to long-term modifications in gene expression and thus to persistent impairments of neuronal functioning. Accordingly, 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 DNA plasmids that increased or decreased Ash2l or Mef2c expression into the cortex of embryos. A decrease in the cortical activity of either Ash2l or 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, overexpression of these genes resulted in changes that mimicked the neurobiological changes promoted by nicotine.

The neurochemical changes reported in this study were observed 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 pregnancy[6], 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 of attentional disorders in children exposed to maternal smoking.

The gene expression changes and subsequent cortical neuroplasticity changes 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 addiction[7] and eating disorders[8]. 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 alteration 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 alterations[9]. It would be of interest to evaluate the possible changes in the density of specific dendritic spines reflecting mature or immature spines 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. Indeed, these subtypes of dendritic spines have different functions in learning and memory processes.

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 organism's 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 lifespans 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.

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PAM helps solve VTA's SHANKless problem

Michael F Priest & Yevgenia Kozorovitskiy

Developmental knockdown of *Shank3* affects excitatory synaptic transmission, activity of midbrain dopamine neurons, and behavior. Optogenetic dopamine release or enhancing metabotropic glutamate receptor signaling rescues these deficits.

Social rewards motivate our daily actions. However, the reward of social interactions is not a fundamental shared by all humanity; it only moves the neurotypical mind. The social motivation theory of autism posits that autism spectrum disorders (ASD) can be construed as severely diminished motivation for social rewards[1].

The ventral striatum and its dopaminergic inputs from the ventral tegmental area (VTA) are strongly implicated in motivating social behavior. Increasingly, studies support the idea that dopaminergic reward circuit function is perturbed in children with ASD[1,2]. However, our understanding of the mechanisms underlying autism remains limited. In this issue of *Nature Neuroscience*, Bariselli, Tzanoulinou *et al.* describe a molecular link between the VTA and autism[3], providing insight into

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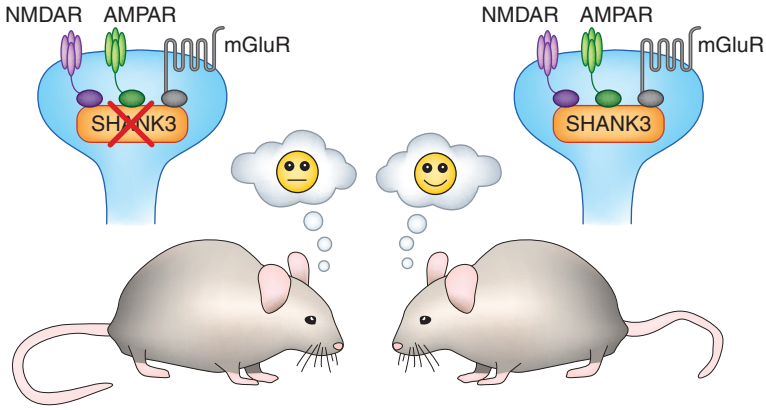

Figure 1 SHANK3, an important protein scaffold at excitatory synapses, is highly expressed during development and is linked to ASD. Now we have learned that even a spatially and temporally delimited insufficiency of *Shank3* in the VTA delays synaptic maturation of dopamine neurons and induces social deficits. NMDAR, NMDA receptor; AMPAR, AMPA receptor; mGluR, metabotropic glutamate receptor.

possible mechanisms underlying ASD and suggesting directions for new therapies.

This molecular link is SHANK3, a dendritic scaffolding protein of the postsynaptic density. This scaffold modulates synaptic activity, clustering ionotropic and metabotropic glutamate receptors at the postsynaptic membrane (**Fig. 1**). The *SHANK3* gene is deleted in a subset of patients with Phelan-McDermid syndrome, also known as 22q13 deletion syndrome. The resulting haploinsufficiency, which can also arise from *de novo* mutations of *SHANK3*, is a common known genetic cause of ASD[4].

Consistently, mouse models with full[5–9] and heterozygous[7] knockouts of *Shank3* exhibit abnormalities in glutamatergic transmission in the basal ganglia[5–9] and behaviors reminiscent of autism, including repetitive grooming and decreased social interactions[5–7,9]. Moreover, reestablishing SHANK3 expression in mice that lacked it during embryonic and perinatal development improves some striatal dysfunction and behavioral deficits, suggesting the potential for treatment in adults[6]. These earlier studies focused on the dorsal striatum, which is more strongly linked to repetitive behaviors than to social reward. Bariselli, Tzanoulinou *et al.*[3] have now taken on the challenge of investigating the link between SHANK3 and glutamatergic transmission development in the reward-associated VTA.

To execute spatiotemporally specific knockdown of *Shank3*, the authors packaged a small hairpin RNA (shRNA) against *Shank3* into an adeno-associated viral (AAV) vector, coupled with a fluorescent reporter. They transduced the VTA of postnatal day 6 pups, and 9 days later, found that the majority of VTA dopaminergic (DA) neurons and about a third of GABAergic neurons expressed the reporter. Western blot analyses confirmed a reduction in SHANK3 protein in the VTA of mice injected with shRNA against *Shank3* (shShank3 mice) as compared to those injected with scrambled sequence shRNA controls (scrShank3).

Would postnatal knockdown of SHANK3 have a lasting influence on excitatory synaptic function? To answer this question, the authors identified putative dopaminergic and GABAergic neurons in the VTA of 18- to 35-day-old mice using passive and active membrane properties. Evoking excitatory responses with electrical stimulation, they pharmacologically isolated currents mediated by AMPA-type (AMPAR) and NMDA-type glutamate receptors. Comparing shShank3 neuron recordings with those from uninfected and scrShank3 cells demonstrated that *Shank3* knockdown resulted in an elevated AMPA/NMDA ratio in both DA and GABA neurons. Given that the paired pulse ratios were unchanged, this observation points to a likely postsynaptic receptor difference.

To better understand the consequences of SHANK3 loss for VTA function and behavior, the authors carried out *in vivo* single-unit recordings. They found that the bursting rate of putative VTA DA neurons was lowered in shShank3 mice and elevated in neighboring GABAergic neurons. Both of these changes should decrease VTA dopamine release and consequently diminish social reward.

The authors hypothesized that behavioral deficits observed in *SHANK3*-associated ASD[4] and in *Shank3* knockout mice would be observed in their spatiotemporally restricted *Shank3* knockdown context. Indeed, in a three-chamber preference test, shShank3-AAV transduced mice quickly lost interest in another mouse and favored the empty enclosure. This loss of social interest was long-lasting, as it was observed in young and adult mice that had been injected perinatally with shShank3. These mice also spent more time grooming, without an overall change in open field locomotion. Thus, developmental loss of VTA SHANK3 recapitulates two critical behavioral elements of ASD: altered interactions with conspecifics and repetitive behaviors.

Bariselli, Tzanoulinou *et al.*[3] used *in vivo* optogenetics to gather more evidence linking SHANK3 loss, dopamine release and social preference attenuation. They delivered sh- or scrShank3-AAV while also expressing channelrhodopsin2 in VTA DA neurons of mice. When the test mouse approached a conspecific, the investigators delivered a stimulus sufficient to evoke a burst of action potentials in DA neurons. This biased all mice toward increased social preference and normalized the behavior of shShank3 mice.

The authors further demonstrate that synaptic maturation of VTA DA neurons is a key contributor to the behavioral effects produced by *Shank3* knockdown. In addition to elevated AMPA/NMDA ratios, AMPA receptor (AMPAR)-mediated currents of DA neurons in shShank3 mice passed more current inwards than outwards, taking into account the difference between the actual membrane potential and the reversal potential. Such rectification is a known property of AMPARs that lack the GluA2 subunit; these GluA2-lacking AMPARs are common in early development, but give way to GluA2-containing AMPARs during postnatal synapse maturation[10]. This observation prompted an important question: can *Shank3* insufficiency 'freeze' VTA DA neurons in a developmentally immature state? Or would blocking *Shank3* have the same consequence at the synapse regardless of developmental stage?

To distinguish between these possibilities, the authors knocked down *Shank3* after weaning age. Remarkably, excitatory transmission remained unaffected by this delayed manipulation. What critical elements guide developmental synaptic maturation and the concomitant increase in GluA2 content in the VTA DA neurons? On the basis of their previous work, the authors knew that positive modulation of type 1 metabotropic glutamate receptors (mGluR1s) could facilitate the insertion of GluA2-containing AMPARs[10]. But is that sufficient to compensate for the delayed maturation associated with loss of a critical scaffold protein during fast-paced neural circuit wiring?

Bariselli, Tzanoulinou *et al.*[3] answered this question in acute brain slices and within the native circuits *in vivo*. After injections of sh- or scrShank3-AAV, mice were treated systemically with Ro 677476, a positive allosteric modulator (PAM) of mGluR1s. At the doses

used, this drug increases mGluR1 activity in the presence of glutamate by binding to a distinct site. Ro 677476 returned the AMPA/NMDA current ratio to normal levels in the shShank3 group, without affecting scrShank3 mice. PAM treatment also abolished the increase in rectification induced by shShank3 injection, suggesting the rescue of abnormal synaptic maturation.

Besides rescuing the molecular phenotype, Ro 677476 compensated for SHANK3 insufficiency at cellular and behavioral levels. Treatment partially restored the normal bursting activity of VTA DA neurons and increased social preference in shShank3 mice. Underscoring the developmental role of SHANK3, treatment with PAM at the appropriate developmental time led to changes lasting into adulthood.

In summary, Bariselli, Tzanoulinou *et al.*[3] provide convincing evidence that SHANK3 is important for synapse maturation in the VTA during early postnatal development[3]. Furthermore, the deficits in DA neurotransmission induced by a reduction of SHANK3 appear to mimic ASD. These findings provide a compelling molecular and circuit mechanism for the losses of social preference observed in the human Phelan-McDermid syndrome.

In addition to the direct impact of VTA SHANK3 loss on excitatory transmission and dopamine release, such a loss also is likely to exert a powerful influence on the development of excitatory synapses in ventral striatum, since dopamine regulates glutamate-dependent striatal synaptogenesis[11]. Coupled with the precocious corticostriatal hyperconnectivity recently reported in *Shank3* knockout mice[8], the total load of synaptic and circuit perturbations in the basal ganglia due to loss of this scaffold protein is clearly substantial. Yet the hope for more effective treatment of ASD is growing, as several positive and negative allosteric modulators of metabotropic glutamate receptors have shown therapeutic promise in ASD[9,12,13]. Bariselli, Tzanoulinou *et al.*[3] have now provided the field with a fundamental explanation for how this could occur, spurring on the development of improved therapies.

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The dynamic nature of value-based decisions

Katherine E Conen & Camillo Padoa-Schioppa

During a binary choice task, neuronal activity in monkey orbitofrontal cortex alternated between two network states. The internal dynamics revealed by a linear decoder correlated with the reaction time and with the eventual choice.

Numerous studies conducted in recent years indicate that key aspects of economic decisions take place in the orbitofrontal cortex (OFC)[1–3]. However, the precise mechanisms through which subjective values are compared during the decision process remain unclear. One difficulty in assessing these mechanisms comes from the uniqueness of every decision. Even when subjects are repeatedly offered the same two options, choices vary. Furthermore, even if the same choice is ultimately made, the time course of the decision process presumably varies from trial to trial, reflecting small changes in subjective values and/or other sources of neuronal variability[4]. These various elements pose a challenge to decision neuroscience. In a study published in this issue of *Nature Neuroscience*, Rich and Wallis[5] begin to address this challenge. By recording simultaneously from small populations of neurons, they were able to decode aspects of the decision dynamics within each trial. Their study shows that value representations in the OFC alternate between network states associated with the two options available in the trial, potentially reflecting internal deliberation.

In the experiments, rhesus monkeys chose between different rewards, which came in two types and in four sizes. Each reward was represented by a particular image, and sessions included choice trials (two rewards available) and non-choice trials (one reward available). The authors recorded from the OFC, collecting data from an average of ten neurons simultaneously. The main results are based on a linear discriminant analysis. Using data from non-choice trials, the authors trained a linear classifier to identify the size of the reward on the basis of population activity. Then the same classifier was run on data collected during choice trials, where the reward size was labeled as chosen, unchosen, or unavailable depending on what rewards were offered to the animal and on the eventual choice. The classifier was trained on one time bin (the time of peak decodability) in non-choice trials and tested separately at different time points in choice trials. Thus, for any given choice trial, any time bin, and each reward size, the authors calculated a posterior probability that represented the likelihood with which the classifier identified that reward size as the one presented to the animal. Finally, on the basis of these posterior probabilities, the authors defined an internal state of the network.

Within each trial, the neural network alternated mainly between the two states corresponding to the available options, the one ultimately chosen and the unchosen one (**Fig. 1**). Furthermore, when the analysis of single cells was conditioned on the state of the network, individual neurons were also found to alternate between the two reward sizes. The alternation between the two states of the network correlated with behavioral measures. Specifically, states associated with the chosen option were slightly more frequent and lasted longer than those associated with the unchosen option.

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