Aug 05, 2024

In vivo microdialysis for striatal DA release

In 2 collections

DOI
dx.doi.org/10.17504/protocols.io.n92ld8q1xv5b/v1

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Protocol Citation: Ariadna Laguna, Miquel Vila 2024. In vivo microdialysis for striatal DA release. **protocols.io https://dx.doi.org/10.17504/protocols.io.n92ld8q1xv5b/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 05, 2024

Last Modified: August 05, 2024

Protocol Integer ID: 104683

Keywords: vivo microdialysis for striatal da release, vivo microdialysi, local effects of nomifensine, nomifensine, net inhibitor

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The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

To assess local effects of nomifensine (DAT and NET inhibitor) on striatal DA release in microdialysis experiments

Troubleshooting

Preparation of reagents

1 Nomifensine (DAT and NET inhibitor) is dissolved in artificial cerebrospinal fluid (aCSF in mM: NaCl, 125; KCl, 2.5; CaCl2, 1.26 and MgCl2 1.18)

2 Concentrated solutions (1 mM; pH adjusted to 6.5–7 with NaHCO3 when necessary) are stored at -80°C and working solutions are prepared daily by dilution in aCSF.

Mouse surgery

3 One concentric dialysis probe (Cuprophan membrane; 6000 Da molecular weight cut-off; 1.5 mm-long) is implanted in the striatum (AP, 0.5; ML, -1.7; DV, -4.5 in mm) of isoflurane-anesthetized mice

4 Microdialysis experiments are performed in freely-moving mice 24h after surgery.

Microdyalisis

5 Probes are perfused with aCSF at 1.5 µL/min

6 Following an initial 100-min stabilization period, 5 or 7 baseline samples are collected (20 min each) before local drug application

7 Nomifensine is administered by reverse dialysis at 10 and 50µM (uncorrected for membrane recovery) and then successive dialysate samples are collected

Dopamine levels determination

8 The concentration of DA in dialysate samples is determined by HPLC coupled to electrochemical detection (+0.7 V, Waters 2465), with 3-fmol detection limit.

9 The mobile phase containing 0.15 M NaH2PO4.H2O, 0.9 mM PICB8, 0.5 mM EDTA (pH 2.8 adjusted with orthophosphoric acid), and 10 % methanol is pumped at 1 ml/min (Waters 515 HPLC pump)

10 DA is separated on a 2.6 mm particle size C18 column (7.5 × 0.46 cm, Kinetex, Phenomenex) at 28°C.

Data representation

11 Microdialysis data is expressed as femtomoles per fraction (uncorrected for recovery) and are shown as percentages of basal values (individual means of 5-7 pre-drug fractions).