



# Analysis of Glutamate, GABA, Noradrenaline, Dopamine and Serotonin using microbore UHPLC with electrochemical detection

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## Introduction

We measured Glutamate, GABA, Noradrenaline, Dopamine and Serotonin concentrations in microdialysis fractions taken from the rat prefrontal cortex, shell and core of the nucleus accumbens, which are acknowledged as having an important role in cognition, reward processes, motivation and extra-pyramidal motor functions.

As most of these neurotransmitters are present only in low concentrations in the small samples, two sensitive analysis methods have been developed with the ALEXYS Neurotransmitter Analyzer.

This poster highlights that the ALEXYS Neurotransmitter Analyzer can be used to comfortably measure these neurotransmitters at basal levels in different brain regions.

## Materials and method

### Microdialysate sample

Probes [dialyzing portion 3.0 mm (PFCX) or 1.5 mm (NAC)] were implanted vertically in the rat PFCX (AP 3.7; L 0.7; V -4.2), or in the shell (AP 1.9; L 0.9; V -7.8) and core (AP 1.6; L 1.5; V -7.5) according to the atlas of Paxinos and Watson (1998). A Ringer solution (147 mM NaCl; 2.2 mM CaCl<sub>2</sub>; 4 mM KCl) was pumped through the dialysis probe at a constant rate of 1 µl/min. Samples were collected every 10/20 min.

### Analysis principle of GABA and Glutamate

- Automated in-needle pre-column derivatisation of amino acids with OPA/sulphite reagent (odorless) into stable and electrochemically-active isoindole sulfonates.
- Step-gradient to wash out late eluting substances
- Separation in 8 min, total analysis time 11 min
- Detection limit GABA about 10 nmole/L
- Required sample volume: >9 µL

**PRECOLUMN DERIVATIZATION**

O=C1C=CC(=O)C=C1 + R1-NH2 >>[SO3^2-][pH=10.4] O=C1C=CC(=O)C=C1N(R1)S(=O)(=O)[O-]

**STEP GRADIENT ELUTION FOR BASELINE CLEAN-UP**

No late eluting peaks  
next analysis can be started after 16 min

ALEXYS Neurotransmitter Analyzer

### Analysis principle of monoamines

- Reversed phase UHPLC with ion pairing separation conditions
- User defined injection program to minimize sample use (5 µL)
- Analysis time < 4 minutes
- Detection limit about 200 pmole/L

## Analysis of Glu and GABA in microdialysates

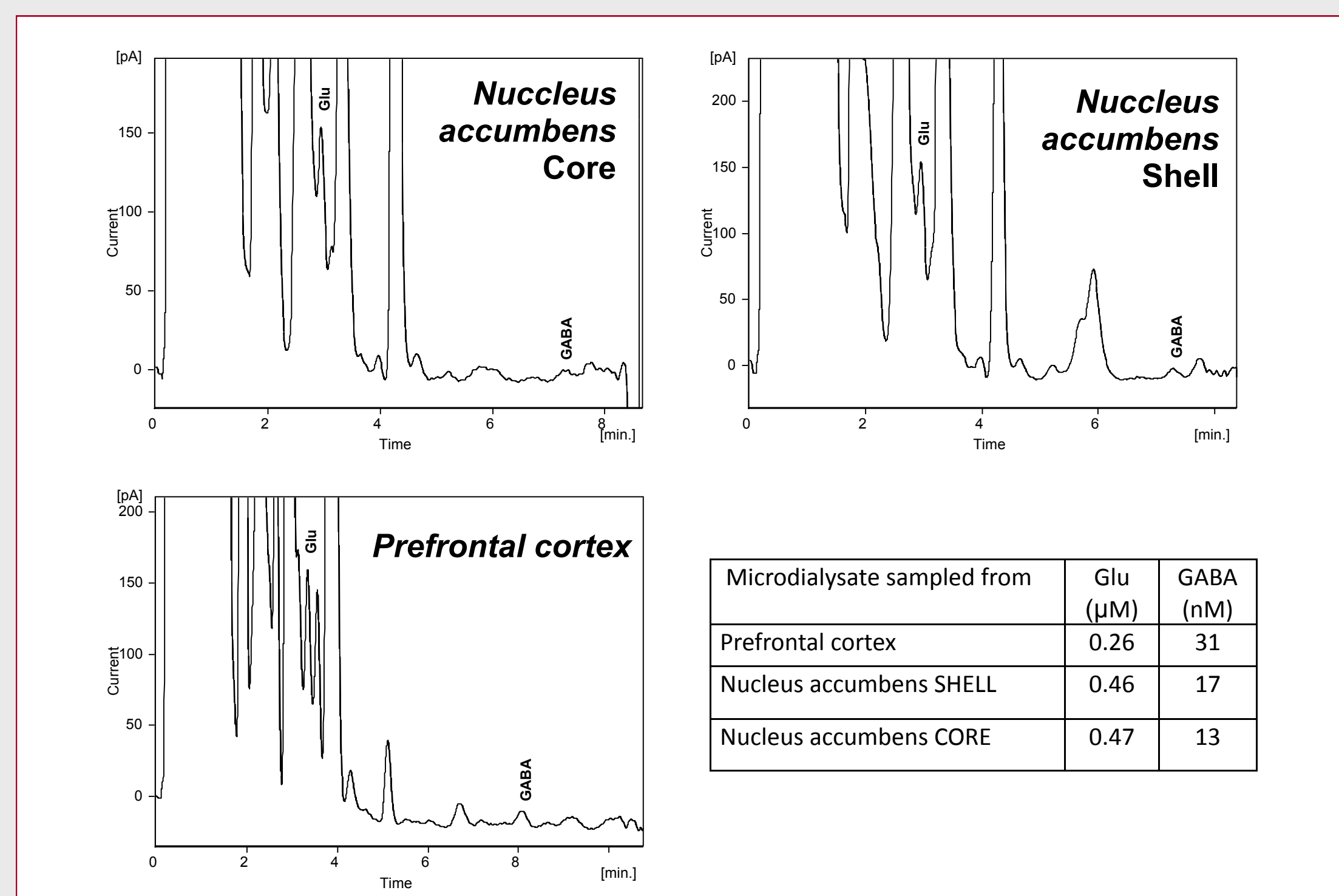


Figure 1. Chromatograms from microdialysate samples taken from different brain regions showing basal levels.

## Analysis of Monoamines in microdialysates

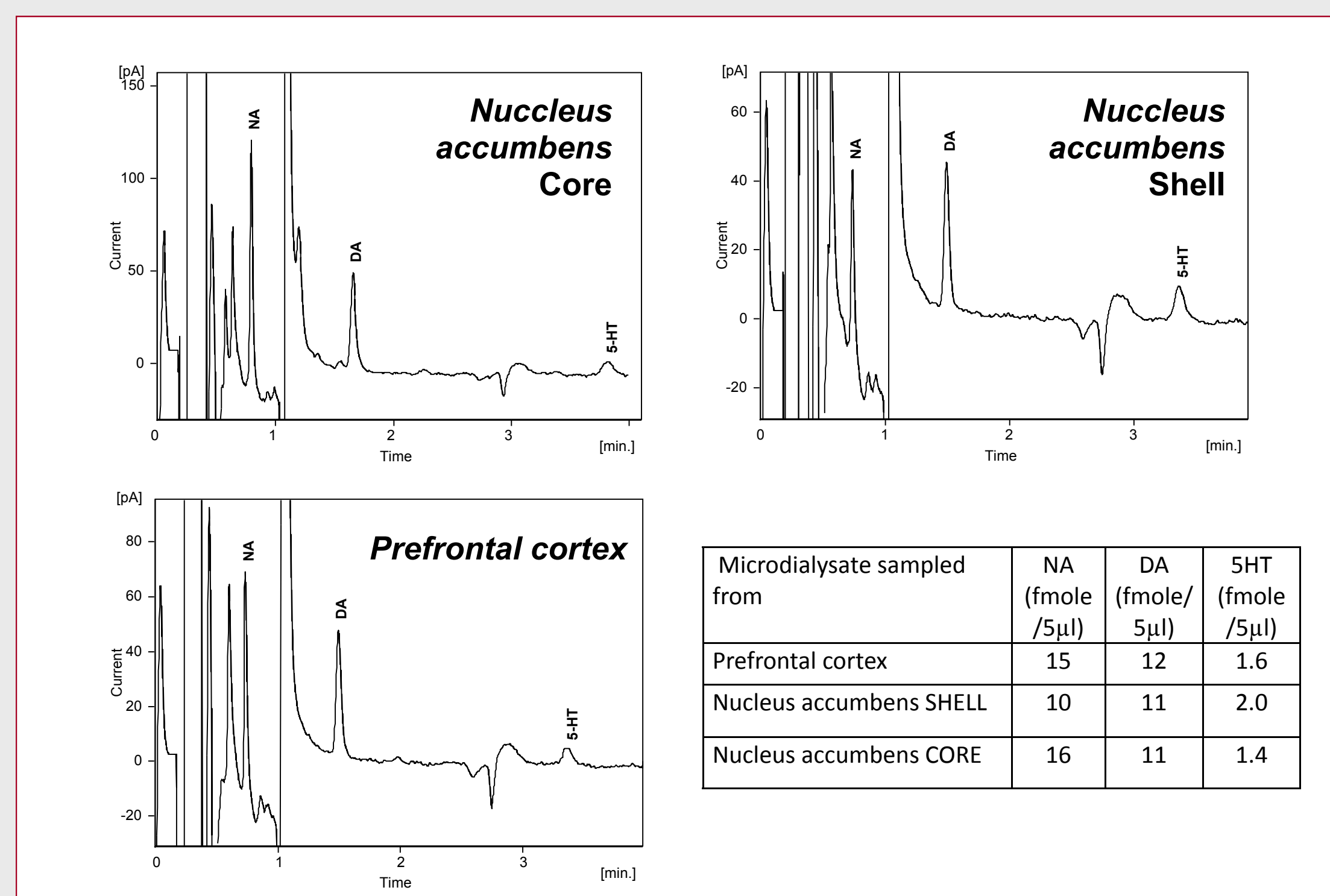


Figure 2. Chromatograms from microdialysate samples taken from different brain regions showing basal levels.

## Conclusion

The ALEXYS Neurotransmitter Analyzer can be used to measure different neurotransmitters at basal levels in different brain regions of rats.

- Monoamines (NA, DA & 5-HT) are analyzed within 4 minutes as well as with an excellent sensitivity of about 200pM, which is well below basal levels.
- GABA and Glu are fully automatically analyzed (OPA "in-needle" derivatization included) within 11 min with a detection limit for GABA of about 10nM