Suman et al., 2017-Failure to replicate antidepressant effect in Swiss mice FST. **Supplementary Data.**

**Section S1- Systematic review of the literature: procedures and results**

The systematic review of the literature aimed to select effective 1-treatment schedule, 2-via and 3-doses of Fluoxetine to male, adult Swiss mice in the FST. The database was Medline (Pubmed), the keywords were “mice AND forced swimming test AND fluoxetine.” From the retrieved literature, we arbitrarily selected the papers between 2009 and 2015 (last five years before collection of our data) containing experiments in male, adult, Swiss mice receiving Fluoxetine through any via of administration. The dose of 20 mg/kg of Fluoxetine was effective in all qualified publications (see list in the Table S6).

**Table S1.1.** Relevant literature retrieved in a Systematic review.

|  |  |  |
| --- | --- | --- |
| AUTHOR | DOSE | Route |
| Haj-Mirzaian et al.(2015) | 20 mg/kg | IP |
| Sales et al.(2015) | 5, 10, 20, 30 mg/kg | IP |
| Thianzu et al.(2015) | 15 mg/kg | O |
| Sashidhara et al.(2014) | 20 mg/kg | IP |
| Li et al.(2014) | 10 mg/kg | IP |
| Ishola et al.(2014) | 30 mg/kg,  | O |
| Bettio et al.(2014) | 10 mg/kg | O |
| Dhingra & Valecha et al.(2014) | 20 mg/kg | O |
| Dhingra & Chhillar et al.(2012) | 20 mg/kg | O |
| Pawar et al.(2009) | 10 mg/kg | O |

IP= intraperitoneal; O= oral, gavage.

**Section S2- Positive control for Fluoxetine treatment: procedures and results.**

Adult, male Wistar rats treated p.o. with sucrose 10% (VEH, n=5) or Fluoxetine 2.5 mg/kg (FLX, n=5) daily for 13 days, submitted to the test (1 hour after the first dose), retest 1 (1 hour the seventh dose) and retest 2 (1 hour after the thirteenth dose) (Table S7). We expected that FLX would reduce significantly the immobility time in the retest 2, as previously observed in Mezadri et al., J Neurosci Methods. 2011;195(2):200-5 and Possamai et al., Prog Neuropsychopharmacol Biol Psychiatry. 2015;58:15-21. No significant differences were observed within test and retests for each treatment (Friedman, non-parametric within-subject analysis). However, between groups analysis revealed reduced immobility time in rats treated with FLX as compared to VEH (Table S8).

**Table S2.1.** Duration of the Immobility (s) in the rFST of individual Wistar rats treated p.o. with VEH or FLX 2.5 mg/kg.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment | Rat | Test | Retest 1 | Retest 2 |
| FLX | 1 | 0 | 93.7 | 99.9 |
| FLX | 2 | 0 | 12 | 20.1 |
| FLX | 3 | 36.8 | 224.3 | 110.7 |
| FLX | 4 | 134.2 | 166.9 | 122.1 |
| FLX | 5 | 72.7 | 144.5 | 86 |
| VEH | 1 | 86.4 | 147.3 | 111.4 |
| VEH | 2 | 62.2 | 96 | 112.1 |
| VEH | 3 | 68.3 | 106.9 | 148 |
| VEH | 4 | 126 | 200.9 | 135 |
| VEH | 5 | 119.8 | 91.6 | 132 |

**Table S2.2.** FLX (2.5 mg/day/13 days) reduced significantly immobility time in retest2:



**Figure S2.1.** FLX (2.5 mg/day/13 days) reduced immobility time in retest 2:Legend for Figure S4.1: Immobility time (Mean+/-SEM) of adult, male Wistar Rat in the test (left), retest 1 (middle) and retest 2 (right) of the repeated FST after treatment with VEH or FLX 2.5 mg/kg. Abbreviations: VEH= vehicle; FLX= fluoxetine.. \* significantly different from VEH at p<0.05, Mann-Whitney U-test.

**Section S3. Experiments 2 to 5: parameters of swimming and climbing.**

**Table S3.1.** Duration of the swimming and climbing (s) of mice submitted to repeated FST under the influence of vehicle or antidepressants in the doses of 1 mg/kg (experiment 2) or 3 mg/kg (experiment 3).

|  |  |  |
| --- | --- | --- |
|  | Experiment 2 | Experiment 3 |
| Swimming | Climbing | Swimming | Climbing |
| VEH | Test | 98.6+17.3 | 18.4+6.8 | 85.7+20.1 | 4.2+1.9 |
| Retest1 | 19.9+5.2 | 12.0+4.9 | 30.5+6.5 | 12.2+6.1 |
| Retest2 | 29.4+6.1 | 9.6+7.3 | 59.9+24.5 | 7.7+4.0 |
| BUP | Test | 74.9+8.2 | 3.7+1.4 | 94.1+27.3 | 8.6+3.0 |
| Retest1 | 57.6+10.8 | 0.1+0.1 | 47.9+21.8 | 5.0+3.6 |
| Retest2 | 37.5+12.3 | 0.2+0.2 | 41.1+14.9 | 1.2+0.9 |
| DMI | Test | 77.1+9.3 | 55.6+25.2 | 96.4+18.1 | 8.5+2.5 |
|  | Retest1 | 25.3+5.0 | 7.1+2.9 | 60.5+16.7 | 10.5+6.9 |
| Retest2 | 14.1+2.0 | 0.5+0.4 | 23.5+5.52 | 0.8+0.5 |
| FLX | Test | 67.7+16.9 | 4.9+1.5 | 72.0+19.0 | 10.2+2.5 |
|  | Retest1 | 21.3+7.2 | 11.4+4.7 | 54.4+22.6 | 1.1+0.9 |
| Retest2 | 16.9+5.6 | 3.4+1.3 | 50.6+30.6 | 4.8+2.9 |
| SB | Test | 87.2+19.8 | 18.7+8.0 | 63.7+11.6 | 7.5+3.0 |
|  | Retest1 | 29.3+9.9 | 11.5+11.2 | 39.7+5.4 | 3 +1.3 |
| Retest2 | 34.7+15.5 | 6.9+3.7 | 18.6+3.,4 | 2.1+0.9 |

Data expressed as mean ± S.E.M of 8 mice *per* group (except for FLX 1 mg/kg (n=6) and BS 1 mg/kg (n=7)). VEH= vehicle; BUP= bupropion; DMI= desipramine; FLX= fluoxetine; SB= sodium butyrate.

**Table S3.2.** Duration of the swimming and climbing (s) of mice submitted to repeated FST under the influence of vehicle or antidepressants in the doses of 10 mg/kg (experiment 4) or 30 mg/kg (experiment 5).

|  |  |  |
| --- | --- | --- |
|  | Experiment 4 | Experiment 5 |
| Swimming | Climbing | Swimming | Climbing |
| VEH | Test | 85.6+7.8 | 7.21+2.5 | 116.1+22.6 | 2.6+1.3 |
| Retest1 | 86.2+20.6 | 0.0+0.0 | 91.4+35.6 | 0.0+0.0 |
| Retest 2 | 37.7+9.9 | 2.5+2.5 | 33.9+8.6 | 4.2+2.0 |
| BUP | Test | 179.2+37.1 | 6.7+3.9 | 154.1+19.0 | 0.5+0.5 |
|  | Retest1 | 109.7+28.2 | 0.0+0.0 | 93.7+36.3 | 0.3+0.3 |
|  | Retest 2 | 49.3+15.1 | 0.0+0.0 | 78.6+28.7 | 7.1+3.1 |
| DMI | Test | 121.2+19.6 | 6,9+3,6 | NT | NT |
|  | Retest1 | 112.5+36.4 | 0.0+0.0 | NT | NT |
| Retest 2 | 67.9+16.6 | 2.,3+2.3 | NT | NT |
| FLX | Test | 112+22 | 1.1+1.1 | 82.9+10.2 | 7.2+5.3 |
|  | Retest1 | 106.4+30.6 | 1.8+1.2 | 58.5+8.3 | 0,1+0,1 |
| Retest 2 | 34,52+5,0 | 0.0+0.0 | 42.5+8.8 | 7,6+2,7 |
| SB | Test | 160.8+42.5 | 1.9+1.9 | 169.5+24.9 | 0.0+0.0 |
|  | Retest1 | 189.9+ 42.7 | 0.0+0.0 | 80.1+18.8 | 0.1+0.1 |
| Retest 2 | 94.2+34.3 | 0.0+0.0 | 85.2+22.,0 | 7.1+3.1 |

Data expressed as mean ± S.E.M of 8 mice *per* group. VEH= vehicle; BUP= bupropion; DMI= desipramine; FLX= fluoxetine; NT= not tested; SB= sodium butyrate.

**Section S4- Confirmatory study: absence of effect or dose-response relationship of Bupropion or Sodium Butyrate in Swiss mice Porsolt test.**

In this confirmatory study, housing and experimental conditions were similar to experiment 05 except for the following characteristics: 1- the behavioural test (rFST vs Porsolt); 2-the animals room (old lab vs new lab); 2- the experimental room (old lab vs new lab); 3- the experimenter (PRS vs NZ).

**Table S4.1.** Duration of the Immobility (s) of individual Swiss mice in the Porsolt test.

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment ID | Dose ID | Mouse ID | TIMOB 1 |
| A1 | 0 | G1A1 | 76,3 |
| A1 | 0 | G2A1 | 156,918 |
| A1 | 0 | G3A1 | 183,101 |
| A2 | 2 | G1A2 | 92,515 |
| A2 | 2 | G2A2 | 194,521 |
| A2 | 2 | G3A2 | 186,596 |
| A3 | 1 | G1A3 | 157,643 |
| A3 | 1 | G2A3 | 190,062 |
| A3 | 1 | G3A3 | 180,937 |
| A4 | 3 | G1A4 | 153,002 |
| A4 | 3 | G2A4 | 184,178 |
| A4 | 3 | G3A4 | 86,278 |
| A5 | 4 | G1A5 | 165,187 |
| A5 | 4 | G2A5 | 67,807 |
| A5 | 4 | G3A5 | 207,179 |
| P1 | 5 | G1P1 | 111,063 |
| P1 | 5 | G2P1 | 168,272 |
| P1 | 5 | G3P1 | 240 |
| P2 | 1 | G1P2 | 185,001 |
| P2 | 1 | G2P2 | 174,18 |
| P2 | 1 | G3P2 | 240 |
| P3 | 2 | G1P3 | 128,64 |
| P3 | 2 | G2P3 | 239,301 |
| P3 | 2 | G3P3 | 173,625 |
| P4 | 3 | G1P4 | 156,632 |
| P4 | 3 | G2P4 | 165,1 |
| P4 | 3 | G3P4 | 203,236 |
| P5 | 4 | G1P5 | 144,783 |
| P5 | 4 | G2P5 | 236,013 |
| P5 | 4 | G3P5 | 163,812 |

05 different doses of Sodium Butyrate (A1-A5, P1) or 04 different doses of Bupropion (P2-P5) administered p.o. 01 h prior Porsolt test (n=3/dose). Immobility time was scored in the last 04 min of the test.

**Figure S4.1.** Different doses of Sodium Butyrate or Bupropion failed to change significantly the immobility time of Swiss mice in the Porsolt test:

Legend for figure S5.1: Duration of the Immobility (s, in the last 04 min, mean +/-SEM) in the Porsolt test of Swiss mice treated with vehicle (0 mg/kg) or 05 different doses of Sodium Butyrate (30, 50, 100, 150, 200, p.o., n=3/dose) or 04 differennt doses of Bupropion (30, 40, 50, 60 mg/kg, p.o., n=3/dose) administered 1 h prior Porsolt test. Statistics (http://www.socscistatistics.com/tests/anova/default2.Aspx): Treatment A-The f-ratio value is 0.63873. The p-value is .639759. The result is not significant at p < .05. Treatment B- The f-ratio value is 0.91982. The p-value is .46934. The result is not significant at p < .05.

**Section S5- Confirmatory study: acute or chronic treatment with Sodium Butyrate failed to chance immobility time of Swiss mice in rFST.**

Housing and experimental conditions were similar to experiment 05 except for the following characteristics: 1- the animals’ room (old lab vs new lab); 2- experimental room (old lab vs new lab); 3- the number of experimental groups (05 vs 02); 4- the experimenter (PRS vs NZ).

**Table S5.1**. Duration of the Immobility (s) of individual Swiss mice in the rFST.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Mouse | Test | Retest1 | Retest2 |
| B | G1A1 | 207,775 | 219,837 | 124,811 |
| B | G1A2 | 216,758 | 213,369 | 235,524 |
| B | G1A4 | 138,657 | 162,207 | 170,725 |
| B | G1A5 | 132,983 | 159,154 | 235,113 |
| B | G2A1 | 218,068 | 201,078 | 146,908 |
| B | G2A2 | 86,392 | 55,598 | 237,251 |
| B | G2A3 | 124,941 | 167,189 | 118,335 |
| B | G2A4 | 166,757 | 161,034 | 198,871 |
| B | G2A5 | 195,205 | 193,514 | 189,928 |
| A | G1P1 | 218,896 | 174,122 | 211,513 |
| A | G1P2 | 203,042 | 118,029 | 86,13 |
| A | G1P3 | 126,282 | 163,488 | 217,204 |
| A | G1P4 | 213,721 | 156,42 | 169,641 |
| A | G2P1 | 155,401 | 236,036 | 221,487 |
| A | G2P2 | 191,533 | 90,313 | 150,87 |
| A | G2P3 | 202,336 | 197,635 | 235,48 |
| A | G2P4 | 188,958 | 97,983 | 167,301 |
| A | G2P5 | 182,255 | 198,468 | 224,129 |

Sodium Butyrate (B, 30 mg/kg/day) or vehicle (A) were administered daily for 13 days whithin rFST. Immobility time was scored in the last 04 min of the test, or retest 1 or retest 2.

**Figure S5.1.** Sodium Butyrate (30 mg/kg/day, p.o., 13 days) failed to change significantly the immobility time of Swiss mice in the rFST.

Duration of the Immobility (s, in the last 04 min) in the rFST of Swiss mice (n=09/group) treated with vehicle (sucrose 10%, white bars) or Sodium Butyrate (30 mg/kg/day p.o., black bars). The p-values were: 0,262662 (test), 0,636173 (retest 1), 0,898155 (retest 2). The results were not significant at p < .05, T-test.

**Section S6-Experiments 2 to 5: mice with high or low immobility time.**

**Table S6.1**. Mean immobility time of the mice classified as LI or HI in the test.

|  |  |  |
| --- | --- | --- |
| DoseTreatment | LI168,05±7.41 (n=93) | HI282,45±2.25 (n=86) |
| 1 mg/kg | VEH | 183.62±8.13 (n=3) | 274.5±9.41 (n=5) |
| BUP | 250.89±2.05 (n=2) | 287.66±8.37 (n=6) |
| DMI | 180.98±19.31(n=4) | 270.61±2.89 (n=4) |
| FLX | 250.89±1.94 (n=3) | 307.5±18.01 (n=4) |
| SB | 227.23±20.74 (n=3) | 276.79±6 (n=3) |
| Total | N= 15 (40.5%) | N=22 (59.4%) |
| 3 mg/kg | VEH | 183.90±28.52 (n=3) | 302.95 ±10.90 (n=5) |
| BUP | 184.65±60.63 (n=3) | 295.433±11.89 (n=5) |
| DMI | 212.45±24.87 (n=4) | 290.66±6.94 (n=4) |
| FLX | 153.44 (n=1) | 291.29±7.99 (n=7) |
| SB | 222.86 (n=1) | 294.18±7.08 (n=7) |
| Total | N= 12 (30%) | N= 28 (70%) |
| 10 mg/kg | VEH | 242.837±6.43 (n=4) | 277.54±3.36 (n=4) |
| BUP | 108.95±34.93 (n=5) | 264.06±1.54 (n=3) |
| DMI | 197.52±18.32 (n=4) | 281.68±21.41 (n=4) |
| FLX | 162.78±23.31 (n=2) | 273.30±6.86 (n=6) |
| SB | 100.26±54.39 (n=4) | 282.42±13.29 (n=4) |
| Total | N=19 (47.5%) | N=21 (52.5%) |
| 20 mg/kg | VEH IP | 75.93±38.15 (n=5) | 277,31±10,67 (n=3) |
| FLX IP | 149.03±25.60 (n=7) | 0±0 (n=0) |
| VEH O | 159.33±35,4 (n=5) | 287.29±33.42 (n=2) |
| FLX O | 137±35.55 (n=7) | 285.05 (n=1) |
| Total | N=24 (80%) | N=6 (20%) |
| 30 mg/kg | VEH | 214.53±26 (n=6) | 295.62±18.2 (n=2) |
| BUP | 190.19±18.76 (n=7) | 266.53±0 (n=1) |
| DMI | NT | NT |
| FLX | 238.15± 8.88(n=3) | 275.49±11.08 (n=5) |
| SB | 171.04±24.71 (n=7) | 274.64 (n=1) |
| Total | n=23 (71.8%) | n=9 (28.12%) |

Data expressed as mean + SEM. n = number of animals per group or subgroup. Percentage (%) is related to the total number of mice in a given dose of the drug (Experiments 2-6). VEH= vehicle; BUP= bupropion; DMI= desipramine; FLX= fluoxetine; IP= intraperitoneal; NT=not tested; O= oral, gavage; SB= sodium butyrate.

**Table S6.2.** Mean immobility time of the mice classified as LI or HI in the retest 1.

|  |  |  |
| --- | --- | --- |
| DoseTreatment | LI  | HI  |
| 230.45±8.63 (n=73) | 328.13±1.42 (n=75) |
| 1mg | VEH | 0±0 (n=0) | 325.54±3.88 (n=8) |
| BUP  | 283.07±8.26 (n=5) | 328.94±12.27 (n=3) |
| DMI | 298.36±7.22(n=2) | 336.89±3.41 (n=6) |
| FLX | 301.05±0 (n=1) | 332.48±7.34 (n=4) |
| SB | 282.14±0.63 (n=3) | 334.52±6.4 (n=4) |
| Total | N=11 (30,55%) | N=25 (69,44%) |
| 3mg | VEH | 287.28±8.02 (n=3) | 331.19 ±4.09 (n=5) |
| BUP  | 153.74±0 (n=1)  | 326.93±3.77 (n=7) |
| DMI | 256.50±18.62 (n=5) | 334.43±2.11 (n=3) |
| FLX | 221.19±69.26(n=2) | 328.09±7.69 (n=6) |
| SB | 295.42±0.92 (n=2) | 318.89±4 (n=6) |
| Total | N=13 (32,5%) | N=27 (67,5%) |
| 10mg | VEH | 248.2±20.83 (n=6) | 311.39±0.14 (n=2) |
| BUP  | 205.48±29.78 (n=6)  | 324.02±2.04 (n=2)  |
| DMI | 220.74±42.66 (n=7) | 305.36±0 (n=1) |
| FLX | 229.71±31.24 (n=7) | 341.31±0(n=1) |
| SB | 107.41±32.23 (n=6)  | 329.45±0.91 (n=2) |
| Total | N=32 (80%) | N=8 (20%) |
| 20mg | VEH IP  | NT | NT |
| FLX IP | NT | NT |
| VEH O  | NT | NT |
| FLX O | NT | NT |
| Total | NT | NT |
| 30mg | VEH | 194.53±48.88 (n=4) | 338.26±3.90 (n=4) |
| BUP  | 173.24±74.02 (n=3) | 317.38±2.46(n=5)  |
| DMI | NT | NT |
| FLX | 283.06±6.64(n=5) | 322.32±3.99 (n=3) |
| SB | 233.53±24.56 (n=5) | 336.73±1.60 (n=3) |
| Total | N=17 (53.12%) | N=15 (46.98%) |

Data expressed as mean + SEM. n = number of animals per group or subgroup. Percentage (%) is related to the total number of mice in a given dose of the drug (Experiments 2-6). VEH= vehicle; BUP= bupropion; DMI= desipramine; FLX= fluoxetine; IP= intraperitoneal; NT=not tested; O= oral, gavage; SB= sodium butyrate.

**Table S6.3:** Mean immobility time of the mice classified as LI or HI in the retest 2.

|  |  |  |
| --- | --- | --- |
| DoseTreatment | LI  | HI  |
| 275.28±6.65 (n=74) | 338.20±1.03 (n=75) |
| 1mg | VEH | 287.52±22.08 (n=3) | 338.31±2.70 (n=5) |
| BUP  | 298.31±20.14 (n=5) | 338.98±5.73 (n=3) |
| DMI | 0±0 (n=0) | 342.79±3 (n=8) |
| FLX | 321.211±0 (n=1) | 342.12±6.02 (n=6) |
| SB | 284.04±35.32 (n=2) | 333.52±4.29 (n=4) |
| Total | N=11 (30,55%) | N=26 (69,44%) |
| 3mg | VEH | 261.12±31.76 (n=5) | 339.73 ±8.28 (n=3) |
| BUP  | 281.42±33.37 (n=3) | 335.87±5.34 (n=5) |
| DMI | 310.78±4.18 (n=2) | 341.29±4.29 (n=6) |
| FLX | 242.73±74.12(n=3) | 337.53±5.34 (n=5) |
| SB | 0±0 (n=0) | 335.98±3.05 (n=8) |
| Total | N=13 (32,5%) | N=27 (67,5%) |
| 10mg | VEH | 288.85±10.43 (n=4) | 338.39±5.48 (n=4) |
| BUP  | 258.42±13.43 (n=4)  | 342.74±2.70 (n=4)  |
| DMI | 274.19±15.56 (n=7) | 333.93±0 (n=1) |
| FLX | 312.39±2.30 (n=6) | 339.09±1.25(n=2) |
| SB | 236.26±41.55 (n=6) | 330.66±6.82 (n=2) |
| Total | N=27 (67.5%) | N=13 (32.5%) |
| 20mg | VEH IP  | NT | NT |
| FLX IP | NT | NT |
| VEH O  | NT | NT |
| FLX O | NT | NT |
| Total | NT | NT |
| 30mg | VEH | 298.98±13.16 (n=4) | 338.6±5.80 (n=4) |
| BUP  | 263.58±31.40 (n=7) | 326.69±0(n=1)  |
| DMI | NT | NT |
| FLX  | 289.47±9.87(n=5) | 337.11±4.74 (n=3) |
| SB | 254.31±24.60 (n=7) | 330.49±0 (n=1) |
| Total | N=23 (71.8%) | N=9 (28.12%) |

Data expressed as mean + SEM. n = number of animals per group or subgroup. Percentage (%) is related to the total number of mice in a given dose of the drug (Experiments 2-6). VEH= vehicle; BUP= bupropion; DMI= desipramine; FLX= fluoxetine; IP= intraperitoneal; NT=not tested; O= oral, gavage; SB= sodium butyrate.