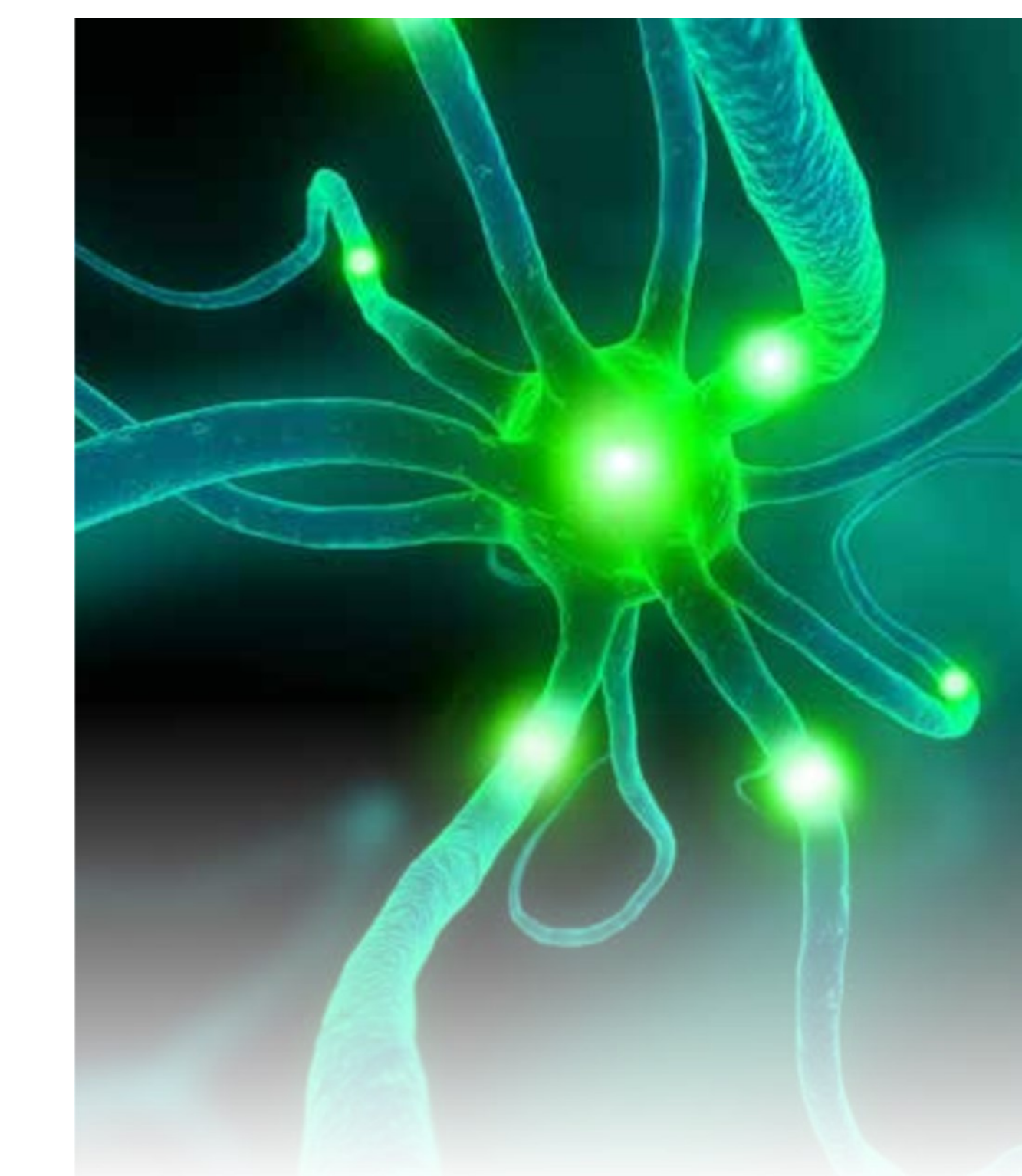


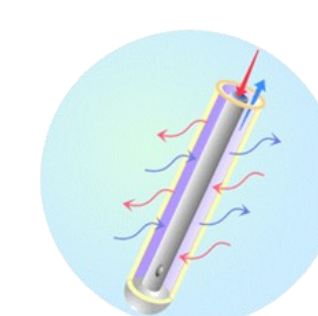
Fast analysis of Dopamine and Serotonin for high time resolution in microdialysis experiments



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Improving time resolution for measurements in brain microdialysates

Microdialysis of neurotransmitters in vivo has become an invaluable tool to study neurotransmission in the living brain. Extracellular fluid of the brain is sampled through a the semipermeable membrane with a microdialysis probe.



HPLC analysis requires fractionation of the sample stream, and the size of the fractions will affect time resolution. To accurately measure fast responses, a high time resolution is necessary as show in Fig.1.

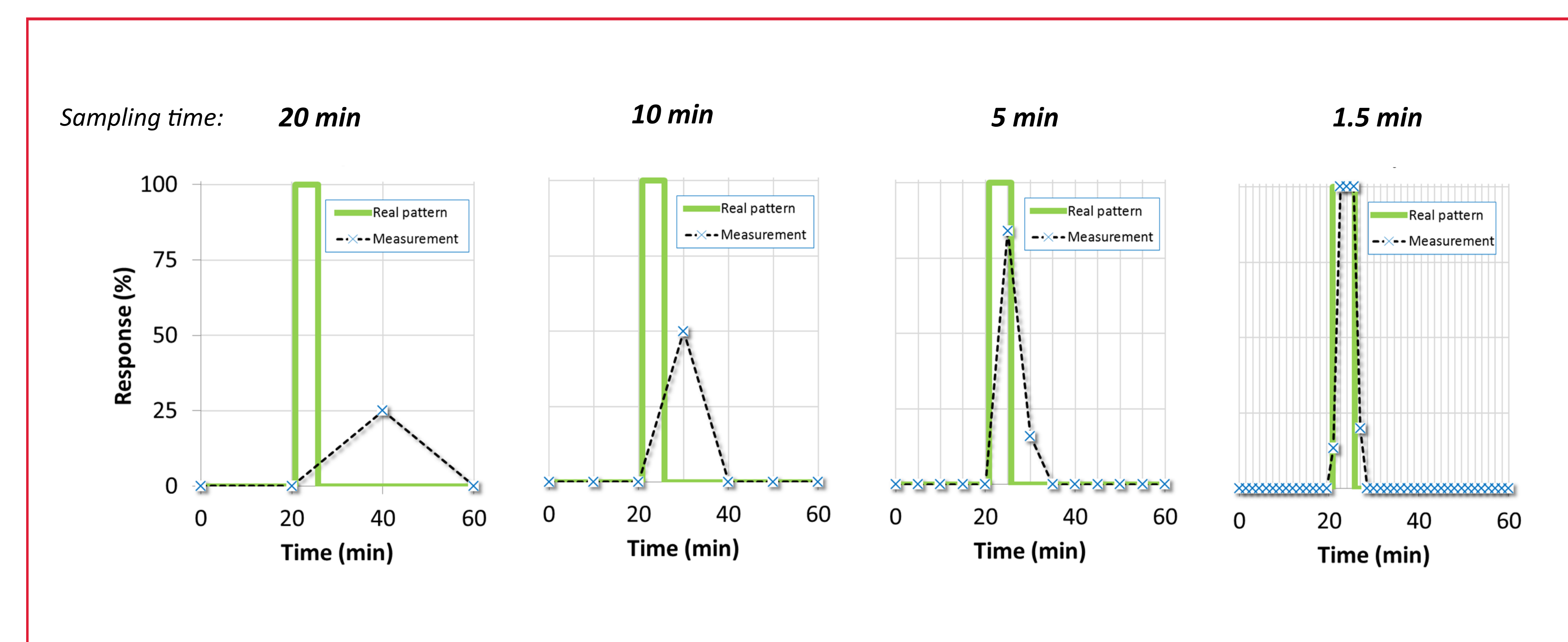


Fig. 1. Higher time resolution more accurately describes fast patterns.

Time resolution is influenced by:

- perfusion rate (typically 1-2 $\mu\text{L}/\text{min}$)
- total analysis time to process a sample
- size and number of serial sample loops

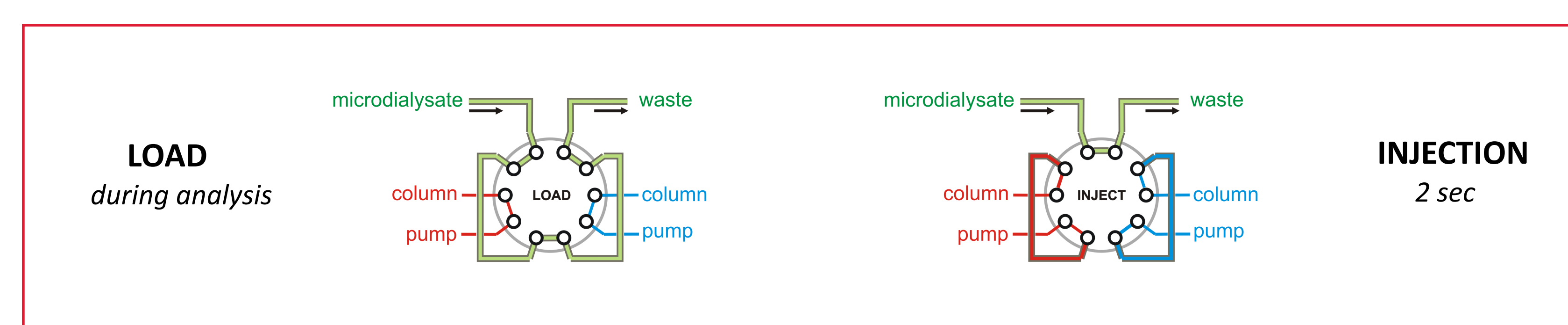
On-line sampling with the ALEXYS Neurotransmitter Analyzer

We developed a robust, commercially available, on-line solution to improve time resolution, as shown in this poster with an example for DA and 5-HT measurements in microdialysate.

The versatile UHPLC ALEXYS Neurotransmitter Analyzer is based on the DECADE Elite electrochemical detector (ECD) with SenCell. Separation and detection of dopamine and serotonin is achieved using two sub-2 μm particle columns in parallel. A fast isocratic LC-ECD method assures high time resolution for low level changes in neurotransmitter response.

Method

The ALEXYS Neurotransmitter Analyzer is equipped with a 10 port valve to simultaneously analyze two different time samples that are collected in two serially installed sample loops.



Two sample loops -> factor 2 better time resolution.

Conditions for analysis of DA and 5-HT

LC ALEXYS[®] Neurotransmitter Analyzer with DECADE Elite and 10-port valve
 Flow cell 2 mm glassy carbon SenCell, saltbridge reference, AST setting '1'
 $V_{\text{injection}}$ 1.5 μL per channel
 Columns UHPLC C18 column, 1.0x100 mm, 1.7 μm particles
 Mobile phase Acetate buffer pH 5.8, ion pairing agent, acetonitrile
 Flow rate 175 $\mu\text{L}/\text{min}$ at 35 $^{\circ}\text{C}$; 280 $\mu\text{L}/\text{min}$ at 60 $^{\circ}\text{C}$ (pressure 480-490 bar in both cases)

Conditions of microdialysis

Perfusion solution 147 mM Na^+ , 1.2 mM Ca^{2+} , 3 mM K^+ , 1.2 mM Mg^{2+} , 152.4 mM Cl^- in water
 Solution flow rate 1.0 $\mu\text{L}/\text{min}$ through probe

Chromatograms from the analysis of DA and 5-HT

The method for quantification of DA and 5-HT shows reproducible results (<2%RSD) and a detection limit of 100 picomol/L.

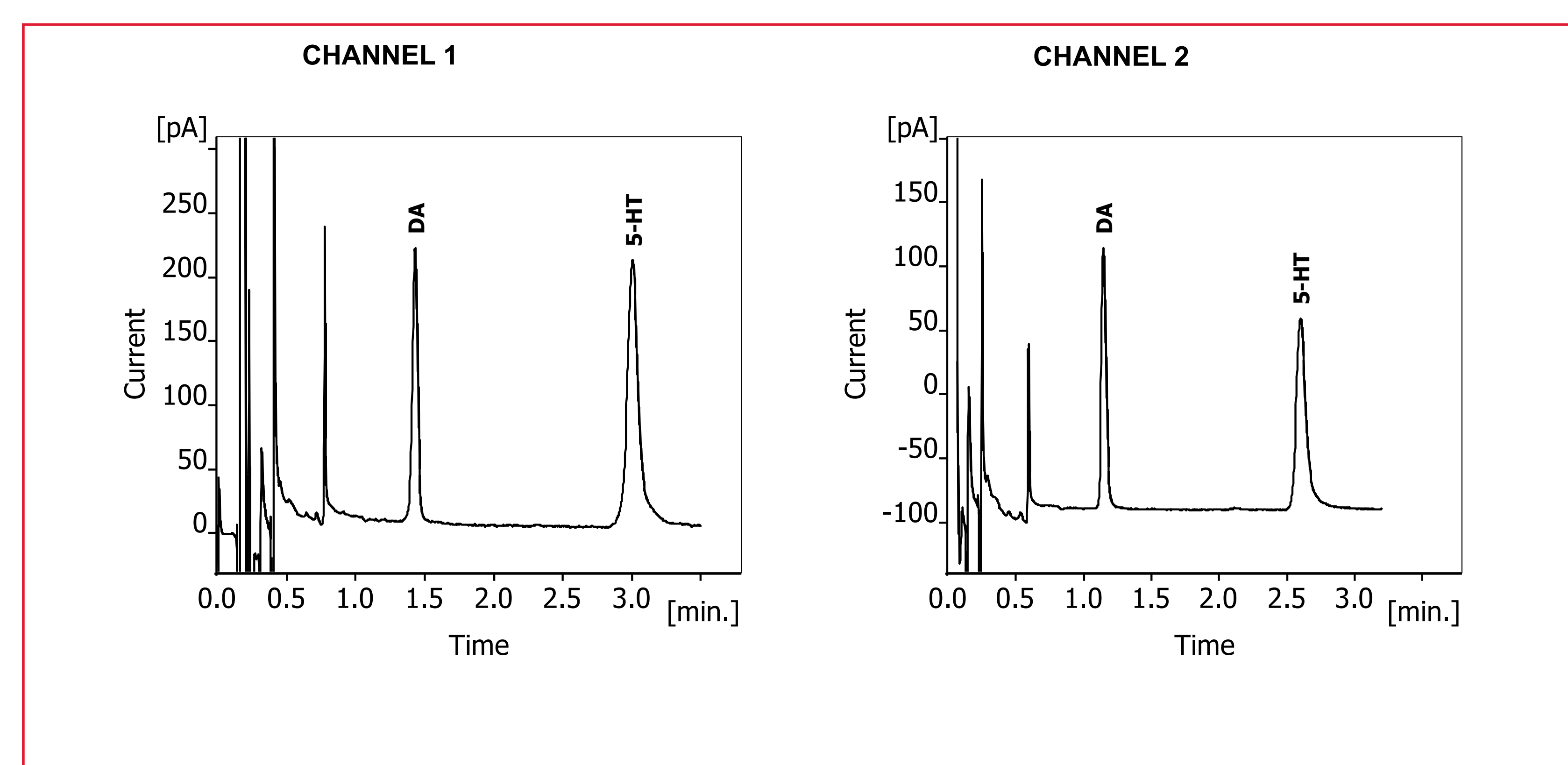


Fig. 2. Simultaneously recorded chromatograms of 10 nM DA and 5-HT standards collected during the previous analysis.

On-line microdialysis experiment

The tip of the probe had been immersed in perfusion fluid and then transferred for 25 min into standard solution (10 nM DA and 5-HT in perfusion fluid). The response was continuously monitored before, during and after the transfer (Fig. 3).

With a time resolution of 1 data point per 1.8 minutes, fast responses are detected. UHPLC/ECD analysis of small samples (down to 1.5 μL) and detection limit down to 0.15 fmol.

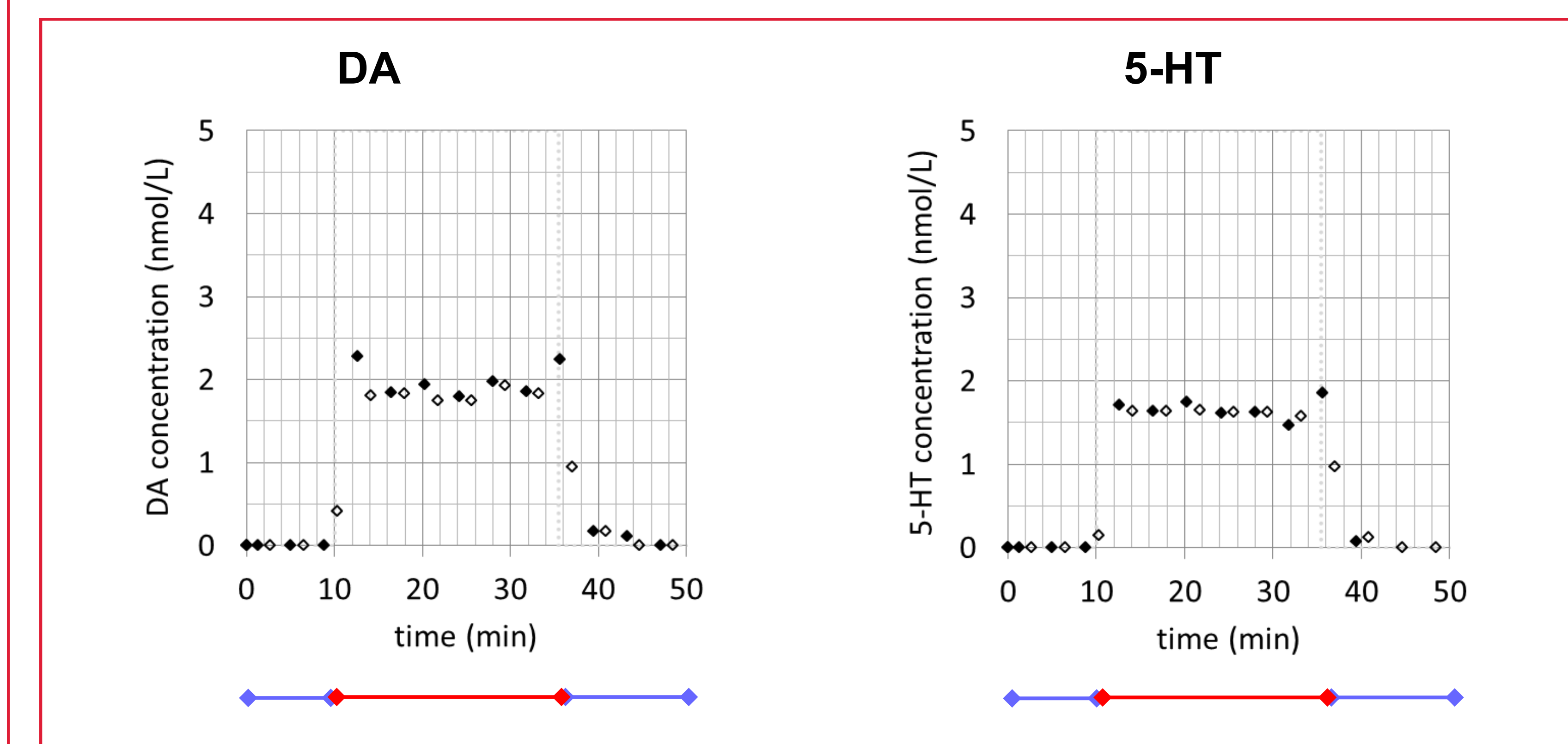
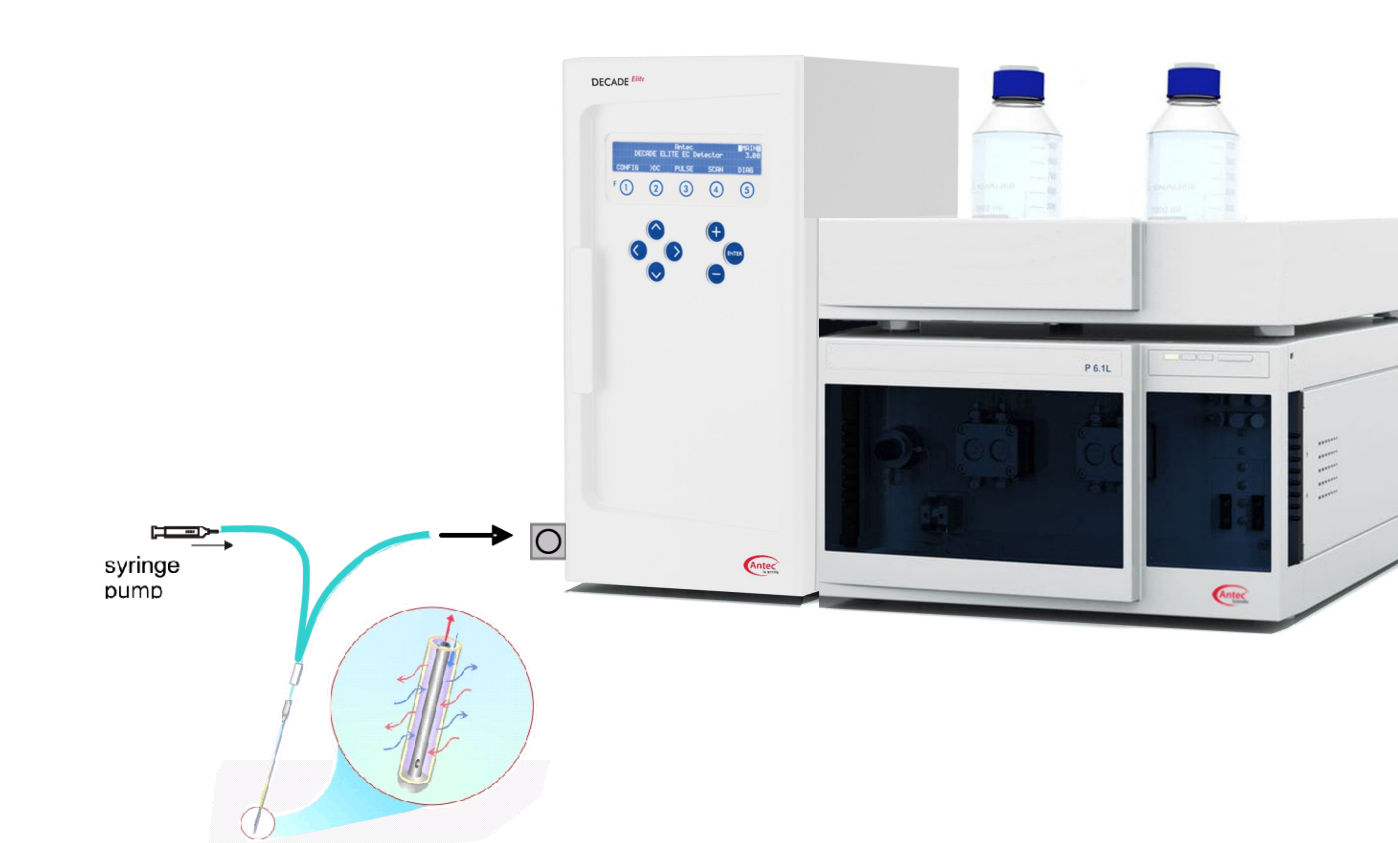


Fig. 3. Reposes from sample loop 1 (closed) and 2 (open). The red line indicates the transfer of the probe from blank to solution containing 10 nM DA and 5-HT.

CONCLUSION

The ALEXYS UHPLC Neurotransmitter Analyzer is a dedicated system solution to sensitively measure neurotransmitters in small samples.

Good sensitivity and a time resolution of < 2 min can now be combined with on-line microdialysis as shown for the analysis of DA and 5-HT.



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