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Neurogenesis in adulthood: a possible role in learning

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The role of the hippocampal formation in learning and memory has long been recognized. However, despite decades of intensive research, the neurobiological basis of this process in the hippocampus remains enigmatic. Over 30 years ago, the production of new neurons was found to occur in the brains of adult rodents. More recently, the documentation of adult neurogenesis in the hippocampal formation of a variety of mammals, including humans, has suggested a novel approach towards understanding the biological bases of hippocampal function. Contemporary theories of hippocampal function include an important role for this brain region in associative learning. The addition of new neurons and consequently, their novel contribution to hippocampal circuitry could conceivably be a mechanism for relating spatially or temporally disparate events. In this review, we examine several lines of evidence suggesting that adult-generated neurons are involved in hippocampal-dependent learning. In particular, we examine the variables that modulate hippocampal neurogenesis in adulthood and their relation to learning and memory.

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The involvement of the hippocampal formation in learning and memory has been recognized for decades[1,2] but despite years of intensive research, virtually nothing is known about the biological basis of this function. A recent series of discoveries about the hippocampal formation suggests a new approach to addressing the cellular basis of learning and memory. Using histological markers of cell proliferation (Box 1), these studies have demonstrated that the production of new neurons, a process termed neurogenesis, occurs in a variety of adult mammals including mice, rats, tree-shrews, marmoset and macaque monkeys, and humans (Box 2). Stereological analyses have demonstrated that several thousand new hippocampal cells are produced each day in adult animals, the majority of which differentiate into granule neurons[3–6]. This article reviews the factors regulating hippocampal neurogenesis in adulthood and considers a possible role for adult-generated neurons.

Factors that regulate neuron production in adulthood

Recent studies have identified a number of factors that modulate adult hippocampal neurogenesis. If granule neurons produced in adulthood are necessary for hippocampal function in certain types of learning and memory, then regulatory factors that diminish the production of new neurons should be associated with impaired learning while those that enhance the production of new neurons should improve

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Box 1. Methods for visualizing new neurons

Demonstrating the production of new neurons in the adult brain requires evidence that a progenitor cell has divided in adulthood and that its daughter cells attain a neuronal phenotype. The classical method of demonstrating cell division is that of ^{3}H-thymidine autoradiography (Ref. a). For *in vivo* studies, this method involves injecting ^{3}H-thymidine into live animals. ^{3}H-thymidine is incorporated into cells that are in the DNA synthetic phase (S phase) of the cell cycle. Thus, depending on the survival time employed after labeling, ^{3}H-thymidine is a marker for proliferating cells (at short survival times) and their progeny (longer survival times). ^{3}H-thymidine incorporation can be visualized using autoradiographic techniques and, when combined with immunocytochemistry for cell-specific markers, can be utilized to determine the phenotype of new cells. A newer method, labeling with the thymidine analog bromodeoxyuridine (BrdU), has been employed recently for studies of adult neurogenesis (Refs b–e). Like thymidine, BrdU is incorporated into cells during S phase, and depending on the survival time, BrdU is a marker of proliferating cells or their progeny. BrdU labeling is a non-isotopic method that can be visualized with immunocytochemical techniques. When ^{3}H-thymidine or BrdU labeling is combined with other methods (see below), the phenotypes of new cells can be determined.

New neurons may be positively identified by the following:

(1) Ultrastructural evidence of synapses on new cells. The presence of synapses on cell bodies and dendrites of ^{3}H-thymidine-labeled cells in the dentate gyrus has been reported (Ref. f).

(2) Evidence of new cells extending axons into target regions. Retrograde tracer placed into the main target of hippocampal granule cells, the CA3 region, is incorporated by adult-generated ^{3}H-thymidine-labeled cells (Ref. g).

(3) Observations of new cells expressing neuronal-specific proteins. The following markers have been immunocytochemically detected in ^{3}H-thymidine or BrdU labeled cells: the markers of immature granule neurons PSA-NCAM (polysialated neural cell adhesion molecule) or TOAD-64 (Turned-On-After-Division 64 kDa), or the markers of mature granule neurons NeuN (Neuronal Nuclei), NSE (Neuron-Specific Enolase), MAP-2 (Microtubule-Associated-Protein 2), calbindin or the NMDA receptor subunit NR1 (Refs d,h,i). Lack of expression of astroglial markers, such as GFAP (glial fibrillary acidic protein) or S-100B, combined with nuclear morphology of granule neurons is considered indirect evidence of a neuronal phenotype (Refs d,h) (Fig. I).

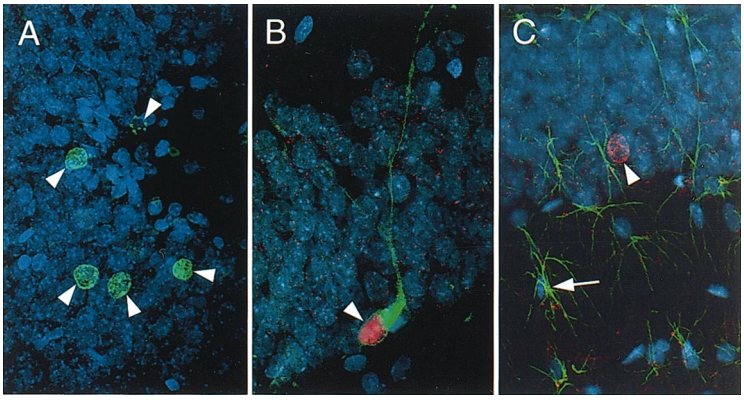

Fig. I. Confocal laser scanning microscopic images of new neurons in the dentate gyrus of adult rats. (A) BrdU labeled nuclei (arrowheads) in the granule cell layer (granule cells not labeled with BrdU are stained blue with the DNA dye Hoechst 33342). **(B)** Cell that is double labeled for BrdU (red) and TOAD-64 (green), a marker of immature granule neurons. **(C)** Cell that is labeled with BrdU (arrowhead, red), has the morphology of a granule neuron and is not co-labeled with the astroglial marker GFAP (arrow).

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learning. Indeed, as summarized in Table 1, available evidence from separate studies examining factors that regulate neurogenesis and those that affect learning are consistent with this view.

When considering factors that regulate adult neuron production, it is important to note that changes in the numbers of new cells are unlikely to have immediate functional consequences. This is particularly true when only the rate of cell proliferation is altered, because newly generated cells require time to become functionally integrated into existing circuitry. Although functional changes might not be immediately evident, factors could exert significant effects throughout the course of differentiation to influence function in the long term. However, these factors might have to act in a chronic, rather than an acute, fashion in order to have significant functional effects. Chronic changes in cell production and survival might be capable of exerting additive effects throughout the continual production, differentiation and integration of adult-generated neurons.

Hormonal levels

Endocrine factors regulate the proliferation of granule cell precursors in the adult dentate gyrus. Experimental elevations in the levels of the glucocorticoid corticosterone diminish the number of proliferating cells whereas removal of adrenal steroids stimulates cell proliferation and ultimately granule cell production in this region[7,8]. The suppressive action of glucocorticoids on neurogenesis is biologically relevant because conditions that are associated with elevated adrenal hormones, such as stress or aging[9], are also accompanied by diminished granule cell production in both rodents and primates[3,4,10]. Furthermore, conditions associated with prolonged inhibition of neurogenesis, such as chronic corticosterone treatment, chronic stress and aging, have been associated with diminished performance on hippocampal-dependent tasks[11–13]. Here it is particularly important to distinguish between acute and chronic studies when exploring the possibility that changes in adult neurogenesis alter hippocampal function. For example, acute

Box 2. History of adult neurogenesis

The first report of neuron production in the adult brain was published over 30 years ago by Joseph Altman and colleagues (Ref. a). A pioneer in the use of ^{3}H-thymidine autoradiography to birthdate neurons in the developing rat brain, Altman applied this technique to adult rats in which he discovered the production of new granule neurons in the dentate gyrus and olfactory bulb. These findings were largely unexplored for a considerable time. Throughout the 1970s and 1980s, a few studies further characterized the neuronal identity of adult-generated hippocampal cells by demonstrating that new cells receive synaptic input and extend axons into the mossy fiber pathway (Refs b,c). However, it was not until very recently that widespread interest and acceptance of adult neurogenesis in the hippocampal formation occurred.

In the 1980s, Fernando Nottebohm and colleagues published groundbreaking work on the existence of substantial neurogenesis in the song system of canaries (Ref. d). These observations were particularly intriguing because the production of new neurons correlated with an important behavior, song learning (Refs d,e), and laid the groundwork for consideration of adult-generated neurons as functionally important cellular entities. However, published reports insisted that neurogenesis is absent in the brains of adult Old World monkeys, *Macaca mulatta* (Refs f–h). In fact, it was argued that neurogenesis was not a feature of the adult primate brain because structural stability was an absolute requirement for the storage of memories over a long period of time. It was perhaps because of these negative reports that study of adult neurogenesis in mammals remained unexplored. In the past several years, however, evidence has been slowly mounting in support of adult neurogenesis in many mammals. In addition to adding further evidence that new cells become neurons in adult rodents, studies using bromodeoxyuridine labeling and stereological methods demonstrated that the number of new neurons produced in adulthood was relatively high. Recently, the existence of granule cell genesis was reported in adult tree shrews, animals considered to be phylogenetically between insectivores and primates (Ref. i) followed by the first reports of neurogenesis in the dentate gyrus of nonhuman primates, marmosets (Ref. j) and macaques (Ref. k). The report by Ericksson and colleagues of a substantial number of new neurons in the dentate gyrus of adult humans (Ref. l) shows that adult hippocampal neurogenesis has been conserved throughout primate evolution.

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stress has been shown to facilitate certain types of learning. Although transient elevations in stress hormones might impact the production and maturation of adult-generated neurons, stress-enhanced learning[14] is more likely to reflect the influence of the adrenal axis on existing neurocircuitry, for example through changes in synaptic efficacy.

In contrast to the suppressive effects of stress and corticosterone on cell proliferation, estrogen treatment has been shown to enhance the proliferation of granule cell precursors[15]. Chronic estrogen treatment, a condition that is likely to increase the pool of immature granule neurons[15], has, in independent studies, been associated with improved performance on spatial navigation tasks[16]. Conversely, low levels of estrogen (a condition known to diminish the proliferation of granule cell precursors[15]) have been linked to age-associated cognitive decline[17]. Here again, it is important to note that these studies focus on chronic changes in estrogen levels.

Environmental complexity

Environmental complexity enhances the survival of new neurons in adult birds and mice[5,18,19]. By injecting the DNA synthesis marker ^{3}H-thymidine and examining newly generated neurons at later time points, Barnea and Nottebohm[18] determined that hippocampal neurons produced in adult black-capped chickadees are more likely to survive in animals living in the wild compared to those living in captivity. Additionally, these investigators found seasonal differences in the maintenance of adult-generated hippocampal neurons that correlate positively with engaging in seasonal changes in seed storage and retrieval, behaviors that involve spatial learning. These findings are supported by numerous studies showing that hippocampal volume is larger in birds that engage in spatial learning behaviors, such as parasitic brooding and long-distance migration[20,21]. Recent studies have extended this view to the mammalian brain. Kempermann *et al.*[5,19] have shown that more hippocampal granule neurons are maintained in mice living in 'enriched environment' conditions compared to laboratory caged controls. In the case of mice, the enhanced survival of new neurons is associated with improved performance on a hippocampal-dependent task, spatial navigation learning in a Morris water maze[5,19]. However, the extent to which the increase in the number of surviving granule cells following environmental enrichment directly contributes to improved hippocampal function remains unknown.

There are many variables that differ between the 'enriched environment' and laboratory-caged controls in the case of mice and between living in the wild and in captivity in the case of birds, including food availability, conspecific interaction and stress level. However, one intriguing possibility is that environmental complexity might rescue new hippocampal neurons by increasing opportunities for learning.

Table 1. Regulators of neurogenesis and their effects on hippocampal-dependent learning

Factor	Proliferation of precursor cells	Survival of new cells	Learning
Glucocorticoids	decrease[8]	–	decrease[12]
Stress	decrease[3,4]	–	decrease[11]
Aging	decrease[10,44]	–	decrease[13]
Estrogen	increase[15]	–	increase[16]
Enriched environment	–	increase[5,19]	increase[5,19]
Learning	–	increase[25]	–

The pool of immature neurons can be enhanced by stimulating cell proliferation or by preventing the death of newly generated cells. Chronic manipulations of these factors lead to diminished performance on hippocampal-dependent tasks when they are associated with decreased numbers of new neurons. Conversely, chronic manipulations of factors that enhance neurogenesis are associated with improved performance on hippocampal-dependent tasks. Dashes represent conditions for which no data are available.

Specific learning experience

Recently, it has been demonstrated that training in hippocampal-dependent learning tasks increases the number of new granule cells. In naive laboratory controls, the majority of new neurons degenerate within two weeks of their production[6]. We found that either of two hippocampal-dependent tasks, place learning in a Morris water maze[22] or trace eyeblink conditioning[23], was sufficient to rescue new granule cells from death (see also Ref. 24). In the Morris water maze, the rat uses extramaze spatial cues to find a hidden platform in a pool of water. In trace eyeblink conditioning, the rat learns to associate two stimuli separated temporally (during the trace interval), a conditioned stimulus (CS; white noise) with an unconditioned stimulus (US; shock to the eyelid). Training for as little as four days on either the spatial water maze or trace conditioning task resulted in the rescue of many new granule neurons from death[25]. Several thousand new cells are produced every day in the dentate gyrus of adult rats; hippocampal-dependent training more than doubled this number. This effect can be attributed to learning, and not merely general experience, because exposure of animals to the same environment and conditions in the absence of overt learning had no effect on the number of new neurons. Control animals exposed to unpaired CS and US presentations (eyeblink conditioning), and those that spent the same amount of time in the water in the absence of a platform (water maze training), showed no difference in the number of new neurons compared to naive animals. Furthermore, learning tasks that do not require the hippocampus, delay eyeblink conditioning and cue learning in a Morris water maze[22,26], did not alter the number of new granule neurons. Delay eyeblink conditioning and cue training on maze tasks result in increased hippocampal neuronal excitability[27,28], although task acquisition does not require hippocampal integrity. Since these changes occur during both hippocampal-dependent and hippocampal-independent tasks, some influence of hippocampal-dependent tasks other than enhanced excitability, must be responsible for the maintenance of new cells.

The results of a recent study examining the effects of a number of different behavioral manipulations on new neurons in the dentate gyrus of adult female mice reported no change in BrdU-labeled cells following spatial training in a Morris water maze[29]. In that study, animals were injected with BrdU during spatial training. These results are essentially consistent with our results in that we also did not observe more labeled cells in the dentate gyrus of animals injected with BrdU during trace eyeblink conditioning[25]. Rather, we only observed an enhanced survival of cells that were generated prior to training. Rescue of adult-generated cells by certain types of learning might occur only during a specific 'sensitive period' following the production of those new cells[24,25]. The results of our study suggest that some new cells require this type of input for survival between one and two weeks after mitosis, a time when adult-generated granule cells might be forming connections with the CA3 region. Hippocampal-dependent learning might enable the integration of new neurons into existing circuitry and ensure their survival.

If new neurons participate in hippocampal-dependent learning, then the increased survival of these neurons as a result of learning might enhance performance on hippocampal-dependent tasks. To date, no evidence supports the view that learning one hippocampal-dependent task enhances performance on another. However, the association between hippocampal-dependent learning and the maintenance of adult-generated neurons suggests that new neurons might be involved in, as well as affected by, these types of learning.

It might be relevant that a substantial number of new neurons persisted in the dentate gyrus of all control groups[25]. The possibility that these cells were maintained by learning of an unspecified nature remains undetermined. Alternatively, basal levels of hippocampal activity might maintain a certain proportion of adult-generated cells. However, these studies indicate that hippocampal-dependent learning, and not merely generalized experience or neuronal activation, is required to enhance the number of adult-generated granule neurons beyond levels observed in control animals.

In summary, these results indicate a strong association between the number of immature neurons and hippocampal function in learning. However, given the extensive action of many of these variables on the adult brain, the possibility that such experimental manipulations alter neurogenesis and learning independently cannot be ruled out. A direct test of the functional role of hippocampal neurons will require experiments designed to examine the behavioral effects of specifically preventing neurogenesis in adulthood.

A role for new neurons in hippocampal-dependent learning?

The prevalent theories of hippocampal function are each compatible with a role for new hippocampal neurons. One view suggests that the hippocampus is an associator of discontiguous events[30]. The presence of a pool of new neurons accompanied by the emergence of new synapses, could play a role in connecting two stimuli with disparate temporal or spatial parameters. Another view of hippocampal function entails long-term changes in synaptic efficacy as a mechanism for learning. Although no studies have examined plasticity at synapses involving adult-generated neurons, it is reasonable to suggest that, given their immature status, synapses of new neurons are at least as modifiable, if not more so, than those of older neurons.

A number of studies support the view that the hippocampus plays a temporary role in storing memories. This theory is supported by data showing that recent, but not remote, memories for hippocampal-dependent tasks are abolished by hippocampal lesions[31]. A rapidly changing population of adult-generated neurons would be particularly suitable as a substrate for such a transient role of the hippocampal formation in memory storage. Neurons produced in adulthood might play a role in information processing related to memory storage during a discrete time after their generation. These cells might then degenerate or undergo changes in connectivity, gene expression, or both, coincident with the end of hippocampal storage of that particular memory. A temporary role for adult-generated granule neurons in learning would be similar to that observed in canaries, in which the seasonal modification of song correlates with recruitment of more new neurons into the song circuitry[32,33].

Do adult-generated neurons influence hippocampal function?

Although substantial granule cell genesis occurs into adulthood, this process begins during gestation and is maximal during the early postnatal period[34]. Thus, in adulthood the hippocampal formation comprises neurons with a wide range of ages (hours to years). Although the number of granule neurons produced in adulthood is large, on the order of a few thousand per day, this is a relatively small percentage when considered against a backdrop of the vast number of mature granule neurons produced during development (the total number of granule neurons is estimated to be between 1.5–2.0 million in the adult rat[35]). However, developmental studies suggest that young neurons have distinct structural and functional characteristics compared to mature neurons and adult-generated neurons probably share these immature features for a discrete period after their generation. It is these immature characteristics that might enable new neurons to exert a substantial influence on hippocampal function.

Adult-generated immature granule neurons might have unusual structural characteristics that have a proportionately larger effect on hippocampal physiology than the same number of mature neurons. For example, because of their presumed ability to form new synapses rapidly, immature neurons might be responsible for more new connections than mature neurons. Developmental studies have shown that granule neurons produced during the embryonic period extend axons rapidly, even while they are migrating[36]. Retrograde tracer studies have reported differences in the axonal terminal fields of granule neurons produced during different developmental time points. Granule cells produced during the early postnatal period have less divergent axonal terminals than those produced during gestation[36]. If this relationship continues into adulthood, adult-generated cells might have a substantial impact on a very small, localized population of CA3 pyramidal neurons to which they might project predominantly. Although synapses on cell bodies and dendrites of adult-generated granule neurons have been reported[37], the source of these inputs remains completely unknown. Neurons generated in adulthood might receive synaptic input from the same afferents as those produced during development but the possibility that these cells receive a differential pattern of inputs cannot be ruled out. Indeed, future studies are needed to determine the extent to which neurons produced in adulthood form circuits that differ from those produced during development.

Another intriguing possibility is that within the adult dentate gyrus, immature neurons differ electrophysiologically from mature neurons. Although it is likely that a neuroblast has very little functional impact immediately after it is formed, many developmental studies suggest that new neurons experience a transient, immature state during which their influence on hippocampal physiology is different, and possibly, greater than that of mature neurons. For example, several studies have demonstrated that LTP at perforant path/granule cell synapses appears relatively early in postnatal development and furthermore, that this change in synaptic strength lasts approximately five times as long as it does in adulthood[38–40]. Thus, when stimulated by perforant path inputs, adult-generated neurons might have a greater effect on their targets than mature neurons. Testing this possibility would require recording electrophysiologically from new granule neurons of known ages and their targets.

Further evidence that immature neurons differ from mature neurons comes from studies demonstrating that hippocampal neurons, including granule neurons, exhibit giant depolarizing action potentials during development[41]. This phenomenon, which is also seen in the pyramidal cell population during its immaturity is at least partially due to the excitatory response that activation of $GABA_A$ receptors has on these young neurons. During early development, GABA is an excitatory neurotransmitter for hippocampal neurons, acting in a similar manner to glutamate at AMPA receptors in maturity. Thus, NMDA receptor responses are dependent on $GABA_A$, as opposed to AMPA, receptor activation. This developmental difference in response to GABA is the result of membrane characteristics that alter the concentration of extracellular chloride ions[42,43]. Although this possibility has not been explored in the adult hippocampal formation, it is possible that adult-generated granule neurons have immature responses to neurotransmitters such that activation of inhibitory interneurons has a profound excitatory effect on these new cells while inhibiting existing mature granule neurons. Future studies will be necessary to determine whether granule neurons generated in adulthood

have a differential, and potentially greater, impact on hippocampal physiology than mature granule neurons.

Conclusions

First reported over 30 years ago, the phenomenon of adult neurogenesis in the hippocampal formation of the rat went virtually unnoticed for some time. Recent studies have re-investigated this issue and provide compelling proof that a substantial number of new cells generated in adulthood differentiate into neurons in the brains of a variety of mammals, including rats, mice, tree shrews, marmoset monkeys, macaque monkeys and humans[3–6,44,45]. The persistence of a high level of neuron production throughout adulthood and its taxonomic conservation suggest that this process is of fundamental biological significance. Although no studies have directly addressed the function of adult-generated neurons, several studies are consistent with a link between these new cells and learning. Negative regulators of neurogenesis, such as glucocorticoids, stress and aging, are associated with impaired performance on hippocampal-dependent learning tasks when applied chronically. On the other hand, positive regulators of neurogenesis, such as estrogen and living in an enriched environment are associated with enhanced performance on hippocampal-dependent learning tasks. The recent observation that hippocampal-dependent learning rescues new granule neurons from death suggests that adult-generated neurons are specifically affected by, and potentially involved in, hippocampal-dependent learning. Future studies designed to assess the behavioral consequences of preventing adult neurogenesis, either with precursor-specific cytotoxins or in transgenic mice, are necessary to determine whether new neurons play an essential role in hippocampal-dependent learning. If new neurons do indeed play a critical role in learning and memory processes, then studies of adult-generated neurons should provide novel insight into the cellular and molecular mechanisms underlying these processes in the adult mammalian brain.

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Outstanding questions

- Are adult-generated neurons involved in learning and memory? If so, are these cells involved in acquisition of hippocampal-dependent tasks, transient memory storage in the hippocampal formation or both?
- Do adult-generated cells form circuits that differ from those produced during development?
- Do other brain regions associated with learning and memory produce new neurons in adulthood? If so, do these neurons serve similar functions in all areas?
- Can specific genes and gene products be identified in adult-generated cells that respond to important regulatory cues?
- Are adult-generated neurons maintained by hippocampal-dependent learning useful for subsequent learning?

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Asperger syndrome: a simple matter of white matter?

Hadyn D. Ellis and Helen L. Gunter

Asperger syndrome, one of the Pervasive Developmental Disorders, is formally diagnosed on the basis of a cluster of cognitive, social and motor signs. It is also associated with poor visuo-spatial skills, good verbal performance, gauche social behaviour and clumsiness. Many of the difficulties evident in those with Asperger syndrome are closely associated with right-hemisphere dysfunction. In this respect they also resemble signs used to diagnose what has been labelled Nonverbal Learning Disorder. Here, these are treated as being the same or closely-related disorders with a possible common underlying aetiology; that is, a neurodevelopmental abnormality affecting white matter. This review examines the ability of this approach to account for a wide range of characteristics of the Asperger syndrome, and contrasts this with a theory-of-mind approach, which, although able to account for the primary features of Asperger syndrome, is less successful at explaining some of its secondary features.

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Asperger syndrome (AS), classed as one of the Pervasive Developmental Disorders, is characterized by a constellation of cognitive, social and motor criteria (World Health Organization ICD-10, 1990; American Psychiatric Association DSM-IV, 1994). These include: normal language development, coupled with problems of empathy and social understanding, stereotyped patterns of behaviour, odd prosody and poor motor skills[1].

In this article we present the thesis that, in many ways, AS can be explained as a deficit that primarily, but not exclusively, affects right-hemisphere performance. We shall also endorse the suggestion that the basic underlying problem is a neurodevelopmental one centred on incomplete or, in some other respect, dysfunctional white matter. Such a deficit, of course, might affect all aspects of brain activity but, we believe, it differentially disadvantages those activities

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