Addiction-like reward dysfunction and compulsive eating in obese rats: Role for dopamine D2 receptors

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Supplementary Figures

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Supplementary Figure 1. Kenny

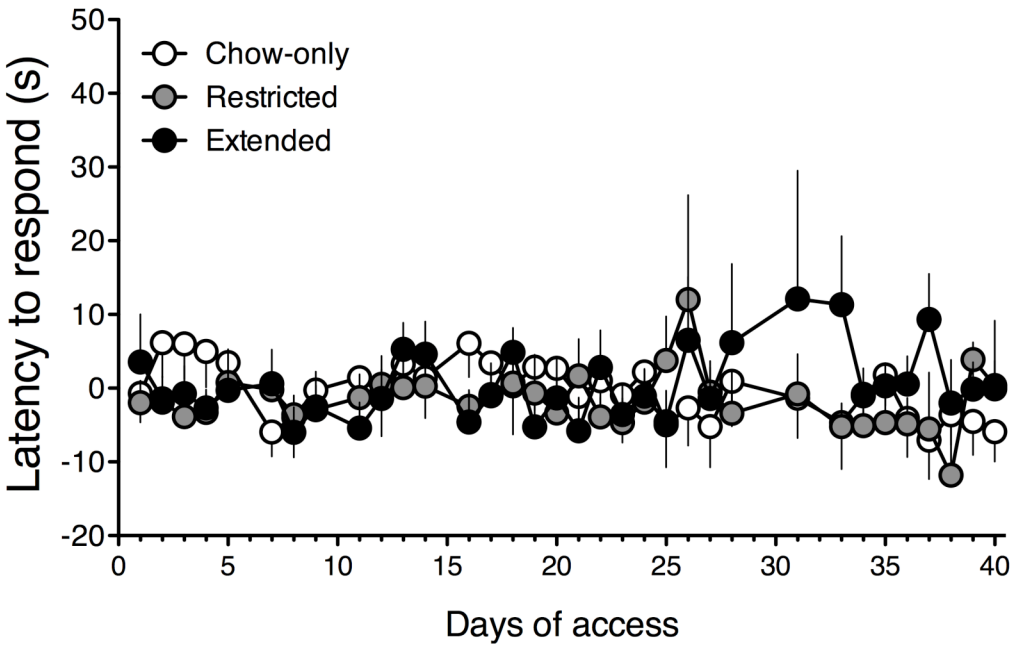

Figure S1. Elevated BSR thresholds are not due to impairments in motor performance. Response latencies in chow-only rats and in rats with restricted or extended daily access to a cafeteria-style diet. Data are expressed as mean (± SEM) percentage change of response latencies (s) each day during the 40-day period of access to the cafeteria diet.

Supplementary Figure 2. Kenny

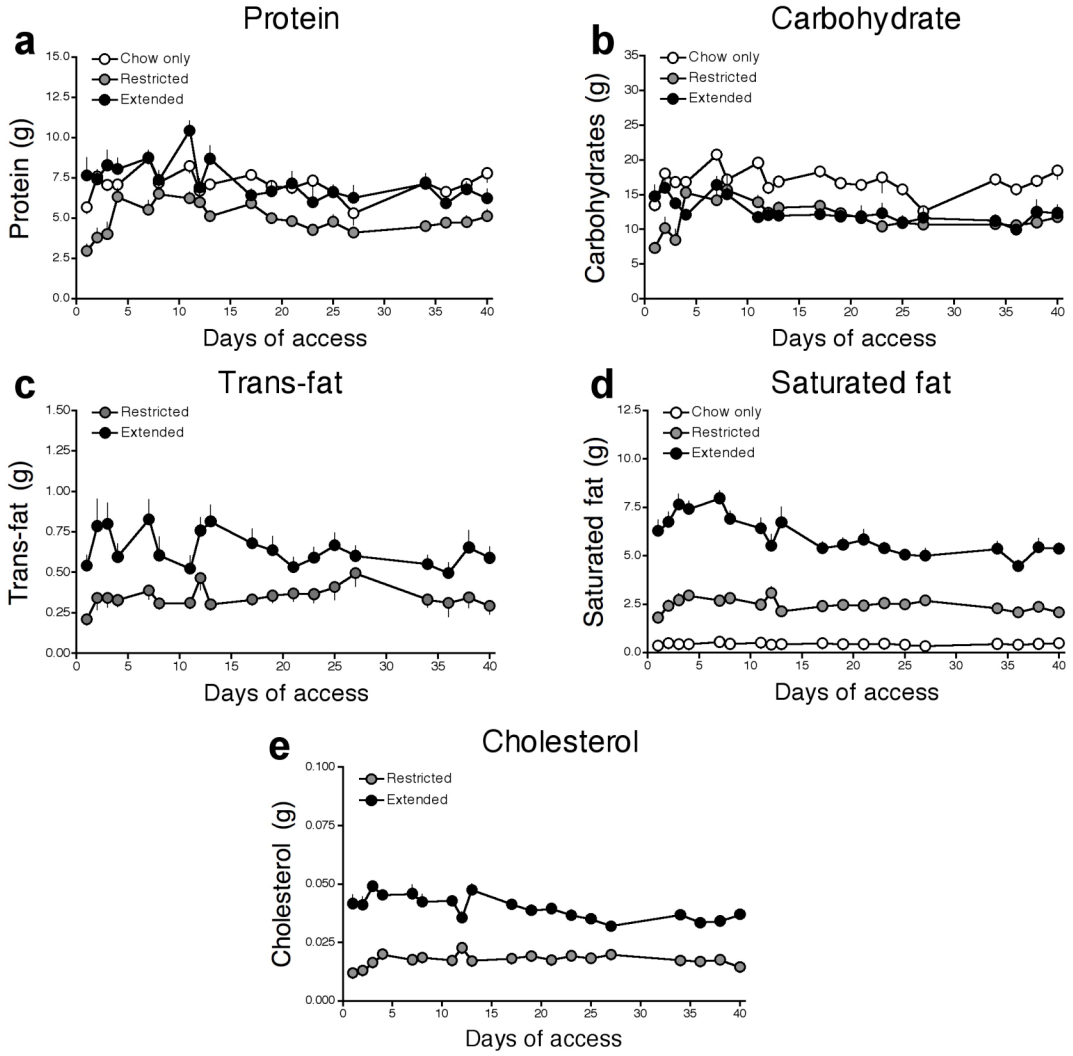

Figure S2. Detailed feeding architecture during 40 days of varied palatable diet access. Summary of macronutrient consumption in chow-only rats and in rats with restricted or extended daily access to a cafeteria-style diet. Mean daily intake (± SEM) of discrete macronutrients (g) was calculated based on nutritional information provided by the vendor. In all cases the calculation of intake includes macronutrients obtained from both standard chow as well as the cafeteria diet.

Supplementary Figure 3. Kenny

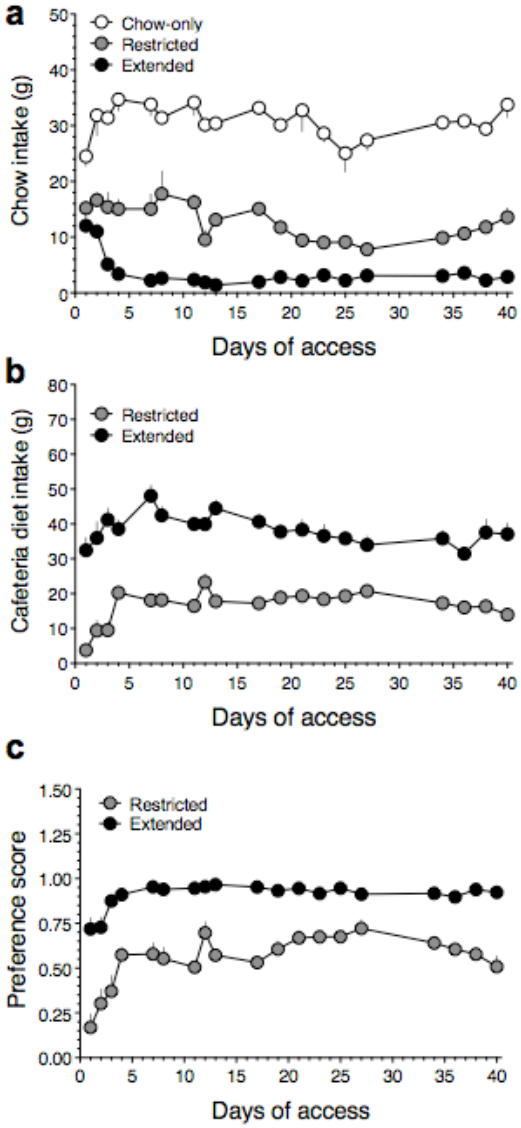

Figure S3. Preference for cafeteria diet remains persistently elevated in restricted and extended access groups. Consumption of chow and palatable food in the chow-only rats and in rats with restricted or extended daily access to a cafeteria-style diet. (**a**) Mean total mass (± SEM) of chow (g) consumed by each group. (**b**) Mean total mass (± SEM) of cafeteria diet food items (g) consumed by the restricted and extended access group. (**c**) The preference ratio for the restricted and extended access rats was calculated by dividing the daily mass of cafeteria diet food items by the total mass of food (chow + cafeteria items) consumed each day.

Supplementary Figure 4. Kenny

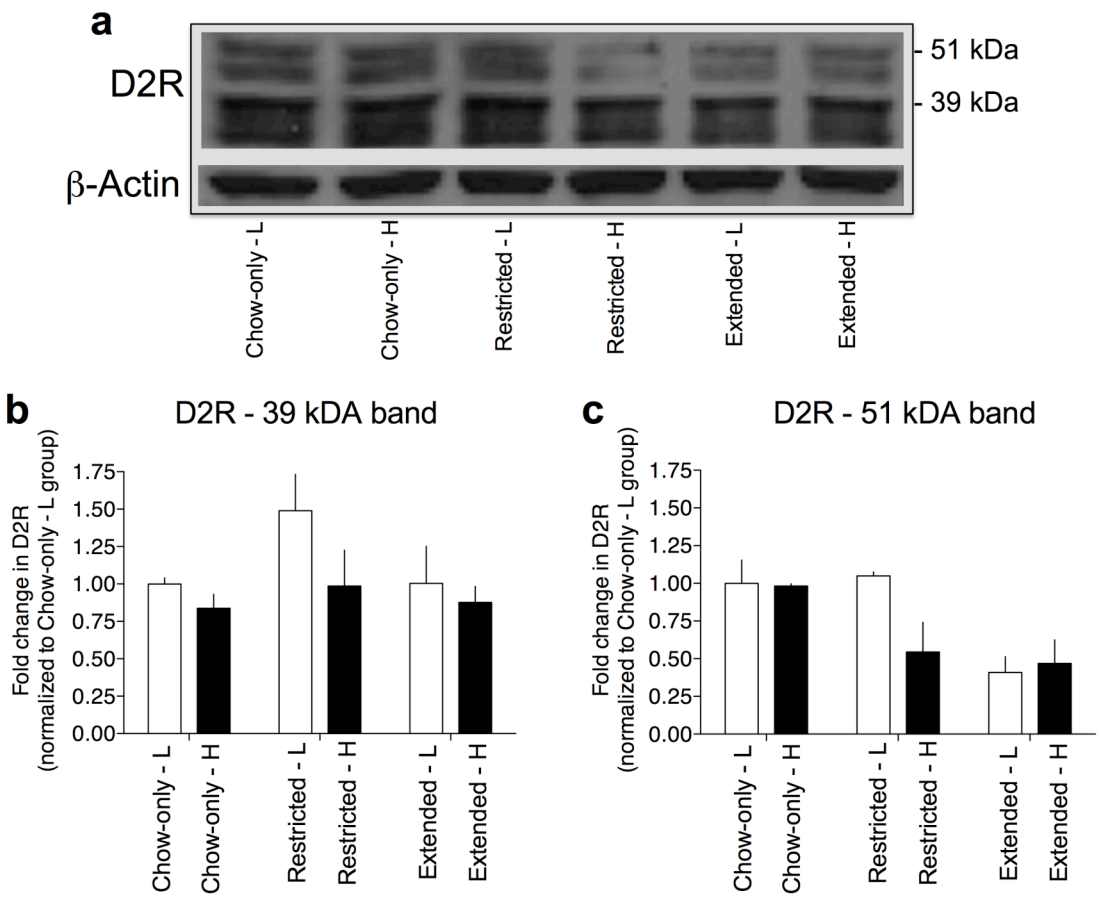

Figure S4. **Nascent striatal D2R expression is unaltered with varied palatable diet access.** Expression levels of additional glycosylation isoforms of D2R in striatum are not altered in obese rats. **(a)** Chow-only, restricted access and extended access rats were sub-divided into two groups per access condition based on a median split of body weights; light (L) or heavy (H). Striatal D2R levels in each group were measured by Western blotting. The immature (unglycosylated) form of the D2R was resolved at 39 kDa, and the glycosylated intracellular form at 51 kDa. **(b)** Relative amounts of immature D2R were quantified by densitometry, and no statistically significant alterations in expression were detected. **(c)** Relative amounts of glycosylated intracellular D2R were quantified by densitometry and no statistically significant alterations in expression were detected, although there was a trend toward decreased expression in the extended access rats.

Supplementary Figure 5. Kenny

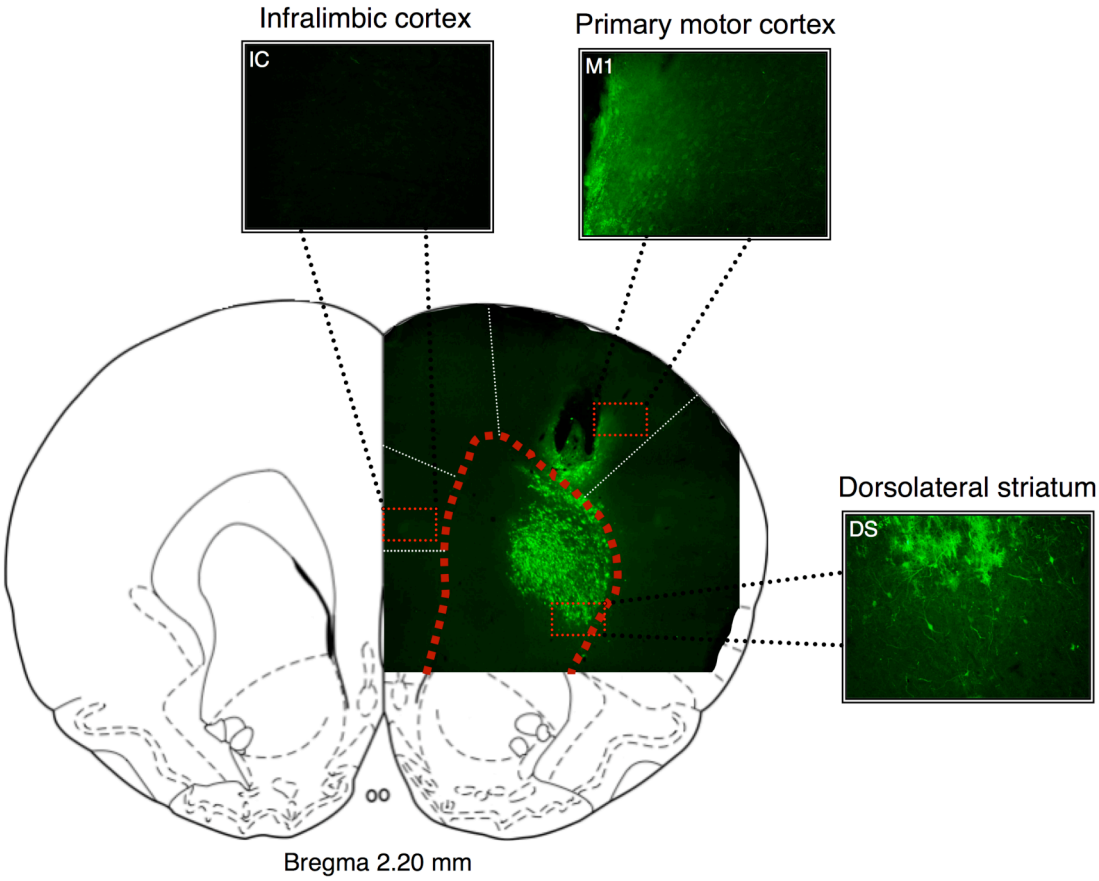

Figure S5. **Viral transduction is spatially restricted to striatal populations.** Graphical representation of striatal and extra-striatal areas exhibiting lentivirus-infected cells. Green staining is representative immunochemistry staining for GFP-positive, lentivirus infected cells. Note the tract of the cannula is clearly visible in the M1 region of the primary motor cortex. GFP-positive cells were found almost exclusively in the dorsal striatum. However, in some cases we also found a small number of GFP-positive cells in the motor cortex surrounding the cannula tract. We found no evidence of diffusion of lentivirus supernatant to surrounding extra-striatal brain regions, including areas of the prefrontal cortex.

Supplementary Figure 6. Kenny

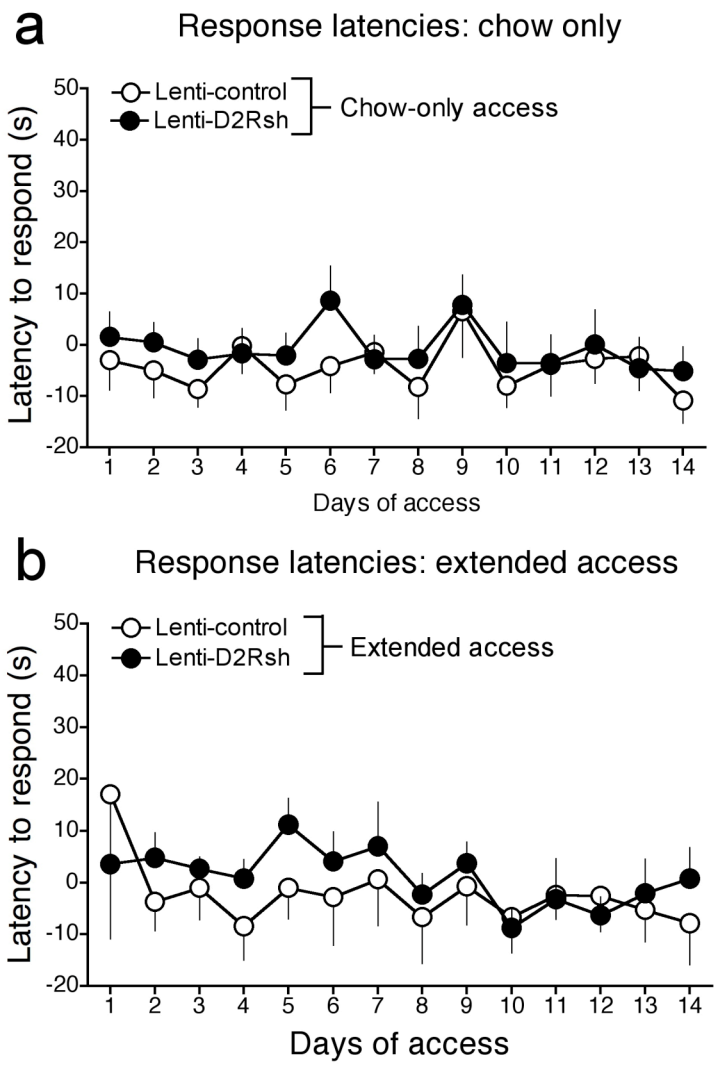

Figure S6. Striatal D2R knockdown does not impair BSR performance. Response latencies are unaltered in D2R knockdown rats. (**a**) Response latencies (± SEM) were unaltered in Lenti-D2Rsh and Lenti-control rats, relative to baseline measurements, under chow-only access conditions. (**b**) Response latencies (± SEM) were unaltered in Lenti-D2Rsh and Lenti-control rats, relative to baseline measurements, under extended access conditions.

Supplementary Figure 7. Kenny

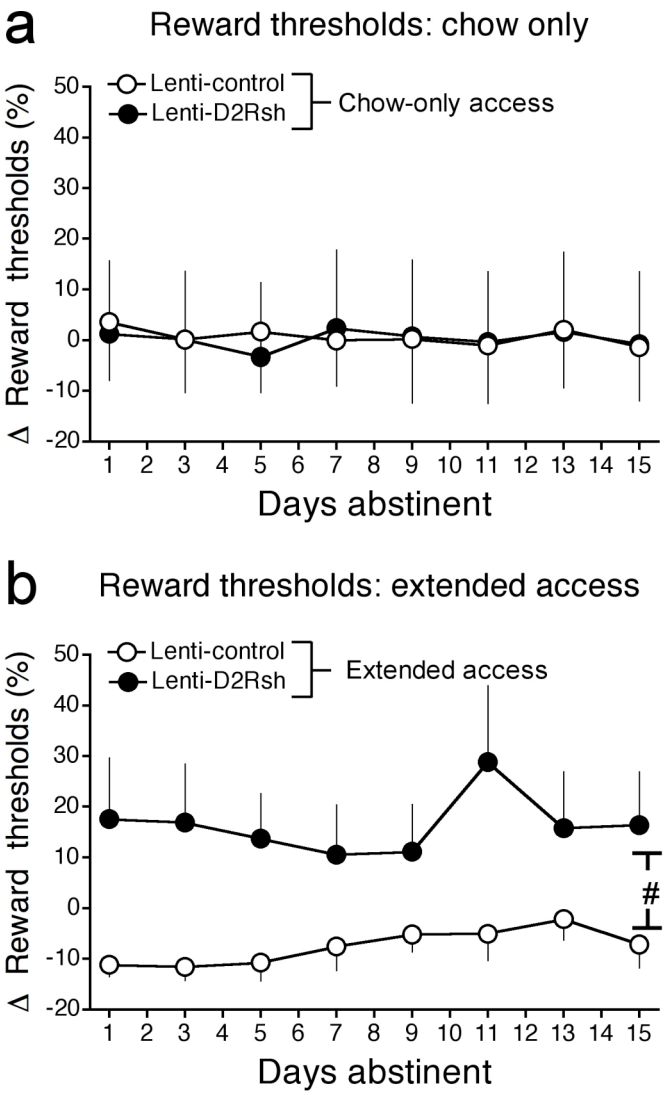

Figure S7. Elevated BSR thresholds are long-lasting in Lenti-D2Rsh animals with a history of extended access to a cafeteria diet. BSR thresholds remain persistently elevated in D2R knockdown rats. (**a**) Mean percentage change from baseline reward thresholds (± SEM) during the abstinence periods in Lenti-Control and Lenti-D2Rsh rats that previously had chow-only access. (**b**) Mean percentage change from baseline reward thresholds (± SEM) during abstinence from a palatable high-fat diet in Lenti-Control and Lenti-D2Rsh rats (Virus: $F_{1,14} = 4.9$, $P < 0.05$; #P < 0.05, main effect of Virus).

Supplementary Figure 8. Kenny

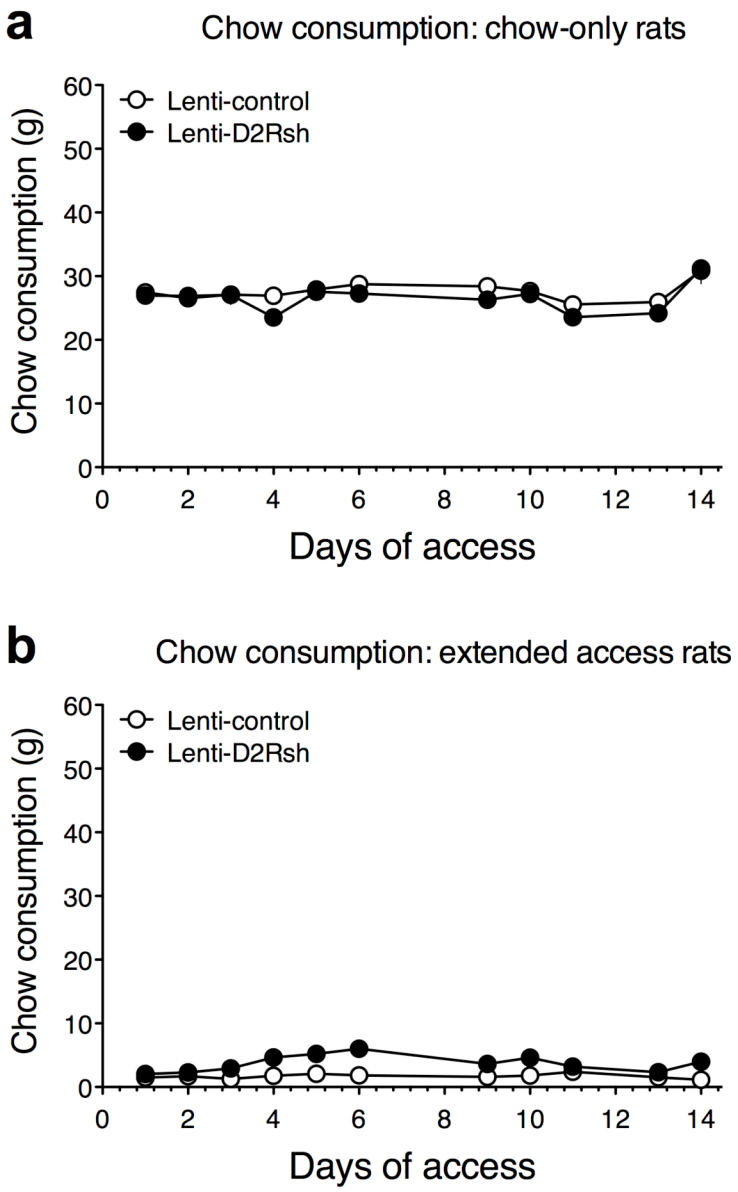

Figure S8. Chow consumption in Lenti-control and Lenti-D2Rsh animals during 14 days of varied palatable diet access. Consumption of chow in the Lenti-Control and Lenti-D2Rsh rats with chow-only or extended access to the cafeteria was recorded throughout the 14 days of access to the cafeteria diet. (**a**) Mean total mass (± SEM) of chow (g) consumed by Lenti-Control and Lenti-D2Rsh rats with chow-only access. (**b**) Mean total mass (± SEM) of chow (g) consumed by Lenti-Control and Lenti-D2Rsh rats with extended access to the cafeteria diet.

Supplementary Figure 9. Kenny

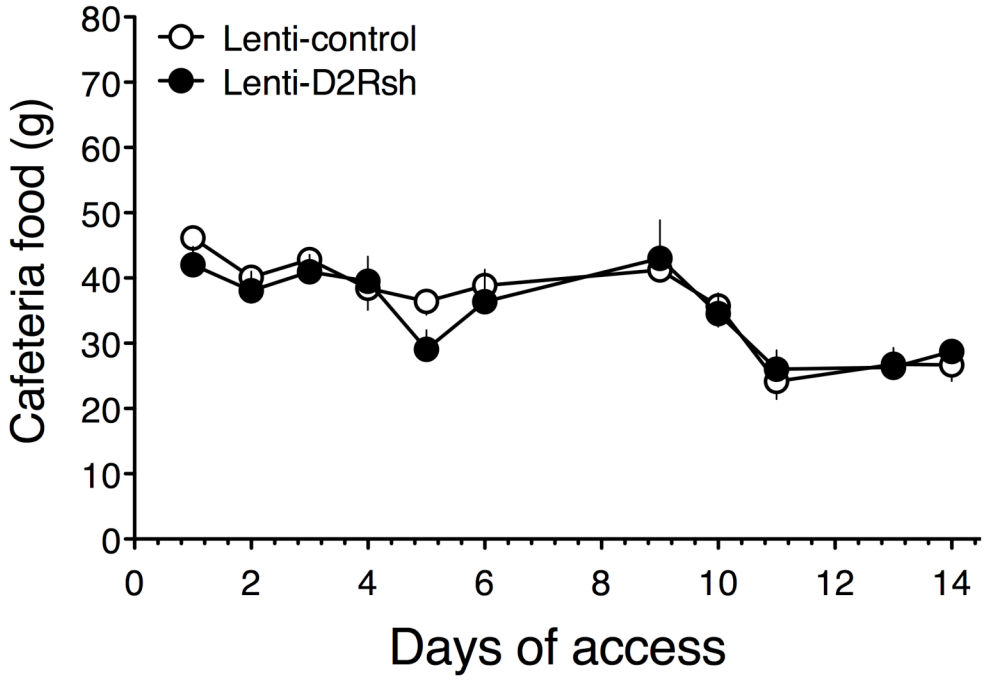

Figure S9. Total cafeteria diet intake of Lenti-control and Lenti-D2Rsh groups during 14 days of extended access. Consumption of cafeteria food items in the Lenti-Control and Lenti-D2Rsh rats with extended access was recorded throughout the 14 days of access to the cafeteria diet. Data are presented as mean total mass (± SEM) of cafeteria food items (g) consumed by each group.

Supplementary Figure 10. Kenny

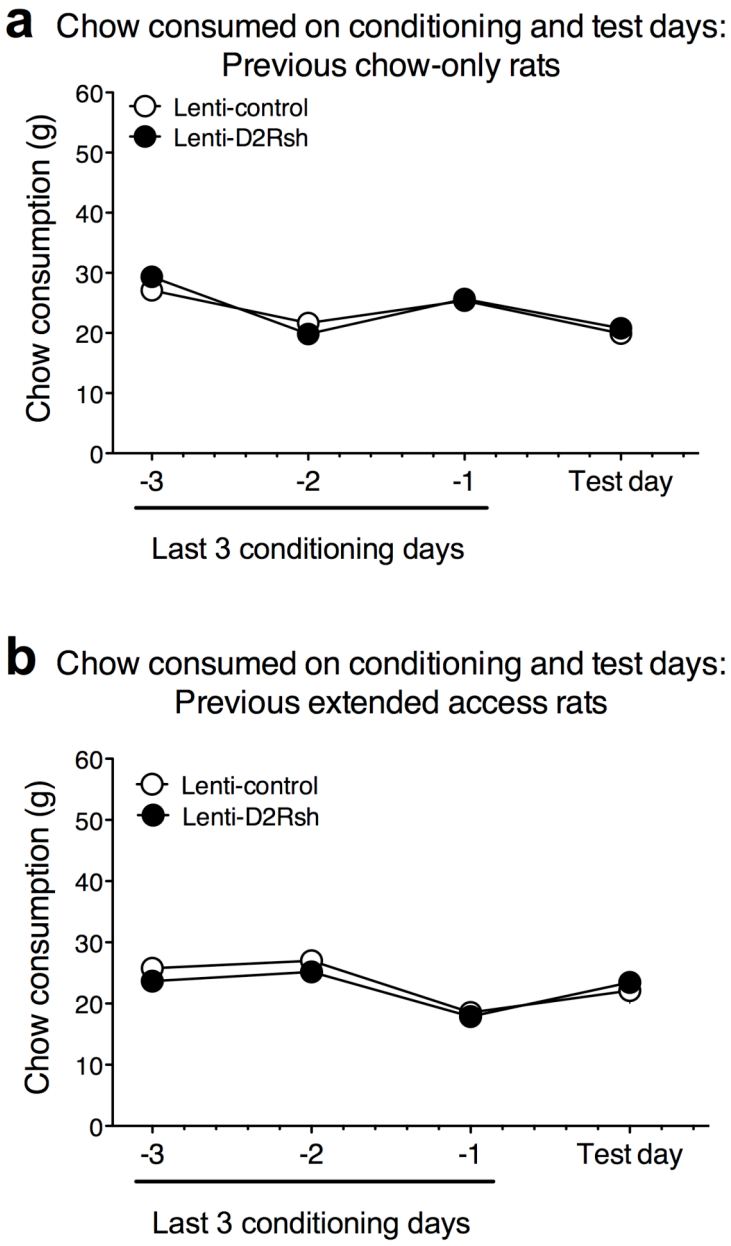

Figure S10. Aversive-cue conditioning does not alter baseline chow intake. Consumption of chow in the Lenti-Control and Lenti-D2Rsh rats with previous access to chow-only or extended access to the cafeteria was recorded on the final three days of conditioning and on the test day. (**a**) Mean daily total mass (± SEM) of chow (g) consumed by Lenti-Control and Lenti-D2Rsh rats with chow-only access. (**b**) Mean daily total mass (± SEM) of chow (g) consumed by Lenti-Control and Lenti-D2Rsh rats with extended access to the cafeteria diet.

Supplementary Figure 11. Kenny

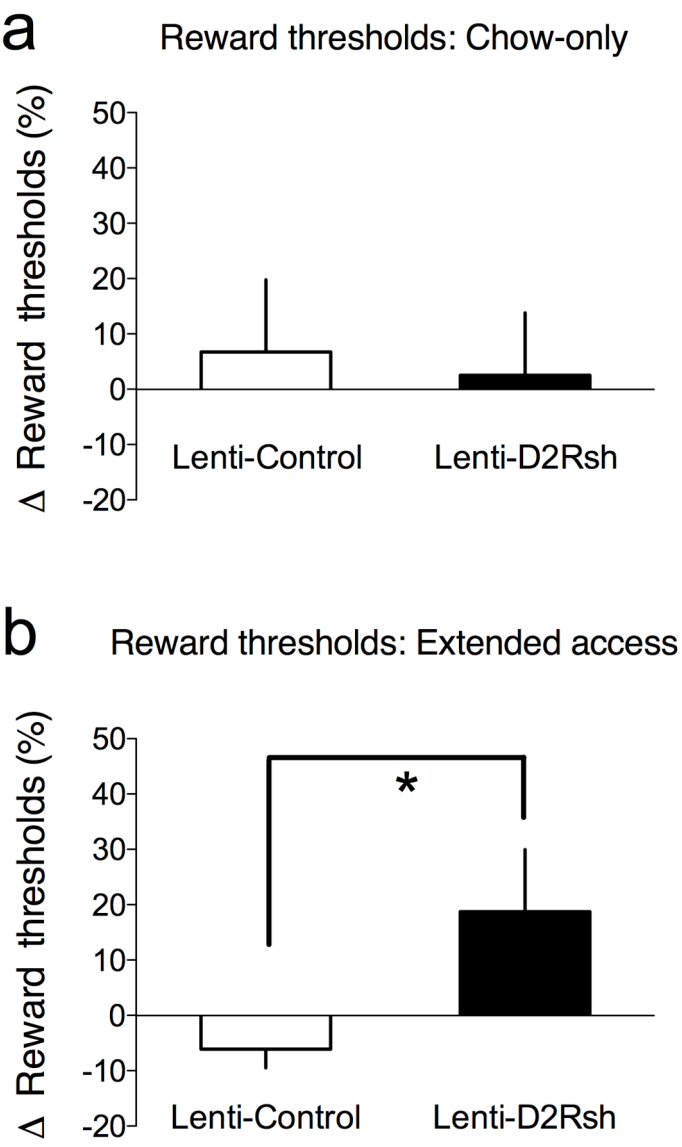

Figure S11. Reward deficits associated with extended cafeteria diet access are long-lasting in Lenti-D2Rsh rats. BSR thresholds remain persistently elevated in D2R knockdown rats. (**a**) Mean percentage change from baseline reward thresholds (± SEM) were assessed 48-h after testing the effects of an aversive CS on palatable food intake in Lenti-Control and Lenti-D2Rsh rats that previously had chow-only access. (**b**) Mean percentage change from baseline reward thresholds (± SEM) were assessed 48-h after testing the effects of an aversive CS on palatable food intake in Lenti-Control and Lenti-D2Rsh rats that previously had access to the cafeteria diet for 14 consecutive days (*P < 0.05, *t*-test).

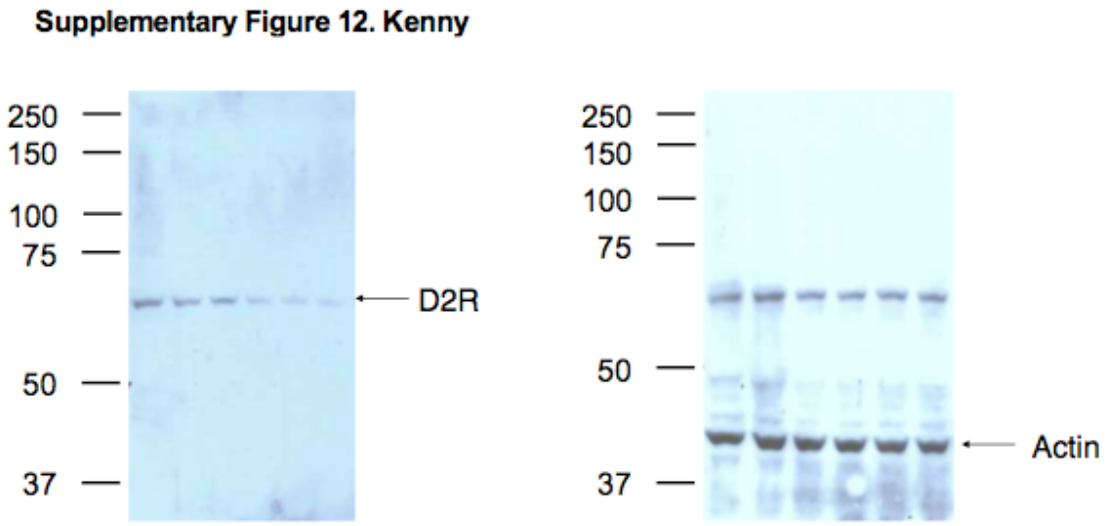

Figure S12. Full-length western blots presented in Fig. 4b.

Supplementary Figure 13. Kenny

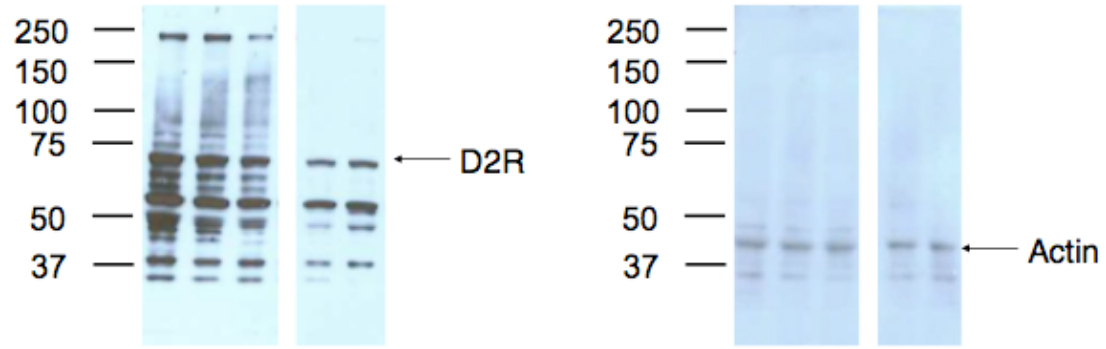

Figure S13. Full-length western blots presented in Fig. 5b.