Epigenetics, behavior and early nicotine

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Early nicotine exposure during brain development may cause long-lasting neurobiological and behavioral alterations in adulthood. Jung *et al.* present insights into the mechanisms mediating these developmental changes.

Exposure to nicotine during prenatal and early postnatal periods of life may impair the normal development of several organs, including the brain, and facilitate the appearance of behavioral

and mental disorders. This is a major public health concern worldwide, since around 10% of pregnant women may smoke or use other forms of nicotine, exposing their future offspring during these critical neurodevelopmental periods¹. Previous preclinical and clinical studies suggest that this early exposure to nicotine may produce long-lasting alterations in neuronal plasticity in different brain areas that could modify neuronal functionality and be involved in the development of nicotine-associated mental disorders^{2,3}. However, little is known about the molecular mechanisms involved in these long-lasting neuroplastic changes induced by nicotine, the long-term consequences of these changes and their involvement in the development of associated psychiatric conditions.

The study published by Jung *et al.* proposes a plausible mechanism to explain long-lasting behavioral impairments produced by exposure to nicotine during early brain development. The authors show that early nicotine exposure modifies neuronal plasticity in several areas of the cortex, as revealed by changes in dendritic branching and spine density. These neuroplastic alterations are mediated by specific changes in histone methylation processes and are responsible of the abnormal behavioral response observed in these animals. Histone methylation can lead to long-lasting modifications in gene expression⁴, and these changes could therefore persist during a lifetime.

The authors exposed mice to nicotine during early development by placental and/or maternal lactational administration, adding the drug in the drinking water of the dam until postnatal day 21. Alterations in neuronal plasticity in different regions of the cortex were subsequently evaluated in the offspring at 3 months of age. This route of administration served to minimize maternal stress during this critical period of mouse development. However, it is likely to expose the animals to a higher nicotine concentration than occurs during fetal nicotine exposure in pregnant women, which should be taken into consideration when extrapolating these results to the human condition.

The results corroborated previous studies showing that early nicotine exposure produces long-lasting alterations in neuronal plasticity—namely, increased spine density and branching in the frontal, medial and caudal cerebral cortex. The authors then used microarray screening to identify the possible gene expression changes that could underlie these alterations. A long-lasting upregulation of two components of the histone methyltransferase complex, Ash2l and Mef2c, was observed in the cortex. Epigenetic changes associated with this alteration in histone methylation may lead to long-term modifications in gene expression and thus to persistent impairments of neuronal functioning. In agreement, the authors also observed increased histone H3K4 methylation in genes involved in synaptic plasticity and dendritic remodeling after nicotine exposure during this early period.

Is there a link between these Ash2l and Mef2c alterations in the cortex, the changes in neuronal plasticity induced by early nicotine exposure and possible associated behavioral changes? To answer this question, the authors used *in utero* electroporation to introduce different DNA plasmids that increased or decreased Ash2l or Mef2c expression into the cortex of embryos. A decrease in the cortical activity of both Ash2l and Mef2c abolished the changes in cortical neuronal plasticity produced by early nicotine exposure and rescued the behavioral effects of this nicotine exposure in aversive learning. In contrast, the overexpression of these genes mimicked the neurobiological changes promoted by nicotine.

The neurochemical changes reported in this study were observed both in mice exposed to nicotine during both prenatal and early postnatal periods and in those receiving nicotine only during the early postnatal period. This finding identifies the early postnatal weeks as a critical period in the mouse for these long-lasting alterations produced by nicotine. The first 2–3 weeks of postnatal cortical synaptic development in the mouse correspond to the third trimester of human pregnancy⁵, which leads to speculation that this pregnancy period in humans would also be critical for the impairment of cortical functioning produced by nicotine

exposure. Moreover, the enhanced aversive learning produced by early nicotine exposure reflects an emotional learning impairment, and the authors suggest that it may be related to an inability to screen out irrelevant sensory information. The authors also suggest a possible link between this behavioral alteration promoted by nicotine in mice and the increased incidence in attentional disorders in children exposed to maternal smoking.

The gene expression changes and subsequent cortical neuroplasticity reported in this study could also contribute to other behavioral alterations induced by early nicotine exposure. Indeed, dendritic plasticity in cortical areas has also been related to drug addiction⁶ and eating disorders⁷. Therefore, the cortical plasticity promoted by early nicotine exposure might also facilitate the development of these behavioral disorders. To further clarify this possibility, it would be productive to investigate whether similar histone methylation and gene expression alterations could occur in the mesolimbic brain reward system, which is also closely related to drug addiction and eating disorders. Changes induced in cortical areas by early nicotine exposure are not likely to be confined to these specific brain regions. This neuroplasticity occurred during a critical period in the development and maturation of the brain and is produced by the activation of heteropentameric nicotinic receptors that are abundant in many brain areas. Thus, other brain regions may be affected by early nicotine exposure and may participate in the development of different behavioral alterations.

Dendritic spines are highly dynamic structures that can alter their morphology as a result of experience-related learning, leading to long-lasting behavioral alterations⁸. It would be of interest to evaluate the possible changes in the density of specific dendritic spines having a different function as a consequence of this early nicotine exposure and how the reported modifications in gene expression and histone methylation could regulate those changes in the cortex and other relevant brain areas.

The fact that the behavioral impairments produced by early nicotine exposure are due to gene expression changes mediated by altered histone methylation suggests that these changes may endure across the lifespan. Although the changes were reported 3 months after mouse exposure to nicotine, it would be worthwhile to clarify the duration of these neurobiological and behavioral alterations across the lifespan of the mice. Similar mechanisms could also be involved in the alterations produced by fetal nicotine exposure in humans, which underlines the possibility of permanent behavioral impairments as a consequence of this nicotine exposure, providing an additional warning concerning tobacco smoking during pregnancy. The mechanism reported in this elegant study opens new perspectives on the consequences of nicotine exposure during early brain development and provides solid arguments for the presence of long-lasting or even permanent behavioral impairments due to this early nicotine exposure.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

References

Jung, Y. *et al. Nat. Neurosci.* **19**, XXX–XXX (2016). [Production: add pp]

1. Suter MA, Aagaard K. *Epigenomics* 4:115–118 (2012).
2. Muhammad A, Mychasiuk R, Nakahashi A, Hossain SR, Gibb R, Kolb B. *Synapse*. 66:950-954 (2012).
3. Blood-Siegfried J, Rende EK. *J Midwifery Womens Health*. 55:143-152 (2010).
4. Rumbaugh G, Miller CA. *Methods Molecular Biology*. 670: 263–274 (2011).
5. Krmpotić-Nemanić J, Kostović I, Kelović Z, Nemanić D, Mrzljak L. *Acta Anat (Basel)*. 116:69-73 (1983).
6. Robinson TE, Kolb B. *Neuropharmacology*. 47 Suppl 1:33-46 (2004).
7. Guegan T, Cutando L, Ayuso E, Santini E, Fisone G, Bosch F, Martinez A, Valjent E, Maldonado R, Martin M. *Eur Neuropsychopharmacol*. 23:146-159 (2013).
8. Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. *Trends Neurosci*. 33:267-176 (2010).

Figure 1. An epigenetic mechanism of the long-term neurobiological and behavioral effects produced by early nicotine exposure. Activation of heteropentameric acetylcholine receptors by nicotine during brain development produces specific modifications in histone methylation in the cerebral cortex and perhaps in other brain regions. These modifications lead to long-term increases in the expression of genes involved in synaptic remodeling and plasticity and the subsequent alteration in the mouse's behavioral responses.