# Presynaptic vesicular accumulation is required for antipsychotic efficacy in psychotic-like rats

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# Supplementary Information

## Chemical synthesis of HAL-F

### 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-hydroxyphenyl)butan-1-one (2)

4-chloro-1-(4-hydroxyphenyl)butan-1-one (**1**) (0.70 g, 3.54 mmol) and 4-(4-chlorophenyl)piperidin-4-ol (1.50 g, 7.09 mmol) were dissolved in dry DMF (20 mL). DIPEA (1.45 mL, 8.50 mmol) and potassium iodide (catalytic amount) were added and the reaction mixture was stirred at 50°C for two days. The reaction mixture was cooled to ambient temperature, quenched by addition of water (50 mL) and extracted with dichloromethane. The combined organic layers were washed with water and brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a mixture of ethyl acetate and methanol (8 : 1) as eluent. The pure product was obtained as pale yellow solid (0.48 g, 1.27 mmol, 36%).

ESI-MS: *m/z* 374.4 $[M+H]^+$

$^1$H NMR (600 MHz, dmso-$d_6$) δ 10.29 (s, 1H), 7.90 – 7.82 (m, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 6.91 – 6.80 (m, 2H), 4.86 (s, 1H), 4.03 (q, J = 7.1 Hz, 1H), 2.97 – 2.85 (m, 2H), 2.78 – 2.58 (m, 2H), 2.42 – 2.20 (m, 1H), 1.99 (s, 1H), 1.90 – 1.69 (m, 4H), 1.61 – 1.42 (m, 2H), 1.17 (t, J = 7.1 Hz, 1H).

### 1-[4-(4-Bromobutoxy)phenyl]-4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]butan-1-one (3)

4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-hydroxyphenyl)butan-1-one (**2**) (0.10 g, 0.27 mmol) was dissolved in acetonitrile (19 mL) and water (1 mL). 1,4-dibromobutane (0.12 g, 0.54 mmol) and potassium carbonate (73.9 mg, 0.54 mmol) were added and the reaction mixture was stirred at 50 °C for one day. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The crude product was taken up in dichloromethane and aqueous sodium bicarbonate solution was added. The aqueous layer was extracted with dichloromethane. The combined organic layers were washed with sodium bicarbonate solution, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a mixture of ethyl acetate and methanol (95 : 5 + 0.5% triethylamine) as eluent to obtain the pure product as pale yellow powder (83.1 mg, 0.16 mmol, 61%).

ESI-MS: *m/z* 510.2 $[M+H]^+$

$^1$H NMR (600 MHz, dmso-$d_6$) δ 7.98 – 7.92 (m, 2H), 7.39 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.09 – 7.01 (m, 2H), 4.82 (s, 1H), 4.10 (t, *J* = 6.3 Hz, 2H), 3.60 (t, *J* = 6.7 Hz, 2H), 2.93 (t, *J* = 6.8 Hz, 2H), 2.67 – 2.54 (m, 2H), 2.43 – 2.25 (m, 4H), 2.02 – 1.93 (m, 2H), 1.91 – 1.77 (m, 4H), 1.77 – 1.66 (m, 2H), 1.48 (d, *J* = 12.4 Hz, 2H).

### 1-[4-(4-Azidobutoxy)phenyl]-4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]butan-1-one (4)

1-[4-(4-bromobutoxy)phenyl]-4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]butan-1-one (**3**) (38.0 mg, 0.075 mmol) was dissolved in acetonitrile (4 mL) and water (0.4 mL). Sodium azide (19.4 mg, 0.30 mmol) was added and the reaction mixture was stirred at 82°C under reflux for one day. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The crude product was taken up in dichloromethane and washed with aqueous sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to obtain the product which was used for the next step without purification (33.8 mg, 0.072 mmol, 96%).

ESI-MS: *m/z* 471.6 $[M+H]^+$

$^1$H NMR (600 MHz, $CDCl_3$) δ 7.99 – 7.93 (m, 2H), 7.42 – 7.36 (m, 2H), 7.32 – 7.28 (m, 2H), 6.95 – 6.89 (m, 2H), 4.06 (t, *J* = 6.1 Hz, 2H), 3.38 (t, *J* = 6.7 Hz, 2H), 2.96 (t, *J* = 7.1 Hz, 2H), 2.85 – 2.74 (m, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 2.41 (td, *J* = 12.2, 2.1 Hz, 4H), 2.07 – 1.95 (m, 4H), 1.94 – 1.86 (m, 2H), 1.84 – 1.76 (m, 2H), 1.71 – 1.65 (m, 2H).

### Hal-F

A solution of triphenylphosphin (8 mg, 0.031 mmol) and **4** (10 mg, 0.021 mmol) in MeOH (2 mL) was stirred at reflux temperature for 2 h. After being cooled to room temperature the solvent was removed by evaporation and 0.1 M aq. HCl (3 mL) was added. The solution was washed with $Et_2O$ (3x 3 mL) and 1 M aq. NaOH (0.5 mL) was added. The solution was extracted with $CH_2Cl_2$ (3x 5 mL) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in DMF (2 mL). After addition of $Et_3N$ (0.015 mL, 0.11 mmol) and Cy3B dye NHS ester (14 mg, 0.021 mml) the mixture was stirred at room temperature for 2 h.  After addition of water (10 mL) and lyophilization the crude product was purified by preparative HPLC, characterized by LC-MS and submitted to biological testing after lyophilization.

ESI-MS: m/z 987.3 $[M+H]^+$