Supplemental Information

Body Weight Analysis

In order to determine whether adolescent nicotine exposure in the parental F_0 generation caused changes in the body weight of their female offspring F_1 generation that were used in this experiment for behavioral testing, an independent-samples *t*-test was used to compare weights of the offspring F_1 generation of the F_0-Control group and the F_0-Nicotine group on P25, which were recorded prior to first injection. The *t*-test revealed that rats whose parents were exposed to nicotine in adolescence weighed significantly more than rats whose parents were not exposed to nicotine in adolescence (M = 57.93 g and M = 53.90 g, respectively) prior to any nicotine injections in the F_1 generation ($t(58) = -2.36$, $p = .022$). These results indicate that rats whose parents were given nicotine in adolescence weighed more than rats whose parents were not exposed.

To determine if this effect was related to parental F_0 generation weight, a 2 (F_0 parental gender) x 2 (F_0 nicotine exposure) ANOVA was conducted on the weights of the F_0-Control and F_0-Nicotine generation on P25. The ANOVA revealed a main effect of F_0 nicotine exposure that approached significance, $F(1,20) = 3.59$, $p = .073$, $\eta^2 = .137$. Thus, males and females assigned to the nicotine group may have weighed more than the males and females assigned to the control group (M = 62.58 g and M =55.75g, respectively), but the nicotine effect on body weight was not distinguishable between males and females ($p > 0.05$). Thus, there was no effect of gender on weight at this age ($p > 0.05$).

To determine if the weight difference found for the F_1-generation at P25 persisted for the duration of injections, through P59, a 2 (F_0 exposure) x 2 (F_1 exposure) x 35 (days) repeated measures ANOVA was performed on offspring F_1 generation weights from P25-P59. The

ANOVA found a significant main effects of F_1 exposure, $F(1, 56) = 20.60$, $p < .001$, $\eta^2 = 0.265$, and days, $F(34, 1904) = 835.88$, $p < .001$, $\eta^2 = 0.929$. Significant interactions included F_0 exposure x days, $F(34, 1904) = 1.53$, $p = .026$, $\eta^2 = 0.002$, and F_1 exposure x days, $F(34, 1904) = 7.31$, $p < .001$, $\eta^2 = 0.008$. As shown in Fig. 1S, rats that received twice-daily nicotine injections gained less weight than rats that received twice-daily saline injections. Planned comparisons based on the appropriate error term from the ANOVA were used to determine when group mean body weights differed significantly. The F_0-Control F_1-Control group weighed more than the F_0-Nicotine F_1-Nicotine group on days 13-15, and 17-35. The F_0-Nicotine F_1-Control weighed more than the F_0-Nicotine F_1-Nicotine group on days 8-35. The F_0-Control F_1-Control weighed more than the F_0-Control F_1-Nicotine group on days 13-15, and 17-35. The F_0-Nicotine F_1-Control group weighed more than the F_0-Control F_1-Nicotine on days 4-35. The F_0-Nicotine F_1-Control weighed less on days 16 and 19 and more on days 34 and 35 than the F_0-Control F_1-Control. All other comparisons were not significant ($p > 0.05$). The results indicate that during the 35 days of injection, offspring F_1 generation nicotine exposure had a larger effect on offspring weight than did parental F_0 generation nicotine exposure. Once nicotine treatment began, parental F_0 generation exposure to nicotine in adolescence had very little effect on offspring F_1 generation weight gain.

Additionally, rats were weighed prior to water deprivation on P90 and once more at P424. Five rats were euthanized due to illness sometime after training ended but prior to the last weighing. Two of these rats were in the F_0-Control F_1-Control group and 3 were in the F_0-Nicotine F_1-Control group. A 2 (F_0 exposure) x 2 (F_1 exposure) ANOVA was performed on offspring F_1 generation weight at P90 and an identical ANOVA was performed on offspring F_1 generation weight at P424. There was no significant difference between groups at either time

point (*p*s > .05). The effect of parental F_0 generation exposure to adolescent nicotine found at P25 was lost once the injection treatment began and did not return until at least P424.

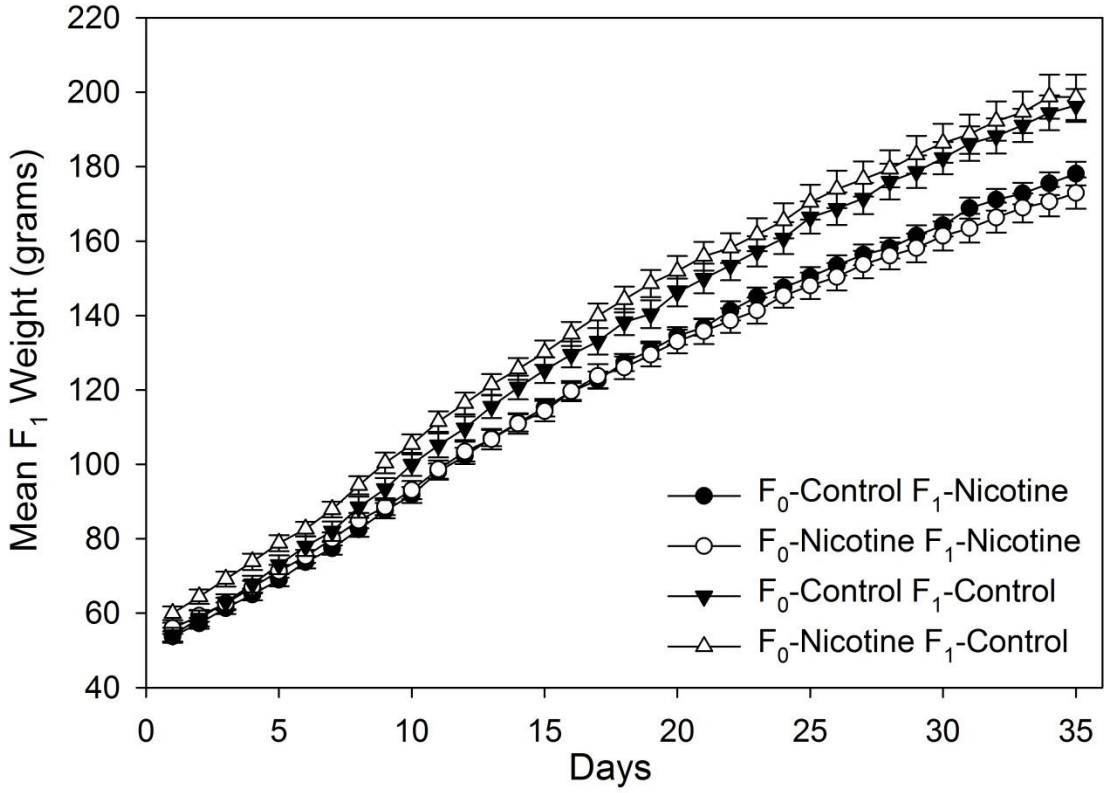

Fig. S1: Daily mean weights for all groups for all 35 days of injections. The F_0-Control F_1-Control group weighed more than the F_0-Nicotine F_1-Nicotine group on days 13-15, and 17-35. The F_0-Nicotine F_1-Control weighed more than the F_0-Nicotine F_1-Nicotine group on days 8-35. The F_0-Control F_1-Control weighed more than the F_0-Control F_1-Nicotine group on days 13-15, and 17-35. The F_0-Nicotine F_1-Control group weighed more than the F_0-Control F_1-Nicotine on days 4-35. The F_0-Nicotine F_1-Control weighed less on

days 16 and 19 and more on days 34 and 35 than the F_0-Control F_1-Control. Error bars: ± SEM.