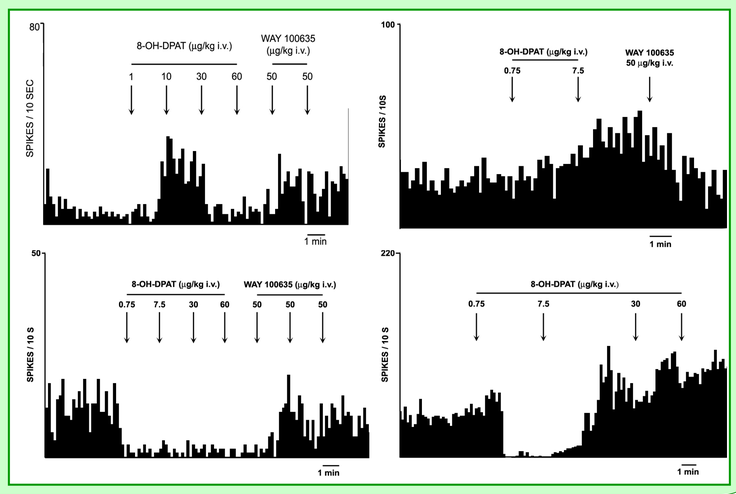
N=15

DISINHIBITION AT LOW DOSES OF 8-OH-DPAT



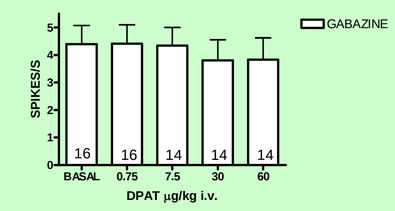
ANOVA F(2,36)=5.47
P<0.0084

* vs basal, p<0.01

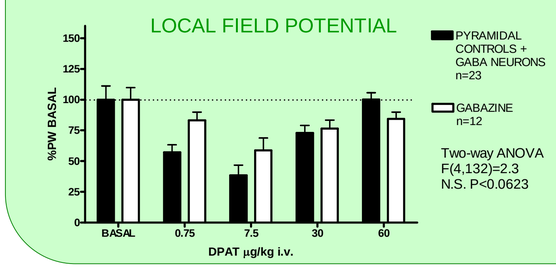


GABAZINE GROUP: LOCAL BLOCKADE OF $GABA_A$ IMPUTS PREVENTS THE DISINHIBITION PRODUCED BY 8-OH-DPAT

PYRAMIDAL NEURON FIRING

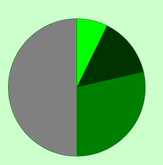


LOCAL FIELD POTENTIAL

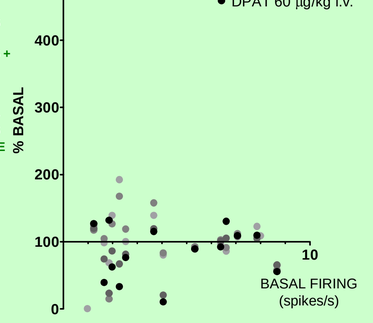


Two-way ANOVA
F(4,132)=2.3
N.S. P<0.0623

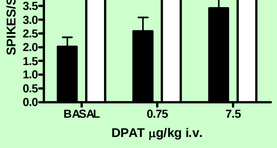
PATTERNS OF RESPONSE



N=14

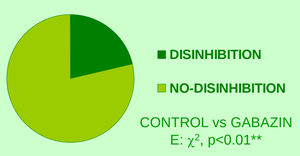


DISINHIBITION AT LOW DOSES OF 8-OH-DPAT

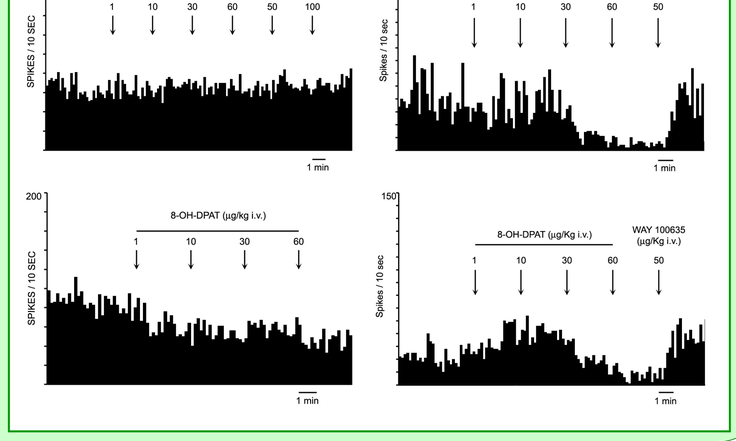


Two-way ANOVA
F(2,62)=3.29
P<0.0438

*vs basal, p<0.01
vs control, p<0.03

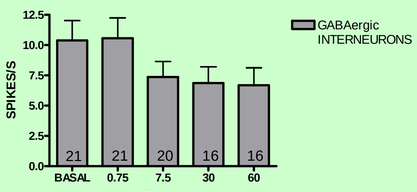


CONTROL vs GABAZIN
E: χ^2, p<0.01**

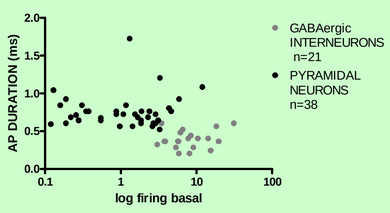


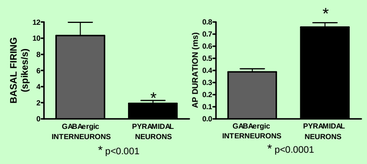
mPFC GABAergic INTERNEURONS ARE INHIBITED BY 8-OH-DPAT WHEN PYRAMIDAL NEURONS ARE DISINHIBITED

GABAergic NEURON FIRING

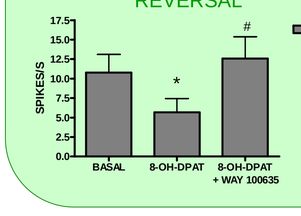


CHARACTERIZATION OF GABAergic NEURONS



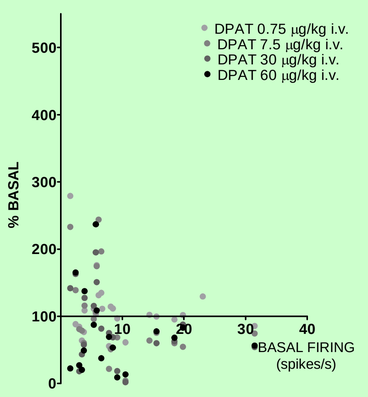


REVERSAL

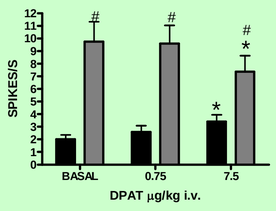


ANOVA F(2,22)=16.83
P<0.0001
* vs basal, p<0.001
# vs 8-OH-DPAT, p<0.001

PATTERNS OF RESPONSE



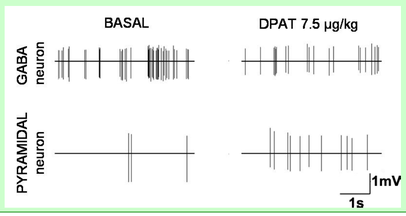
LOW DOSES OF 8-OH-DPAT

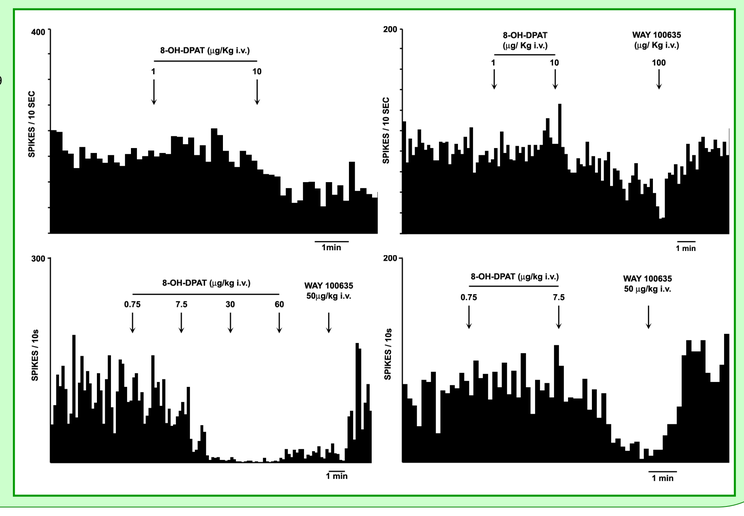


Two-way ANOVA
F(2,74)=8.44
P<0.0005

* vs basal, p<0.05
# vs control, p<0.000000

DUAL RECORDINGS





HIPPOCAMPUS

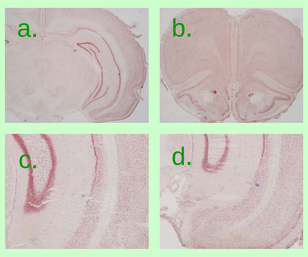
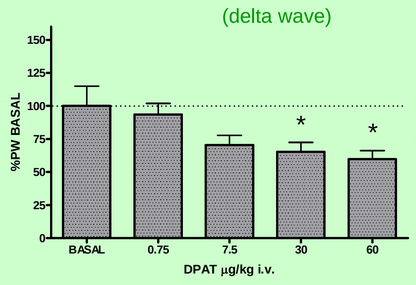


Fig1. Exmaples of recording sites (a, c,d) and stimulation sites (b).

Hippocampal pyramidal neurons (n=7) projecting to the mPFC are silent in our model and the administration of 8-OH-DPAT does not change their firing rate.

LOCAL FIELD POTENTIAL (delta wave)

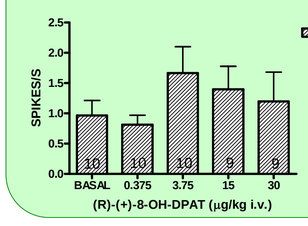


ANOVA F(4,24)=3.35
P<0.0259
* vs basal, p<0.03

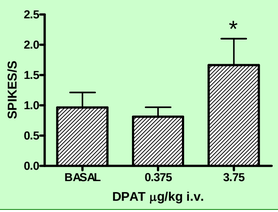
Fig 2. *Schematic representation of the location of $5HT_{1A}$ receptors in rat brain*

(*R*)-(+)8-OH-DPAT ALSO DISINHIBITS mPFC PYRAMIDAL NEURONS

PYRAMIDAL FIRING

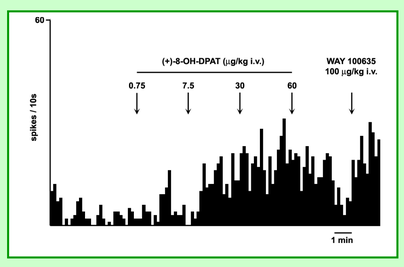


DISINHIBITION AT LOW DOSES (*R*)-(+)-8-OH-DPAT



ANOVA F(2,18)=5.46
P<0.014

* vs basal, p<0.03



CONCLUSIONS

8-OH-DPAT appears to act on two different populations of cortical $5HT_{1A}$ receptors to modulate mPFC pyramidal activity, located on GABAergic interneurons and pyramidal neurons.

The hippomcapus does not seem to be involved in the effects of 8-OH-DPAT on mPFC, although the involvement of other mPFC afferent areas cannot be discarded.

There are not apparent differencies between the (R)-(+)-8-OH-DPAT enantiomer and (±)-8-OH-DPAT.

Drugs with a preferential effect on GABAergic 5-HT_{1A} receptors could have a favourable therapeutic profile for the treatment of negative symptomatology in schizophrenic patients.

REFERENCES

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(2) Díaz-Mataix L. *et al* (2005), *Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action*. J Neurosci 25(47): 10831-10843.

(3) Santana N. *et al* (2004), *Expression of Serotonin1A and Serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex*. Cerebral Cortex 14: 1100-1109.

(4) Amargós-Bosch M. *et al* (2004), *Co-expression and "in vivo" interaction of $Serotonin_{1A}$ and $Serotonin_{2A}$ receptors in pyramidal neurons of prefrontal cortex*. Cereb Cortex 14: 281-299.

(5) Borsini F. *et al* (1995) *BIMT-17 a 5HT2A receptor antagonist and 5HT1A receptor full agonist in rat cerebral cortex*. Naunyn-Schmiedeberg's Arch Pharmacol 352:276-282.

METHODS

Animals: Male Wistar rats (250-300 g) (Iffa-Credo, Lyon, France).

Drugs:+- *8-OH-DPAT* (5-HT_{1A} agonist, Sigma, injected i.v.), *(R)-(+)-8-OH-DPAT* (active isomer $5HT_{1A}$ agonist, Sigma, injected i.v.), *WAY 100635* (5-HT_{1A} antagonist, Sigma-RBI, injected i.v.), *GABAZINE* (SR95531, $GABA_A$ antagonist, Sigma-RBI, 20mM in 0.2M saline in the recording electrode in the experimental group Gabazine)

EXTRACELLULAR SINGLE UNIT RECORDINGS AND LOCAL FIELD POTENTIALS (LFP) IN mPFC: Anaesthesia: Initial chloral hydrate 400 mg/kg i.p.. Maintenance: Chloral hydrate ~1mg/kg/min i.p.. **Analysis:** *FIRING RATE* (spikes per second), *LOCAL FIELD POTENTIAL* (power spectra, values from 0.3 to 4Hz, low frequency oscillations).

Experimental groups: *CONTROL and (+)8-OH-DPAT:* Saline 2M in the recording electrode. Recordings of mPFC pyramidal neurons (AP +3.2 to +3.4, L -0.5 to -1, DV -1 to -4 mm) projecting to the VTA. Identification of neurons by antidromic stimulation (0.4-1.5 mA, 0.2 ms) from the VTA (AP -5.8, L -0.4, DV -8 mm). *GABAZINE:* Gabazine 20mM in 0.2M saline in the recording electrode. Recordings of mPFC pyramidal neurons projecting to the VTA. Identification by antidromic activation from the VTA. *mPFC GABAergic INTERNEURONS:* Neurobiotin 2% in saline 0.5M in the recording electrode. Recordings of mPFC fast spiking GABAergic interneurons identified by electrophysiologic characteristics and subsequent IHQ for neurobiotin and ISH for GAD. *HIPPOCAMPUS:* 2% Pontamine Sky Blue in saline 2M in the recording electrode for the identification of the recording site. Recordings of hippocampal pyramidal neurons (AP -6.3 to -7, L -4, DV 15º -4 to -7 mm) projecting to the mPFc (AP+3, L-0.8, DV -3.5 mm). Identification of neurons by antidromic stimulation (1mA, 0.5Hz, 300ms) from the mPFC.

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