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Constant Potential Amperometry in vitro

In 1 collection

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ABSTRACT

Protocol for Constant Potential Amperometry recordings in vitro of evoked dopamine release in the rat striatum

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Slice preparation

1 Anesthetize the animal by isofluorane inhalation and decapitate the animal.

2 Revome quickly and carefully the brain from the skull, and slice the brain in 250–300 µm thick coronal slices containing the striatum by a vibratome immersed in cooled bubbled (95% O2, 5% CO2) 'sucrose-based' artificial CSF (aCSF) solution containing (in mM): KCl3, NaH2PO4 (1.25), NaHCO3 (26), MgSO4 (10), CaCl2 (0.5), glucose (25), sucrose (185); ~300 mOsm, pH 7.4

3 Transfer slices in standard aCSF solution containing (in mM): NaCl (126), KCl (2.5), NaH2PO4 (1.2), NaHCO3 (24), MgCl2 (1.3), CaCl2 (2.4), glucose 10; ~290 mOsm, pH 7.4, bubbling (95% O2, 5% CO2); at 32 °C, and let them recovery for at least 40 min

4 Transfer one slice for amperometric recordings into the recording chamber under an optical microscope, and superfuse (2-3 ml/min) the slice with standard aCSF solution kept at 32°C

Amperometric recordings

5 Place a bipolar Ni/Cr insulated stimulating electrode into the striatal slice. To monitor the electrically evoked dopamine overflow, gently place a carbon fiber electrode (active surface 30 µm in diameter and 100 µm long; World Precision Instruments, CF10) into the striatal slice to a depth of 100–150 µm near the stimulating electrode, connected to a potentiostat (MicroC, World Precision Instruments) to apply voltage and measure current.

5.1 Impose 0.55 V between the carbon fiber electrode and the Ag/AgCl pellet

6 Apply every 5 min a single rectangular electrical pulse through a bipolar stimulating electrode placed on the striatal slice by using a Digitimer DS3 stimulator (80–500 µA, 20–40 µs duration)

6.1 Apply increasing electrical pulse step until maximum evoked dopamine outflow

7 At the end of each experiment, perform electrode calibration in aCSF containing known dopamine concentration (300 nmol/L–3 µmol/L)

Drug application

1h

8 Monitor the extracellular dopamine response to electrical stimulation is stable for four or five successive stimulations

9 Superfuse the striatal slice with drugs (0.3 µM cocaine, 1 µM cocaine, or 0.03 µM quinpirole, dissolved in standard aCSF) for 10 min (cocaine) and 5 min (quinpirole)

9.1 Wash out with standard aCSF for 1h (cocaine) or 30 min (quinpirole)

10 At the end of each experiment, perform electrode calibration in aCSF containing known dopamine concentration (300 nmol/L–3 µmol/L)