Published in final edited form as:
Biochem Pharmacol. 2007 April 15; 73(8): 1225–1236.

Comparison of the Maturation of the Adrenergic and Serotonergic Neurotransmitter Systems in the Brain: Implications for Differential Drug Effects on Juveniles and Adults

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Abstract

Our understanding of the development of neurotransmitter systems in the central nervous system has increased greatly over the past three decades and it has become apparent that drug effects on the developing nervous system may differ considerably from effects on the mature nervous system. Recently it has become clear there are significant differences in the effectiveness of antidepressant drug classes in children and adolescents compared to adults. Whereas the selective serotonin reuptake inhibitors are effective in treating all ages from children to adults, the tricyclic antidepressants, many of which inhibit norepinephrine reuptake, have been shown to be ineffective in treating children and adolescents even though they are effective in adults. We review here the development of the noradrenergic and serotonergic nervous systems, both in terms of neurotransmitter system markers and function. Both of these neurotransmitter systems are primary targets of antidepressant medications as well as of central nervous system stimulants. It is clear from a comparison of their development that the serotonin system reaches maturity much earlier than the norepinephrine system. We suggest this may help explain the differences in response to antidepressants in children and adolescents compared to adults. In addition, these differences suggest that drugs acting preferentially on either neurotransmitter system may impact the normal course of CNS development at different time points. Consideration of such differences in the development of neurotransmitter systems may be of significance in optimizing treatments for a variety of centrally mediated disorders.

Keywords

norepinephrine; serotonin; development; adolescent; depression; brain

Introduction

Understanding the development of central nervous system neurotransmitter systems is important for several reasons. This information will help in determining not only the sequence in which these systems develop, but also how neurotransmitters regulate other developmental processes, as well as how their development may be influenced or altered by exposure to xenobiotics or other abnormal conditions present at critical periods during development. This

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knowledge is also important to assess why drugs have differential clinical effects at different stages of development. For example, the tricyclic antidepressants are ineffective in children and adolescents, but are effective in adults, whereas the serotonin-selective reuptake inhibitor (SSRI) antidepressants are effective at all ages [1–3]. This has significant implications for the treatment of depression, a major psychiatric illness, during childhood and adolescence. Whereas age-dependent differences in the response to drugs is not a new concept [4], the realization that this applies to antidepressant drugs is relatively new. Depression is manifest in 4% or more of the population during childhood and adolescence each year. However, our treatment of this disorder has been inadequate, to a great extent because it was only recently demonstrated clearly that there are major differences in response to antidepressant drug classes when comparing children and adolescents to adults.

We here review the development of the norepinephrine and serotonin systems. These two neurotransmitter systems are of particular interest because both have been postulated to be important in regulating central nervous system development and because both are implicated in the pathophysiology of depression. Because children and adolescents respond to antidepressant medications acting on these two systems in a different pattern from adults, it seems likely that at least part of the explanation for this discrepancy may lie in differences in how these systems develop. Understanding these differences may lead the way to developing or determining optimum antidepressant therapies for each age group.

Differences in maturation of these two systems may also impact not only the clinical efficacy of drugs that act on them differentially, but also their long term consequences and safety. It has been postulated that during development the brain becomes wired to match the needs of or to compensate for its environment [4]. Chronic drug exposure represents an important environmental factor in this situation. As a result, exposure to psychoactive drugs, particularly during vulnerable periods of development, could produce changes in final development that would be reflected in subtle, or possibly obvious, differences in mature response to the environment.

Another important aspect of the noradrenergic and serotonergic systems is their possible involvement in the development of sensitization to the effects of drugs, including many antidepressants and psychomotor stimulants such as the amphetamines and cocaine [5–7]. Both antidepressants and stimulants produce a similar effect, an increase in monoamine concentrations in the synapse. While dopamine has been the focus of most studies of sensitization of the monoamines, there is increasing evidence that norepinephrine and serotonin are involved. In addition, there are differences in the type and duration of sensitization produced by drugs when comparing juvenile and adult animals [8], supporting the idea that the monamine systems are to some degree functionally different in juveniles compared to adults. This also suggests that the differences in sensitization could be contributors to the differences in response to antidepressants and to long term effects of many drugs.

Most of the information available concerning development of neurotransmitter systems is derived from laboratory animal studies, particularly rats. Data from humans are limited. Given this, it important to keep in mind that brain development relative to time of birth differs greatly between species [9]. For example, at birth brain development in rats is equivalent in relative weight to development in humans at the end of the second trimester. Rats become sexually mature at about 5 weeks of age, corresponding roughly to puberty or early adolescence in humans. In contrast, brain development in rhesus monkeys at birth is similar to that found in humans at 10–15 years of age [9]. As a result, it is necessary to be cautious in making time comparisons across species. Nevertheless, the basic developmental processes, and the impact of alterations in normal development, appear to be very similar across mammalian species.

In this review, we present first the development of the biochemical aspects of the norepinephrine and serotonin neuronal systems. This provides the foundation for analysis and understanding of the functional development of these systems. The general pattern that emerges from consideration of studies in this area is that the norepinephrine system develops more slowly than the serotonergic system. This may provide at least a partial explanation for why the classical tricyclic antidepressants, many of which act selectively on noradrenergic neurons, are not efficacious in children and adolescents. Since this system is not as fully developed, the drugs that act on it do not produce the same effects as found in adults. In contrast, SSRIs act on a more rapidly maturing system and would be expected to produce clinical antidepressant effects similar to adults at earlier ages.

Biochemical and Pharmacological Development of Neurotransmitter Systems

Norepinephrine

Cell bodies expressing tyrosine hydroxylase, the rate-limiting enzyme for norepinephrine synthesis, can be detected by 4 weeks of gestation in humans and norepinephrine itself is detectable by 5–6 weeks [10;11]. By 8 weeks of gestation, noradrenergic axons have penetrated the cortical anlage [11]. A similar pattern is seen in rhesus monkeys, with tyrosine hydroxylase-containing cell bodies appearing in the locus coeruleus by weeks 4–5 of gestation and axons appearing in the cortical anlage by week 10 [11]. In prefrontal cortex of rhesus monkey, tyrosine hydroxylase-positive axons mature quite slowly. At 6 months of age they are only half the adult density and it takes two years to finally reach full maturity [12]. This contrasts with serotonergic innervation, which develops much more rapidly.

Norepinephrine levels in humans increase throughout the first trimester, especially from about two months of gestation forward. Following the initial increase in spinal norepinephrine, a decrease of 30–40% in concentration occurs between 6 months of gestation and early childhood [13]. Similar to nerve terminals, development of norepinephrine content is slower than that for serotonin or dopamine, based on studies in rhesus monkeys. It takes at least two to three years for norepinephrine content to reach adult levels in most regions of the monkey cortex [14; 15].

Noradrenergic neurons differentiate in rats between gestational day (GD) 10 and 13 [16;17]. At the end of noradrenergic cellular division, a time when the full complement of cells appears to be present, these neurons express the synthetic enzymes for norepinephrine and norepinephrine itself. From this point forward there is a steady differentiation and a nearly linear development of markers for noradrenergic neurons in CNS, increasing approximately 100- to 1000-fold by adulthood [18]. Axonal projections reach most regions of the fetal brain by a week before birth and start to form terminal varicosities. Well before one week of age the number of monoamine cell bodies has reached adult levels, but the corresponding nerve terminals continue to proliferate for several more weeks [19].

The most detailed analysis of noradrenergic neuron terminal development in rats has focused on the cortex. Noradrenergic projections to cortex appear between GD 16 and 18 [16;20]. The timing of noradrenergic cortical innervation coincides with many important events relevant to cortical development. Cortical neurogenesis, for example, begins in the deep layers at GD13, with more superficial layers exhibiting neurogenesis until GD20 [21]. The migration of cortical neurons begins about GD13 and continues through early postnatal development. Further maturation of cortical neurons, i.e., sprouting of cellular processes and formation of synaptic contacts with neighboring neurons, occurs largely within the first three weeks of postnatal

development, a time period in which noradrenergic innervation is increasing to adult levels [21;22].

In parallel with primates, development of norepinephrine concentrations to adult levels in rat brain is relatively slow compared to serotonin. While norepinephrine levels increase steadily from birth onward, full adult concentrations are not reached until 30–40 days postnatally [23–25]. In lower brainstem and cerebellum norepinephrine reaches adult levels somewhat earlier, at 2–3 weeks postnatally, but these regions are the exception to the general rule [25].

Serotonin

Serotonin is one of the first neurotransmitter systems to develop in the mammalian brain [26; 27]. Serotonin content, serotonin uptake sites, and serotonin receptor binding measured in animal studies are all generally higher in the developing brain as compared with adult values, and then decline before puberty.

In humans, serotonin immunoreactive neuroblasts (5-HT-IR) are evident at 5 weeks of gestation [10;28]. In the pons a bilaterally symmetrical very dense group of 5-HT-IR neuroblasts is located close to the midline. At 11 weeks gestational age, these 5-HT-IR neurons form a dense group covering two-thirds of the dorsal to the ventral surface. A similar developmental pattern is seen in the rostral medulla oblongata [10].

In humans serotonin is detected by 5 weeks of gestation [10]. In the pons, there is a steady increase up to 8 weeks followed by a steep rise between 8 and 12 weeks. The levels of serotonin are significantly higher than those of norepinephrine. The amount of serotonin in medulla oblongata, on the other hand, is lower [10].

The serotonin inputs to pyramidal and nonpyramidal cells in prefrontal cortex have been quantified in rhesus monkeys ranging in age from 2 weeks to 10 years [12]. The density of serotonin appositions on these cells reaches the adult level before the second week after birth and remains constant over the age range studied. By contrast, the density of catecholamine appositions matures slowly, reaching only half the adult level by 6 months of age and thereafter rising gradually to adult levels by 2 years of age [12].

The development of serotonin content has been studied in some detail in various regions of the cerebral cortex of rhesus monkey [15]. In the prefrontal, visual, somatosensory, posterior parietal and motor cortices, serotonin content appears to reach adult values by 2 months of age and to show little change after this point. By comparison to norepinephrine and dopamine, serotonin content shows the least dramatic and most rapid development [15]. In the cat occipital cortex, the levels at birth are approximately 25% of adult levels and peak at levels higher than the adult at 3–6 weeks [29]. By contrast, norepinephrine levels are at less than 10% of adult levels at birth and increase slowly, only reaching about 50% of adult levels by 11 weeks after birth.

In the rat, early neuroembryonic studies compared the appearance of fluorescent cells containing serotonin, dopamine and norepinephrine. Neurons containing serotonin first were observed in the 8 mm embryo, dopamine at 9 mm, and norepinephrine at 11 mm [30]. In more detailed studies, serotonin positive neurons are first evident as early as E12–E13 and by El9 they are distributed in groups that are similar to those seen in the adult [31;32]. Serotonin fiber projections can be detected by E14 and they reach the frontal neocortical pole by E17 [32]. Up to the end of the first postnatal week there is rapid growth of serotonin dendrites into the form seen in the adult rat, and the adult pattern is well established by the 3rd postnatal week [24; 33]. By contrast, for norepinephrine neurons, the adult pattern is not obtained until the 4th to 5th postnatal week [24]. By 15 days of age, synaptogenesis is at approximately 75% of adult

levels in the raphe nucleus (serotonin) where as it is only at 55% of adult levels in the locus coeruleus (norepinephrine) [34]. In the cerebral cortex of the rat, the serotonergic innervation pattern characteristic of the adult cortex is attained by the end of the 3rd postnatal week [35]. In the visual cortex the proportion of serotonin-containing axonal varicosities forming synapses increased gradually from birth to reach a peak at the end of the 2nd week, coinciding with the period of maturation of the neuronal circuitry of this cortical area. In the basal forebrain the percentage of serotonin varicosities engaged in synaptic junctions increased from birth to adult levels by the end of the 2nd postnatal week, then declined in the following week before increasing again [36]. By contrast, the motor cortex showed a continuous increase in the proportion of serotonin varicosities engaged in synaptic contacts from birth to 5 weeks of age [35]. Serotonin levels in rat CNS are low at birth and generally peak around PND 21–30 and then decline somewhat to adult levels [19;37].

Development of Neurotransmitter Synthetic Enzymes

Norepinephrine

As would be anticipated, the appearance of the synthetic enzymes for norepinephrine during development parallels the appearance of the neurotransmitter itself. None of the enzymes, tyrosine hydroxylase (TH), aromatic amino acid decarboxylase (AADC) or dopamine β-hydroxylase (DBH), are specific to just the norepinephrine synthetic pathway, although DBH comes close since there is very little epinephrine in the brain. Several studies have found that immunohistochemical analysis of TH identifies primarily dopaminergic neurons and that DBH is the more reliable for noradrenergic neurons.

The early gestational development of TH-expressing cells in humans prior to establishment of the noradrenergic neuronal system has been described in some detail [11], although additional information is still needed, especially for the late gestation period and later [38]. Studies in humans and rhesus monkeys indicate that catecholamine neuron cell bodies, including noradrenergic cell bodies, appear very early in CNS development [11;39]. However, following this initial period, the complete development of the noradrenergic system is quite protracted, requiring at least two years to reach adult levels in rhesus cortex [14;39]. In contrast, the serotonin system develops more rapidly in these same primates, reaching stable serotonin concentrations in some cortical regions, such as parietal and visual cortex, by eight months of age [14]. Presumably humans follow a similar, but more extended, pattern.

TH can be detected in fetal rat brain at 15 days of gestation, right after the norepinephrine neurons have finished dividing [40]. There is a burst of development between days 15 and 17 of gestation and a shift in distribution of TH from regions containing cell bodies to regions that contain only terminals. There are also regional differences in TH appearance. In striatum, where TH is primarily associated with DA terminals, the enzyme increases dramatically in the second postnatal week. In cortex, where TH is primarily associated with norepinephrine neurons, the greatest increase occurs after one month of age. This parallels the appearance of norepinephrine in developing brain [41]. The two enzymes following the rate-limiting TH in norepinephrine synthesis are AADC and DBH. The developmental pattern of both follows closely the patterns for TH and norepinephrine [42;43].

Serotonin

Studies on the development of the serotonin synthetic system are limited. The serotonin synthesis capacity in humans (as determined using α[^{11}C]methyl-tryptophan and positron emission tomography) is highest between ages 2 and 5, being approximately twice that of the adult, and then declines to adult levels by about 11 years of age. [44]. In the rat, tryptophan hydroxylase (the rate limiting step in the synthesis of serotonin) is found in early developing

neurons [26], and reaches peak levels of activity well before tyrosine hydroxylase [45]. Other studies demonstrating serotonin-containing cells by E12–E13, although not directly examining the enzymes, imply that both tryptophan hydroxylase and AADC are present [30;32]. These findings are consistent with the idea that the serotonin system develops early and plays an important role in regulation of development [46;47].

Development of Neurotransmitter Receptors

Adrenergic Receptors

The adrenergic receptor family consists of three types, alpha-1, alpha-2 and beta. These receptor families have distinct pharmacological characteristics, molecular structures and signal transduction pathways. Each of the three main types of adrenergic receptors in turn have three subtypes (alpha-1A, -1B, -1D; alpha-2A, -2B, -2C; beta-1, -2, -3) [48;49]. All are G protein-coupled receptors, with alpha-1 receptors coupled to G_q, alpha-2 receptors coupled to $G_{i/o}$ and beta receptors coupled to G_s.

The developmental profiles of the three major groups of adrenergic receptors have been studied to varying extents, primarily in rodents. Considerable attention has been paid to developmental issues concerning beta adrenergic receptors, both in CNS and in peripheral tissues, especially heart. Alpha-2 adrenergic receptors have also been examined extensively during development, particularly in the CNS, while alpha-1 adrenergic receptors have received less attention, even though they are densely expressed in CNS. Due to difficulties in obtaining tissue and maintaining it in suitable condition for receptors studies, few, if any, studies have been carried out in humans. Development of more sophisticated and more sensitive *in vivo* imaging techniques may alleviate this discrepancy in the future.

Alpha-1 adrenergic receptors—In early studies, alpha-1 adrenergic receptors were found at very low levels in rat brain at time of birth. They subsequently increase to greater than adult levels by PND20, with a particularly rapid increase between PND 15 and PND 20 [23]. Alpha-1 receptors then decrease to adult levels in the following weeks. More detailed analyses indicate there are regional differences in developmental patterns, similar to the findings with many other receptors. In some brain regions, such as globus pallidus, there are significant levels of alpha-1 adrenergic receptors at birth, peaking in density in the first two weeks postnatally and subsequently decreasing to much lower levels in adults [50]. Most regions, however, follow the pattern for whole brain, increasing to adult levels or greater than adult levels in the first three weeks postnatally and then stabilizing at adult levels over the next month [51]. A similar pattern has been found in kitten visual cortex, although the increase to maximum density and the subsequent decrease to adult levels are much more dramatic [52], indicating significant species differences in alpha-1 adrenergic receptor development. A detailed analysis of alpha-1 adrenergic receptor development in CNS of any species has yet to be published.

Alpha-2 adrenergic receptors—Alpha-2 adrenergic receptors have three subtypes, alpha-2A, alpha-2B and alpha-2C, and are widely distributed throughout the central nervous system [49;53–55]. Each of these subtypes exhibit a distinct transcriptional profile in developing brain.

As early as E19 alpha-2A mRNA is expressed at high levels in the rat cortex and olfactory system, equivalent to what is seen in the adult brain. In areas such as the basal ganglia, amygdala, thalamus, spinal cord and brainstem of the rat, alpha-2A mRNA is expressed at high levels in early development and then decreases with subsequent maturation of the nervous system [56].

In contrast to alpha-2A, alpha-2B mRNA is not present in most rat brain structures in early development, but slowly increases with CNS maturation. Alpha-2B is differentially regulated in sensory and non-sensory thalamic nuclei with sensory nuclei exhibiting expression as early as PND 3. Non-sensory nuclei lag and express mRNA at PND 14. Alpha-2B mRNA is transiently expressed at high levels in developing cerebellum and striatum and is enriched in cerebellar Purkinje cells as early as E21, then disappears with subsequent development [57].

Alpha-2C mRNA is highly expressed within the CA1 pyramidal cell layer of the rat at PND1 and decreases slightly by adulthood. This subtype also displays transient developmental enrichments in the molecular layer of the cerebellum that disappear with adulthood [58]. In many areas of the developing brain, including the olfactory system and hippocampus, alpha2-C mRNA is expressed at adult levels at birth [58].

In the majority of rat brain regions alpha-2 adrenergic receptor protein increases progressively during the postnatal period [23;59;60]. Alpha-2 adrenergic receptor density in most regions increases after birth and reaches maximum levels at about PND 15 [61]. The period between PND 10 to 15 appears to be particularly important because nearly all brain regions exhibit a major increase in alpha-2 receptor levels in the rat. This is a period of intense synaptogenesis in rat brain and the increase in alpha-2 adrenergic receptor densities is consistent with this. Thereafter receptor levels remain the same or slightly decrease to adult levels.

A more detailed anatomical analysis indicates there is considerable variation from region to region in development of alpha-2 adrenergic receptors [61]. Although alpha-2 adrenergic receptors reach their highest levels in many brain regions during the second postnatal week, a time of intense synaptogenesis in the rat, the timing and pattern of alpha-2 receptor expression during development varies from region to region. In some regions, such as some cortical and limbic system areas, receptor density increases beyond adult levels and then declines later in the postnatal period to adult levels. In a few regions, such as the striatum, there is a transient spike in receptor expression two to three weeks postnatally. In other rat brain regions, such as locus coeruleus and some midbrain nuclei, alpha-2 adrenergic receptors show significant early development and have near adult receptor densities at birth. Finally there are regions in which receptor levels are transiently expressed at very high levels early in the postnatal period and then decline to very low levels in the adult or disappear entirely. These areas include the cerebellum, several white matter tracts and many brain stem nuclei [61;62].

Particularly interesting examples of the latter are the corpus callosum and anterior commissure [62]. Alpha-2 adrenergic receptors are present in these fiber tracts as early as gestational day (GD20), and are expressed strongly throughout the first postnatal week. These receptors appear to be expressed in axons and perhaps in glia. On the other hand, *in situ* hybridization studies indicate the receptors are synthesized at sites distant from the white matter tracts. They are functionally linked to G proteins, as demonstrated with [^{35}S]GTPγS autoradiography, and so apparently play a role in the early development of these tracts, even though there is no obvious source of norepinephrine to stimulate them. By PND 14 their expression is largely eliminated and very few remain in white matter tracts of the adult animal, suggesting their function in these structures is limited to the developmental period.

Beta Adrenergic receptors—The postnatal development of beta adrenergic receptors has been analyzed primarily with membrane binding techniques. As a result, there is relatively little anatomical information on development and apparently no quantitative anatomical data. Beta adrenergic receptors have been detected in the forebrain of rats very shortly after final differentiation of noradrenergic neurons [63]. However, their levels remain relatively constant until birth, after which beta adrenergic receptors develop rapidly, reaching adults levels within two to three weeks of age.

In the rat cerebral cortex, beta adrenergic receptors are very low in density shortly after birth. In the second postnatal week there is a very rapid increase in receptor density and greater than adult levels of receptor are reached by PND 15 to 21 [64;65]. This increase is followed by a slow decline to adult levels over the next several months [65;66]. Throughout cortical development, the proportion of beta-1 to beta-2 receptors remains relatively constant, with beta-1 receptors making up about 80% of the total [65].

The increase in beta receptor density is paralleled by an increase in catecholamine-stimulated adenylyl cyclase activity [64;67], a marker for beta adrenergic receptor function. Total adenylyl cyclase activity develops steadily and more slowly than beta adrenergic receptor expression from the prenatal period on, reaching adult levels at about one month of age. Norepinephrine-stimulated adenylate cyclase activity correlates with beta adrenergic receptor development rather than adenylyl cyclase activity development, indicating that the receptors are fully functional throughout development and that receptor expression governs development of receptor function [64;67].

Cerebellar beta adrenergic receptor development follows a slower time course, as would be expected for a late developing brain region. Beta adrenergic receptor levels in the rat are very low at PND 5 and increase gradually but steadily, attaining adult levels at about 6 weeks of age [65]. In cerebellum development of beta-1 and beta-2 receptors follows distinct patterns, in contrast to cortex. Although beta-2 receptors are always the dominant subtype, in the early postnatal period beta1 receptors make up almost 20% of the total. After peaking in density at day 20, beta-1 receptors decline slowly in cerebellum, reaching their final low density at PND 90, accounting for about 2% of the total beta adrenergic receptor population at that time [65]. Thus, total beta adrenergic receptor and subtype developmental patterns are distinct for different rat brain regions. Similar findings for beta-1 and beta-2 receptors have been reported by others [66], although differences in methodology and brain dissections make comparisons difficult. The time course of beta adrenergic receptor development is similar in other species, although due to the scarcity of studies and differences in methods it is difficult to make exact comparisons. The development of beta adrenergic receptors in human brain has not been reported.

Serotonergic Receptors

The family of serotonin receptors consists of seven types (5HT1–7), classified according to their pharmacological, structural and signal transduction characteristics. Several of these main types have multiple subtypes (i.e., 1A, 1B, 1D, 1E, 1F, 2A, 2B, 2C, 5A, 5B). One, the 5HT3, is a ligand-gated ion channel, and the others are all G protein-coupled receptors [68]. As a general rule, serotonergic receptors in both humans and rats attain peak levels in fetal or early neonatal life and then decrease to adult levels [69].

High densities of 5-HT1A receptors ([^{3}H]8-OH-DPAT binding), 3- to 6-fold higher than adult levels, are present the human fetal brain between the 16th and 22nd weeks of gestation, but the neuroanatomical distribution of the receptor is similar to the adult pattern [70]. For example, at 16 weeks of gestation in the frontal cortex and the CA 1 field of the hippocampus, the receptor levels are 4- and 5-fold higher respectively than in the adult. In the hippocampal dentate gyrus the 5-HT1A receptor density at 16 weeks of gestation is 6-fold higher than in the adult [70].

Receptors for serotonin also exhibit high densities during development in the rat and cat. At birth, rat 5-HT1 receptors (serotonin binding) in the brainstem are expressed at higher than adult levels and appear to be fully functional [71]. By day 15 the levels are similar to those in the adult. In the striatum, [^{3}H]5-HT binding is only 12 % of adult levels at birth but increases rapidly to 150% of adult levels by 3 weeks of age, whereas in the hippocampus and cortex, the levels increase to 75% of adult levels by 3 weeks [72]. In the rat visual cortex, levels of [^{3}H]

5-HT binding is twice that of the adult on the fourth day after birth, and then decrease to adult levels [73]. Similarly in cat occipital cortex the amount of serotonin binding is close that seen in the adult at birth and peaks at over twice adult levels at week 4 after birth [29].

In the rat lateral lemniscus, 5-HT1A receptor levels ([^{3}H]8-OH-DPAT binding) are higher at birth than in the adult, and peak at 2 weeks of age before declining to adult levels [74]. By contrast, the 5-HT1A receptor in the dentate gyrus is at about 30% of adult levels at birth and increases to adult levels by 3 weeks of age [74].

Rat 5-HT 2 receptors (whole brain), measured with [^{3}H]ketanserin increased 8-fold between embryonic day 17 and postnatal day 13, and then declined to adult levels [75]. The ontogenetic distribution of 5-HT2C, 5-HT5A, and 5-HT7 receptors had been reported in four regions of the rat hippocampus using immunocytochemical techniques. In general, the receptor levels are highest at birth and then decrease to adult levels. In several brain regions for the 5-HT5A, and 5-HT7, the lowest level was observed at postnatal day 11, and then the recover slightly to adult levels [76]. Studies of the development of other serotonin receptors have not been published.

The expression of the mRNA for the $G_{i/o}$ coupled 5-HT1 serotonin receptor subtypes (5-HT1A, 1B, 1D and 1F) during fetal and early postnatal mouse forebrain development has been determined by in situ hybridization [77]. All 5-HT1 receptor subtypes are expressed by embryonic day (E) 14.5 in the forebrain. The 5-HT1A transcript is expressed densely in E14.5–16.5 thalamus, in hippocampus, and in a medial to lateral gradient in cortex, whereas the 5-HT1B receptor mRNA is expressed in more lateral parts of the dorsal thalamus and in the striatum at these ages. The 5-HT1D message is also expressed heavily in E14.5–E16.5 thalamus and then decreases by birth. The 5-HT1F mRNA is present in proliferative regions such as the cortical ventricular zone, ganglionic eminences, and medial aspects of the thalamus at E14.5–16.5 [77].

While limited both in terms of the number of receptors and species examined, the findings on norepinephrine and serotonin receptor development parallel the findings with other markers for these neuronal systems. In general, the serotonin receptors develop more rapidly and reach adult levels earlier compared to norepinephrine receptors. This is again consistent with the earlier clinical effectiveness of antidepressant drugs which preferentially act on serotonin neurons compared to drugs acting preferentially on norepinephrine neurons.

Development of Neurotransmitter Transporters

Norepinephrine Transporter

The innervation of the CNS by noradrenergic neurons is widespread and these neurons are known to be involved in many aspects of CNS physiology [78]. The norepinephrine transporter (NET) plays a major role in regulating noradrenergic signaling. It is, therefore, not surprising that NET is the target of several drugs, including antidepressants and many drugs of abuse. Since NET is the target of several antidepressants that are ineffective in juveniles but highly effective in adults, the developmental expression of this protein is naturally of interest in analysis of this differential response in depression treatment. Similar to studies on receptors, analysis of the development of NET has, to present, been carried out almost exclusively in rodents.

NET density of expression in rats increases with development in forebrain regions [79]. At PND 5 and 10 the transporter is expressed at very low levels throughout the brain, but by PND 15 NET density increases greatly in most forebrain regions. From PND 15 to 25 a higher level of NET expression is evident that subsequently decreases slightly in many areas of the adult brain. By contrast, brainstem and cerebellum NET expression is highest during early postnatal

development and decreases dramatically in the adult brain. This was true for the brainstem as a whole and for discrete brainstem regions, such as the noradrenergic nucleus A5 and the locus coeruleus.

Development of NET temporally coincides with the development of adult noradrenergic innervation. It has been reported, for example, that noradrenergic innervation attains adult patterns and densities during the second to third week of postnatal development in the rat [80] and an adult-like pattern of transporter expression emerges during this same time frame. NET expression increases most dramatically between PND 10 and PND 15, the same time frame during which forebrain alpha-2 adrenergic receptors demonstrate a large increase [61].

Studies of noradrenergic innervation of the rat brain have found a steady increase in noradrenergic fiber density and synapses during the first two postnatal weeks [81]. The lack of synchrony between these two markers for noradrenergic neurons suggests that the developmental increases in NET during this five day period (PND 10 to PND 15) may be attributable to the robust synaptogenesis that exceeds the appearance of new terminals during this stage of brain development. Large increases in immediate early gene expression have also been documented within this period in the rat and are attributed to synaptogenesis [82]. The finding that forebrain NET densities slightly decrease from PND 25 to adulthood [79] is consistent with studies which found a similar decrease in NET between PND 25 and adulthood within the frontal cortex [83]. It suggests a final developmental pruning of noradrenergic innervation takes place during this time.

In summary, studies reveal a dynamic CNS ontogeny of the NET. There is sparse forebrain NET expression during the first ten days of postnatal development. At PND 15 NET density has increased substantially, developmentally coinciding with adult noradrenergic terminal distribution and locus coeruleus activity. Developmental increases in forebrain contrast with decreases in hindbrain structures. The early presence of NET in hindbrain suggests an important developmental role for the NET and/or norepinephrine in these structures and suggests that the development of this region may be susceptible to antidepressants and CNS stimulants with high affinities for the NET.

Serotonin Transporter

The high-affinity serotonin transporter (SERT) is a marker of serotoninergic axons and allows visualization of serotonin afferents. There are few developmental studies of this serotonergic marker. The development of the serotonin transporter in the human cerebral cortex has been investigated using antibodies to SERT. SERT-positive fibers reach the cortical anlage at gestational week 8, reach the subplate at gestational week 10 and enter the cortical plate at gestational week 13 [38].

In rats mRNA for SERT can be found as early as E10, making it one of the earliest markers for neurotransmitters to be detected [84]. By E13 there is strong expression of message for SERT in the raphe nuclei and this continues into adulthood. There is also a coincident strong expression of tryptophan hydroxylase immunoreactivity from birth onward, supporting the identification of these neurons as serotonergic [84]. The uptake of [^{14}C]5-HT is fully at adult levels at birth in rat brain synaptosomes. By five weeks of age, the amount of uptake is double that seen in the adult and then decreases to adult levels [85].

In addition to development in serotonergic neurons, there is transient expression of SERT mRNA in many brain regions in neurons that do not appear to be serotonergic [84;86]. This transient expression appears to be exclusive to SERT among the monoamine transporters. Whether all of these regions also express the protein has not yet been determined and the significance of this transient expression is not clear. Nevertheless, since this expression occurs

during prenatal and postanal development, it suggests additional brain regions in which exposure to drugs acting on SERT may alter the normal course of development.

Even though the data for NET are very limited in terms of species and for SERT in the number and extent of studies published, the results are again consistent with the idea that the serotonin system, in this case SERT, develops more quickly and reaches maturity at an earlier time point than the norepinephrine system. This would lead to drugs acting on SERT producing effects similar to those found in adults earlier in the course of development compared to drugs acting on NET. It also suggests that drugs acting on SERT could produce changes in CNS development at an earlier stage than would be found for drugs acting on NET.

Functional development of Neurotransmitter Systems

Norepinephrine

Characterization of the functional development of neurotransmitter systems in the CNS depends not only on the neurotransmitter, its receptors and the metabolic machinery involved, but also upon the behavior, functional linkage or signaling pathway under consideration. Because signal transduction assays for some receptors, such as the alpha-1 adrenergic receptors, are difficult and/or not highly sensitive, particularly when the amount of tissue available is very small, there have been few studies of the functional development of adrenergic systems. To date, studies of the functional development of alpha-1 adrenergic receptors have not been published.

Studies of electrical activity in the developing locus coeruleus have shown that the spontaneous firing rate of noradrenergic neurons in rats of PND 7 to 18 is significantly greater than that seen in PND 1 to 6. Furthermore the adult spontaneous firing rate in locus coeruleus is reached by PND 20 [80]. This study illustrates a timeline of emerging LC activity which is consistent with emergence of adult innervation patterns and profiles of norepinephrine transporter expression.

Because the adrenergic receptors are G protein-coupled receptors, the first sites of interaction for the receptors are with the G proteins themselves. This linkage can be assayed using [^{35}S]GTPγS assays. However, because [^{35}S]GTPγS assays of receptors linked to $G_{i/o}$ proteins are much more sensitive than assays for receptors linked to other G proteins, nearly all developmental work has focused on $G_{i/o}$ receptors. In the case of the adrenergic receptors, these are the alpha-2 adrenergic receptors. In forebrain the alpha-2 adrenergic receptor agonist-stimulated [^{35}S]GTPγS binding increases rapidly after PND 7, reaching highest levels at PND 21 and then declining slightly to adult levels [59]. This binding increases more slowly than receptor number, suggesting the appearance of G proteins, rather than alpha-2 adrenergic receptors, determines the developmental appearance of functional receptors in forebrain. In agreement with this, basal [^{35}S]GTPγS binding and [^{35}S]GTPγS binding stimulated by other neurotransmitter receptor systems (GABA-B, mu opiate and muscarinic) increase with a time course similar to alpha-2 adrenergic receptor-stimulated [^{35}S]GTPγS binding. In contrast, in hindbrain alpha-2 adrenergic receptor-stimulated [^{35}S]GTPγS binding decreases during postnatal development in parallel with the decrease in alpha-2 adrenergic receptor levels, whereas [^{35}S]GTPγS binding stimulated by other neurotransmitter receptor systems increases in parallel with basal [^{35}S]GTPγS binding. Functional receptor-G protein coupling in hindbrain, as in forebrain, appears dependent on the developmental appearance of G proteins for most neurotransmitter systems. However, for alpha-2 adrenergic receptors the decrease in receptor density is the overriding factor [59]. Other studies have shown a functional linkage for alpha-2 adrenergic receptors as early as GD 20 using the [^{35}S]GTPγS autoradiographic approach [62].

We and others have shown that developing and mature brains are differentially regulated by the noradrenergic system. The developing brain responds to increased norepinephrine levels by up-regulating the density of alpha-2 adrenergic receptors. This response differs from that in the adult brain, which down-regulates alpha-2 adrenergic receptors in response to increased synaptic norepinephrine, and up-regulates alpha-2 adrenergic receptors in its absence [87–92]. The developing brain further differs from the adult in the regulation of immediate early genes by norepinephrine. Depletion of norepinephrine produces little or no change in cortical IEG levels throughout most of postnatal development, whereas in adults the loss of norepinephrine leads to a decrease in IEG expression (J. Sanders, unpublished data). Another difference in function of alpha-2 adrenergic receptors in developing and mature animals has been shown in studies that demonstrate that stimulation of alpha-2 adrenergic receptors activates ERK in developing but not in adult hippocampus [93].

On a behavioral level, the alpha-2 adrenergic receptor agonist clonidine produces a dose-dependent hyperactivity (increases in locomotion, wall climbing and head raising) in 7-day old rats, and to a lesser extent in 14-day old rats [94–96]. A dramatic shift occurs at day 21 when the same doses of clonidine now induce catalepsy. The cataleptic effect of clonidine is also observed at day 28, but declines markedly in frequency by day 35 [94]. In the adult rat, clonidine produces a depression of activity without catalepsy. This represents another example in which a drug acting on the noradrenergic system produces effects that differ in juveniles compared to adults.

Functional development of beta adrenergic receptors has been examined, albeit briefly, via analysis of stimulation of adenylyl cyclase by beta adrenergic receptors, a second step in the signal transduction pathway. Increased cyclic AMP production by beta adrenergic receptor agonists increases dramatically in the first two weeks postnatally. This change parallels changes in beta adrenergic receptor density and is not tightly correlated with increases in basal adenylyl cyclase activity, indicating that functional linkage to regulation of cyclic AMP levels is dependent on the receptors themselves and not on the appearance of adenylyl cyclase [64].

The observed timeline for mature noradrenergic regulation correlates roughly with the attainment of adult synaptic densities [22;97]. The rat brain exhibits very few synapses until PND 10. Then, between PND 10 and 15 and PND 20 and 30 the brain undergoes robust increases in synapse density. Around PND 25–30 this peaks, and a small decline occurs between PND 30 and 60, at which point the adult density of synapses is achieved [22]. The characteristic adult regulation of the brain by norepinephrine does not occur until after peak synaptogenesis has occurred. Therefore it appears that cellular changes conferred by the process of synapse formation and elimination are necessary prerequisites for the adult regulation of the brain by norepinephrine.

Serotonin

Postsynaptic serotonin-sensitive adenylate cyclase is found in various structures of the central nervous system in the rat and the guinea pig [98]. At birth, the regional distribution of this enzyme in the rat brain was closely correlated with the topographical distribution of the serotonergic innervation in young (9-day-old) as well as adult animals, and appears to be associated with postsynaptic serotonin receptors in the brain. During development, in both the rat and the guinea pig, the amount of cyclic AMP formed in response to an optimal concentration of serotonin in a given area remained constant, indicating that this functional system in these species is essentially mature at birth [98]. In both the rat hippocampus and cerebral cortex, serotonin-sensitive adenylate cyclase is higher than adult levels at birth and peaks at about 3 times adult levels between 2 and 3 weeks of age [72]. By contrast in the striatum, the serotonin-sensitive cyclase is only 20% of adult levels at birth and increases to 150% of birth levels by 3 weeks of age [72].

Activation of 5-HT1A receptors in rats produces hypothermia and several behaviors known collectively as the serotonin syndrome. The age of onset for most serotonin syndrome behaviors induced by 8-OH-DPAT is the first week of life. These behaviors attain maximal intensities at ages 7 to 14 days, and then decrease [99]. By contrast, agonist-induced hypothermia is relatively constant beginning at one week of age. Stimulation of 5-HT2A receptors produces a characteristic behavior known as wet-dog shakes. The onset of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced wet-dog shakes occurs after PD 14, it reaches its maximal intensity by PD 18, which persists up to PD 60, and then declines [99].

As would be expected, the data examining functions of noradrenergic and serotonergic systems parallels studies examining the biochemical foundations for these functions. In general, the function of serotonergic systems develops earlier and attains adult status much more quickly than function of noradrenergic systems.

Conclusion

The dominant pattern that emerges from the studies summarized here that is relevant to the effect of drugs on the central nervous system is that serotonergic systems generally develop more quickly and attain adult configurations earlier in development than noradrenergic systems. Table 1 compares the time during development that various parameters associated with the central adrenergic and serotonergic systems of several species reach adult levels. In all cases, the serotonergic parameters reach adult levels much sooner than the adrenergic parameters, indicating the more rapid maturation of the serotonergic system. In addition to this general pattern, there are both regional differences in the development of these neurotransmitters as well as transient expression of some markers, particularly the serotonin transporter, which may impact the effects of drugs on CNS function. Consideration of these detailed differences is beyond the scope of this review.

There is considerable evidence that both the noradrenergic and serotonergic systems play important roles in regulating the development of the central nervous system. The differences in development between these two systems suggest that drugs acting on serotonergic neurons may be more likely to have adverse effects during earlier stages of development when compared to drugs acting primarily on noradrenergic systems. This could be an important factor in treatment of depression during pregnancy.

Tricyclic antidepressants, monoamine oxidase inhibitors and venlafaxine are effective in the treatment of adult depression, but do not appear to be effective in children [1–3;100]. Much of the clinical efficacy of these agents is attributable to their actions on the noradrenergic system. In contrast, antidepressants that work primarily on the serotonergic system, such as the serotonin selective reuptake inhibitors (SSRIs), are effective in treating childhood and adolescent depression [3;100]. The temporal differences in development of these two neurotransmitter systems may provide at least a partial explanation for this. Markers for the serotonergic system reach adult stages of development more rapidly than markers for the noradrenergic system, in some cases far more rapidly. If the beneficial effects of antidepressant drugs in the treatment of depression require the neurotransmitter system on which they act predominantly to be fully mature, it would be expected that drugs acting on the serotonergic system would be effective earlier in development than drugs acting primarily on noradrenergic systems. These insights may lead to new and better therapeutic approaches for the treatment of childhood and adolescent depression, as well as other disorders for this age group.

Acknowledgements

Supported by grants from the National Institutes of Health MH064772 and MH066959.

Reference List

1. Geller B, Reising D, Leonard HL, Riddle MA, Walsh BT. Critical review of tricyclic antidepressant use in children and adolescents. J Am Acad Child Adolesc Psychiatry 1999;38:513–516. [PubMed: 10230182]
2. Hazell P, O'Connell D, Heathcote D, Henry D. Tricyclic drugs for depression in children and adolescents. Cochrane Database Syst Rev 2000:CD002317. [PubMed: 10908557]
3. Whittington CJ, Kendall T, Fonagy P, Cottrell D, Cotgrove A, Boddington E. Selective serotonin reuptake inhibitors in childhood depression: systematic review of published versus unpublished data. Lancet 2004;363:1341–1345. [PubMed: 15110490]
4. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev 2003;27:3–18. [PubMed: 12732219]
5. Antelman SM, Levine J, Gershon S. Time-dependent sensitization: the odyssey of a scientific heresy from the laboratory to the door of the clinic. Mol Psychiatry 2000;5:350–356. [PubMed: 10889544]
6. Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 1997;25:192–216. [PubMed: 9403138]
7. Steketee JD. Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. Brain Res Brain Res Rev 2003;41:203–228. [PubMed: 12663081]
8. Tirelli E, Laviola G, Adriani W. Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neurosci Biobehav Rev 2003;27:163–178. [PubMed: 12732232]
9. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3:79–83. [PubMed: 118862]
10. Sundstrom E, Kolare S, Souverbie F, Samuelsson EB, Pschera H, Lunell NO, Seiger A. Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. Brain Res Dev Brain Res 1993;75:1–12.
11. Verney C. Distribution of the catecholaminergic neurons in the central nervous system of human embryos and fetuses. Microscopy Research and Technique 1999;46:24–47. [PubMed: 10402270]
12. Lambe EK, Krimer LS, Goldman-Rakic PS. Differential postnatal development of catecholamine and serotonin inputs to identified neurons in prefrontal cortex of rhesus monkey. J Neurosci 2000;20:8780–8787. [PubMed: 11102486]
13. Lackovic Z, Jakupcevic M, Bunarevic A, Damjanov I, Relja M, Kostovic I. Serotonin and norepinephrine in the spinal cord of man. Brain Res 1988;443:199–203. [PubMed: 2451990]
14. Brown RM, Goldman PS. Catecholamines in neocortex of rhesus monkeys: regional distribution and ontogenetic development. Brain Research 1977;124:576–580. [PubMed: 404000]
15. Goldman-Rakic PS, Brown RM. Postnatal development of monoamine content and synthesis in the cerebral cortex of rhesus monkeys. Developmental Brain Research 1982;4:339–349.
16. Lauder JM, Bloom FE. Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. J Comp Neurol 1974;155:469–481. [PubMed: 4847734]
17. Thomas SA, Matsumoto AM, Palmiter RD. Noradrenaline is essential for mouse fetal development. Nature 1995;374:643–646. [PubMed: 7715704]
18. Coyle JT. Biochemical aspects of neurotransmission in the developing brain. Int Rev Neurobiol 1977;20:65–103. [PubMed: 22512]
19. Loizou LA. The postnatal ontogeny of monoamine-containing neurones in the central nervous system of the albino rat. Brain Res 1972;40:395–418. [PubMed: 4537284]
20. Coyle JT, Molliver ME. Major innervation of newborn rat cortex by monoaminergic neurons. Science 1977;196:444–447. [PubMed: 850788]
21. Berger-Sweeney J, Hohmann CF. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. Behavioral Brain Research 1997;86:121–142.
22. Markus EJ, Petit TL. Neocortical synaptogenesis, aging, and behavior: lifespan development in the motor-sensory system of the rat. Exp Neurol 1987;96:262–278. [PubMed: 3569455]

23. Morris MJ, Dausse JP, Devynck MA, Meyer P. Ontogeny of a_1 and a_2-adrenoceptors in rat brain. Brain Res 1980;190:268–271. [PubMed: 6247010]
24. Loizou LA, Salt P. Regional changes in monoamines of the rat brain during postnatal development. Brain Res 1970;20:467–470. [PubMed: 5433101]
25. Konkol RJ, Bendeich EG, Breese GR. A biochemical and morphological study of the altered growth pattern of central catecholamine neurons following 6-hydroxydopamine. Brain Research 1978;140:125–135. [PubMed: 626876]
26. Rho JM, Storey TW. Molecular ontogeny of major neurotransmitter receptor systems in the mammalian central nervous system: norepinephrine, dopamine, serotonin, acetylcholine, and glycine. J Child Neurol 2001;16:271–280. [PubMed: 11332462]
27. Rubenstein JL. Development of serotonergic neurons and their projections. Biol Psychiatry 1998;44:145–150. [PubMed: 9693386]
28. Levallois C, Valence C, Baldet P, Privat A. Morphological and morphometric analysis of serotonin-containing neurons in primary dissociated cultures of human rhombencephalon: A study of development. Brain Res Dev Brain Res 1997;99:243–252.
29. Jonsson G, Kasamatsu T. Maturation of monoamine neurotransmitters and receptors in cat occipital cortex during postnatal critical period. Exp Brain Res 1983;50:449–458. [PubMed: 6315470]
30. Golden GS. A review of the neuroembryology of monoamine systems. Brain Res Bull 1982;9:553–558. [PubMed: 6129048]
31. Aitken AR, Tork I. Early development of serotonin-containing neurons and pathways as seen in wholemount preparations of the fetal rat brain. J Comp Neurol 1988;274:32–47. [PubMed: 3047187]
32. Wallace JA, Lauder JM. Development of the serotonergic system in the rat embryo: an immunocytochemical study. Brain Res Bull 1983;10:459–479. [PubMed: 6344960]
33. Lidov HG, Molliver ME. Immunohistochemical study of the development of serotonergic neurons in the rat CNS. Brain Res Bull 1982;9:559–604. [PubMed: 6756556]
34. Lauder JM, Bloom FE. Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. II. Synaptogenesis. J Comp Neurol 1975;163:251–264. [PubMed: 240873]
35. Dori I, Dinopoulos A, Blue ME, Parnavelas JG. Regional differences in the ontogeny of the serotonergic projection to the cerebral cortex. Exp Neurol 1996;138:1–14. [PubMed: 8593886]
36. Dinopoulos A, Dori I, Parnavelas JG. The serotonin innervation of the basal forebrain shows a transient phase during development. Brain Res Dev Brain Res 1997;99:38–52.
37. Whitaker-Azmitia PM. Behavioral and cellular consequences of increasing serotonergic activity during brain development: A role in autism? Int J Dev Neurosci 2005;23:75–83. [PubMed: 15730889]
38. Verney C, Lebrand C, Gaspar P. Changing distribution of monoaminergic markers in the developing human cerebral cortex with special emphasis on the serotonin transporter. Anatomic Record 2002;267:87–93.
39. Levitt P, Rakic P. The time of genesis, embryonic origin and differentiation of the brain stem monoamine neurons in the rhesus monkey. Developmental Brain Research 1982;4:35–57.
40. Coyle JT, Axelrod J. Tyrosine hydroxylase in rat brain: developmental characteristics. J Neurochem 1972;19:1117–1123. [PubMed: 4401682]
41. Coyle JT, Henry D. Catecholamines in fetal and newborn rat brain. J Neurochem 1973;21:61–67. [PubMed: 4146461]
42. Coyle JT, Axelrod J. Dopamine-beta-hydroxylase in the rat brain: developmental characteristics. J Neurochem 1972;19:449–459. [PubMed: 4621978]
43. Lamprecht F, Coyle JT. Dopa decarboxylase in the developing rat brain. Brain es 1972;41:503–506.
44. Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Ann Neurol 1999;45:287–295. [PubMed: 10072042]
45. Kato T, Yamaguchi T, Togari A, Nagatsu T, Yajima T, Maeda N, Kumegawa M. Ontogenesis of monoamine-synthesizing enzyme activities and biopterin levels in rat brain or salivary glands, and the effects of thyroxine administration. J Neurochem 1982;38:896–901. [PubMed: 6121004]
46. Lauder JM. Ontogeny of the serotonergic system in the rat: serotonin as a developmental signal. Ann NY Acad Sci 1990;600:297–314. [PubMed: 2252317]

47. Lauder JM. Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci 1993;16:233–240. [PubMed: 7688165]
48. Hein L, Kobilka BK. Adrenergic receptors - From molecular structure to in vivo function. Trends Cardiovasc Med 1997;7:137–145.
49. Bylund DB. Subtypes of a_1- and a_2-adrenergic receptor. FASEB J 1992;6:832–839. [PubMed: 1346768]
50. Jones LS, Gauger LL, Davis JN, Slotkin TA, Bartolme JV. Postnatal development of brain alpha$_1$-adrenergic receptors: in vitro autoradiography with [^{125}I]HEAT in normal rats and rats treated with alpha-difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase. Neuroscience 1985;15:1195–1202. [PubMed: 2864658]
51. Slotkin TA, Kudlacz EM, Lappi SE, Tayyeb MI, Seidler FJ. Fetal terbutaline exposure causes selective postnatal increases in cerebellar a-adrenergic receptor binding. Life Sci 1990;47:2051–2057. [PubMed: 2177130]
52. Jia WWG, Liu Y, Lepore F, Ptito M, Cynader M. Development and regulation of alpha adrenoceptors in kitten visual cortex. Neuroscience 1994;63:179–190. [PubMed: 7898647]
53. Kable JW, Murrin LC, Bylund DB. In vivo gene modification elucidates subtype-specific functions of a_2-adrenergic receptors. J Pharmacol Exp Ther 2000;293:1–7. [PubMed: 10734146]
54. Venkatesan C, Song XZ, Go CG, Kurose H, Aoki C. Cellular and subcellular distribution of a_{2A}-adrenergic receptors in the visual cortex of neonatal and adult rats. J Comp Neurol 1996;365:79–95. [PubMed: 8821443]
55. Bylund DB, Blaxall HS, Iversen LJ, Caron MG, Lefkowitz RJ, Lomasney JW. Pharmacological characteristics of a_2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. Mol Pharm 1992;42:1–5.
56. Winzer-Serhan UH, Raymon HK, Broide RS, Chen Y, Leslie FM. Expression of a_2 adrenoceptors during rat brain development - I. a_{2A} messenger RNA expression. Neuroscience 1997;76:241–260. [PubMed: 8971775]
57. Winzer-Serhan UH, Leslie FM. a_{2B} Adrenoceptor mRNA expression during rat brain development. Developmental Brain Research 1997;100:90–100. [PubMed: 9174250]
58. Winzer-Serhan UH, Raymon HK, Broide RS, Chen Y, Leslie FM. Expression of a_2 adrenoceptors during rat brain development - II. a_{2C} messenger RNA expression and [^{3}H]rauwolscine binding. Neuroscience 1997;76:261–272. [PubMed: 8971776]
59. Happe HK, Bylund DB, Murrin LC. *Alpha*-2 adrenergic receptor functional coupling to G proteins in rat brain during postnatal development. J Pharmacol Exp Ther 1999;288:1134–1142. [PubMed: 10027851]
60. Bartolome JV, Kavlock RJ, Cowdery T, Orband-Miller L, Slotkin TA. Development of adrenergic receptor binding sites in brain regions of the neonatal rat: effects of prenatal or postnatal exposure to methylmercury. Neurotoxicol 1987;8:1–14.
61. Happe HK, Coulter CL, Gerety ME, Sanders JD, O'Rourke M, Bylund DB, Murrin LC. Alpha-2 adrenergic receptor development in rat CNS: an autoradiographic study. Neuroscience 2004;123:167–178. [PubMed: 14667451]
62. Sanders JD, Happe HK, Murrin LC. A transient expression of functional alpha2-adrenergic receptors in white matter of the developing brain. Synapse 2005;57:213–222. [PubMed: 15986363]
63. Schlumpf M, Bruinink A, Lichtensteiger W, Cortes R, Palacios JM, Pazos A. Beta-adrenergic binding sites in fetal rat central nervous system and pineal gland: their relation to other receptor sites. Dev Pharmacol Ther 1987;10:422–435. [PubMed: 2824144]
64. Harden TK, Wolfe BB, Sporn JR, Perkins JP, Molinoff PB. Ontogeny of b-adrenergic receptors in rat cerebral cortex. Brain Res 1977;125:99–108. [PubMed: 192417]
65. Pittman RN, Minneman KP, Molinoff PB. Ontogeny of b_1- and b_2-adrenergic receptors in rat cerebellum and cerebral cortex. Brain Res 1980;188:357–368. [PubMed: 6245756]
66. Erdtsieck-Ernste BHW, Feenstra MGP, Boer GJ. Pre- and postnatal developmental changes of adrenoceptor subtypes in rat brain. J Neurochem 1991;57:897–903. [PubMed: 1677680]
67. Bruinink A, Lichtensteiger W. b-Adrenergic binding sites in fetal rat brain. Journal of Neurochemistry 1984;43:578–581. [PubMed: 6330304]

68. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacology Biochemistry and Behavior 2002;71:533–554.
69. Whitaker-Azmitia PM. Role of serotonin and other neurotransmitter receptors in brain development: Basis for developmental pharmacology. Pharmacol Rev 1991;43:553–561. [PubMed: 1663620]
70. Bar-Peled O, Gross-Isseroff R, Ben-Hur H, Hoskins I, Groner Y, Biegon A. Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT1A receptors. Neurosci Lett 1991;127:173–176. [PubMed: 1831889]
71. Whitaker-Azmitia PM, Lauder JM, Shemmer A, Azmitia EC. Postnatal changes in serotonin receptors following prenatal alterations in serotonin levels: Further evidence for functional fetal serotonin receptors. Brain Res 1987;430:285–289. [PubMed: 2955853]
72. Nelson DL, Herbet A, Adrien J, Bockaert J, Hamon M. Serotonin-sensitive adenylate cyclase and [3H]serotonin binding sites in the CNS of the rat--II. Respective regional and subcellular distributions and ontogenetic developments. Biochem Pharmacol 1980;29:2455–2463. [PubMed: 7426052]
73. Uzbekov MG, Murphy S, Rose SP. Ontogenesis of serotonin ‘receptors’ in different regions of rat brain. Brain Res 1979;168:195–199. [PubMed: 455080]
74. Daval G, Verge D, Becerril A, Gozlan H, Spampinato U, Hamon M. Transient expression of 5-HT1A receptor binding sites in some areas of the rat CNS during postnatal development. Int J Dev Neurosci 1987;5:171–180. [PubMed: 2972174]
75. Roth BL, Hamblin MW, Ciaranello RD. Developmental regulation of 5-HT2 and 5-HT1c mRNA and receptor levels. Brain Res Dev Brain Res 1991;58:51–58.
76. Garcia-Alcocer G, Segura LC, Garcia PM, Martinez-Torres A, Miledi R. Ontogenetic distribution of 5-HT2C, 5-HT5A, and 5-HT7 receptors in the rat hippocampus. Gene Expr 2006;13:53–57. [PubMed: 16572590]
77. Bonnin A, Peng W, Hewlett W, Levitt P. Expression mapping of 5-HT1 serotonin receptor subtypes during fetal and early postnatal mouse forebrain development. Neuroscience 2006;141:781–794. [PubMed: 16824687]
78. Foote, SL.; Aston-Jones, GS. Pharmacology and physiology of central noradrenergic systems. In: Bloom, FE.; Kupfer, DJ., editors. Psychopharmacology: The Fourth Generation of Progress. Raven Press; New York: 1995. p. 334-345.
79. Sanders JD, Happe HK, Bylund DB, Murrin LC. Development of the norepinephrine transporter in the rat CNS. Neuroscience 2005;130:107–117. [PubMed: 15561429]
80. Nakamura S, Kimura F, Sakaguchi T. Postnatal development of electrical activity in the locus ceruleus. J Neurophysiol 1987;58:510–524. [PubMed: 3655880]
81. Latsari M, Dori I, Antonopoulos J, Chiotelli M, Dinopoulos A. Noradrenergic innervation of the developing and mature visual and motor cortex of the rat brain: a light and electron microscopic immunocytochemical analysis. J Comp Neurol 2002;445:145–158. [PubMed: 11891659]
82. Herms J, Zurmohle U, Schlingensiepen R, Brysch W, Schlingensiepen KH. Developmental expression of the transcription factor zif268 in rat brain. Neurosci Lett 1994;165:171–174. [PubMed: 8015720]
83. Moll GH, Mehnert C, Wicker M, Bock N, Rothenberger A, Ruther E, Huether G. Age-associated changes in the densities of presynaptic monoamine transporters in different regions of the rat brain from early juvenile life to late adulthood. Brain Res Dev Brain Res 2000;119:251–257.
84. Hansson SR, Mezey E, Hoffman BJ. Serotonin transporter messenger RNA in the developing rat brain: early expression in serotonergic neurons and transient expression in non-serotonergic neurons. Neuroscience 1998;83:1185–1201. [PubMed: 9502257]
85. Tissari AH. Pharmacological and ultrastructural maturation of serotonergic synapses during ontogeny. Med Biol 1975;53:1–14. [PubMed: 1095838]
86. Lebrand C, Cases O, Wehrle R, Blakely RD, Edwards RH, Gaspar P. Transient developmental expression of monoamine transporters in the rodent forebrain. J Comp Neurol 1998;401:506–524. [PubMed: 9826275]
87. Zahniser NR, Weiner GR, Worth T, Philpott K, Yasuda RP, Jonsson G, Dunwiddie TV. DSP4-induced noradrenergic lesions increase beta-adrenergic receptors and hippocampal electrophysiological responsiveness. Pharmacol Biochem Behav 1986;24:1397–1402. [PubMed: 3014567]

88. Dooley DJ, Bittiger H, Hauser KL, Bischoff SF, Waldmeier PC. Alteration of central alpha 2- and beta-adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin. Neuroscience 1983;9:889–898. [PubMed: 6312376]

89. Ribas C, Miralles A, Busquets X, Garcia-Sevilla JA. Brain alpha(2)-adrenoceptors in monoamine-depleted rats: increased receptor density, G coupling proteins, receptor turnover and receptor mRNA. Br J Pharmacol 2001;132:1467–1476. [PubMed: 11264240]

90. Tiong AH, Richardson JS. Beta-adrenoceptor and post-receptor components show different rates of desensitization to desipramine. Eur J Pharmacol 1990;188:411–415. [PubMed: 2164943]

91. Subhash MN, Nagaraja MR, Sharada S, Vinod KY. Cortical alpha-adrenoceptor downregulation by tricyclic antidepressants in the rat brain. Neurochem Int 2003;43:603–609. [PubMed: 12892647]

92. Daws LC, Lopez R, Frazer A. Effects of antidepressant treatment on inhibitory avoidance behavior and amygdaloid beta-adrenoceptors in rats. Neuropsychopharmacology 1998;19:300–313. [PubMed: 9718593]

93. Vanhoose AM, Emery M, Jimenez L, Winder DG. ERK activation by G-protein-coupled receptors in mouse brain is receptor identity-specific. J Biol Chem 2002;277:9049–9053. [PubMed: 11782465]

94. Reinstein DK, Isaacson RL. Clonidine sensitivity in the developing rat. Brain Research 1977;135:378–382. [PubMed: 562700]

95. Nomura Y, Segawa T. The effect of alpha-adrenoceptor antagonists and metiamide on clonidine-induced locomotor stimulation in the infant rat. Br J Pharmacol 1979;66:531–535. [PubMed: 37963]

96. Spear LP, Brick J. Cocaine-induced behavior in the developing rat. Behav Neural Biol 1979;26:401–415. [PubMed: 574000]

97. Nakamura H, Kobayashi S, Ohashi Y, Ando S. Age-changes of brain synapses and synaptic plasticity in response to an enriched environment. J Neurosci Res 1999;56:307–315. [PubMed: 10336260]

98. Enjalbert A, Bourgoin S, Hamon M, Adrien J, Bockaert J. Postsynaptic serotonin-sensitive adenylate cyclase in the central nervous system. I. Development and distribution of serotonin and dopamine-sensitive adenylate cyclases in rat and guinea pig brain. Mol Pharmacol 1978;14:2–10. [PubMed: 625285]

99. Darmani NA, Ahmad B. Long-term sequential determination of behavioral ontogeny of 5-HT1A and 5-HT2 receptor functions in the rat. J Pharmacol Exp Ther 1999;288:247–253. [PubMed: 9862777]

100. Varley CK. Psychopharmacological treatment of major depressive disorder in children and adolescents. JAMA 2003;290:1091–1093. [PubMed: 12941683]

Table 1

Indicators of maturation of adrenergic and serotonergic systems in the mammalian brain

Parameter / Species	Reaches Adult Levels	
	Norepinephrine	Serotonin
Innervation / Rat	5 weeks	3 weeks
Innervation / Monkey	2 years	2 weeks
Neurotransmitter / Rat	5 weeks	3 weeks
Neurotransmitter / monkey	2 years	2 months
Neurotransmitter / Cat	> 11 weeks	3 weeks
Transporters / Rat	3 weeks	birth