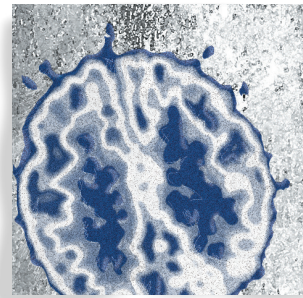


## *Serotonin-related pathways and developmental plasticity: relevance for psychiatric disorders*

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*Risk for adult psychiatric disorders is partially determined by early-life alterations occurring during neural circuit formation and maturation. In this perspective, recent data show that the serotonin system regulates key cellular processes involved in the construction of cortical circuits. Translational data for rodents indicate that early-life serotonin dysregulation leads to a wide range of behavioral alterations, ranging from stress-related phenotypes to social deficits. Studies in humans have revealed that serotonin-related genetic variants interact with early-life stress to regulate stress-induced cortisol responsiveness and activate the neural circuits involved in mood and anxiety disorders. Emerging data demonstrate that early-life adversity induces epigenetic modifications in serotonin-related genes. Finally, recent findings reveal that selective serotonin reuptake inhibitors can reinstate juvenile-like forms of neural plasticity, thus allowing the erasure of long-lasting fear memories. These approaches are providing new insights on the biological mechanisms and clinical application of antidepressants.*

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### Introduction

**E**arly-life alterations in neural circuit formation increasingly appear to underlie the risk for the emergence of psychiatric disorders later in life.<sup>1,2</sup> Rare genomic alterations have been associated with an increased risk for schizophrenia, and preferentially target a variety of neurodevelopmental pathways.<sup>3,5</sup> Recent data from whole-genome sequencing in the field of schizophrenia have identified a large diversity of genetic variants that may confer risk by acting during the early stages of embryonic brain development.<sup>6</sup> In addition to genetic risks conferred during embryogenesis, early-life stress has increasingly been shown to constitute an important risk factor involved in the emergence of psychiatric-related phenotypes.<sup>7-9</sup> Given the complexity of brain development and the compensatory adaptations that occur after early-life insults, it is still difficult to link specific cellular events involved in altered neural circuit formation with psychiatric-relevant phenotypes emerging later in life. Among the molecular pathways involved in determining early-life vulnerability to psychiatric phenotypes, the serotonin system is an important system to consider for several reasons. First, large amounts of pre-clinical data have revealed that early-life serotonin (5-HT) regulates a variety of developmental processes involved in neural circuit formation. We will specifically

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## Selected abbreviations and acronyms

<b>SSRI</b>	<i>selective serotonin reuptake inhibitor</i>
<b>5-HT</b>	<i>serotonin</i>
<b>SERT</b>	<i>serotonin transporter</i>
<b>MAOA</b>	<i>monoamine oxidase A</i>
<b>CR</b>	<i>Cajal-Retzius</i>
<b>HPA</b>	<i>hypothalamic-pituitary axis</i>

review the data regarding early-life serotonin control of key cellular processes for normal circuit formation in the mammalian neocortex. Second, studies conducted in rodents exposed to selective serotonin reuptake inhibitors (SSRIs) during brain development have revealed that early-life serotonin dysregulation induces a wide spectrum of psychiatric-relevant phenotypes. Human exposure to SSRIs in utero impacts fetal physiology, is associated with poor neonatal adaptation, and increases the risk for autism spectrum disorders. Third, genetic variants in serotonin-related genes interact with different early-life stressors to modulate functional circuit responses to emotional stimuli, stress axis responsiveness, and risk for stress-related psychopathology. These early-life interactions are among the best examples of gene-environment interactions in contemporary psychiatry. Finally, recent data in the field supports the novel hypothesis that serotonin plays an important role in regulating developmental plasticity during critical time periods of neural development, thereby opening up possibilities for new therapeutic applications for antidepressants.

## Source of serotonin during embryonic cortical development

Serotonin is detected at the onset of cortical development as early as embryonic day E10.5 in the mouse.<sup>10</sup> Initially, it was thought that serotonin originated from the maternal blood because during early development the fetal serotonergic raphe neurons have not been specified and are not functional yet. Therefore, it was suggested that at this stage the placenta could take up serotonin from the maternal blood and transfer it into the fetal bloodstream where it could reach the developing forebrain prior to the formation of the blood-brain barrier.<sup>11,12</sup> Recently, data has shown that the placenta plays a key role in producing the serotonin that accumulates in the embryonic forebrain during the early phases of telencephalic development.<sup>10</sup> Serotonin is synthesized

after tryptophan uptake in the placenta by using the tryptophan hydroxylase 1 and aromatic amino acid decarboxylase enzymes. As development continues, the serotonergic raphe neurons are specified through the action of specific transcriptional programs involving PET1, a serotonin raphe neuron transcription factor, and progressively acquire the molecular machinery to synthesize and uptake serotonin.<sup>13</sup> By day E15 or E16, serotonergic raphe neurons have extended their axons into the marginal zone and the subplate region of the developing cortex.<sup>14</sup> At this stage, the fetus becomes progressively autonomous in its ability to generate serotonin, and during the last phase of embryonic development, the raphe neurons, and not the placenta, are the main source of serotonin. In addition to the placenta and central raphe neurons, other sources of brain serotonin have been suggested such as gut enterochromaffin cells, mast cells, or platelets,<sup>15</sup> but these sources are likely to play only a minor role in the regulation of cortical development.

## Molecular targets of early-life serotonin

The presence of serotonin in the forebrain during the very early stages of development is associated with the dynamic developmental expression of different subtypes of serotonin receptors and transporters in a variety of neuronal cell types. At least 14 genes encode the different serotonin receptors, and further functional diversity is obtained through RNA editing and alternative splicing of various 5-HT receptor subtypes.<sup>16</sup> Although the developmental pattern of each serotonin receptor subtype is not available, mapping the developmental embryonic expression of the 5-HT1 receptor subtype in mice revealed that all members of the 5-HT1 subtype are strongly, but transiently, expressed in the developing thalamus.<sup>17</sup> Embryonic expression of serotonin-related genes can occur in neurons that cease to express these genes later in adulthood. For example, the serotonin transporter, SERT, is expressed during embryogenesis in thalamic and cortical neurons that do not normally express SERT in adulthood,<sup>18-20</sup> and the expression of SERT is observed as early as gestational week 8 in the developing human cortex.<sup>21</sup> Different subtypes of serotonin receptors are specifically expressed in distinct neuronal subtypes during cortical development. For example, the ionotropic serotonin receptor (5-HT3A) is expressed in Cajal-Retzius (CR) neurons and in a spe-

cific subtype of cortical interneurons derived from caudal ganglionic eminences that preferentially populate the upper layers of the cortex.<sup>22</sup> In humans, the developmental expression pattern of serotonin-related genes remains to be fully described. Mapping of the expression levels of human 5-HT receptors in postmortem prefrontal cortex across postnatal life and in neonates revealed distinct developmental expression patterns of the different subclasses of serotonin receptors.<sup>23</sup>

### Impact of early-life serotonin dysregulation on cortical circuit formation

An important focus of animal research in the field is to understand the impact of early-life serotonin on specific cellular events that are involved in the construction of neural circuits. In this section, we will review the key findings that have emerged over recent years that support the view that early-life serotonin regulates different cellular processes involved in cortical circuit formation. A seminal observation in the field was the discovery that excess serotonin disrupts the normal wiring of the rodent somatosensory cortex. In mice deficient for either *monoamine oxidase A (MAOA)* or *SERT*, it was shown that thalamocortical axons (TCAs) fail to segregate normally and do not form normal barrel-like structures.<sup>20,24</sup> This process was found to be under the control of the serotonin receptor 1B (5-HT1B) since segregation and barrel formation were normal in MAOA/5-HT1B receptor double knockout (KO) mice.<sup>25,26</sup> Abnormal TCA segregation was rescued in MAOA KO mice by specifically decreasing serotonin levels during the early postnatal days using a pharmacological approach.<sup>20</sup> At earlier developmental steps, when TCAs navigate to the cortex, serotonin was shown to regulate their responsiveness to the guidance cue, netrin-1, and this process required functional 5-HT1B and 5HT1C receptors.<sup>27</sup> Taken together, these data indicate that serotonin regulates thalamocortical pathfinding and wiring during the embryonic and early postnatal period.

The assembly of cortical circuits relies on the proper migration and laminar positioning of different subtypes of inhibitory  $\gamma$ -aminobutyric acid (GABA)ergic neurons and excitatory cortical neurons. Inhibitory GABAergic interneurons are generated in the ganglionic eminences of the ventral pallidum and migrate tangentially toward the developing cortex.<sup>28</sup> In contrast, excitatory projection

neurons are generated in the ventricular zone of the dorsal pallidum and migrate radially following an inside-outside pattern, where early pyramidal neurons first form in the deep cortical layers and the late-born neurons populate superficial layers.<sup>28</sup> Alterations in the migration and integration of GABAergic interneurons in cortical circuits have emerged as key processes involved in the susceptibility to psychiatric disorders.<sup>29,30</sup> In addition to genetic alterations, early-life stress affects the migration of cortical interneurons.<sup>31</sup> Recent work using time-lapse imaging of cortical slices has revealed that excess serotonin decreases the migration speed of cortical interneurons as well as the velocity of the pyramidal neuron in the superficial layer.<sup>32,33</sup> Furthermore, the distribution of both cortical interneurons and projection neurons was altered in the somatosensory cortex of neonatal SERT KO mice.<sup>32,33</sup> Alterations in neuronal migration due to a developmental excess of serotonin could contribute to the subtle changes in the thickness of cortical layers observed in different cortical regions of SERT KO mice.<sup>34</sup> In vitro studies combined with pharmacological approaches using time-lapse imaging revealed that serotonin receptor 6 (5-HT6R) is involved in regulating cortical neuronal migration.<sup>32,33</sup> Interestingly, proteomic approaches indicate that 5-HT6R forms a complex with a set of proteins involved in regulating developmental processes such as the mTOR pathway,<sup>35</sup> and 5-HT6R-mediated mTOR signaling is affected in the medial frontal cortex of mice exposed to postweaning social isolation, a developmental model that induces schizophrenia-like phenotypes.<sup>35</sup> The mTOR pathway has been shown to be an important signaling hub involved in autism spectrum disorders.<sup>36</sup>

Following their migration to specific cortical layers, pyramidal neurons progressively develop a dendritic arborization and receive synaptic inputs. Morphological investigation of pyramidal neurons in the ventromedial infralimbic prefrontal cortex of SERT KO mice has revealed conflicting results with either decreased<sup>37</sup> or increased<sup>38</sup> apical dendritic morphologies in SERT KO mice. More studies are clearly necessary to understand these dendritic structural changes, which have been shown to be very sensitive to stress.<sup>39</sup> Dendritic growth of cortical neurons has been shown to be regulated by serotonin fibers, creating synapses on CR cells.<sup>40</sup> Genetically deleting the 5-HT3A receptor increases apical dendritic arborization of upper layer pyramidal neurons in the somatosensory cortex, whereas pharmaco-

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logically blocking SERT during pregnancy decreases their dendritic complexity.<sup>40,41</sup> In CR neurons lacking 5-HT3A, serotonin is unable to stimulate the secretion of reelin, a glycoprotein that helps regulate neuronal migration and inhibits the growth of apical dendrites. Therefore, a reduction in reelin secretion has been proposed to lead to an abnormal hypercomplexity of apical dendrites.<sup>40</sup>

Alterations in the different cellular processes involved in cortical circuit formation have mainly been observed in either SERT KO or MAOA KO mouse models, and induce a relative excess of serotonin. Several mouse models of central serotonin depletion have been investigated. Surprisingly, no major alterations in cortical development were observed, although behavioral alterations such as increased aggression were reported,<sup>42-46</sup> ie, TCA segregation in the mouse barrel cortex was normal in serotonin-depleted mouse models.<sup>45</sup> Serotonin depletion after *tryptophan hydroxylase 2* (*TPH2*) deletion does not affect the specification of serotonin raphe neurons,<sup>46</sup> although abnormal growth of serotonin raphe neurons in specific brain regions such as the hippocampus and nucleus accumbens were reported.<sup>47</sup> Therefore, it is possible that subtle developmental abnormalities remain to be discovered in serotonin-depleted mouse models (ie, decreases in the density of GABAergic cortical interneuron populations have been observed in *TPH2* KO mice).<sup>48</sup> Finally, it should also be noted that during the early stages of embryonic cortical development a lack of central serotonin production by raphe neurons could be partially compensated for by the placenta.

## Impact of early-life serotonin dysregulation on psychiatric-relevant phenotypes

### Rodent studies

A large number of studies in rodents have investigated the behavioral consequences of blocking early-life SERT during specific developmental periods by administering SSRIs. Pharmacological blocking of SERT during the prenatal period<sup>41,49</sup> or the early postnatal period<sup>49-51</sup> has been shown to induce long-term anxiety-like and depressive-like phenotypes. Long-term stress-related behavioral effects of early-life antidepressant exposure were specific for SSRIs because antidepressants specifically blocking the norepinephrine transporter did not

induce similar anxiety-like behaviors.<sup>52</sup> SERT KO mice<sup>53</sup> and rats<sup>54</sup> exhibited similar types of stress-related behavioral phenotypes including increased hypothalamo-pituitary-adrenal (HPA) reactivity to stressors and impaired fear extinction.<sup>38,55,56</sup> Blocking the 5-HT1A receptor during the early postnatal period<sup>57</sup> reversed the depression-like phenotypes and sleep disturbances observed in SERT KO mice, suggesting an important role for this receptor in mediating the developmental effects of serotonin. In addition to these findings, conditional deletion of the 5-HT1A receptor during development but not during adulthood induces anxiety-like behaviors. The contribution of 5-HT1A presynaptic autoreceptors located on serotonin raphe neurons versus postsynaptic heteroreceptors remains to be fully established in these models.<sup>58</sup>

In addition to anxiety-like and depressive-like phenotypes, autism-related behavioral dimensions (eg, reduced social interactions, increased self-grooming, and impaired sensory-motor integration) have also been reported in genetic and pharmacological rodent models of early-life SERT blockade.<sup>54,59-61</sup> A large body of research has shown that early-life SERT deficiency leads to the emergence of a broad spectrum of psychiatrically relevant phenotypes that affect social, cognitive, and emotional domains.

### Human studies

Pregnancy is associated with an increased risk for mood and anxiety episodes. The fraction of pregnant women that present the diagnostic criteria for major depression ranges from 7% to 26%<sup>62,63</sup> and about 40% of patients with a history of major depression relapse during pregnancy.<sup>64</sup> Given the deleterious effects of maternal depression on fetal development, an increasing fraction of women (up to 13% of pregnant women in some studies) are treated with antidepressants during pregnancy.<sup>62,65</sup> Unfortunately, SSRIs cross the placenta<sup>49,66</sup> and have been shown to impact the developing fetus.<sup>63,67</sup> Ultrasonographic observations of fetuses throughout gestation revealed that exposure to SSRIs altered the emergence of quiet nonrapid eye movement sleep during the last trimester and decreased the inhibitory motor control during this sleep phase.<sup>68</sup> Furthermore, exposure to SSRIs reduced fetal middle cerebral artery blood flow as well as fetal heart rate variability.<sup>69</sup> Exposure to SSRIs during pregnancy is associated with lower APGAR

scores, with poor neonatal adjustment, increased risk for neonatal respiratory distress, jaundice, feeding problems,<sup>62,70-74</sup> delayed head growth,<sup>75</sup> pulmonary hypertension, and preterm birth.<sup>70,75,76</sup> Newborns exposed to SSRIs during late gestation more frequently display symptoms such as myoclonus, restlessness, tremor, hyperreflexia, shivering, and rigidity.<sup>73</sup> Neonatal symptoms were usually mild and disappeared within 2 weeks of age.<sup>77</sup> Adverse neonatal outcomes were generally attributed to a withdrawal or a toxicity effect from SSRI exposure. However, a recent study indicates that infants exposed to SSRIs during gestation, but for whom the drug was stopped 14 days before delivery, still displayed an increased risk for adverse neonatal outcomes, suggesting that exposure to SSRIs during late gestation resulted in more enduring effects.<sup>78</sup> At later developmental time points, gestational SSRI exposure was associated with blunted pain reactivity,<sup>79</sup> a slight delay in motor development,<sup>71,74</sup> and increased internalizing behaviors.<sup>80</sup> More worrisome findings come from two recent studies showing that antidepressant exposure may increase the risk for autism spectrum disorder<sup>81,82</sup>; however, it should be noted that in retrospective studies that examined the long-term effects of SSRI exposure it is often difficult to control for the severity of maternal depression and associated psychiatric comorbidities. Thus, some of the developmental consequences attributed to SSRI exposure could be due to the effects of increased maternal stress in the context of complex psychiatric psychopathology.

### Serotonin and stressor controllability

Stress acts across different developmental time periods and can have a profound impact on the functional maturation of different sets of neural circuits.<sup>9</sup> The physiological response to stress involves the coordinated activation of a network of brain regions that controls learning, memory, decision making, and emotional responses, and includes the hippocampus, amygdala, and prefrontal cortex.<sup>83</sup> The complex activation of these neural networks lead to autonomic and hormonal responses such as the activation of the HPA axis and cortisol secretion in the blood of humans and corticosterone secretion in rodents. Multiple mediators have been shown to be involved in the stress response including neuropeptides such as corticotropin-releasing hormone, vasopressin, dynorphin, steroids, and monoamines.<sup>83,84</sup> Activation of the serotonin dorsal raphe

(DR) is strongly implicated in stressor controllability. It has been shown that stress-induced activation of the DR is dependent on the medial prefrontal cortex (mPFC), which can detect whether a stressor is under the animal's control. When an organism is confronted by an uncontrollable stressor, the mPFC normally does not inhibit the stress-induced activation of the DR, thus leading to psychiatric-relevant behavioral sequelae.<sup>85</sup> In addition, the experience of actively controlling a stressor has been shown to increase the animal's resilience to subsequent uncontrollable stressors. Interestingly, stress-induced activation of the DR is a key event that impairs resilience to subsequent stressors.<sup>86</sup> The mechanisms underlying these effects are beginning to be elucidated. For example, a recent study has shown that the behavioral consequences of stress-induced activation of the DR is linked to a functional desensitization of the 5-HT1A autoreceptors, thus leading to a state of serotonin raphe hypersensitivity to subsequent stressors.<sup>87</sup>

### Interaction between early-life stress and serotonin-related pathways

#### Rodent studies

Multiple lines of evidence from rodents (and primates) support the view that early-life stress interacts with the serotonin system. Studies in rodents have shown that prenatal stress affects the development of serotonin raphe neurons as well as the long-term expression of serotonin receptors in different brain structures (eg, hippocampus and frontal cortex).<sup>88-91</sup> Exposure to glucocorticoids during the prenatal period modifies the developmental expression and function of the SERT and serotonin receptors in a dose-dependent manner.<sup>92,93</sup> During the early postnatal period, the impact of early-life stress on developing pups is highly dependent on maternal care, and normal maternal care is dependent on the serotonin system. Female PET1 KO mice are serotonin-deficient and present a phenotype characterized by a pattern of severe maternal neglect, leading to the death of their pups.<sup>94</sup> In addition to these genetic factors, maternal care is very sensitive to stress because mothers exposed to unpredictable stressors during the postnatal period displayed altered patterns of maternal care.<sup>95</sup> The offspring of these stressed mothers displayed long-term alterations in serotonin system reactivity as well as depressive-like and anxiety-like behaviors.<sup>95</sup> Mechanistic data suggest

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that low levels of maternal care leads to decreased serotonin signaling in the hippocampus of developing pups,<sup>96</sup> and has been linked to a decreased activation of the 5-HT7 receptor, which leads to long-term molecular adaptations (eg, increased methylation of the glucocorticoid receptor [GR], decreased expression of GR, and alterations in the regulation of the HPA axis).<sup>97</sup> Stress occurring later in life such as during childhood and the peripubertal period has also been shown to alter the function of the serotonin system. In these models, peripubertal stress was shown to decrease the activation of the serotonin raphe neurons and the expression of serotonin-related genes such as MAOA in the frontal cortex.<sup>98</sup> In addition to the effects of early-life stress on the expression of serotonin-related pathways; early-life gene-environment interactions have been investigated using heterozygous SERT KO mice. Using these models, it has been shown that prenatal stress or decreased maternal care leads to increased depressive-like and anxiety-like behaviors as well as social deficits in the offspring of stressed heterozygous SERT KO mice compared with the wild-type controls.<sup>99-101</sup> Finally, in rats, a single-nucleotide polymorphism in the SERT gene was found to interact with prenatal stressors to increase the HPA axis stress reactivity.<sup>102</sup>

## Primate studies

In humans and nonhuman primates, a large amount of research has investigated the interaction between early-life stress and a genetic variant in the promoter region of the SERT gene.<sup>103,104</sup> The common short (s) allele variant in the regulatory region of the SERT gene was shown to decrease the levels of SERT mRNA expression in vitro and to decrease serotonin reuptake.<sup>103,105</sup> Using functional imaging in healthy subjects, multiple studies have found that s allele carriers display increased amygdala responses to emotional stimuli.<sup>106</sup> The absence of a correlation between SERT genotypes and positron emission tomography (PET) binding in the amygdala of adults suggests that developmental mechanisms are likely to mediate the effects of SERT variants on brain function.<sup>107</sup> From this perspective, it is interesting to note that the impact of the s allele appears to be already detectable in children who display increased activation of limbic neural networks after viewing sad film excerpts.<sup>108</sup> Furthermore, SERT gene variants have been shown to modulate HPA responsiveness as early as birth. Newborn babies carrying the s allele carriers dis-

play increased stress-induced cortisol secretion when compared with long (l) allele carriers.<sup>109</sup> Modulation of stress-induced release of cortisol was observed in children carrying the s allele and exposed to a social stressor.<sup>110</sup> In line with these findings, recent meta-analysis also supported the modulatory role of SERT genotypes on HPA axis regulation.<sup>111</sup> A mechanistic link between SERT genotypes and early-life stress could possibly involve glucocorticoids. In vitro studies have shown that glucocorticoids regulate the expression of SERT and that the modulatory effects of glucocorticoid-induced SERT expression are decreased in s allele carriers.<sup>112</sup> At a behavioral level, interactions between SERT genotypes and early-life stress have been shown to occur during early development. For example, high levels of maternal anxiety during pregnancy interact with the s allele genotype to increase levels of negative emotionality in infants.<sup>113</sup> During the perinatal period, the quality of attachment between the mother and her baby plays a critical role in controlling the development of emotional regulation in the baby. In conditions of insecure attachment, toddlers carrying the s allele were found to develop poor self-regulation capacities, a measure indicative of the child's ability to deliberately control his or her affect and behavior.<sup>114</sup> In conditions of low maternal responsiveness infants carrying the s allele displayed decreased levels of attachment to their mothers.<sup>115</sup> Maternal care is also modulated by SERT genotypes. Mothers carrying the s allele display increased maternal sensitivity associated with higher scores on their perceived attachment to their baby,<sup>116</sup> supporting the view that the s allele genotype may be linked to increased sensitivity and vigilance to external stimuli and that during development s allele carriers could be more sensitive to the deleterious effects of early-life adversity. Indeed, a meta-analysis reported a significant interaction between childhood maltreatment and the s allele genotype, which can increase the risk for depression later in life,<sup>117</sup> although negative results have been reported.<sup>118</sup> Discrepancies in the field could be linked to the timing of the gene-environment interaction and the outcome measure. A recent study indicates that the s allele moderates the risk for persistent depressive episodes, but not for single episodes.<sup>119</sup> Finally, supportive evidence for interactions between the s allele genotype and early-life stress comes from studies on macaques using a maternal separation design. In these experimental models, monkeys were separated from their mothers at birth and

peer-reared for 6 months. Peer-reared macaques carrying the *s* allele exhibited more aggressive and fearful phenotypes as well as higher levels of HPA stress reactivity and alcohol consumption compared with *l* allele carriers.<sup>104,120,121</sup>

In addition to SERT, increasing numbers of genetic variants have been shown to interact with early-life stressors and modulate the risk for stress-related psychopathology, including the corticotrophin receptor 1, GR, and FKBP5, a co-chaperone of the GR.<sup>122</sup> Among serotonin-related genes, studies have shown that the low activity allele of the MAOA gene interacts with early-life stress to increase risk for aggressive and impulsive phenotypes.<sup>123,124</sup> Among serotonin receptors, a functional variant in the regulatory region of the 5-HT3A receptor gene, has been shown to interact with childhood adversity to increase the risk for depressive symptoms, increase emotion-induced heart rate, and modify electroencephalogram activation patterns and brain structures involved in emotional processing.<sup>125,126</sup> Interestingly, 5-HT3 receptor variants were initially associated with bipolar disorder, a finding that has been recently replicated in genome-wide association studies.<sup>127,128</sup>

### Epigenetic modulation of serotonin-related genes by early-life stress

Rodent and human studies indicate that early-life adversity helps program responsiveness to stressors by inducing long-term epigenetic modifications in several genes regulating the HPA axis such as the *NR3C1* gene coding for GR.<sup>122</sup> The best studied epigenetic marker, with regards to early-life adversity, is DNA methylation of cytosine-guanine dinucleotides. In rodents, prenatal stress<sup>129</sup> as well as low maternal care<sup>130,131</sup> has been shown to increase methylation in the *NR3C1* promoter region, thereby leading to decreased expression and function of GR in the hippocampus. In humans, increased *NR3C1* methylation in blood cells, cord cells, or in hippocampal postmortem tissue have been observed in individuals exposed to prenatal adversity<sup>132,133</sup> or high levels of childhood maltreatment.<sup>134-136</sup> In addition, increased *NR3C1* methylation was linked to increased stress-induced cortisol reactivity in humans<sup>132</sup> and rodents.<sup>130,131</sup>

To date, few studies have explored the impact of early-life stress on the methylation status of serotonin-related genes. In humans, methylation in the promoter region of SERT decreases its expression and this effect

is dependent on the genotype of the serotonin transporter gene-linked polymorphic region (5-HTTLPR).<sup>137</sup> The methylation status of SERT was increased in females compared with males<sup>138</sup> and was associated with increased scores for unresolved loss and trauma, a risk factor for psychopathology, in *s* allele carriers.<sup>139</sup> Furthermore, an association between increased SERT methylation and depressive scores was observed in individuals carrying the *s* allele.<sup>140</sup> In a monozygotic twin sample, bullying victimization during childhood was found to be associated with increased SERT methylation and a blunted cortisol response to stress.<sup>141</sup> Increased SERT methylation was also associated with childhood sexual abuse and to an increased risk for antisocial behavior in women.<sup>142</sup> Relevant to human studies, macaque models of early-life stress indicate that increased SERT methylation is associated with lower SERT expression in the peripheral blood and increased behavioral stress reactivity in infants subjected to early maternal separation<sup>143</sup> or in adults exposed during infancy to early-life stress.<sup>144</sup> Emerging data suggests that the methylation pattern of other serotonin-related genes could be associated with psychiatric disorders and related to expression levels in the brain. For example, peripheral white blood cell methylation of the MAOA promoter has been associated with changes in brain MAOA levels as measured by in vivo PET imaging.<sup>145</sup> The MAOA promoter methylation was decreased in females with depression or panic disorder compared with controls.<sup>146,147</sup> Finally, among serotonin receptors, increased 5-HT3A receptor methylation in the promoter region was associated with alcohol exposure in humans<sup>148</sup> and mice.<sup>149</sup> More studies are necessary to determine the impact of early-life stress on these novel serotonin-related epigenetic targets.

### Serotonin and the reinstatement of juvenile forms of plasticity

Early-life experiences permanently shape neural circuit wiring and function during critical time periods of development.<sup>150</sup> In mammals, monocular deprivation during the juvenile critical time period leads to permanent changes in the wiring of the visual cortex, which leads to amblyopia in the deprived eye. Chronic administration of the SSRI fluoxetine in adulthood has been shown to reinstate a form of critical time period plasticity in the visual cortex.<sup>151</sup> Reinstatement of this type of juvenile-

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like plasticity promoted the recovery of visual function in amblyopic animals that had been visually deprived during the juvenile period.<sup>151</sup> Positive effects of fluoxetine on the recovery of visual function were blocked by cortical administration of diazepam, indicating that increased cortical excitation is necessary to mediate the rejuvenating effects of fluoxetine. The mechanisms that underlie these effects were dependent on 5-HT<sub>1A</sub> receptor-dependent serotonin and brain-derived neurotrophic factor signaling and were involved in downstream epigenetic changes.<sup>152</sup> Environmental enrichment in adulthood has also been shown to reactivate juvenile-like plasticity in the visual cortex. Rejuvenating effects of environmental enrichment on visual plasticity were also dependent on the activation of serotonin signaling pathways.<sup>153</sup>

In other systems, a critical time period for fear memory erasure was described in juvenile mice.<sup>154</sup> During this critical time period, which occurs before postnatal day 16 in mice, extinction training followed by an initial phase of fear conditioning led to a permanent erasure of the fear memory.<sup>154</sup> The closure of this juvenile plasticity period depends on increased formation of perineuronal nets surrounding a specific subtype of parvalbumin-expressing interneurons in the basolateral amygdala. Following the closure of this critical time period, fear conditioning induces an enduring memory that cannot be erased through extinction training.<sup>154</sup> Recent data indicates that the combined administration of fluoxetine with extinction learning has the ability to reactivate critical period-like plasticity in the basolateral amygdala by decreasing the percentage of parvalbumin-expressing interneurons surrounded by perineuronal nets.<sup>155</sup> Reinstatement of this critical time period-like plasticity in the basolateral amygdala of adult animals led to an erasure of the fear memory similarly to what is observed in juvenile animals.<sup>155</sup> These data provide novel biological insights to clinical studies supporting the view that the combination of behavioral therapy with antidepressants has synergistic effects in promoting functional recovery in mood and anxiety disorders.<sup>156</sup>

Since SSRIs and serotonin appear to modulate critical time period-like plasticity in mice, it is possible that exposure to SSRIs during early stages of human devel-

opment could lead to modifications in developmental plasticity in humans. This hypothesis has recently been tested using a paradigm that probes sensitive periods in human language development. Infants with gestational exposure to SSRIs exhibited a more mature pattern of language discrimination than non-exposed infants,<sup>157</sup> suggesting that in utero SSRI exposure accelerates the closure of a critical time period in the speech perception system. Interestingly, maternal depression appeared to have the opposite effect by inducing a delay in the maturation of language discrimination.<sup>157</sup>

## Conclusions

Current translational research has revealed novel roles for the serotonin system in regulating the formation of cortical circuits and modulating plasticity during critical time periods of development. This has provided new insights on the impact of early-life serotonin programming in determining the risk for a wide range of behavioral phenotypes ranging from stress-related dimensions to alterations in social domains. Genetic studies in humans have revealed that serotonin-related gene variants interact with early-life stress and modulate activation of neural circuits involved in mood and anxiety disorders as well as HPA axis responsiveness to stressors. Vulnerability or resilience to the detrimental consequences of early-life stress is likely to depend on the complex interactions between early-life adversity and serotonin-related genetic variants. In addition, data demonstrates a novel level of transcriptional regulation suggesting that early-life stress modifies the methylation status of serotonin-related genes. Further work is needed to explore the impact of early-life stress on these novel epigenetic targets and its consequences on neural circuit activation patterns and psychiatric-relevant dimensions. Finally, the discovery that SSRIs can reinstate juvenile-like forms of neural plasticity, in conjunction with behavioral learning, is providing new insights on the biological mechanisms and clinical applications of antidepressants. □

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### Vías relacionadas con serotonina y plasticidad del desarrollo: su importancia para los trastornos psiquiátricos

El riesgo para los trastornos psiquiátricos del adulto está determinado en parte por alteraciones de los primeros años de vida que ocurren durante la formación y maduración de los circuitos neurales. Desde esta perspectiva, hay información reciente que muestra que el sistema serotoninérgico regula los procesos celulares que participan en la construcción de los circuitos corticales. Hay información traslacional de roedores que indica que la falta de regulación de serotonina en etapas precoces de la vida conduce a una amplia gama de alteraciones conductuales, que van desde fenotipos relacionados con el estrés a déficits sociales. Los estudios en humanos han revelado que las variantes genéticas relacionadas con la serotonina interactúan con el estrés de los primeros años para regular la respuesta de cortisol inducida por el estrés y activar los circuitos neurales involucrados en los trastornos del ánimo y de ansiedad. Han aparecido datos que demuestran que la adversidad en los primeros años de vida induce modificaciones epigenéticas en los genes relacionados con la serotonina. Finalmente, hay hallazgos recientes que revelan que los inhibidores selectivos de la recaptura de serotonina pueden restituir formas de tipo juvenil de plasticidad neural, lo que permite la supresión de memorias de temor de larga duración. Estas aproximaciones están aportando nuevas perspectivas acerca de los mecanismos biológicos y la aplicación clínica de los antidepresivos.

### Les voies de la sérotonine et la plasticité du développement : pertinence pour les troubles psychiatriques

Le risque de développer des maladies psychiatriques à l'âge adulte est en partie déterminé par des altérations précoces touchant la formation et la maturation des circuits neuronaux. Dans cette perspective, des données récentes indiquent que le système sérotoninergique régule des événements cellulaires impliqués dans la construction des circuits corticaux. Des recherches translationnelles chez l'animal révèlent qu'une dysrégulation précoce du système sérotoninergique induit un large éventail d'altérations comportementales allant de phénotypes anxieux-dépressifs liés au stress à des déficits sociaux. Chez l'homme, des études ont permis de mettre en évidence que des variants génétiques du système sérotoninergique interagissent avec l'adversité précoce et régulent la sécrétion du cortisol induite par le stress et l'activation de circuits cérébraux impliqués dans les troubles de l'humeur. De plus, des données récentes révèlent que l'adversité précoce induit des modifications épigénétiques dans des gènes du système sérotoninergique. Finalement, il a pu être récemment démontré que des inhibiteurs sélectifs de la recapture de la sérotonine peuvent réinstaurer des formes de neuroplasticité juvénile permettant d'effacer des souvenirs liés à la peur. Ces approches donnent un éclairage novateur sur les effets biologiques des antidépresseurs et leur application clinique.

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