Supplemental methods

Leptin and Insulin plasma measurements

On the last day of restricted or extended access to a cafeteria diet or chow (40-47d), tail blood (300 µL in 0.1M EGTA) was collected from each unfasted rat 1 h into its dark cycle prior to replenishment of the cafeteria diet. Samples were centrifuged at 14,000 r.p.m. for 10 min at 4 °C to separate plasma. The concentration of plasma insulin was determined using an enzyme-linked immunosorbent assay (ELISA) according to manufacturing instructions from the Mouse Ultrasensitive Insulin ELISA kit from Alpco (Salem, NH). Plasma leptin concentration was assayed with a Rat Leptin ELISA kit according to manufacturers instructions (Crystal Chem, Downers Grove, IL).

Freezing and appetitive conditioning

In a subgroup of rats used for electrophysiology experiments, freezing was measured on the 4th conditioning session where a light was paired with foot shocks on an intermittent, 10 min on (foot shocks), 10 min off (no foot shocks), 10 min on (foot shocks), schedule. At the beginning of the session, the light is activated for 1 min prior to foot shock delivery during which freezing activity was recorded. Freezing, defined as the absence of all movement other than that required for respiration, was assessed manually from video with a minimum freezing duration of 2s.

In separate cohort of cafeteria-diet or chow-fed rats, *ad libitum* rats on their respective diets were trained on a fixed ratio 1 (FR1) schedule of reinforcement for sucrose pellets (Bioserve, Frenchtown NJ). After 40 days on the cafeteria diet or chow, rats were placed in the operant chamber for 2h each day for 5 consecutive days. Pressing on the active lever delivered one 45 mg pellet as well as a cue light illumination and tone where as pressing on the inactive lever had no consequence. Each reinforcer was followed by an 18s timeout. Animals were not food restricted for these experiments.

Parvalbumin Immunohistochemistry

The day after the conditioned suppression test, a separate cohort of animals was perfused transcardially with 4% paraformaldehyde solution and brains were extracted. The brains were cryoprotected in 30% sucrose before collecting 30 μm sections containing the lateral OFC on a cryostat (Leica Microsystems). OFC sections were blocked (2% normal goat serum) and then reacted with the primary antibody for parvalbumin (PV+) (1:1000, AbCAM ab11427), a marker for a cortical interneuron subtype, in blocking solution for 24 h at 4°C. After washing, secondary antibody was applied (goat anti-rabbit IgG-Alexa 488; 1:2000) in blocking solution for 3 hrs. Slices were then washed and mounted with DAPI. Images were taken with a confocal microscope (Nikon Eclipse C1 si spectral) with a 20x PLAN FLUOR (NA 0.75) objective. Four slices of the OFC were taken from each rat. Within each slice, 4 non-overlapping images/fields of view (512 x512 pixel resolution) were taken of the lateral OFC. The number of PV+ neurons was counted in each of the 16 regions of interest within the lateral OFC and averaged across each animal. All PV+ neurons were counted blind to the condition of the animal.

Supplemental table 1

Macronutrient composition of the cafeteria diet or chow. Values represent the percentage of macronutrient composition of each food per gram. Right most column represents the energy density of each food.

Food	Fat (%)	Protein (%)	Carbohydrate (%)	kCal/g
Kraft™ Peanut Butter	53	20	27	6.0
Kellogg's™ Froot Loops	4	4	89	4.1
Doritos™	26	6	64	5.2
Kirkland™ Hot Dogs	25	12	4	3.0
Tim Hortons™ Tim Bits	16	5	53	3.7
Lab Diet (5P14) Rat Chow	5	24	49	3.3

Figure legends

Supplemental Figure 1. Plasma insulin and leptin are increased with access to cafeteria diet. a. Plasma insulin concentration was elevated in rats with restricted and extended access to a cafeteria diet. (One-Way ANOVA; $F(2,42) = 48.69$, $P< 0.0001$). **b**. Plasma leptin concentration was elevated in rats with restricted and extended access to a cafeteria diet. (One-Way ANOVA; $F(2,26) = 97.90$, $P<0.0001$). Number on bars refers to N (rats). Bars or symbols represent mean ± s.e.m. Error bars may be occluded by symbols. $^{**}P < 0.01$, $^{***}P < 0.0001$.

Supplemental Figure 2. Access to a cafeteria diet does not alter Pavlovian or instrumental appetitive or aversive conditioning.

a. Rats with extended or restricted access are not different from chow-fed rats in appetitive conditioning. Rats were placed in an operant chamber daily for 2 h for 5 consecutive days to learn sucrose-self administration on a fixed ratio 1 schedule. There was no effect of diet on the percentage of active responses over the total number of responses (2-way ANOVA, Interaction: $F (8, 36) = 0.8333$, $P = 0.6$, Diet: $F (2, 9) = 0.4368$, $P = 0.7$). **b.** The total number of pellets received was not significantly different between chow-fed, restricted access or extended access rats (2-way ANOVA, lever

press x diet interaction, $F(8,36) = 0.70$, $P = 0.69$, n = 4/group). All rats reached a learning criteria of >80% responses on the active lever by day 3.

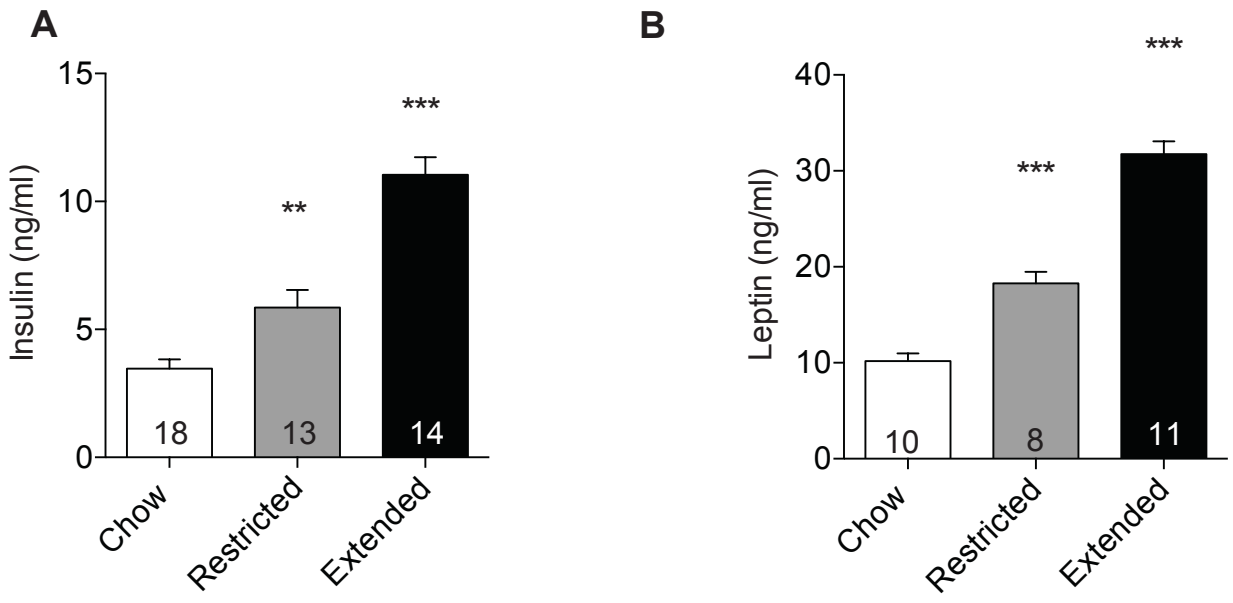
c. To assess aversive learning, time spent freezing was measured during the first minute the rats were placed in the foot-shock context in the presence of the conditioned cue-light (CS+) or the unconditioned cue-light (UCS), prior to receiving any foot shocks (or nothing in the case of UCS) on the 4th conditioning session. There was no significant interaction of CS on diet, but a main effect of CS+ vs UCS (2-Way ANOVA; Interaction: $F(2,38)=0.44$, $P = 0.64$; CS+ vs UCS: $F(1,38) = 78.85$, $P <0.0001$).
d. Startle responses on the 4th foot-shock conditioning session were not significantly different between chow-fed, restricted access or extended access rats (one-way ANOVA, $F(2,22) = 1.97$, $P = 0.16$). Number on bars refers to N (rats). Bars or symbols represent mean + s.e.m. * $P < 0.05$.

Supplemental 3. Excitatory synaptic transmission onto lateral OFC pyramidal neurons is not changed with access to a cafeteria diet.
a. Example traces of sEPSC recorded at -70 mV from lateral OFC pyramidal neurons from chow-fed, restricted or extended access rats.
b. sEPSC amplitude onto lateral OFC pyramidal neurons was not significantly different between feeding groups (one-way ANOVA, $F(2,28 = 0.16$, $P = 0.85$).
c. Frequency of sEPSCs onto lateral OFC neurons are not significantly different between groups. (one-way ANOVA, $F(2,28) = 1.08$, $P = 0.35$)
d. mEPSC amplitude was not significantly different between feeding groups (one-way ANOVA, $F(2,24) = 0.45$, $P = 0.64$).
e. mEPSC frequency was not significantly different between feeding groups although there was a trend of increased mEPSC frequency in pyramidal neurons from extended access rats (one-way ANOVA, $F(2,24) = 3.41$, $P = 0.05$).
f. Paired pulse ratio (50 ms interval) of AMPA receptor evoked EPSCs from lateral OFC neurons indicate no change in glutamate release probability between feeding groups (one-way ANOVA $F(2, 23) = 0.08$, $P = 0.92$).
g. Example traces of paired pulses evoked at -70 mV demonstrating high release probability of synapses onto lateral OFC pyramidal neurons. Number on bars refers to N (cells/rats). Bars represent mean ± s.e.m.

Supplemental 4. Extended access to a cafeteria diet does not alter number of PV+ interneurons in the OFC.

a. Example images of PV+ (green) neurons and DAPI (blue). Scale bar, 50 μm.

b. The number of PV+ neurons in the lateral OFC was not different between feeding groups (one-way ANOVA, $F(2,11)= 0.28$, $P = 0.97$). Number on bars refers to N (rats). Bars represent mean ± s.e.m.



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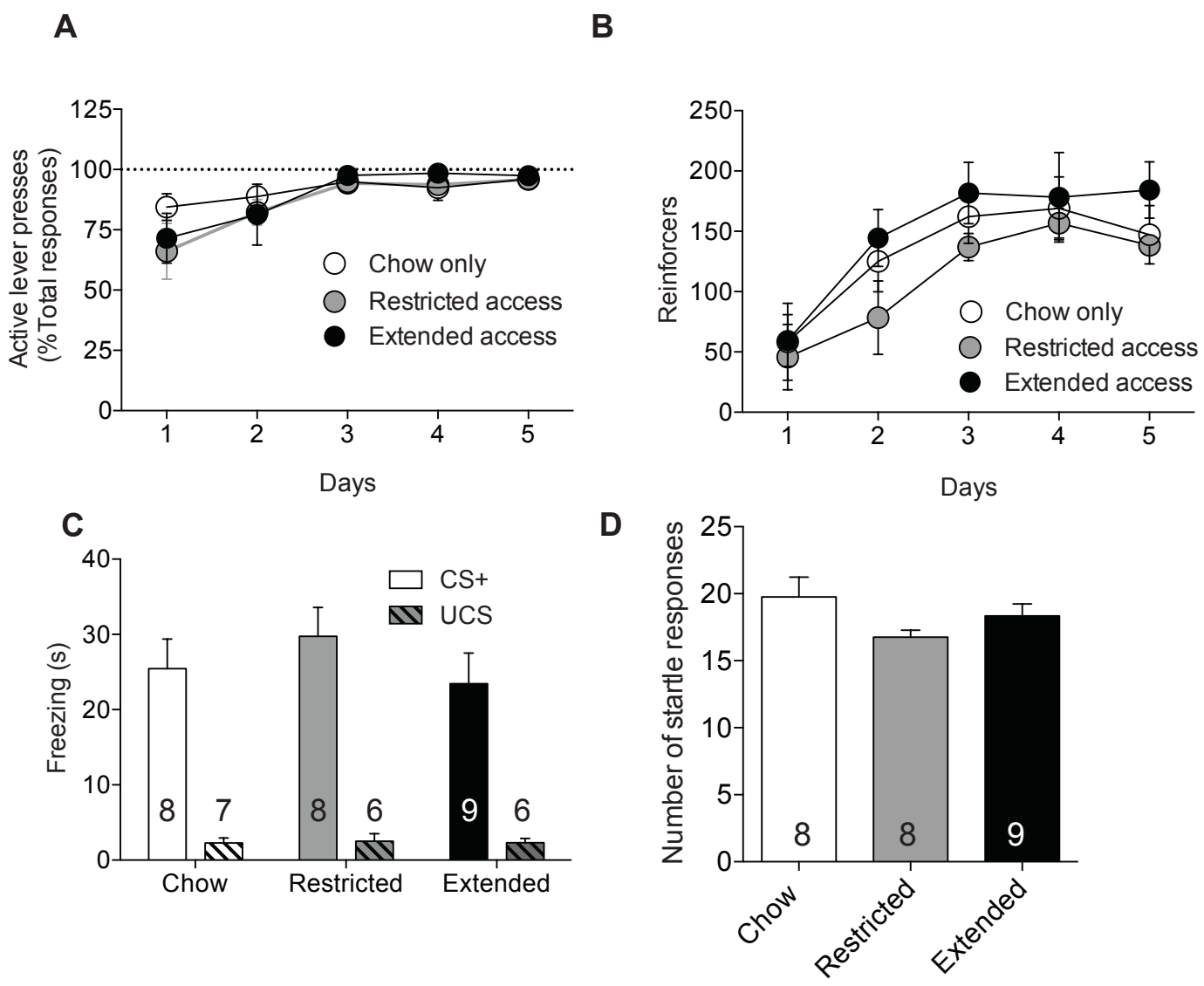

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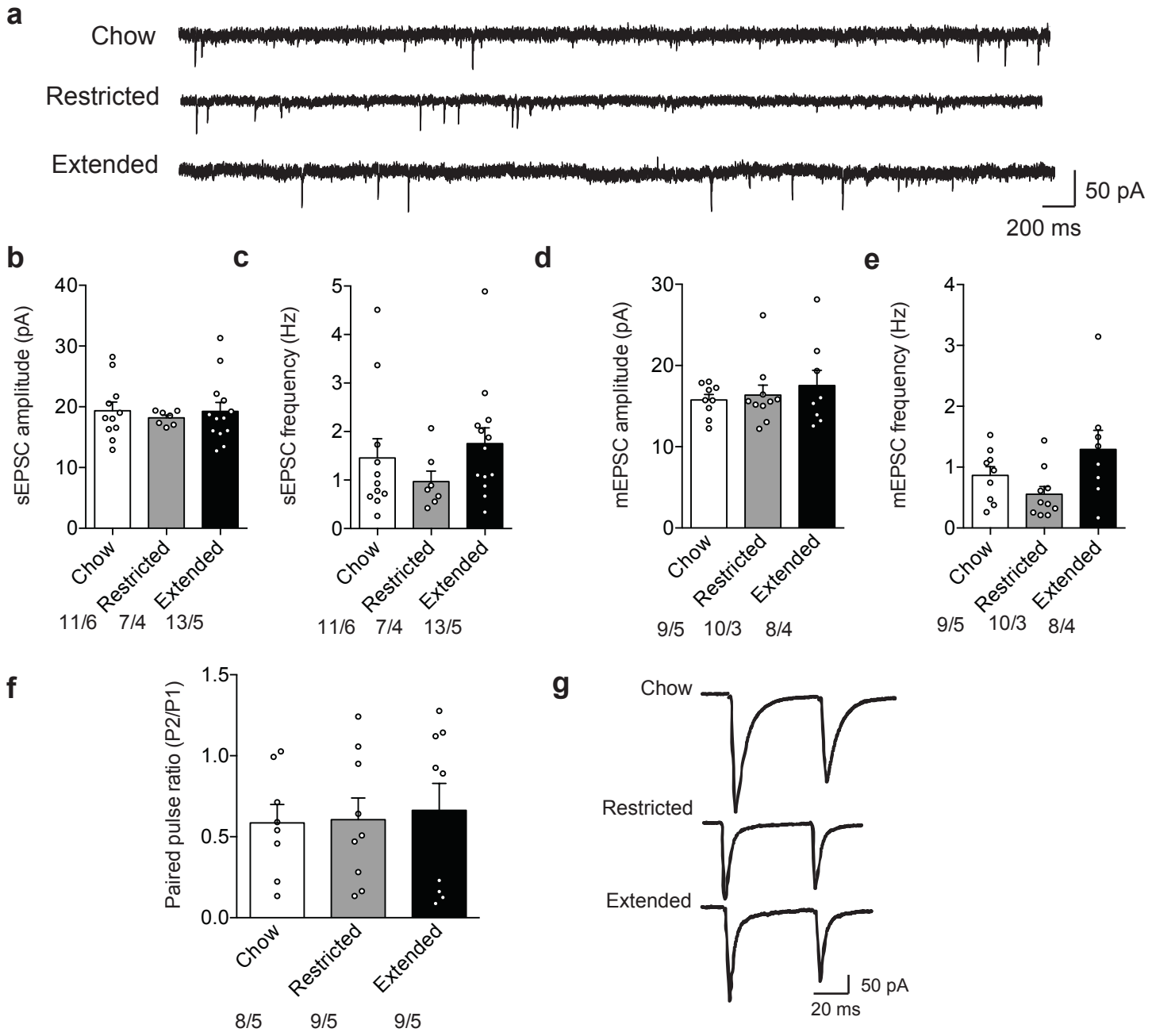

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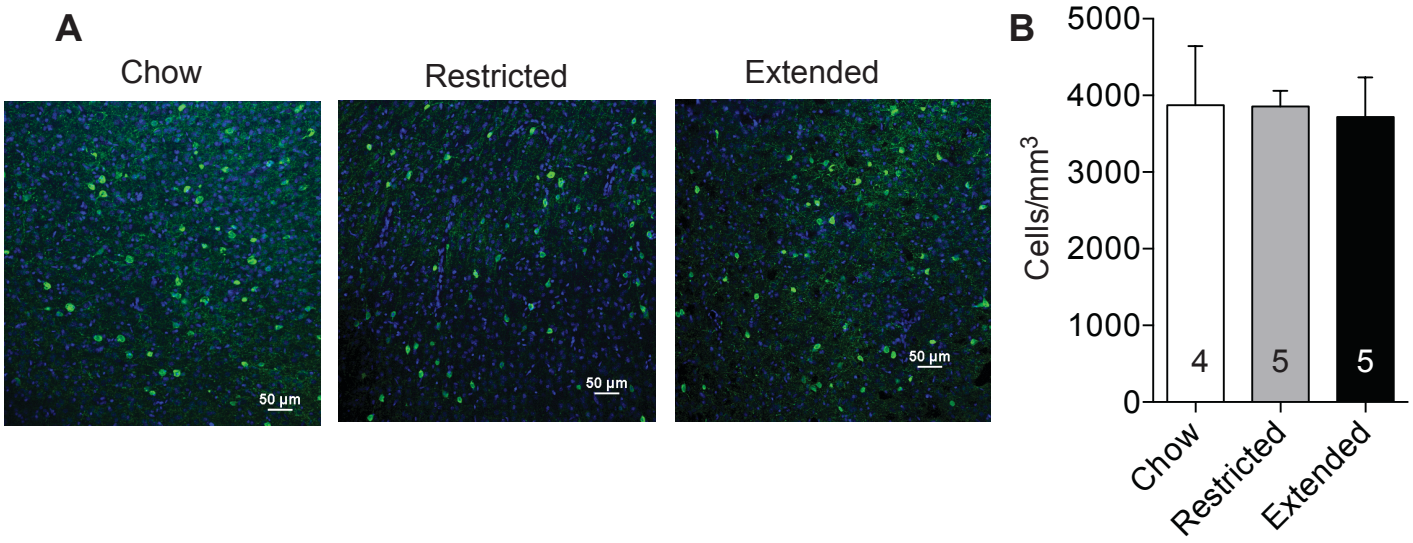

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