SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Other behavioral repertoires are comparable between naïve and experienced males. (A) MD assays of singly reared male. Newly eclosed CS males were signly reared in each vial for 5 days (naïve). Two virgin females were introduced to each vial 1d, 2d, or 3d before assay. Females were replaced with fresh virgins every day. (B) Courtship index of naïve and experienced males. See **EXPERIMENTAL PROCEDURES** section for detailed methods. (C) Courtship latency of naïve and experienced males. See **EXPERIMENTAL PROCEDURES** section for detailed methods. (D) Mating initiation time of naïve and experienced males. (E-G) Locomotion of naïve and experienced male flies were quantified as (D) velocity by climbing assay paradigm, (E) locomotion activity by horizontal paradigm, and (G) stop frequency by horizontal paradigm. See **EXPERIMENTAL PROCEDURES** section for detailed methods. See **EXPERIMENTAL PROCEDURES** section for detailed methods.

Figure S2. (A and B) Electrical silencing or hyperexcitation of *Gr5a*-positive neurons abolish SMD behavior. Flies expressing (A) potassium channel *UAS-Kir2.1* with the *tub-GAL80^{ts}*, so that Kir2.1 expression could be triggered by temperature shifts were crossed with flies expressing *Gr5a-* or *Gr66a-GAL4* drivers. Flies were reared at 29℃ for the first 2 days to strongly induce *UAS-Kir2.1* expression and then shifted to 25℃ for the last 3 days for mild induction of *UAS-Kir2.1* transgenes. (B) MD assays of different *snmp* alleles' combination. Genotypes are specified below the graphs.

Figure S3. (A) Brains of female flies expressing *Gr5a-GAL4* together with *UAS[stop]mCD8GFP; fru*^{FLP} were immunostained with anti-GFP (green) and nc82 (magenta) antibodies. (B) Brains of male flies expressing *Gr5a-GAL4* together with *UAS[stop]DscamGFP; fru*^{FLP} were immunostained with anti-GFP (green) and nc82 (magenta) antibodies. Scale bars represent 100 μm. (C) MD assays of *Gr5a-* and *Gr66a-GAL4* drivers for inactivation of synaptic transmission via expression of *UAS-TNT* transgene.

Figure S4. (A) Electrical silencing of mushroom body or ellipsoid body neurons abolishes SMD behavior. Flies expressing potassium channel *UAS-Kir2.1* together with the *tub-GAL80*^{ts}, so that Kir2.1 expression could be triggered by temperature shifts were crossed with flies expressing various drivers the label subset of clock neurons (*pdf-GAL4*), mushroom body neurons (*ok107-GAL4*), fan-shape body neurons (14-94-*GAL4*), and ellipsoid body neurons (*c547-GAL4*). Flies were reared at 29℃ for the first 2 days to strongly induce *UAS-Kir2.1* expression and then shifted to 25℃ for the last 3 days for mild induction of *UAS-Kir2.1* transgene. (B) MD assays of CS males after isolated from 1 day of female experience. Males were reared with sufficient numbers of virgin females for 1 day to be assured to show SMD phenotype then isolated. Assay times after isolation are described below the boxes. Boxes labeled naïve and exp. represent standard rearing conditions described in **Figure 1A.** (C) MD assays of Orb2 mutants.

Blue bar represents singly reared condition to compare to group reared condition (white), which is performed to check the Orb2 mutants show intact LMD behaviour. (D) MD assays of *14-94-GAL4* driver for persistent depolarization of neurons by expressing *UAS-NachBac* transgene combined with *tub-GAL80^{ts}* to express UAS transgene in adult-specific manner.

Figure S5. (A) MD assays of *npfR1* mutants. Genotypes are specified below the graphs. (B) Brain regions labeled by *GAL4* drivers used for screening the requirement of sNPF signaling in SMD. (sLNv: small ventral lateral neurons, lLNv: large ventral lateral neurons, 5th sLNv: 5th small ventral lateral neurons, LNd: dorsal lateral neurons, DN1-3: dorsal neurons 1-3, EB: ellipsoid body, MB: mushroom body, FB: fan-shape body). (C) MD assays of *GAL4* mediated sNPF overexpression via *UAS-sNPF*. Names of *GAL4* drivers are described below the graphs. (D and E) Brains of male flies expressing *sNPF-GAL4* together with *UAS-Denmark* (a dendritic marker) together with *UAS-sytGFP* (a presynaptic marker) were immunostained with (D) anti-GFP (green), anti-DsRed (red) and nc82 (blue) antibodies or (E) anti-GFP (green), anti-DsRed (red) and anti-PDF (blue) antibodies. White and yellow boxes indicate the magnified regions of interest presented next right panels. Scale bars represent 100 μm. (F) MD assays for *GR5a-* or *Gr66a-GAL4* mediated knockdown of sNPF via *UAS-sNPF-IR; UAS-dicer (sNPF-RNAi)*.

Figure S6. (A-E) Brain of flies expressing *sNPF-* or *sNPF-R-GAL4* drivers together with UAS-mCD8GFP, UAS-RedStinger and with (A) *elav-GAL80* (suppress *GAL4* expression

in neuronal population), (B) *MB-GAL80* (suppress *GAL4* expression in mushroom body neurons), (C) *repo-GAL80* (suppress *GAL4* expression in glial cell population) (D) *cry-GAL80* (suppress *GAL4* expression in broad subset of clock neurons) (E) *MB-GAL80 with cry-GAL80* (suppress *GAL4* expression in both mushroom body and broad subset of clock neurons) were immunostained with anti-GFP (green), anti-DsRed (red), and anti-nc82 (blue) antibodies. White boxes indicate the magnified regions of interest presented next right or bottom panels. Scale bars represent 100 μm. Dashed circles in (A) indicate labeled cells by each *GAL4* drivers after suppression via *elav-GAL80* suggesting that non-neuronal populations. (F) Brain of flies expressing *sNPF-R-GAL4* together with *UAS-tra*^{F}; *UAS-RedStinger, UAS-mCD8GFP* were dissected and immunostained with anti-GFP (green), anti-DsRed (red), and anti-nc82 (blue) antibodies. Dashed circles indicate that disappeared GFP signals after feminization via *UAS-tra*^{F} compared to **Figure 5G**. Scale bars represent 100 μm. (G) MD assays for *sNPF-* or *sNPF-R-GAL4* drivers for ablation of cells only in *fruitless*-positive cells via *UAS[stop]RicinA; fru*^{FLP} (Hidalgo et al., 1995).

Figure S7. (A) Brain of flies expressing *sNPF-GAL4* with *UAS-n-sybGFP* were immunostained with anti-GFP (red hot) and nc82 (grey) antibodies. GFP is pseudocolored "red hot." Scale bars represent 100 μm. (B) Flies expressing *sNPF-GAL4* with *UAS[stop]DscamGFP; fru*^{FLP} were naively reared (upper panels) or experienced with virgin females 1 day before dissection (bottom panels). Brains of flies were immunostained with anti-GFP (red hot) and nc82 (grey) antibodies. GFP is

pseudocolored “red hot.” Dashed circles represent the clearly increased GFP signals compared to upper panel. Scale bars represent 100 μm. (C) Number of particles shown in (B) was quantified using *ImageJ* software. (D) Average size of particles shown in (B) was quantified. (E) Percent of brain area covered by particles shown in (B) are quantified. See **EXPERIMENTAL PROCEDURES** for details. (F) Brains of naïve male (top), experienced male (middle), and female (bottom) flies expressing *sNPF-R-GAL4* along with *LexAop-CD2-GFP; UAS-mLexA-VP16-NFAT, LexAop-CD8-GFP-2A-CD8-GFP* were dissected then immunostained with anti-GFP (green) and anti-nc82 (magenta) antibodies. GFP is pseudocolored “fire” in the middle and the right panels. Warmer colors reflect increased signal intensity. Cells showing strong GFP signals were labeled “a and b”. (G) GFP fluorescence of “b” region is normalized by “a” region. See **EXPERIMENTAL PROCEDURES** for detailed quantification methods. (H) Brains of naïve male (top panels) and experienced male (bottom panels) flies expressing *pdf-GAL4* (left panels) or *npf-GAL4* (right panels) along with *LexAop-CD2-GFP; UAS-mLexA-VP16-NFAT, LexAop-CD8-GFP-2A-CD8-GFP (CalexA)* were dissected then immunostained with anti-GFP (green) and anti-nc82 (magenta) antibodies. GFP is pseudocolored “fire”. Warmer colors reflect increased signal intensity. (I and J) Relative GFP fluorescence shown in (H) is quantified. (I) For *pdf-GAL4*, GFP fluorescence of l-LNv region is normalized by s-LNv region. (J) For *npf-GAL4*, GFP fluorescence of LNd region is normalized by D2 region. See **EXPERIMENTAL PROCEDURES** and previous report (Kim et al., 2013) for detailed quantification methods.

Table 1. The differences between LMD and SMD behavior.

[a] For the information about LMD, see our previous two papers (Kim et al., 2012, 2013).

REFERENCES

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