Supplemental Information

Supplemental Results

Further Analysis of the Deprivation Group Manipulation

Tests for Deprivation Group differences for Wisconsin Smoking Withdrawal scale (WSWS) and CO level at the start of the experimental session confirm that 24-hours of nicotine deprivation significantly increased withdrawal symptoms (p's $\leq$ .014 and .041, respectively, for Experiments 1 & 2) and decreased CO level (p's $\leq$ .001) in each experiment (bottom section, Table 1). Deprived smokers in both experiments displayed large decreases in CO level from screening to experimental sessions measured in raw ppm units (for Experiment 1, mean decrease: 19.1 ppm, inter-quartile range: 9.5-27.5 ppm; for Experiment 2, mean decrease: 20.4 ppm, inter-quartile range: 11.5-26.0 ppm) and percent decrease (for Experiment 1, mean decrease: 75%, inter-quartile range: 57%-90%; for Experiment 2, mean decrease: 78%, inter-quartile range: 69%-88%).

Analysis of Shock Sensitization of the Startle Response

Both experiments were designed to minimize concerns that electric shock administration would produce sensitization of the startle response. Specifically, a minimum 15.4 s separated electric shock administration from subsequent measurement of the startle response to acoustic startle probes across trials. The mean duration between shock administration and startle response measurement was 33.7 s. Similar matching has been used by other laboratories that use shock and startle response. For example, Grillon and colleagues (1) report a 10 s minimum separation between shocks and startle response measurements. Furthermore, we matched the overall density/number of shocks (as well as number of cues, number of startle probes, and total experiment duration) across Experiments 1 and 2. Specifically, each experiment contained 12 administrations of electric shock, 24 acoustic "startle" probes, and 36 colored square cues. Despite this matching of shock density and number of shock administrations across experiments, a significant Deprivation Group effect was observed only for Experiment 1 that involved unpredictable shocks.

We also explicitly tested for differential sensitization to the electric shocks in our deprived and non-deprived smokers. Specifically, we recorded and analyzed startle response to the electric shocks (rather than the acoustic probes) in a general linear model (GLM) with Deprivation Group as a between subject factor and Shock number (1-12) as a within subject factor. This provided for a test of sensitization (or retarded habituation) of the startle response across the 12 electric shock administrations in Experiment 1. Overall, all smokers displayed significant (primarily linear) habitation of their startle response across the 12 electric shocks, $F(11,605) = 4.32$, $p < .001$. More importantly, there was no

evidence that the startle response to the shocks habituated differentially across deprived and non-deprived smokers, $F(11,605) = 0.44$, $p < .885$. Thus, it is unlikely that the significant Deprivation Group effect reported in Experiment 1 resulted from differential shock sensitization across deprived and non-deprived smokers.

Analysis of Objective Shock Levels

Calibration of shock level to participants' individual shock tolerance is an important design feature of experiments that administer electric shock as stressors to elicit negative affective response. There are large individual differences in subjective response to shock and this procedure is designed to minimize these individual differences such that all participants receive a shock stimulus that they judge to be comparably aversive. To accomplish this, participants report their subjective response to a series of increasing intensity electric shocks in a pre-experimental assessment of shock sensitivity. The series is terminated when they reach the maximum shock that they indicate they can tolerate (rated 100 on a 0-100 point rating scale). They are not informed that these ratings will affect the intensity of the shocks administered in the main experiment. This is a standard procedure that we have used in published research using shock for the past decade (2-7). It is also regularly employed in other labs that administer electric shock to elicit negative affect (1, 8-10). To increase confidence that this procedure did not bias our analysis of Deprivation Group effects in either experiment, we formally analyzed the objective shock levels administered to participants.

To start, the interpretation of Deprivation Group effects are not confounded by systematic differences in shock levels. If we aggregate participants across the two experiments, there is no significant difference in the shock levels administered to deprived vs. non-deprived smokers, $t(115) = 0.05$, $p \leq .964$. Furthermore, there are no significant Deprivation Group effects on shock levels administered in either Experiment 1, $t(55) = 0.05$, $p \leq .961$, or Experiment 2, $t(58) = 0.04$, $p \leq .967$.

Objective shock level is not related to individual differences in overall startle response or fear-potentiated startle in our experiments as would be expected if participants were administered varying shock levels to achieve the same subjectively aversive stimulus across participants. Specifically, there are no significant correlations between shock level and overall startle magnitude across cues due to unpredictable shocks administered in Experiment 1, $r = -0.09$, $p \leq .504$ or fear-potentiated startle due to predictable shocks administered in Experiment 2, $r = 0.08$, $p \leq .559$.

Finally, if we include shock levels as an additional covariate in the main analyses of startle response in both experiments, we reach the same conclusions. Most importantly, in Experiment 1, the overall significant effect of Deprivation Group remains significant (and close to identical to primary analysis) when shock levels are controlled, $F(1,55) = 8.30$, $\eta_p^2 = .13$, $p \leq .006$, with overall startle response

magnitude 28.4 μV higher in the 24-hour deprived smokers than the non-deprived smokers during unpredictable shock administration. Similarly, the non-significant Deprivation Group X Cue Type interaction in Experiment 2 remains non-significant ($p \leq .597$) with the addition of shock level as a covariate to that analysis.

Screening Session Startle Response Covariate Issues

Screening session resting startle response is included as a covariate in analyses of startle response during the main task in Experiments 1 and 2. It is included to increase the power to detect Deprivation Group effects by accounting for extraneous variance associated with expected large individual differences in startle response magnitude (11) in the main tasks (see 12-13 for technical treatments of the gain in efficiency from this appropriate use of covariates in ANCOVA). Miller and Chapman (2001) indicate that group effects are most clearly interpreted in ANCOVA when there is no systematic relationship between group and the covariate (14). If this criterion is met, the magnitude of the group effect is not substantively altered by including the covariate; only the precision of its estimation is increased (i.e., the standard error for the group effect is reduced). Given that screening startle response was measured prior to random assignment to Deprivation Group, these two variables should not be systematically related. However, to be rigorous, we confirmed that no significant effect of Deprivation Group was observed for screening startle response in either experiment (p's $\leq .645$ and .531, Experiments 1 & 2, respectively). Screening startle response was strongly related to overall startle response in both experiments (p's $< .001$). Therefore, including screening startle response as a covariate substantially improved the power of the GLM by reducing standard errors for all effects. For Deprivation Group, the standard error was reduced from 16.3 μV to 9.9 μV by including screening startle as a covariate.

A comparable significant effect of Deprivation Group during unpredictable shock is observed (startle response increased by 15 μV in deprived vs. non-deprives smokers; $p \leq .05$) if baseline startle response on the day of the experiment is substituted for screening session startle response as the covariate. However, screening startle response is preferred as a covariate because it was measured prior to random assignment (14). A Deprivation Group effect of 20 μV was observed in a GLM that include no covariate, but as noted above, the precision of its estimate was compromised due to extraneous individual difference variance in overall startle response. All effects of Deprivation Group were non-significant in Experiment 2 (predictable shocks) for GLMs with either screening or experiment day baseline startle as covariates and with no covariate.

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