Supplementary Material

Sex- and age-dependent differences in nicotine susceptibility evoked by developmental exposure to tobacco smoke and/or ethanol in mice

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Data Analysis

The Kolmogorov–Smirnov one sample test (K–S) was used to assess the normality of the distributions of each of the variables. To reduce the likelihood of type 1 statistical errors that might result from repeated testing, results on each variable were evaluated first by a global analysis of variance (ANOVA) or a repeated-measures analysis of variance (rANOVA). ANOVAs were performed for adolescent and adult body mass and locomotor activity in the OF. rANOVAs were performed for the analyses of dams' and pups' postnatal body mass, as well as %Time NPref data (within-subject factor: Day). Exposure (VEH, SMK, ETOH and SMK+ETOH), Challenge (SAL and NIC), Age and Sex were between-subject factors. Whenever Age or Sex interactions were identified, lower order ANOVAs on each age and sex were performed.

With this one-dimensional design (from now on described as 1-d), in which just one factor accounts for all groups of Exposure, lower-order ANOVAs were followed by Fisher's Protected Least Significant Difference (FPLSD) *post hoc* tests to investigate which groups were affected by the early drugs of abuse exposure (pairwise comparisons between SAL groups; e.g. $VEH_{SAL} \times SMK_{SAL}$). In addition, pairwise comparisons between NIC groups (e.g. $VEH_{NIC} \times SMK_{NIC}$) and between SAL and NIC groups (e.g. $SMK_{SAL} \times SMK_{NIC}$) were used to investigate the impact of nicotine re-exposure. Specifically, for the CPP, significant challenge effects or interactions were followed by lower-order ANOVAs on each challenge group (SAL and NIC) and by FPLSD pairwise comparisons. Paired *t* tests were further used to compare a given group before and after conditioning in the CPP.

In order to assess the possibility that tobacco smoke and ethanol interacted, resulting in effects that were either more-than-additive (synergistic) or less-than-additive, a two-dimensional ANOVA design was used (from now on described as 2-d) (Abreu-Villaça *et al.*, 2007, 2018, 2019; Ribeiro-Carvalho *et al.*, 2009; Slotkin *et al.*, 2019. In this design, Smoke (exposed: SMK and SMK+ETOH; non-exposed: VEH and ETOH) and Ethanol (exposed: ETOH and SMK+ETOH; non-exposed: VEH and SMK) are used as two independent between-factors in the analyses. More-than-additive and less-than-additive effects are indicated by significant *p*-values regarding Smoke × Ethanol interactions in the ANOVAs. The distinction between more- and less-than-additive effects is provided by the comparison of the effect observed for the SMK+ETOH group with the summation of the effects observed for the SMK and ETOH groups. In a two-dimensional ANOVA, an additive effect is indicated by a non-significant *p*-value associated with Smoke × Ethanol interaction.

Data are compiled as means and standard errors of the means. For the body mass analysis, data from males and females of the same litter were averaged separately within each exposure/challenge group to minimize litter effects and avoid over-sampling (Wainwright, 1998). For CPP and OF analyses, each litter contributed with no more than 1 male and 1 female to any of the groups. Effects were considered significant when $p < 0.05$ (two-tailed).

References

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