Addiction-Like Synaptic Impairments in Diet-Induced Obesity

Supplemental Information

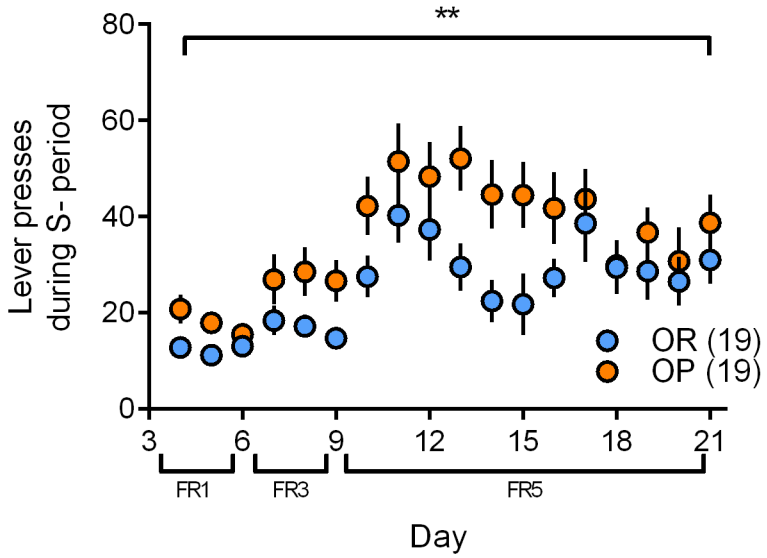

Figure S1. Timecourse of lever pressing during reward unavailable (S-) periods. OP rats pressed more than OR rats during the periods of reward unavailability (S-) throughout the entire operant protocol (two-way ANOVA, $F_{(1,36)} = 10.88$, $p = 0.0022$ for weight gain main effect).

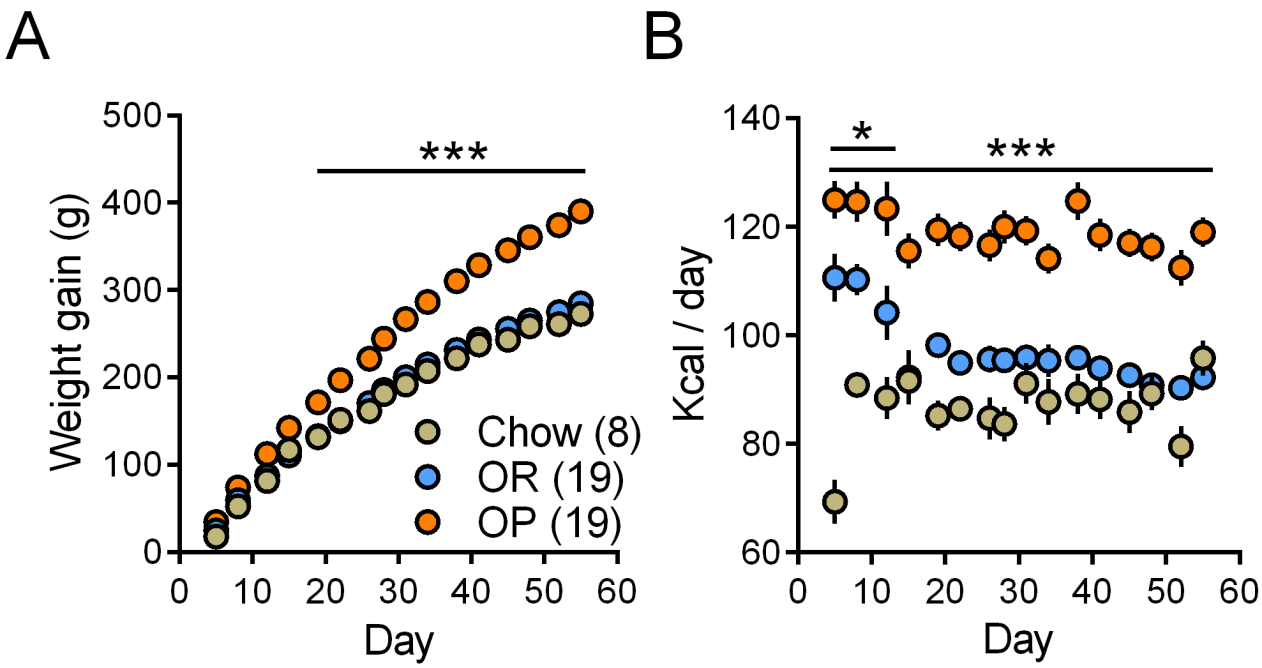

Figure S2. Weight and kcal over the 8 week diet period in chow rats versus OP and OR rats. (A) Weight gain of chow rats mirrored that of OR rats over the homecage diet period as both were significantly different from OP rats from day 19 onwards. **(B)** Kcal consumption of the chow diet was significantly lower than consumption of the palatable diet by both OP and OR rats up until day 12 of the diet period after there was no difference between OR and chow rats and both consumed significantly less kcal than OP rats (*$p < 0.05$ as compared to OR for that day, ***$p < 0.001$ as compared to OP for that day; two-way ANOVA with treatment and day as factors). Rats belong to the same cohort. Data from OP and OR rats of this cohort comprise the figures of the main text.

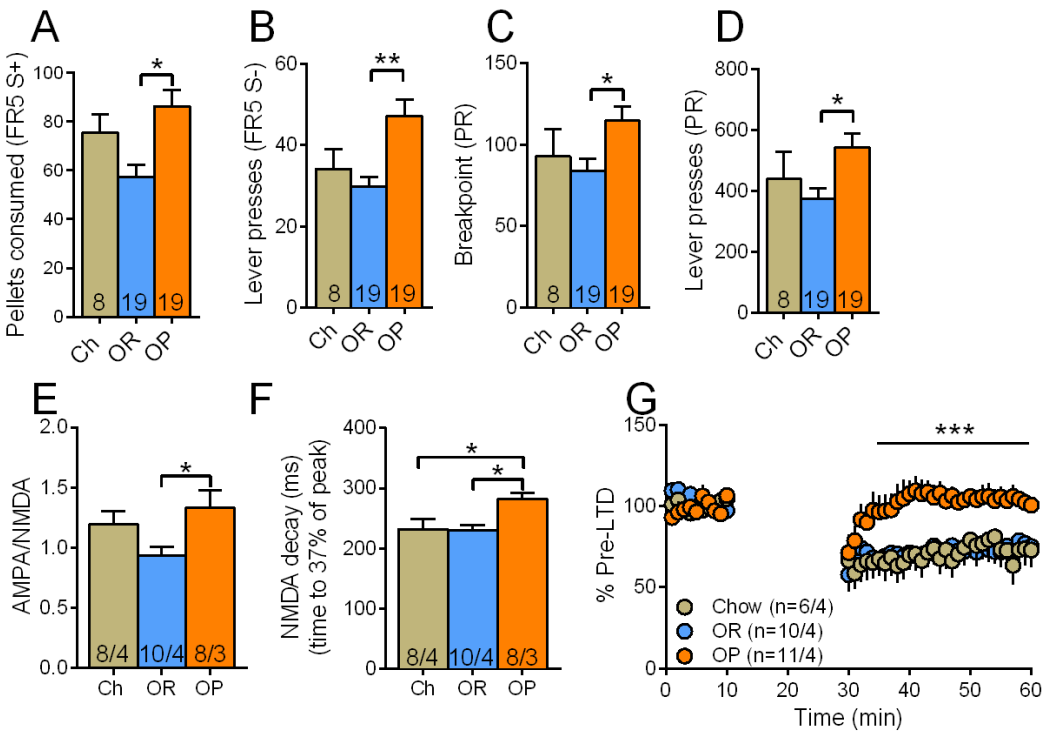

Figure S3. Behavioral and electrophysiological endpoints in rats that were on homecage chow diet before going through operant protocol as compared to OP and OR rats. (A-D) Chow diet group sat in between OP and OR rats on the measurements of 'addiction-like' behavior when pressing for palatable food pellets and therefore was not significantly different to either group. This was also the case for AMPA/NMDA ratio **(E)** but in the case of the NMDA decay time **(F)** and LTD **(G)** the chow group was significantly different to OP and mirrored more the obesity resistant rats (**$p < 0.01$, *$p < 0.05$, one way ANOVA with Tukey post hoc analysis; ***$p < 0.001$, two way ANOVA with time and treatment as factors). Rats belong to the same cohort. Data from OP and OR rats of this cohort comprise the figures of the main text.

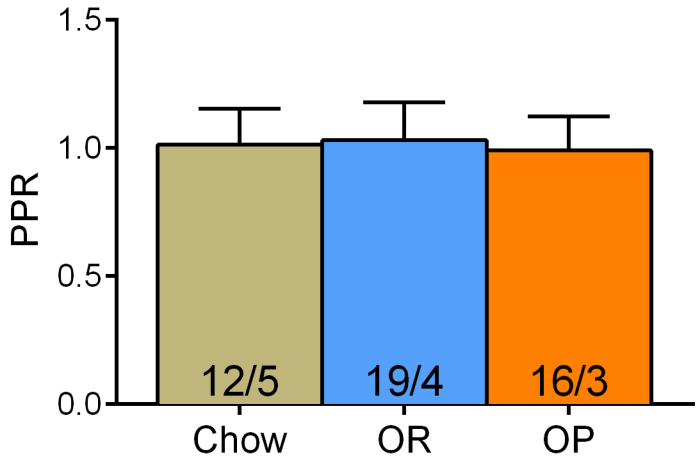

Figure S4. No difference in paired pulse ratio in chow, OP and OR rats. A pair of stimulation was administered with an interpulse interval of 50 ms. PPR was determined as the ratio between the peak of the second and first EPSCs. Rats belong to the same cohort.

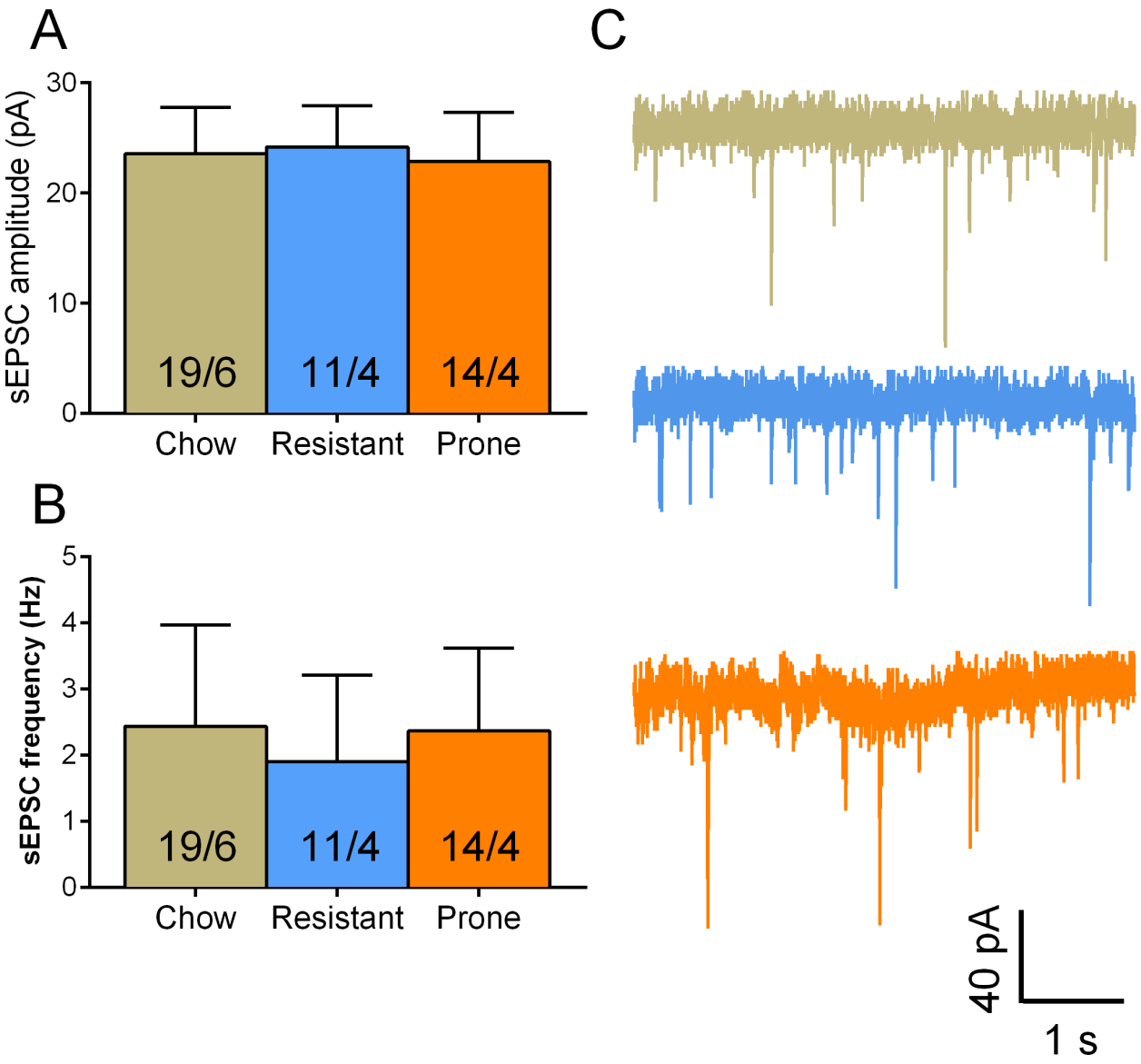

Figure S5. Spontaneous EPSCs in nucleus accumbens slices of chow, OP and OR rats. No difference was observed in either amplitude **(A)** or frequency **(B)**. **(C)** Representative traces, colors correspond to colors in (A) and (B). Rats belong to the same cohort.

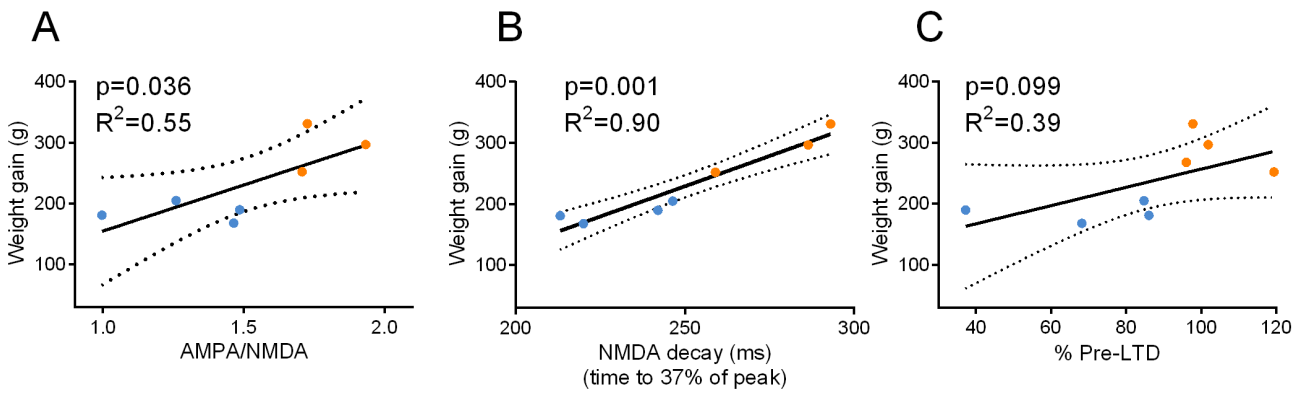

Figure S6. Weight gain is positively correlated with electrophysiological hallmarks of addiction. Weight gain was positively correlated with AMPA/NMDA ($F_{(1,5)} = 7.29$) **(A)** and NMDA current decay ($F_{(1,5)} = 45.64$) **(B)**. Weight gain also correlated with the degree of LTD that was produced ($F_{(1,6)} = 3.81$) but this correlation did not reach significance **(C)**. Blue – OR. Orange – OP.

Supplemental Methods

Operant Self-Administration Protocol

Operant training occurred in chambers (ENV-008, Med Associates, St Albans, VT) that were fitted with two retractable levers on either side of a central food receptacle where reinforcement was delivered by a pellet dispenser. For rats to learn the operant task they experienced an overnight training session (no cues) before being placed on the palatable diet whereby reinforcement consisted of a 45 mg standard chow pellet (F0165, Bioserv Inc, Frenchtown, NJ, USA). Rats were restricted to 20-25 g of food 24 h prior to this task, after which no further food restriction occurred. After the 8 week palatable diet period all rats were placed on standard chow *ad libitum* in their home cage and their access to palatable food restricted to 45 min daily during an operant session (rats experienced 3 d on standard chow before operant testing began). Rats were tested 6-7 days per week between 7:00 a.m. and 10:00 a.m. during their dark phase. The operant session, a modified version of those previously designed to identify 'addiction vulnerable' versus 'addiction resilient' subjects (1, 2) consisted of alternating 'reward available' (designated S+, 15 min x 3) and 'reward unavailable' (designated 'S-', 5 min x 3) periods that were paired with distinct discriminative stimuli. S+ periods were signaled by an illuminated house light and S- periods by the lack of house light illumination as well as a very brief (0.1 s) non-contingent tone/light presentation, which occurred every 5 s during the 5 min period. During S+ periods lever pressing on the active lever resulted in the dispensing of a 45 mg palatable food pellet (F06162, 45% kcal from fat, total density = 4.6 kcal g^{-1}; Bioserv Inc, Frenchtown, NJ) which was followed by illumination of the stimulus light above the active lever, the presentation of a tone (4500 Hz) and the switching off of the house light. The light/tone cue presentation was 5 s in duration which coincided with a timeout period whereby responding on the active lever would result in no programmed consequence. Responding on the active lever during S-, as well as responding on the inactive lever during either S+ or S- periods also

resulted in no programmed consequence. Rats received one ‘free’ 45 mg pellet at the initiation of each daily session.

Progressive Ratio. Successive pellets were earned at the completion of steps that required increasing ratios of lever presses, according to the logarithmic function $[5e^{(\text{step number} \times 0.2)}] - 5$ (3, 4). However, to reduce satiation on the food reward some of the earlier PR steps were omitted (steps 2, 3, 5, and 6), so that a modified step sequence was used which resulted in the following schedule: 1, 6, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603…. The PR breakpoint was taken as the last step completed prior to a lapse of 1 h during which no pellets were earned or the last step completed in 5 h, whichever occurred first. For the purposes of correlational analysis and computation of the ‘addiction score’ total lever pressing during the PR session was the parameter used as it was normally distributed and provided more detail of the differences between rats. The other two parameters used to compute the addiction score were also normally distributed.

Addiction Score. The ‘addiction score’ was calculated using an approach previously described (2): for each of the three behavioral criteria (lever presses during PR, pellets consumed, and lever pressing during S-) the average and standard deviation were calculated. Then, each dataset was standardized by subtracting the average from each data point and dividing by the standard deviation. The addiction score for each rat equals the sum of the standardized scores in the three behaviors.

Electrophysiology

Slice Preparation. Slices were prepared from OP and OR animals 24 h after their final self-administration session. Rats were anesthetized with ketamine HCl (1 mg/kg Ketaset, Fort Dodge Animal Health) and decapitated. The brain was removed and coronal accumbens brain slices (220 μm) (VT1200S Leica vibratome) were collected into a vial containing aCSF (in mM:

126 NaCl, 1.4 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 2.5 KCl, 2.0 NaPyruvate, 0.4 ascorbic acid, bubbled with 95% O_2 and 5% CO_2) and a mixture of 5 mM kynurenic acid and 50 µM D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5). Slices were stored at room temperature until recording.

***In vitro* Whole Cell Recording.** All recordings were collected at 32˚C (TC-344B, Warner Instrument Corporation) in the dorsomedial NAcore, where the prefrontal inputs are most dense (5). Medium spiny neurons were visualized with an Olympus BX51WI microscope. Inhibitory synaptic transmission was blocked with picrotoxin (50 µM). Multiclamp 700B (Axon Instruments, Union City, CA) was used to record excitatory postsynaptic currents (EPSCs) in whole cell configuration. Glass microelectrodes (1.3-2 MΩ) were filled with cesium-based internal solution (in mM: 124 cesium methanesulfonate, 10 HEPES potassium, 1 EGTA, 1 $MgCl_2$, 10 NaCl, 2.0 MgATP, and 0.3 NaGTP, 1 QX-314, pH 7.2-7.3, 275 mOsm). Recordings started no earlier than 10 min after the cell membrane was ruptured, to allow diffusion of the internal solution into the cell. Data were acquired at 10 kHz, and filtered at 2 kHz using AxoGraph X software (AxoGraph Scientific, Sydney). To evoke EPSCs, a bipolar stimulating electrode was placed ~100-200 µm dorsomedial of the recorded cell to maximize chances of stimulating PFC afferents. The stimulation intensity chosen evoked a 30-70% of maximal EPSC. Recordings were collected every 20 s (in the AMPA/NMDA experiments) or 10 sec (in the LTD experiments). Series resistance (Rs) measured with a -2 mV depolarizing step (10 ms) given with each stimulus and holding current were always monitored online. Recordings with unstable Rs, or when Rs exceeded 20 MΩ were aborted. AMPA/NMDA ratios and LTD measurements were never obtained from the same slice.

LTD Measurements*.* Baseline EPSCs were first measured for 10 minutes (0.1 Hz). After obtaining a stable baseline, we applied the LTD protocol described in (6). LTD was induced by applying three 5 Hz trains, each for 3 minutes, with 5 minutes inter-train interval. The trains were

paired with depolarization of the cell to -50 mV while during the inter-train intervals the membrane potential was brought back to -80 mV. After the last train membrane potential was returned to -80 mV and recording at 0.1 Hz was resumed for 30 minutes.

Measuring the AMPA/NMDA Ratio. Ten minutes after achieving whole-cell patch clamp configuration AMPA currents were first measured at -80 mV to ensure stability of response. Then the membrane potential was gradually increased until +40 mV. Recording of currents was resumed 5 min after reaching +40 mV to allow stabilization of cell parameters. Currents composed of both AMPA and NMDA components were then obtained. Then (2*R*)-amino-5-phosphonovaleric acid (D-AP5), an NMDA receptor antagonist, was bath-applied (50 μM) to block the NMDA currents and recording of AMPA currents at +40 mV was started after 2 min. NMDA currents were obtained by subtracting the average AMPA current from the average total current at +40 mV. To estimate the time-course of the decay of the NMDA current we measured the time in which the NMDA current decayed to 37% of its peak.

Supplemental References

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