**Circuits that encode and guide alcohol associated preference**

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|  |
| --- |
| UAS-mcd GFP |
| 40B | **42B** | **HL9** | **R5802** |
| n=6 | n=6 | n=6 | n=4 |
| 44.83 ±5.26 | 41.00 ±1.37 | 60.10 ±07.54\* | 101 ±3.43 |

Supplementary Table 1 . PAM dopamine cell counts per hemisphere. \*HL9 numbers from Claridge-Chang et al. 2009 (Claridge-Chang et al., 2009)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Odor1 |  |  | Odor 2 |  |
| GAL4 lines | **+/GAL4** | **+/UAS** | **GAL4/UAS** | **+/GAL4** | **+/UAS** | **GAL4/UAS** |
| R58E02 | 0.73 ±0.05 | 0.83 ±0.06 | 0.92 ±0.04 | 0.36 ±0.05 | 0.51 ±0.11 | 0.66 ±0.07 |
| HL9 | 0.53 ±0.11 | 0.49 ±0.08 | 0.62 ±0.08 | 0.49 ±0.10 | 0.44 ±0.13 | 0.33 ±0.07 |
| MB109B | 0.71 ±0.06 | 0.68 ±0.05 | 0.80 ±0.04 | 0.37 ±0.05 | 0.67 ±0.03 | 0.68 ±0.06 |
| MB058B | 0.69 ±0.04 | 0.78 ±0.04 | 0.90 ±0.02 | 0.56 ±0.04 | 0.57 ±0.04 | 0.67 ±0.03 |
| MB399B | 0.67 ±0.06 | 0.62 ±0.05 | 0.92 ±0.02 | 0.49 ±0.05 | 0.59 ±0.03 | 0.67 ±0.04 |
| MB018B |  0.65 ±0.06 |  0.74 ±0.06 |  0.57 ±0.04 | 0.47 ±0.07 | 0.41 ±0.09 | 0.48 ±0.04 |

Supplementary Table 2. Odor Controls at 30C. Odor 1: Isoamyl Acetate or Ethyl Acetate. Odor 2: Isoamyl Alcohol. Naïve flies were presented with either odor 1 vs air or odor 2 vs air in the Y maze.

|  |  |  |
| --- | --- | --- |
| **Split-GAL4 lines** | **Target Expression Pattern with Intensity** | **Off Target Expression** |
| **MB040B** | PAM-1 (2), PAM-`1ap (2), PAM-`1m (2), PAM-`2a (1), PAM-`2m (2), PAM-`2p (2), PAM-2 (3), PAM-3 (3), PAM-4 (3), PAM-5 (3)  | Diffuse central brain expression  |
| **MB042B** | PAM-1(1), PAM-`1ap (1), PAM-`1m (1), PAM-`2a (1), PAM-`2m (2), PAM-`2p (1), PAM-1 (1), PAM-3 (3), PAM-4 (3), PAM-4<12 (1), PAM-5 (3) | Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB188B** | PAM-`1ap (5), PAM-`1m (5), PAM-3 (3), PAM-4 (2) | Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB032B** | PAM-’2m (4), PAM-`2p (1), PAM-2`2a (1), PAM-3 (1) | Limited central brain expression with reporter: pJFRC2-10xUAS-IVS-mCD8::GFP in VK00005 |
| **MB301B** | PAM-’2m (1), PAM-2`2a (3) | VNC expression with reporter: pJFRC200-10XUAS-IVS-myr::smGFP-HA in attP18 |
| **MB109B** | PAM-`2a (5), PAM-5 (1) | Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB315C** | PAM-5 (5) | Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB299B** | PAM-1 (3), PAM-1 (1), PAM-2 (1) |  Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB210B** | MBON-`2mp (4), MBON-`2mp\_bilateral (1), MBON-5`2a (5) | Limited posterior VNC expression with reporters: 20xUAS-IVS-CsChrimson-mVenus in attP18 pJFRC2-10xUAS-IVS-mCD8::GFP in VK00005Posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB011B** | MBON-`2mp (3), MBON-`2mp\_bilateral (3), MBON- 5`2a (4) | Limited central brain expression with reporter: pJFRC2-10xUAS-IVS-mCD8::GFP in VK00005Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB399B** | MBON-2`2a (2) | Limited posterior VNC expression with reporter: pJFRC2-10xUAS-IVS-mCD8::GFP in VK00005Central brain expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB074C** | MBON-`2mp (4), MBON-2`2a (3), MBON- 5`2a (1) | Limited posterior VNC expression with reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |
| **MB002B** | MBON-`2mp (4), MBON- 5`2a (2) | Limited posterior VNC expression reporter: pJFRC206-5xUAS-IVS-myr::smGFP-FLAG in VK00005 |

Supplementary Table 3. Summary of all target and off target expression of each split-GAL4 line used in the paper as described on FlyLight (<https://www.janelia.org/project-team/flylight>). Intensity of expression reported as low (1) to high (5).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Figure** | **Experiment** | **n** | **Statistical Test** | **Result** | **Post-hoc** | **Result** |
| 1B | Acquisition | +/R58E02 (n=23)+/shits (n=23)shits/R58E02 (n=23) | One-way ANOVA | F(2, 66)=5.355, p=0.007 | Tukey  | +/ shits vs +/R58E02 p=0.87+/ shits vs shits/R58E02 p=0.009+/R58E02 vs shits/R58E02 p=0.04 |
| 1B | Consolidation | +/R58E02 (n=13)+/shits (n=15)shits/R58E02 (n=13) | One-way ANOVA | F(2,38)=5.964, p=0.00559 | Tukey | +/ shits vs +/R58E02 p=0.004+/ shits vs shits/R58E02 p=0.18+/R58E02 vs shits/R58E02 p=0.26 |
| 1B | Retrieval | +/R58E02 (n=25)+/shits (n=24)shits/R58E02 (n=25) | One-way ANOVA | F(2,71)=5.707, p=0.005 | Tukey | +/ shits vs +/R58E02 p=0.65+/ shits vs shits/R58E02 p=0.05+/R58E02 vs shits/R58E02 p=0.005 |
| 1C | D2R | +/R58E02 (n=37)+/D2Ri (n=37)D2Ri/R58E02 (n=37) | One-way ANOVA | F(2,89)=6.441, p=0.002 | Tukey | +/ D2Rivs +/R58E02 p=0.980+/D2Ri vs D2Ri/R58E02 p=0.0099+/R58E02 vs D2Ri/R58E02 p=0.0056 |
| 1F | Calcium Imaging Early Epoch | GCaMP6m/R58E02 (n=6) | Repeated Measures ANOVA | F(1,5)=8.705, p=0.03 | N/A | N/A |
| 1G | Calcium ImagingLate Epoch | GCaMP6m/R58E02 (n=6) | Repeated Measures ANOVA | F(1,5)=24,177, p=0.004 | N/A | N/A |
| 2A | Retrieval | +/40B (n=7)+/shits (n=6)shits/40B (n=7) | One-way ANOVA | F(2,17)=2.43, p=0.12 | N/A | N/A |
| 2B | Retrieval | +/42B (n=16)+/shits (n=16)shits/42B (n=14) | One-way ANOVA | F(2,68)=0.995, p=0.38 | N/A | N/A |
| 2C | Retrieval | +/188B (n=24)+/shits (n=27)shits/188B (n=25) | One-way ANOVA | F(2,73)=0.044, p=0.96 | N/A | N/A |
| 2D | Retrieval | +/32B (n=20)+/shits (n=20)shits/32B (n=20) | One-way ANOVA | F(2,57)=1.164, p=0.32 | N/A | N/A |
| 2E | Retrieval | +/301B (n=23)+/shits (n=24)shits/301B (n=24) | One-way ANOVA | F(2,78)=0.389, p=0.68 | N/A | N/A |
| 2F | Retrieval | +/109B (n=20)+/shits (n=24)shits/109B (n=24) | One-way ANOVA | F(2,65)=14.18, p= 7.78x10^-6 | Tukey | +/ shits vs +/MB109B p=0.07+/ shits vs shits/MB109B p=0.007+/MB109B vs shits/MB109B p=0.000005 |
| 2G | Retrieval | +/315C (n=20)+/shits (n=19)shits/315C (n=20) | One-way ANOVA | F(2,56)=0.109, p=0.90 | N/A | N/A |
| 2H | Retrieval | +/299B (n=9)+/shits (n=13)shits/MB299B (n=13) | One-way ANOVA | F(2,32)=1.468, p=0.246 | N/A | N/A |
| 3A | Acquisition | +/2B (n=16)+/shits (n=17)shits/2B (n=17) | One-way ANOVA | F(2,47)=0.31, p=0.73 | N/A | N/A |
| 3B | Acquisition | +/210B (n=26)+/shits (n=26)shits/210B (n=27) | One-way ANOVA | F(2,76)=1.59, p=0.21 | N/A | N/A |
| 3C | Acquisition | +/11B (n=17)+/shits (n=15)shits/11B (n=15) | One-way ANOVA | F(2,44)=0.09, p=0.92 | N/A | N/A |
| 3D | Acquisition | +/399B (n=25)+/shits (n=26)shits/399B (n=25) | One-way ANOVA | F(2,73)=0.90, p=0.42 | N/A | N/A |
| 3E | Acquisition | +/74C (n=11)+/shits (n=12)shits/74C (n=12) | One-way ANOVA | F(2,32)=0.30, p=0.75 | Tukey | N/A |
| 3F | Retrieval | +/2B (n=19)+/shits (n=19)shits/2B (n=19) | One-way ANOVA | F(2,54)=2.05, p=0.14 | N/A | N/A |
| 3G | Retrieval | +/210B (n=29)+/shits (n=29)shits/210B (n=26) | One-way ANOVA | F(2,81)=0.52, p=0.60 | N/A | N/A |
| 3H | Retrieval | +/11B (n=19)+/shits (n=19)shits/11B (n=18) | One-way ANOVA | F(2,53)=0.40, p=0.67 | N/A | N/A |
| 3I | Retrieval | +/399B (n=22)+/shits (n=19)shits/399B (n=21) | One-way ANOVA | F(2,59)=5.62, p=0.006 | Tukey | +/ shits vs +/MB399B p=0.93+/ shits vs shits/MB399B p=0.010+/MB399B vs shits/MB399B p=0.02 |
| 3J | Retrieval | +/MB074C (n=32)+/shits (n=30)shits/MB074C (n=32) | One-way ANOVA | F(2,91)=2.22, p=0.11 | Tukey | N/A |
| 4C | Consolidation | +/MB002B (n=20)+/shits (n=18)shits/MB002B (n=19)+/MB074C (n=21)+/shits (n=21)shits/MB074C (n=21) | One-way ANOVA | F(2,54)= 9.287, p=0.0003F(2,71)= 3.51, p=0.04 | TukeyTukey | +/ shits vs +/MB002B p=0.989+/ shits vs shits/MB002B p=0.001+/MB002B vs shits/MB002B p=0.001+/ shits vs +/MB074C p=0.46+/MB074C vs shits/MB074C p=0.14+/ shits vs shits/MB074C p=0.008 |
| 4D | D2R | +/MB002B (n=22)+/D2Ri(n=22)D2Ri/MB002B (n=22)+/MB074C (n=26)+/D2Ri(n=23)D2Ri/MB074C (n=25) | One-way ANOVA | F(2,63)=12.77, p=2.22x10^-05F(2,71)=3.51, p=0.04 | TukeyTukey | +/ D2Rivs +/MB002B p=0.07+/ D2Ri vs D2Ri/MB002B p=0.00001+/MB002B vs D2Ri/MB002B p=0.019+/ D2Rivs +/74C p=0.47+/ D2Ri vs D2Ri/74C p=0.03+/74C vs D2Ri/74C p=0.29 |
| 5C | Acquisition | +/MB018B (n=20)+/shits (n=21)shits/MB018B (n=25) | One-way ANOVA | F(2,63)=2.18, p=0.12 | N/A | N/A |
| 5D | Retrieval | +/MB018B (n=36)+/shits (n=38)shits/MB018B (n=45) | One-way ANOVA | F(2,116)=19.46, p=5.17x10^-08 | Tukey | +/ shits vs +/MB018B p=0.40+/ shits vs shits/MB018B p=0.00004+/18B vs shits/18B p=0.0000001 |
| 5E | Acquisition | +/MB058B (n=28)+/shits (n=30)shits/MB058B (n=30) | One-way ANOVA | F(2,85)=0.202, p=0.817 | N/A | N/A |
| 5F | Retrieval | +/MB058B (n=18)+/shits (n=20)shits/MB958B (n=22) | One-way ANOVA | F(2,54)=5.103, p=0.009 | Tukey | +/ shits vs +/MB058B p=0.81+/ shits vs shits/MB058B p=0.049+/MB058B vs shits/MB058B p=0.013 |
| F1.S1 | Group Activity | +/R58E02 (n=15)+/shits (n=15)shits/R58E02 (n=15) | Repeated Measures ANOVA Mauchly’s test | F(3.38, 76)= 16.21, p=0.002(5)=90.51, p=0.00; =0.563 | Bonferonni | Baseline: +/ shits vs +/R58E02 p=1.00+/ shits vs shits/R58E02 p=0.00+/R58E02 vs shits/R58E02 p=0.00Early EtOH:+/ shits vs +/R58E02 p=0.00+/ shits vs shits/R58E02 p=0.232+/R58E02 vs shits/R58E02 p=0.002Late EtOH:+/ shits vs +/R58E02 p=0.00+/ shits vs shits/R58E02 p=0.016+/R58E02 vs shits/R58E02 p=0.380Recovery:+/ shits vs +/R58E02 p=0.127+/ shits vs shits/R58E02 p=0.075+/R58E02 vs shits/R58E02 p=1.000 |
| F1.S2 | Dopamine Fluorescence  | Air (n=7)Ethanol (n=11) | One-way ANOVA | F(1,16)=2.947, p=0.105 | N/A | N/A |
| F1.S3D | Calcium Imaging Early Epoch Odor 1 vs 2 | GCaMP6m/R58E02 (n=6) | F(1,5)=0.144, p=0.720 | N/A | N/A | N/A |
| F1.S3G | Calcium Imaging Late Epoch Odor 1 vs 2 | GCaMP6m/R58E02 (n=6) | F(1,5)=3.437, p=0.123 | N/A | N/A | N/A |
| F1.S3K | Calcium Imaging Early Ethanol vs Late Ethanol | GCaMP6m/R58E02 (n=6) | F(1,5)=0.390, p=0.560 | N/A | N/A | N/A |
| F1.S4A | Dopamine Acquisition and Retrieval | shits/MB042B (n=11)shits/MB196B (n=11)shits/MB299B (n=6)shits/MB047B (n=11)shits/MB195B (n=12)shits/MB316B (n=12)shits/MB312B (n=10)shits/MB194B (n=12)shits/MB025B (n=11)shits/MB043B (n=11)shits/MB213B (n=11)shits/MB301B (n=12)shits/MB040B (n=11)shits/MB087C (n=12)shits/MB315C (n=12)shits/MB109B (n=12)shits/MB188B (n=10)shits/MB032B (n=11)shits/PBP (n=11) | Kruskal-Wallis | χ2(18)=30.81, p=0.03 | Dunnett’s Test | shits/MB042B vs shits/PBP p=0.38shits/MB196B vs shits/PBP p=0.64shits/MB299B vs shits/PBP p=0.55shits/MB047B vs shits/PBP p=0.71shits/MB195B vs shits/PBP p=0.79shits/MB316B vs shits/PBP p=0.71shits/MB312B vs shits/PBP p=0.49shits/MB194B vs shits/PBP p=0.71shits/MB025B vs shits/PBP p=0.17shits/MB043B vs shits/PBP p=0.86shits/MB213B vs shits/PBP p=0.67shits/MB301B vs shits/PBP p=0.27shits/MB040B vs shits/PBP p=0.54shits/MB087C vs shits/PBP p=0.17shits/MB315C vs shits/PBP p=0.04shits/MB109B vs shits/PBP p=0.03shits/MB188B vs shits/PBP p=0.008shits/MB032B vs shits/PBP p=0.01 |
| F1.S4B | MB Acquisition and Retrieval | shits/MB010B (n=10)shits/MB152B (n=12)shits/MB364B (n=11)shits/MB009B (n=12)shits/MB417B (n=11)shits/MB005B (n=12)shits/MB370B (n=12)shits/MB461B (n=12)shits/MB008B (n=11)shits/MB371B (n=12)shits/PBP (n=12) | Kruskal-Wallis | χ2(10)=27.97, p=0.002 | Dunnett’s Test | shits/MB010B vs shits/PBP p=0.04shits/MB152B vs shits/PBP p=3.69x10^-05shits/MB364B vs shits/PBP p=0.04shits/MB009B vs shits/PBP p=4.86x10^-04shits/MB417B vs shits/PBP p=2.68x10^-04shits/MB005B vs shits/PBP p=0.002shits/MB370B vs shits/PBP p=0.04shits/MB461B vs shits/PBP p=0.04shits/MB008B vs shits/PBP p=1.87x10^-04shits/MB371B vs shits/PBP p=0.045 |
| F1.S6A | Acquisition | +/HL9 (n=8)+/shits (n=8)shits /HL9 (n=8) | One-way ANOVA | F(2,21)=0.24, p=0.788 | N/A | N/A |
| F1.S6B | Consolidation | +/HL9 (n=8)+/shits (n=8)shits/HL9 (n=8) | One-way ANOVA | F(2,21)=0.698, p=0.509 | N/A | N/A |
| F1.S6C | Retrieval | +/HL9 (n=8)+/shits (n=8)shits /HL9 (n=8) | One-way ANOVA | F(2,21)=8.596, p=0.00187 | Tukey | +/ shits vs +/HL9 p=0.92+/ shits vs shits/HL9 p=0.003+/HL9 vs shits/HL9 p=0.007 |
| F1.S6A | Acquisition | +/MB040B (n=23)+/shits (n=23)shits/MB040B (n=21) | One-way ANOVA | F(2,64)=1.262, p=0.39 | N/A | N/A |
| F1.S6B | Acquisition | +/MB042B (n=24)+/shits (n=24)shits/MB042B (n=23) | One-way ANOVA | F(2,68)=0.995, p=0.38 | N/A | N/A |
| F1.S6C | Acquisition | +/MB188B (n=10)+/shits (n=11)shits/MB188B (n=12) | One-way ANOVA | F(2,30)=0.084, p=0.92 | N/A | N/A |
| F1.S6D | Acquisition | +/MB032B (n=22)+/shits (n=21)shits/MB032B (n=20) | One-way ANOVA | F(2,60)=1.52, p=0.23 | N/A | N/A |
| F1.S6E | Acquisition | +/MB301B (n=27)+/shits (n=27)shits/MB301B (n=27) | One-way ANOVA | F(2,78)=0.389, p=0.68 | N/A | N/A |
| F1.S6F | Acquisition | +/MB109B (n=24)+/shits (n=24)shits/MB109B (n=24) | One-way ANOVA | F(2,69)=0.091, p=0.91 | N/A | N/A |
| F1.S6G | Acquisition | +/315C (n=20)+/shits (n=23)shits/315C (n=24) | One-way ANOVA | F(2,64)=0.24, p=0.79 | N/A | N/A |
| F1.S6H | Acquisition | +/299B (n=20)+/shits (n=23)shits/299B (n=24) | One-way ANOVA | F(2,31)=0.6, p=0.555 | N/A | N/A |
| F1.S7 | mRNA PCR | D1R1/elav (n=6)+/D1R1 (n=6)D1R2/elav (n=6)+/D1R2 (n=6)D2R/elav (n=3)+/D2R (n=3) | One-way ANOVA | F(1,10)=20.05, p=0.001F(1,10)=30.31, p=0.0003F(1,4)=19.14, p=0.011 | N/A | N/A |
| F1.S8A | R58E02 Temperature Controls | +/R58E02 (n=15)+/ shits (n=15)shits/R58E02 (n=15) | One-way ANOVA | F(2,42)=1.953, p=0.155 | N/A | N/A |
| F1.S8B | HL9 Temperature Controls | +/HL9 (n=8)+/ shits (n=8)shits/HL9 (n=8) | One-way ANOVA | F(2,21)=0.823, p=0.453 | N/A | N/A |
| F1.S8C | MB109B Temperature Controls | +/MB109B (n=35)+/MB109B(n=35)shits/MB109B (n=35) | One-way ANOVA | F(2,102)=0.411, p=0.664 | N/A | N/A |
| F4.S7B | D2R | +/MB399B (n=23)+/dD2R (n=23)dD2R/MB399B (n=23) | One-way ANOVA | F(2,65)=0.032, p=0.968 | N/A | N/A |
| F4.S7C | D1R1;D1R2 | +/MB399B (n=14)+/dD1R1;dD1R2 (n=15)dD1R1;dD1R2/MB399B (n=15) | One-way ANOVA | F(2,41)=0.223, p=0.801 | N/A | N/A |
| F4.S7D | D1R1i;D1R2i | +/MB074C (n=26)+/D1R1;D1R2 (n=28)D1R1;D1R2/MB074C (n=28) | One- way ANOVA | F(2,79)=0.123, p=0.884 | N/A | N/A |
| F4.S8 | 399B Temperature Controls | +/399B (n=18)+/shits (n=18)shits/399B (n=18) | One-way ANOVA | F(2,51)=1.039, p=0.361 | N/A | N/A |
| F5.S1A | D1R1;D1R2 | +/MB018B (n=14)+/D1R1i;D1R2i(n=14)D1R1iD1R2i/MB018B (n=12) | One-way ANOVA | F(2,37)=2.00, p=0.15 | N/A | N/A |
| F5.S1B | D2R | +/MB018B (n=20)+/D2Ri(n=20)D2Ri/MB018B (n=20) | One-way ANOVA | F(2,57)=0.113, p=0.90 | N/A | N/A |
| F5.S2A | 18B Temperature Controls | +/18B (n=24)+/shits (n=25)shits/18B (n=25) | One-way ANOVA | F(2,71)=0.225, p=0.799 | N/A | N/A |
| F5.S2B | MB058B Temperature Controls | +/MB058B (n=18)+/MB058B(n=18)shits/MB058B (n=18) | One-way ANOVA | F(2,50)=0.516, p=0.6 | N/A | N/A |
|  |  |  |  |  |  |  |

Supplementary Table 4. Statistical Analysis Summary for Main Figures and Supplemental Figures

|  |  |  |
| --- | --- | --- |
| Target Gene | Stock # | Citation |
| *Dop1R1* | VDRC-KK-107058 | (Wang et al., 2016, Wang et al., 2013, Lark et al., 2017, Ferguson et al., 2017, Agrawal and Hasan, 2015) |
| *Dop1R2* | VDRC-GD-3391 | (Wang et al., 2016, Regna et al., 2016, Dietzl et al., 2007) |
| *D2R* | VDRC-GD-11471 | (Andreatta et al., 2018, Bang et al., 2011, Neckameyer and White, 1993, Petruccelli et al., 2018, Wang et al., 2016, Dietzl et al., 2007, Agrawal and Hasan, 2015, Shang et al., 2011) |

Supplementary Table 5. Previous publications using RNAi lines in this paper.

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