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### **Supplemental Material**

#### **Neural mechanisms underlying the disruption of male courtship behavior by adult exposure to di-(2-ethylhexyl)phthalate in mice**

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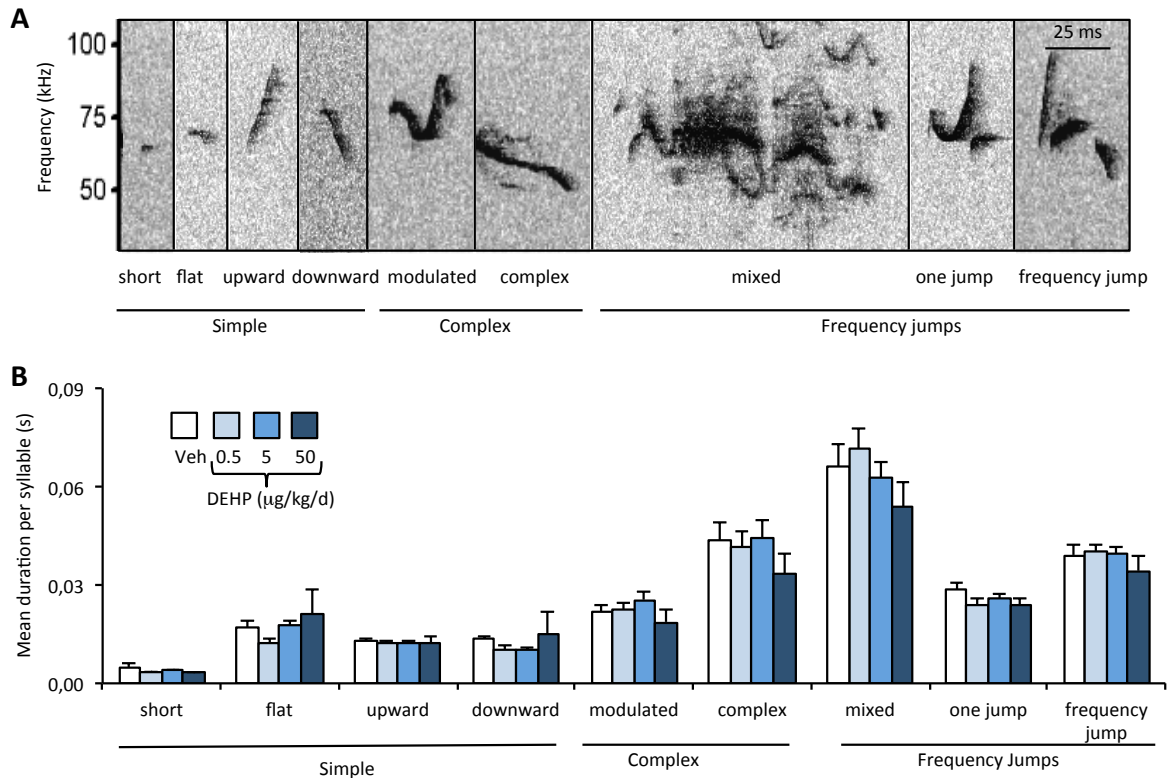
**Figure S2. A.** Dimensions of the three-chamber paradigm used for the mate preference test. The total time spent by females in the two male compartments was similar for the three experimental conditions (vehicle vs DEHP-0.5:  $485.99 \pm 6.22$  sec; vehicle vs DEHP-5:  $474.90 \pm 7.32$  sec and vehicle vs DEHP-50:  $483.24 \pm 10.37$  sec). Females spent a comparable amount of time in investigation of male stimuli (vehicle vs DEHP-0.5:  $134.84 \pm 7.55$  sec; vehicle vs DEHP-5:  $151.43 \pm 6.70$  sec; vehicle vs DEHP-50:  $134.10 \pm 6.16$  sec). **B.** Locomotor activity was recorded in a computed circular corridor during the light phase for 2 h and expressed as cumulative activity every 20 min for males exposed to the vehicle (Veh) or to DEHP at 0.5, 5, or 50  $\mu\text{g}/\text{kg}/\text{d}$ . Data are expressed as the means  $\pm$  S.E.M. of 11-12 males per treatment group. There was an effect of time ( $F_{(5, 215)} = 79.97$ ,  $p < 0.0001$ ) but not of treatment ( $F_{(3, 43)} = 0.75$ ,  $p = 0.53$ ). Locomotor activity decreased similarly for the four groups during the 2 h-test. **C.** Activity in the circular corridor was recorded for 10 h for another group of males (7 exposed to the vehicle (Veh), 9 to DEHP-5 and 8 to DEHP-50). The light (yellow) and dark (black) phases are indicated. The red arrow head indicates the time at which started sexual behavior tests. Data are expressed as the means  $\pm$  S.E.M. There was an effect of time ( $F_{(19, 437)} = 33.06$ ,  $p < 0.0001$ ) but not of treatment ( $F_{(2, 23)} = 0.62$ ,  $p = 0.55$ ). **D.** Balance and coordination of the same males as in (C) were assessed in the rotarod task. One-way ANOVA showed no effect of DEHP exposure on the latency to fall off accelerating rotarod ( $p = 0.70$ ).

**Figure S3.** Representative high magnification confocal optical sections through the preoptic area (**A**) and arcuate nucleus (**B**) of Kiss-creGFP mice, illustrating double immunofluorescent labelling of GFP (green) and ER $\alpha$  (red) (top images) and of GFP (green) and AR (red) (bottom images). Note the nuclear localization of both markers confirmed by Hoechst counterstaining. Kiss-creGFP cells exhibiting nuclear ER $\alpha$  or AR immunoreactivity were found in both regions (white arrows).

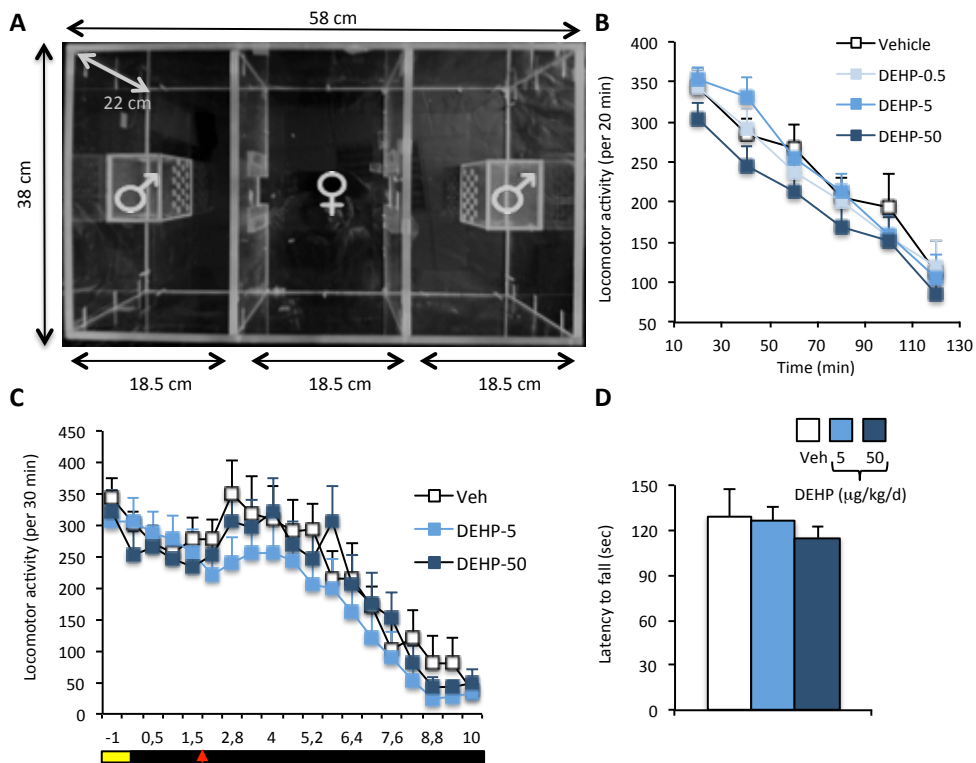
**Figure S4. A.** Representative Western blots of NDRG2 and GAPDH in the medial preoptic nucleus of the Veh, DEHP-5, and DEHP-50 groups. **B.** Quantification of the protein levels normalized to GAPDH. Data are expressed as the means  $\pm$  S.E.M. of 4 males per treatment group, \* $p < 0.05$  compared to the vehicle group.

**Figure S5. A.** Representative AR-immunolabeling in the medial amygdala (MeA) and bed nucleus of stria terminalis (BNST) of males exposed to the vehicle (Veh) or DEHP at 5 or 50  $\mu\text{g}/\text{kg}/\text{d}$ . Opt: optical tract; AC: anterior commissure. **B-C.** Quantitative analyses of the number of AR- (**B**) and ER $\alpha$ -immunoreactive neurons (**C**). Data are expressed as the means  $\pm$  S.E.M. of 4-6 males per treatment group, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.01$  vs the vehicle group.

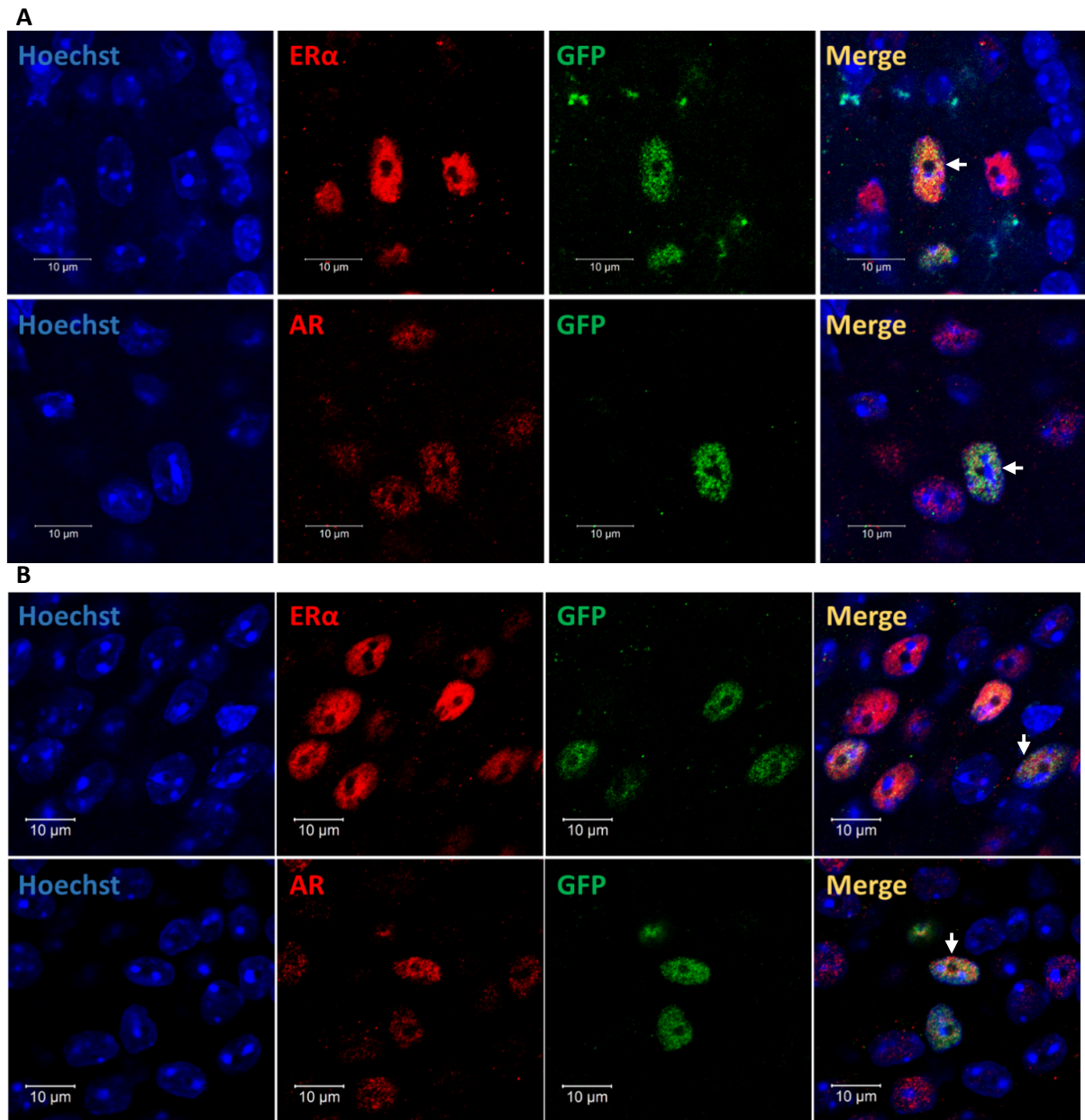
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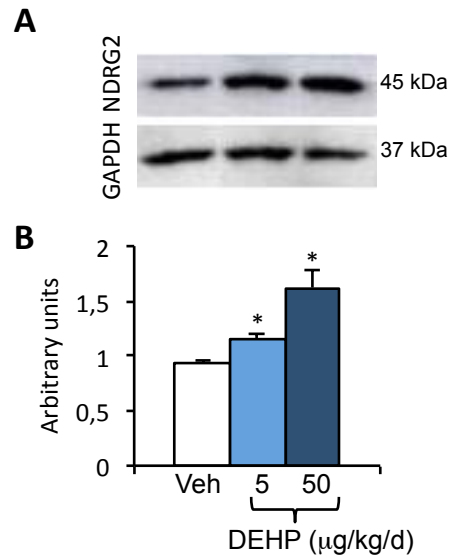
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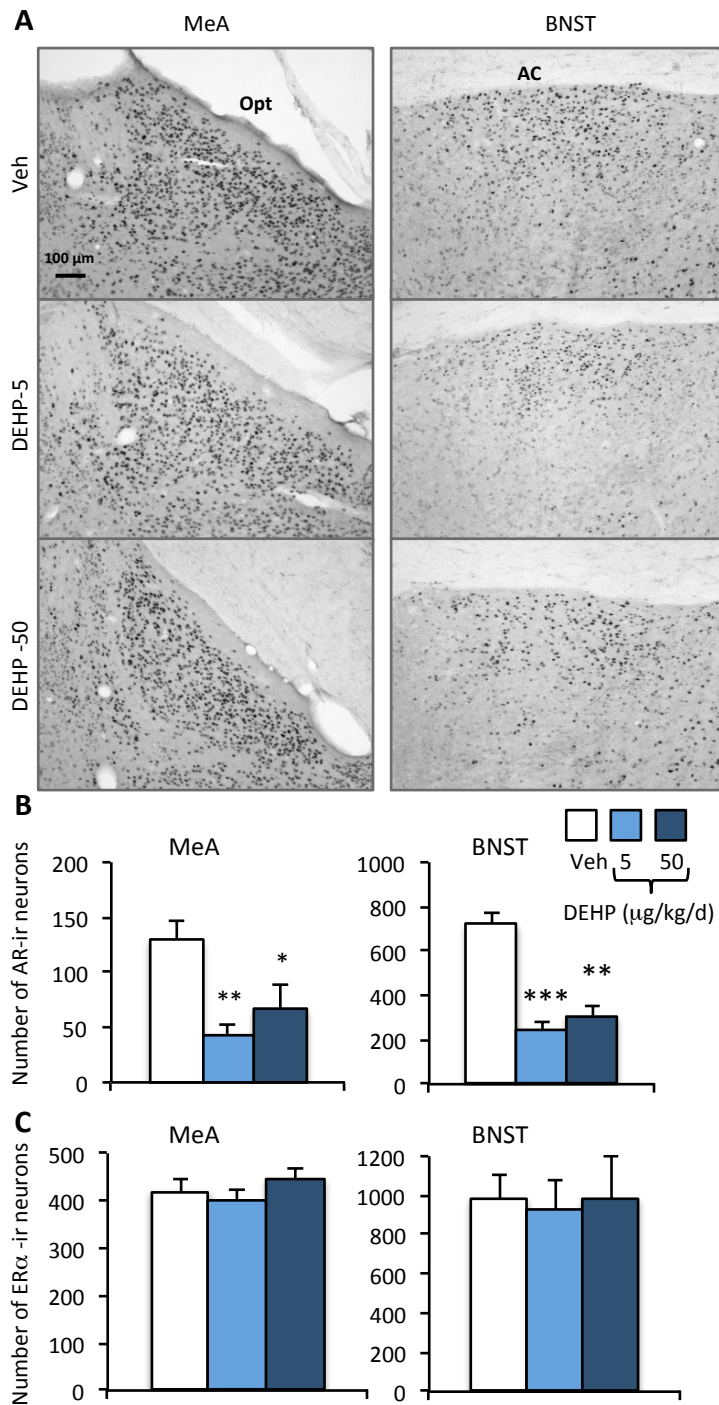
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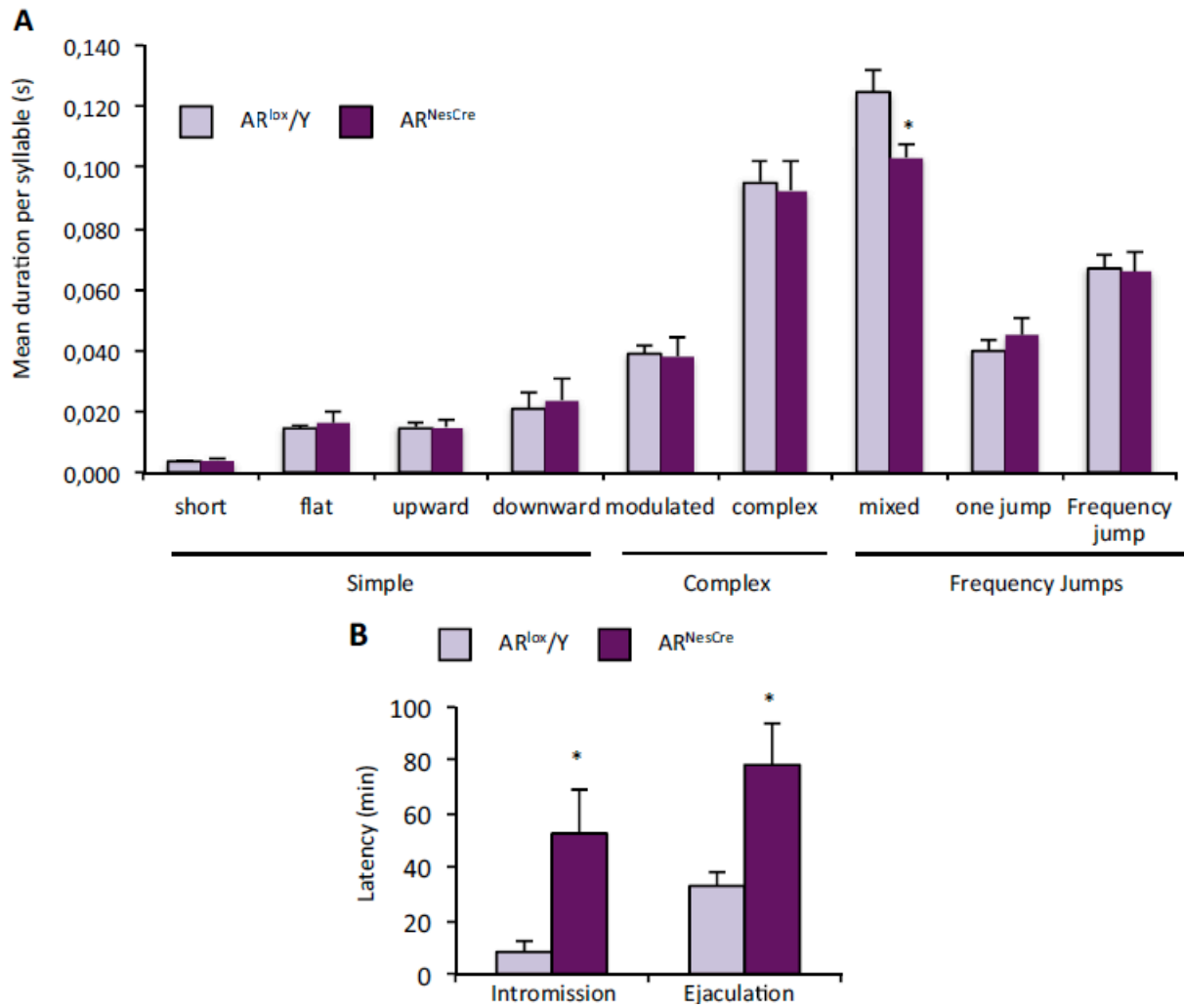
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