

Radioligand Binding Autoradiography for dopamine D1-like and D2-like receptor sites (1/1/08 H Yamaguchi)

- (1)** Remove the mouse brain and cut the brain in sagittal or coronal plane. The brains are then embedded in OCT compound (Sakura) on the ice. (If you have control brains and KO brains, put the brains together in the same embedding block. For example, 2 control brains + 2 KO brains.) Freeze the brains on the powdered dry ice and stored at -80C until use.
- (2)** Cut the sections on a cryostat at a thickness of 20 μm , thaw-mounted on to glass slides and stored at -80C until use.
- (3)** Pick up the serial coronal sections every 100 μm and put the slides at room temperature and dried.

Wash slides for 5 minutes in the working buffer to remove endogenous ligands.

D1-like receptor sites

Incubate brain sections for 1 hr at room temperature in 50 mM Tris-HCl (pH 7.4) containing 4 mM MgCl_2 and 1 nM $^3\text{H-SCH23390}^*$ (85.0 Ci/mmol; PerkinElmer) with 0.3 μM ketanserin (Sigma) (to prevent the labeling of 5-HT₂ receptors). Incubate some sections with 1 μM unlabeled SCH23390 (Sigma) to assess nonspecific binding.

D2-like receptor sites

Incubate brain sections for 1 hr at room temperature in 50 mM Tris-HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 1 mM ascorbic acid and 0.6 nM of $^3\text{H-spiperone}^*$, (15.0 Ci/mmol; PerkinElmer) with 0.3 μM ketanserin. Incubate some sections with 10 μM unlabeled spiperone (Sigma) to assess nonspecific binding.

*Concentration of radioligand may be variable depending on the radioactivity/unit of each ligand.

Rinse brain sections twice in the respective incubation buffer for five min each time, then dip the sections in water and dry the sections completely.

Place the sections on imaging plates (BAS-TR2025, Fuji film) with tritium activity standards (Microscales, Amersham Biosciences) for 3-7 days.

- (4)** Scan the imaging plates with a high-performance imaging plate reader (BAS5000, Fuji film) and quantify the radioactivity* by the image analysis software (Multi Gauge V3.0, Fuji film).

*Set a region of interest on the part of dorsal striata for each section. Use seven to ten sections from each mouse to calculate the mean radioactivity of the striatum.

(References)

- (1)** Kitada T, Pisani A, Porter DR, Yamaguchi H, Tscherter A, Martella G, Bonsi P, Zhang C, Pothos EN, Shen J. Proc Natl Acad Sci U S A. 2007 Jul 3;104(27):11441-6.
- (2)** Yamaguchi H, Aiba A, Nakamura K, Nakao K, Sakagami H, Goto K, Kondo H, Katsuki M. Genes Cells. 1996;1:253-268.