Supplemental material for:

µ-Opioid receptors in the stimulation of mesolimbic dopamine activity by ethanol and morphine: a delayed effect of ethanol.

Psychopharmacology

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In this supplement we offer additional details regarding baseline dialysate dopamine concentrations, high-performance liquid chromatography, gas chromatography, and microdialysis probe placements

Basal dialysate dopamine concentrations and the effect of saline infusion

In β-funaltrexamine experiments (experiments 4 & 5), rats were pretreated subcutaneously with saline or β-funaltrexamine (20 mg/kg) followed by saline (i.v.) 20 - 25 hours later and then (at least 1.5 hours later) by morphine or ethanol.

Baseline concentrations of dialysate dopamine just prior to the “day 2” intravenous saline infusion were 1.4 ± 0.1 (n=6) and 1.5 ± 0.2 (n=10) nM for the saline and β-funaltrexamine pretreated rats, respectively. In other words, there was no significant effect of β-funaltrexamine pretreatment on baseline dopamine (t=0.22, df=14, p>0.05). Five minutes after the saline infusion the dopamine concentrations were 1.4 ± 0.2 and 1.6 ± 0.2 nM for the saline and β-funaltrexamine groups, respectively. There was no significant effect of i.v. saline infusion for either group compared to baseline (F_{5,70} =1.05, P > 0.05).

The baseline dopamine levels, before morphine administration, were 1.2±0.3 (n=6) and 1.7±0.5 (n=5) nM for control and rats pretreated with β-funaltrexamine, respectively. The baseline dopamine levels, before ethanol infusion, were 0.9±0.1 (n=6) and 1.4±0.3 (n=6) nM for control and rats pretreated with β-funaltrexamine, respectively.

HPLC Details

Reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection was used to analyze dialysate dopamine concentrations. The experiments were carried out over a period of approximately 5 years and various configurations of HPLC systems were used. Early in the course of these studies a typical analysis was carried out using the following procedure: seven microliters of the dialysate were mixed with a solution of ascorbate oxidase and a portion of this mixture was injected into the system with an autosampler (FAMOS, Thermo Scientific, Bannockburn, IL). Separation of dopamine occurred with a C18 column (Polaris 50 x 2 mm, 3 µm particle size; Agilent, Santa Clara, CA) using a mobile phase consisting of 0.50 g octanesulfonic acid, 0.05 g sodium hydroxide, and 0.1 mL/L L-ascorbic acid.
decanesulfonic acid, 0.13 g ethylenediaminetetraacetic acid, 11.08 g NaH₂PO₄, 4.47 g KCl and 150 mL methanol in 1 L of deionized water. The pH was adjusted to 5.6 before addition of the methanol. Detection of dopamine took place with a VT-03 flow cell with a 2 mm glassy carbon working electrode (ISAAC reference, Antec Leyden, Zoeterwoude, The Netherlands) using an oxidizing potential of +345 mV. The flow cell was controlled using an INTRO (Antec). More recently the HPLC configuration allows us to use 7 microliter injection volumes with an 8125 manual injector (Rheodyne, Cotati, CA) without ascorbate oxidase. The column is C18 (50 x 1 mm, 3 μm particle size, Phenomenex, Torrance, CA), and the flow cell is a VT-03 with a salt bridge reference electrode (Ag/AgCl) and a 2 mm working electrode at a potential of +450 mV. The flow cell is controlled using a Model 400 detector originally from EG&G Princeton Applied Electronics (Princeton, NJ). EZChrom Elite software (Agilent, Wilmington, DE) was used to record and analyze all chromatograms. External dopamine standards were used to determine the concentration of dopamine in each sample. The signal to noise ratios were calculated and recorded for all samples, and only dopamine peaks with a signal to noise ratio above 3 were included in the analyses.

Gas Chromatography Details

Ethanol was analyzed in all dialysis samples collected after administration of ethanol. Before the dialysis sample was frozen, 2 μl was transferred into a glass vial and sealed with a septum for ethanol analysis later that day. The following parameters were used: A gas chromatograph (Varian CP 3800, Varian, Walnut Creek, CA) equipped with a flame ionization detector (220°C) measured the dialysate ethanol. The sealed vial was heated for at least 20 min to vaporize the liquid sample before injection. An autosampler equipped with a solid-phase microextraction fiber assembly (75 μm of carboxen-PDMS (polydimethyl siloxane); Supelco, Bellefonte, PA) injected the samples (3-min absorption and 1-min desorption) into the heated injection port (175 or 220°C). Helium was the carrier gas (8.5 ml/min), and separation occurred under isothermal conditions (65°C) with an HP Innowax capillary column (30 m x 0.53 mm x 1.0 μm film thickness; Agilent, Santa Clara, CA). The limit of detection was 0.03 mM (signal to noise > 3), and quantitation of ethanol in dialysates was performed by comparison of peak heights obtained from the Star chromatographic analysis system (Varian) with external standards.

Histology

The microdialysis probe placements for the systemic naltrexone experiments can be found in Figure S1. The microdialysis probe placements for the β-funaltrexamine experiments can be found in Figure S2. The microdialysis probe placements for the intracranial naltrexone experiments can be found in Figure S3. The microinjector placements for the VTA experiments can be found in Figure S4. Only rodents that had the tip of the microinjector within the VTA were included in the analysis. Coordinates are mm from bregma.
**Fig. S2** The microdialysis probe placements for the β-funaltrexamine experiments.  
(a) Placements for the subcutaneous β-funaltrexamine effect on morphine-stimulated dopamine release (experiment 4).  
(b) Placements for the subcutaneous β-funaltrexamine effect on ethanol-stimulated dopamine release (experiment 5).  
Only rodents that had probes predominantly (> 50%) within the shell of the NAcc were included in the analysis.  
Coordinates are mm from bregma.

**Fig. S3** The microdialysis placements for the intracranial naltrexone experiments.  
(a) Placements for the intra-ventral tegmental area microinjection of naltrexone effect on morphine-stimulated dopamine release (experiment 6).  
(b) Placements for the intra-ventral tegmental area microinjection of naltrexone effect on ethanol-stimulated dopamine release (experiment 7).  
Only rodents that had probes predominantly (> 50%) within the shell of the NAcc were included in the analysis.  
Coordinates are mm from bregma.
Fig. S4 The microinjector placements for the VTA experiments (experiments 6 & 7). Only rodents that had the tip of the microinjector within the VTA were included in the analysis. Coordinates are mm from bregma.